Mapping of Disease Resistance in Ornamentals: a Long Haul

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Abstract

Although disease control is of importance in ornamental production, breeding efforts to reach higher levels of resistance in most ornamental breeding programs have been relatively limited. If resistance is considered as a selection criterion it is often used at a relative late stage in the breeding process or when cultivars are in the trialling stage. This is due to a number of specific problems related to ornamental breeding such as the many different ornamental crops and ploidy level in a number of the most important ornamentals. Nevertheless, in the last few years a number of new developments have changed the roadmaps for research in the life sciences and also the feasibility of disease resistance mapping and marker assisted breeding in ornamentals. A number of examples of these new developments will be presented as well as some direct applications for disease mapping research in tulip will be shown.

INTRODUCTION

Genetic mapping of disease resistance and, as a result, the use of molecular markers in breeding of ornamentals is still at a modest level compared to other sectors in plant breeding. In most breeding schemes disease resistance is included at a late stage in the selection process leading to new cultivars. This may be due to a number of specific properties of ornamental crops (Debener, 2009). However, besides the large number of ornamental crops and relative small size of breeding companies which limit resources, there are also a number of crop specific limitations in ornamentals that have hindered the enrolment of molecular selection schemes. Quite a number of ornamentals are polyploids and/or have large genomes which are characteristics that need more input in time and resources for mapping of resistances. As few companies and research groups work on a particular crop these resources have been limited and could not be shared in larger scientific communities like in for instance vegetable research which would have speeded things up. Therefore advances, even in a big ornamental crop like rose with a single resistance gene as blackspot, have been extending over several years with the recent description of the causal gene as the latest step (Terefe-Ayana, 2011). Clearly disease mapping in ornamentals has been a matter of perseverance. Recent developments in phenotyping, genome sequencing and, marker development and genotyping, enable more opportunities for disease mapping studies in ornamentals and show a promise for the future. Some developments on genomic resources and mapping will be presented and two examples of phenotyping for disease resistance in tulip will be shown.

RESISTANCE BREEDING

Mapping of disease resistance and ultimately the use of markers in breeding need a number of steps to be made both on the disease causing organism as well as the disease host crop. There has to be knowledge on host diversity and specificity of the pathogen to understand whether there are different pathotypes (e.g., see Backer et al., 2011 for *Puccinia horiana* pathotypes in chrysanthemum white rust resistance). Simultaneously, resistant germplasm has to be identified and the genetics of the resistance has to be studied in the host plant. Of course the development of a disease test is essential for this. Based on these results a concerted choice can be made on the feasibility to proceed with genetic mapping and the use of markers for breeding. Especially resistance traits that are

difficult or lengthy c.q. costly to select for, are amendable for genetic selection. This could be resistances that are based on a number of QTLs, for which disease tests are difficult to reproduce due to the high influence of environmental factors or for disease caused be quarantine organisms (e.g., rust in chrysanthemum), in crops with a very long generation time and low reproduction factor (e.g., tulip) or polyploid crops in which presence of recessive alleles are often hidden (e.g., rose, chrysanthemum and alstroemeria).

GENETIC RESOURCE DEVELOPMENT: SEQUENCING PROGRESS

In the last five years a revolution in sequencing technologies has appeared with the massive parallel sequencing methods that be typed as Next Generation Sequencing (NGS) (Egan et al., 2012). These sequencing methods do not need prior information or cloning and can be performed parallel for many targets simultaneously at affordable costs. Even more, these costs have been steadily decreasing while output in numbers of reads and read length has been increasing. The sequencing capacity can be applied for the development of all kind of data that can be valuable for breeding efforts like for markers retrieval, EST resources (Hsiao et al., 2011) and expression studies.

New Developments in Molecular Markers and Genotyping

The development of these sequencing methods has led to a cascade of sequence data which enables to mine these sequences for polymorphisms that form the basis of the development of molecular markers. Single Nucleotide Polymorphism (SNP) markers as such will be the marker of choice for new molecular breeding programs as they are versatile, abundant and easily obtainable once bioinformatics pipelines are available. At the moment SNP retrieval and subsequent SNP genotyping is the most effective approach, for mapping applications in high-density maps or mapping polyploids Genotyping-by-Sequencing (GBS) strategies like Restriction-site Associated DNA (RAD) tag sequencing (Miller, 2007) or SureSelect in combination to Illumina sequencing may be used to combine SNP retrieval and genotyping.

Many ornamental genomes are too large to completely sequence to sufficient depth with the current techniques. Besides, it is not necessary to generate so much sequence information for SNP retrieval. An easy way to establish a so-called ‘complexity reduction’ is to sequence not genomic DNA but cDNA made from the mRNAs that are being expressed in one or more tissues (commonly referred to as RNA-seq or Whole Transcriptome Shotgun Sequencing). With the use of RNA-seq only a part of the whole genome will be sequenced whereas such an approach will also generate EST data and establish genetic resources that can be used for other purposes as well. Assembly is an important step for SNP retrieval (Shahin et al., 2012a) especially in case of large genomes that likely contain paralogs. Development of pipelines, in which both assembly and SNP calling and selection is incorporated, is in progress. Similarly, for SNP genotyping systems like GoldenGate and Infinium (Illumina) and KASP (Kbioscience/LCG) software is developed and will be further improved in tetraploids so that scores will be assigned to one of the five possible classes (AAAA, Aaaa, AAaa, AAAa and aaaa) to enable dosage designation. Whereas the first genetic maps in tetraploid rose that are recently published (Gar et al., 2011; Koning-Boucoiran et al., 2012) had to rely on single marker data input, the development of massive numbers of SNPs and the sequencing of larger stretches of sequence with GBS will enable the use of SNP haplotypes that may give a higher resolution in mapping by the identification of individual chromosomes.

CANDIDATE GENE APPROACHES

One of the problems in ornamental research in disease resistance mapping was the low number of groups that work on similar topics and could share molecular resources. The becoming available of genome sequences or gene space (e.g., for *Phalaenopsis*) of more and more species, especially those that have been model species for genetic studies can be very helpful in the annotation of de novo sequencing of transcriptomes in other

species and the indication of Candidate Genes (CGs) for certain traits. For instance the research in *Arabidopsis* has already led to new insights into the regulation of flowering and stress responses. Simultaneously, several genomes of pathogens have been sequenced that gave more insights into effector genes and genes determining pathogenicity. Also proteomics and metabolomics studies add data to the mechanisms that give or responses that lead to plant defence against pathogens. In gene-for-gene resistance effector screens have shown effective in searching for resistance genes that work in different ways (Vleeshouwers et al., 2011) to prevent resistance breakthrough by stacking these different genes. All this research that has led to better understanding of the genetic regulation of defence pathways or the presence of susceptibility genes (Pavan, 2010) can lead us to the identification of candidate genes (CG) for resistance or molecular switches for defence pathways in ornamentals. Recently published examples of CGs are for instance the study of Koskela et al. (2011) on gerbera *Botrytis* resistance or the studies of Cho et al. (2012) and Birkenbihl et al. (2012) on a WRKY transcription factor that seems to be a molecular switch to control defence against a number of pathogens. In the study of Koskela et al. (2011), information on biochemical pathways and previously obtained sequence data is combined and an antisense approach is used to show that the chalcone synthase-like polyketide synthase, 2-pyrone synthase (2PS) is involved in the defence pathway against *Botrytis*. Sequence data from EST resources can be used to further analyse this gene and related genes in mapping studies and expression studies to validate the role of these genes in resistance against *Botrytis*.

DEVELOPMENT OF TULIP BREEDING RESEARCH IN MAIN DISEASES

Tulips have a very long generation time of 5 years between seed and forcible bulb whereas the subsequent clonal production needed for further selection of promising genotypes takes another 10 to 15 years. Therefore, breeding of tulips would benefit from marker assisted selection. Disease resistance research at Wageningen UR in tulip started around 35 years ago. In tulip three main diseases are identified; *Fusarium oxysporum* f. sp. *tulipae*, *Botrytis tulipea* and *Tulip breaking virus* (TBV).

Fusarium Resistance in Tulip

Fusarium oxysporum is a very important fungal, soil-born disease in many flower bulb crops where it causes bulb rotting. The fungus occurs all over the world. The host range of *F. oxysporum* is possibly the widest of all known fungal plant pathogens. Individual strains have the ability to infect one or a few plant species only (Mes, 1999). Specialized forms of this fungus exist for *Tulipa*, *Gladiolus*, *Crocus*, *Hyacinthus*, *Narcissus*, lily and cyclamen and many more plant species. The fact that some of those specialized forms can also infect other plant species makes distinction sometimes difficult (Baayen et al., 1998).

The first step in *Fusarium* resistance breeding was made by Van Eijk et al. (1978) in which study a disease test was developed that lasted a whole growing season. Within *T. gesneriana* cultivars large differences in resistance were found. These results were subsequently used by Van Eijk et al. (1979, 1983) in juvenile and adults of the same genotype and in crossing populations to study the genetics of the resistance. Resistance in juveniles and adults showed a high correlation indicating the test can be used for pre-selection although a final selection at clonal stage remains required. *Fusarium oxysporum* resistance in tulip is assessed by counting the numbers of affected plants which needs the evaluation of large numbers of plants during a whole growing season. Later on this test has been replaced by a test on bulbs during the storage period in a climate room that was subsequently further optimized to a reliable test in which *Fusarium* can be scored in about 10 weeks and scoring is performed along a scale of 0 to 5 (unpublished).

Most recently, a new test has been developed by transforming *Fusarium* strains with Green Fluorescent Protein (Van der Lee, unpublished) and inoculating bulbs with these strains. This test only requires an incubation for 2 weeks at high humidity. Using camera imaging of fluorescent signals (Fig. 1) and automatic scoring, quantitative

assessments can be made of the growing speed of the fungus on individual bulbs. First results show a good correlation with previous results and these quantitative measurements will be used (hopefully) in mapping QTLs for *Fusarium*.

Botrytis tulipae Resistance

B. tulipae is a necrotropic pathogen which is able to kill its host tulip actively and is also able to live on dead tissue subsequently (Staats, 2007). As a leaf pathogen, it severely affects tulip bulb production. It has been shown that the developmental stage of the tulip plant has an enormous effect on the level of the resistance (Straathof et al., 2002). Therefore, *B. tulipae* experiments were all performed at the same developmental stage. A greenhouse resistance test was developed by Straathof et al. (2002) which involved rubbing of the leaf surface to remove the wax layer before infection. Water vaporisers were used to obtain leaf wetness and a high humidity. Spreading lesions on the inoculated leaves were rated on a scale of 1-5. Per plant an average disease rating (ADR) was calculated.

Most recently, a detached leaf assay was set-up in which the first (bottom, 4 inoculation spots) and second (middle, two inoculation spots) leaf of each plant was infected and stored in a climate chamber. Besides measuring the length and width of lesions with a ruler, lesions were measured using Chlorophyl activity assessment (H. Jalink, unpublished) in which dead and affected leaf tissue can be exactly distinguished from healthy leaf tissue (Fig. 2) giving an precise and quantitative measure of the infected area that can be used for QTL analyses. The test results on a number of reference cultivars show good concordance to previous observations and further analysis is in progress.

Tulip Breaking Virus

Tulip breaking virus is a serious problem in growing and forcing tulips. The virus causes breaking of the flower colour and reduction of bulb yield (Eikelboom et al., 1992). Transmission takes place through aphids in a non-persistent way, and can therefore spread through the field in a short period of time. After infection in the field in spring, the virus will be detectable in the bulb during the next storing period. The next growing season the whole plant is infected and shows leaf and flower symptoms. To screen cultivars for resistance, detection of the natural occurring virus is sometimes done by ELISA. To prevent virus infection fields are sprayed with mineral oil and insecticides against aphids. Furthermore, to keep the disease under control in production visual inspection of fields and removal of sick plants has to be done, which is very labour intensive. Therefore, researchers started to look for TBV-resistant cultivars using mechanic or aphid infection in a greenhouse test. Seven tulip cultivars, which are all members of the *T. fosteriana* cultivar group, show different levels of resistance to TBV (Eikelboom et al., 1992). A very high degree of resistance was found in the cultivars ‘Cantata’ and ‘Princeps’ (Straathof et al., 1996). These two cultivars are now used in introgression breeding of the virus resistance to *T. gesneriana*. The use of a field test, which is a good and cheap alternative to the previous greenhouse test for resistance is tested nowadays. Individual plants are planted in a randomized way, surrounded by TBV infected tulips. No aphid control is applied and aphid are deliberately introduced in the field. After one growing season individuals are checked for TBV infection visually and if necessary checked by ELISA.

Genetic Map for Tulip

From transcriptome sequencing using a 454 sequencing a set of around 80,000 unigenes was derived from which SNPs are identified (Shahin et al., 2012b). A first set of around 250 SNPs were mapped together with previously derived AFLP and NBS-profiling markers. The map is being made in a population from a cross between cultivars ‘Kees Nelis’ and ‘Cantate’ in which all three resistances are segregating.

CONCLUSIONS

A number of developments in especially genotyping and in the identification of possible Candidate Genes for disease resistance together with existing data on pathogens and progress in digital phenotyping will allow a faster turnaround time for disease mapping and increase the feasibility of the use of molecular markers in ornamental breeding.

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Figures

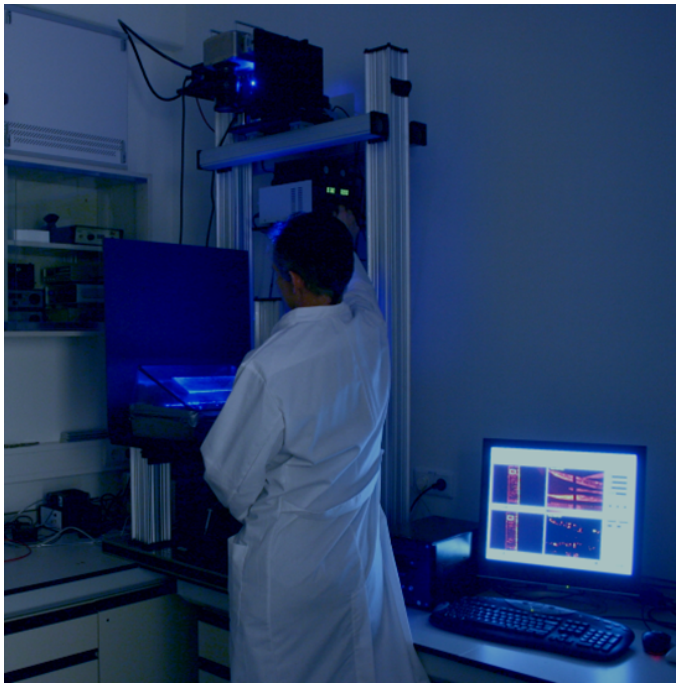
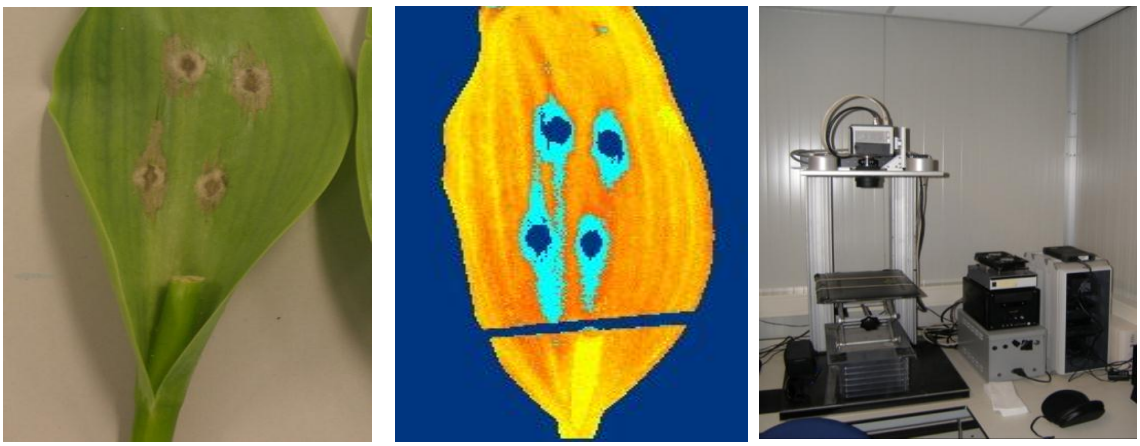


Fig. 1. Camera setting for Green Fluoresent Protein measurements (picture courtesy T. van der Lee).



a b c

Fig. 2. *Botrytis tulipea* test on bottom leaf under normal light (a), image from chlorophyl activity measurement (b) and camera setting (c) (picture courtesy H. Jalink).