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Resistance of Cool Season Food Legumes to *Ascochyta* Blight

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Summary: Legume cultivation is strongly hampered by the occurrence of Assochyta blights. Strategies of control have been developed but only marginal successes have been achieved. In the present paper we present and re-evaluate some recent developments in control in the light of recent developments in crop breeding and molecular genetics. The current focus in applied breeding is leveraging biotechnological tools to develop more and better markers to speed up the delivery of improved cultivars to the farmer. To date, however, progress in marker development and delivery of useful markers has been slow. The application of knowledge gained from basic genomic research and genetic engineering will contribute to more rapid crop legume improvement for resistance against Assochyta blight.

Key words: Ascochyta spp., biotechnology, crop management, food legumes, resistance

Introduction

Cool season food legumes provide an excellent source of high quality plant protein and have a key role in arable crop rotations, reducing the need for fertiliser application and acting as break-crops. However, cool season food legumes are affected by a number of foliar and root diseases that cause wide spread damage and in severe cases cause complete crop loss. The most important foliar diseases worldwide are Ascochyta blights. A number of strategies of Ascochyta blight control have been developed including cultural practices and chemical control. However, only marginal successes have been achieved, most control methods being uneconomical, hard to achieve or resulting in incomplete protection. Breeding for resistance is the most economic and environmentally friendly control method.

Breeding for Resistance

Sources of incomplete resistance have been identified in all of the cool season food legumes and the currently available resistance is being used in breeding programmes designed to develop cultivars with improved resistance. Since there are some recent reviews on breeding methods, screening procedures, the *Ascochyta*

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diseases and biology of the pathogens (Ye et al. 2002, Pande et al. 2005, Bretag et al. 2006, Tivoli et al. 2006, Sillero et al. 2010), in this review we will mainly focus on the recent developments in understanding the genetics of host resistance for each of the major cool season food legumes and point out immediate needs in research that in our opinion will further advance deployment of resistance in managing *Ascochyta* blight in cool season food legumes.

Ascochyta blight of pea is a disease complex caused by three pathogens: Ascochyta pisi which causes well-defined lesions (spots) on leaves, stems and pods; Phoma medicaginis var. pinodella, previously Ascochyta pinodella, which causes lesions on leaves and stems, and foot rot; and Didymella pinodes, previously Mycosphaerella pinodes which causes blight starting with small purple to black spots, enlarging and turning brown to black.

Resistance to *A. pisi* race C is controlled by a mayor gene modified by minor genes or QTLs (Darby et al. 1985, Dirlewanger et al. 1994). Some levels of incomplete resistance against *M. pinodes* and *P. medicaginis* have been reported (Ali et al. 1978, Kraft 1998, Wroth 1998, Prioul et al. 2003, Fondevilla et al. 2005). The majority of the genetic studies concerning resistance to *M. pinodes* in pea have concluded that resistance is a polygenic trait (Fondevilla et al. 2007). Numerous QTLs explaining from low to moderate percentage of the variation of the trait have been identified (Timmerman-Vaughan et al. 2002, Tar'an et

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al. 2003, Prioul et al. 2004, Fondevilla et al. 2008). In addition, candidate genes approached and comparative mapping have revealed the co-localization of QTLs for resistance to *M. pinodes* and resistance gene analogs, the putative transcription factor *PsDof1* and the pea defensin DRR230-b (Timmerman-Vaughan et at. 2002; Prioul-Gervais et al. 2007).

Incomplete levels of resistance to the Ascochyta blight of lentil (Ascochyta lentis, teleomorph Didymella lentis) is also available in lentil germplasm. Single genes, either dominant (Ford et al. 1999) or recessive (Chowdhury et al. 2001) have been reported together with molecular markers flanking the resistance gene. In addition, at least five QTL for blight resistance have been mapped that together accounted for 50% of phenotypic variation (Rubeena et al. 2006).

Ascochyta blight, caused by Ascochyta rabiei (teleomorph: Didymella rabiei), is responsible for widespread damage to chickpea crops worldwide. Incomplete resistance is available in chickpea germplasm (Reddy & Singh 1984, Singh & Reddy 1994) and is being used in breeding programs. Early inheritance studies concluded that resistance could be controlled by one, two or three genes (Singh & Reddy 1993, Collard et al. 2001, Chen et al. 2004). More recently, two major QTLs have been identified in LG4, conferring resistance to pathotype II. Other QTLs have been identified in LG2, mainly associated with resistance to pathotype I, (Santra et al. 2000, Tekeoglu et al. 2002, Collard et al. 2003, Rakshit et al. 2003, Udupa and Baum 2003, Cho et al. 2004, Iruela et al. 2006). Current efforts are focused in saturating these regions with additional markers. Some genes involved in defence co-localizing with resistance QTLs have been reported (Winter et al. 2000, Huettel et al. 2002, Flandez-Galvez et al. 2003, Cho et al. 2004, Iruela et al. 2009).

Ascochyta blight of faba bean is caused by Ascochyta fabae (teleomorph Didymella fabae). Incomplete levels of resistance have been identified in faba bean germplasm (Tivoli et al. 2006, Sillero et al. 2010) that are being used in breeding programmes to develop improved cultivars. The information about the genetic basis of the resistance has been conflicting since both polygenic and major gene inheritance have been suggested. Rashid et al. (1991) proposed a model consisting of 7 major genes controlling the resistance to 5 different isolates of A. fabae. Kophina et al. (2000) identified a major dominant gene as well as a complex of minor genes controlling resistance. More recently, Kharrat et al. (2006) suggested dominant monogenic control at the level of leaves and, in addition, a recessive gene controlling resistance of stems. Román et al. (2003) reported a polygenic control determined by at least two QTLs. In a subsequent study Avila et al. (2004) detected 6 additional QTLs.

Lessons to Learn from Model Legumes

Although genomic regions involved in resistance to Ascochyta blight and candidate genes located in these regions have been identified, still very little is known about the mechanisms of response to Ascochyta at the histological, molecular and biochemical level. Utilisation of model systems, together with the modern molecular biology tools will be of great help for understanding the physiological and genetic nature of the Ascochyta-host interaction (Dita et al. 2006, Rispail et al. 2010). Considering the high level of synteny between the genomes of the model plant Medicago truncatula and the economically important grain legume crops, the results obtained in this model legume should be transferred between species and used in breeding programs.

In M. truncatula several microarray platforms have been developed including an Affymetrix system that can be used to identify genes involved in M. truncatula defence against Ascochyta. Furthermore, this microarray can also be hybridized to cDNA from other legumes and has been already used to identify genes differentially expressed in response to M. pinodes in pea (Fondevilla et al. 2009). Other tools for this model legume include a library of more than 1000 transcription factors available at the Max-Planck Institute of Molecular Plant Physiology (Potsdam, Germany) that could be used to quantify the level of expression of these transcription factors in the interactions Medicago -M. pinodes by real-time quantitative PCR and high resolution genetic maps under construction that could facilitate the identification and cloning of genes and QTLs involved in resistance to Ascochyta by comparative mapping with crop legumes maps.

The omic tools developed for *M. truncatula* will allow us to investigate whether genes expressed in different processes in the model plant *M. truncatula*, including interaction with pathogens, are involved in the resistance to *Ascochyta* blight in crop legumes. However, these techniques demand previous sequence information knowledge, being limited to known genes. As a consequence, genes specifically involved in the *Ascochyta*-crop legumes interaction may not be detected. For this reason

these approaches could be complemented with large scale expression techniques such as cDNA-AFLPs, Supersage or Suppression Subtractive Hybridisation cDNA libraries that are able to identify novel genes.

Conclusions

Though limited in efficacy in many cases, the control methods available today represent a major progress when compared to the lack of any means for the control of these plants one or two decades ago. Crops can be protected by resistance, by selective fungicides, by biocontrol agents, and by cultural methods that did not existed before.

The current focus in applied breeding is leveraging biotechnological tools to develop more and better markers to allow marker –assisted selection with the hope that this will speed up the delivery of improved cultivars to the farmer. To date, however, progress in marker development and delivery of useful markers has been slow. We are now also facing an accelerated progress in the genomic and biotechnological research, which should soon provide important understanding of some crucial developmental mechanisms in both the pathogen and their host plants. The application of knowledge gained from basic genomic research and genetic engineering will contribute to more rapid legume improvement.

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Otpornost povrtarskih mahunarki umerenih klimata prema parazitima iz roda Ascochyta

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Izvod: Gajenje mahunarki je u velikoj meri otežano prisustvom bolesti koje prouzrokuju gljive iz roda Ascochyta. Razvijane su strategije u cilju njihovog suzbijanja, ali sa ograničenim uspehom. Rad prikazuje i ponovo ocenjuje neka od savremenih dostignuća mera suzbijanja u svetlu skorašnjih pomaka u oplemenjivanju biljaka i molekularnoj genetici. Primenjeno oplemenjivanje je trenutno usredsređeno na primenu biotehnoloških metoda u razvoju većeg broja boljih markera koji treba da ubrzaju stvaranje poboljšanih sorti. Međutim, do danas je napredak u razvoju markera uopšte i stvaranju korisnih markera bio spor. Primena znanja dobijenog putem osnovnih genomskih istraživanja i genetičkog inženjeringa doprineće bržem oplemenjivanju gajenih mahunarki na otpornosti prema parazitima iz roda Ascochyta.

Ključne reči: Ascochyta spp., biotehnologija, otpornost, povrtarske mahunarke, zaštita useva