Session III
Bob Henson Award
Student Competition
A COMPARISON OF PHENOTYPIC AND MARKER-ASSISTED SELECTION FOR ASCOCHYTA BLIGHT RESISTANCE IN CHICKPEA.

P. Castro1, M.D. Fernández-Romero2, T. Millán2, J. Gil2 and J. Rubio1, 1IFAPA, Córdoba Spain, 2Córdoba University, Córdoba, Spain. Email: patriciar castro@juntadeandalucia.es

Student presenter

Introduction

Ascochyta blight caused by Ascochyta rabiei (teleomorph: Didymella rabiei) is one of the most serious diseases of chickpea. QTL for resistance to blight have been located on linkage group 4 (LG4) (QTLAR1 and QTLAR2) and LG2 (QTLAR3) of the chickpea map (Iruela et al., 2007). Molecular markers have been associated with these QTLs for resistance to blight. Marker assisted selection (MAS) for Ascochyta blight resistance would greatly accelerate the development of new chickpea cultivars. In this study, MAS and phenotypic selection were employed to select blight resistant chickpea genotypes comparing the effectiveness of both methods.

Materials and Methods

600 F2 plants derived from the cross ILC3279 x WR315, resistant and susceptible to blight, were used to MAS and phenotypic selection. The genotyped of F2 plants was performed in a multiplex PCR. STMS GAA47 linked to QTLAR1, STMS TA72 and the SCAR SCY17 to QTLAR2 and the STMS TA194, TS82 and TRS8 to QTLAR3 were analyzed. Early generation testing method of F2-derived lines (Fehr, 1987) was used for resistance selection. Ascochyta blight was evaluated in the field using a 1 to 9 rating scale as proposed by Singh et al (1981). F2.5 resistant lines were also genotyped to confirm the presence of the alleles associated with the resistance.

Results and Discussion

The AUDPC data distribution in the F2:3 was skewed toward the susceptible parental. Only 58 out of the 558 F2:3 families evaluated were resistant. This data suggest that resistance to Ascochyta blight is recessive. Resistance was also confirmed in selected F2:4 lines. However, the markers TA72 and SCY17 (QTLAR2) exhibited a strong distorted segregation toward the susceptible parental with respect to the expected Mendelian inheritance (1:2:1) in F2 plants, GAA47 marker (QTLAR1) showed also distorted segregation although in less extension. All of these markers are located in LG4. Most of resistant F2:3 lines selected in the field were derived from heterozygous F2 plants according to the mentioned markers. These results suggest that resistance may be dominant. Markers linked to QTLAR3 were not associated to resistance. The GAA47 allele associated to resistance is being fixed in the F2:5 resistant lines. Markers linked to QTLAR2 were not clearly associated to resistance in the selected F2:5 lines probably due to the strong distorted segregation. In this study STMS GAA47 linked to QTLAR1 was the most reliable marker to predict resistant phenotype and it would be an useful marker in MAS for Ascochyta blight.

References


PARTIAL CLONING OF TWO POLYKETIDE SYNTHASE GENES ASSOCIATED WITH PATHOGENICITY OF ASCOCHYTA RABIEI.

J. A. Delgado, S. W. Meinhardt, S. G. Markell, and R. S. Goswami. Department of Plant Pathology, North Dakota State University, Fargo, ND, USA. Email: rubella.goswami@ndsu.edu. Student presenter.

Introduction
Ascochyla rabiei (Pass.) Labr., is the most important chickpea fungal pathogen in North Dakota. According to previous studies, solanapyrone (mycotoxin) and melanin have been associated with virulence in this pathogen. Solanapyrone crude extracts produce blight symptoms on chickpea leaflets (Hölh et al. 1991) and melanin deficient mutants are non pathogenic (Chen et al. 2004). Both metabolites are synthesized via the polyketide synthase pathway. Polyketide synthases (PKSs) are multdomain proteins and their minimum functional domain structure consists of beta-ketoacyl synthase (KS), acyltransferase (AT), and acyl carrier protein (ACP) domains (Hopwood and Sherman 1990). PKSs have been studied in other fungi, however, to date, little is known about the genetics of these pathogenicity determinants in A. rabiei. The work to be presented is part of a larger study that focuses on cloning PKS genes involved in A. rabiei pathogenicity.

Materials and Methods
A pathogenic pathotype II, A. rabiei isolate from chickpea fields in North Dakota was used for this study. An A. rabiei PKS nucleotide sequence available in Genbank was used to initiate the melanin PKS (PKS-Mel) cloning. However, no information of the solanapyrone PKS (PKS-Sol) nucleotide sequence was available. Therefore, initial portions of the PKS-Sol sequences were amplified using degenerate primers designed by alignment of reducing type I PKSs following which, both PKS nucleotide sequences were extended towards the N-terminus using new degenerate primers. Subsequently, a genome walking approach involving construction and screening of four genomic libraries with adaptor specific and gene specific primers was used to extend both PKS sequences towards the N- and C-terminus. The significant PCR products were then cloned and sequenced.

Results and Discussion
We have cloned a 3098 bp region likely to be related to PKS-Sol using degenerate primers and genome walking. The sequence matched several reducing type I PKSs involved in secondary metabolite biosynthesis. The most significant BLASTX match was to a PKS from Cochliobolus heterotrophus involved in the biosynthesis of T-toxin. Similarly, a 2466 bp region of a related PKS-Mel was amplified. It had a BLASTX match to several non-reducing type I PKSs involved in melanin biosynthesis with the most significant match being a PKS from C. heterotrophus. The translated amino acid sequences of both PKS-Sol and PKS-Mel matched the KS and AT domains using the Conserved Domain Search at the NCBI website. Thus, to date we have partially cloned two different PKS genes involved in the biosynthesis of secondary metabolites and melanin respectively from A. rabiei. These are believed to be associated with pathogenicity and further genome walking to obtain the complete gene sequences is in progress.

References
O16
COMPARATIVE POPULATION STUDY OF DIDYMELLA RABIEI IN TURKEY AND ISRAEL
H. Ozkilinc, O. Frenkel**, C. Can*, S. Abbo, D. Shtienberg*, A. Sherman*
*Department of Biology, University of Gaziantep, 27310, Turkey; ** The Hebrew University of Jerusalem, Rehovot 76100, Israel; "Department of Plant Pathology and Weed Research, ARO, The Volcani Center, Bet-Dagan 50250 and *Genomics Department, ARO, The Volcani Center, Bet-Dagan 50250, Israel E-mail:hilalozkilinc@hotmail.com
Student Presenter

Introduction
To properly infer the evolutionary history of an agricultural pathogen, it is necessary to sample pathogen populations from both domesticated and the wild hosts, especially from the host/pathogen's center of origin. D. rabiei isolates were sampled from domesticated chickpea and wild Cicer spp. in Israel and southeastern Turkey where chickpea was first domesticated. Different seasonality of the hosts and conditions in natural and agro-ecosystems may affect their pathogens. The main objectives of this study was to compare D. rabiei populations from wild and domesticated Cicer spp. in Israel and Turkey in view of population genetic structure and in-vitro temperature growth response of the pathogen. The results were evaluated using integrated genetic, ecological and evolutionary approaches.

Materials and Methods
A total of 128 D. rabiei isolates from naturally infected Turkish and Israeli domesticated chickpea and wild Cicer species (C. pinnatifidum and C. judaicum, respectively) were screened for variation at six STMS loci (1). The in vitro hyphal growth response of the 80 D. rabiei isolates was determined at 15°C and 25°C (2).

Results
The majority of the microsatellite variation occured within populations. The highest genetic diversity was detected within the Turkish D. rabiei populations from domesticated chickpea (H=0.68). Genetic distance analysis based on pooled allele frequencies within populations presented two main clusters of isolates from wild and domesticated Cicer spp. The model based Bayesian algorithm demonstrated the highest posterior probability for three populations among all isolates: while Turkish and Israeli isolates from domesticated chickpea took part in population 1 or population 2, most of the isolates from the wild Cicer spp. were strongly assigned to population 3.
Isolates from domesticated chickpea were significantly better adapted to 25°C and many isolates from wild host C. judaicum were adapted to both 15°C and 25°C.

Discussion
The six STMS markers and in vitro temperature responses of colony hyphal growth exposed a distinction between D. rabiei from the domesticated and wild Cicer spp. hosts. D. rabiei populations of domesticated chickpea may have diverged from its ancestral population on wild Cicer spp. and the pathogen populations evolved on their wild and domesticated hosts seperately in accord with ecological divergence. Turkish areas of the pathogen that exhibit high genetic diversity are likely to be important sources of host resistance genes, both among wild and domesticated Cicer spp. Studying the effect of the temperature on hyphal growth under controlled conditions provided useful information supporting the hypothesis regarding the evolutionary and ecologically effect of the cropping shift of chickpea on its pathogen D. rabiei.

References
O17
CLONING AND CHARACTERIZATION OF ANONYMOUS REGIONS OF ASCOCHYTA LENTIS AND A. FABAE GENOMES AND SUITABILITY OF THESE REGIONS FOR PHYLOGENETIC ANALYSIS OF ASCOCHYTA SPECIES.
J.E. Stewart, R.N. Attanayake, E.N. Njambere, T. Drader*, and T.L. Peever, Department of Plant Pathology, Department of Crops and Soils, Washington State University, Pullman, WA USA. Email:jestewart@wsu.edu
Student Presenter

Introduction
Ascochyta species cause blights on a number of wild and cultivated cool-season legume hosts, including chickpea (Cicer arietinum L.), faba bean (Vicia faba L.), lentil (Lens culinaris Medik.), pea (Pisum sativum L.), and vetches (Vicia spp.). Ascochyta blight of faba bean and lentil are caused by the host-specific fungi A. fabae Speg. and A. lentis Vassiljevsky, respectively. Identification of these species has been primarily based on host specificity because they are morphologically indistinguishable (Gossen et al. 1986). Previous studies have demonstrated that each species have distinct RAPD-PCR banding patterns (Kaiser et al. 1997), and each form a monophyletic group in a combined phylogeny estimated from glyceraldehyde-3-phosphate-dehydrogenase (G3PD), translation elongation factor alpha (EF), and chitin synthase (CHS) genes (Peever et al. 2007). No additional fast-evolving markers have been identified for these fungi that would facilitate research at the population/species interface. Therefore, the objective of this research was to develop sequence characterized anonymous region (SCAR) markers for identification of A. fabae and A. lentis, for estimating genetic variation within and among species, and for inferring phylogenetic relationships.

Materials and Methods
Two isolates of each species were used. Random Amplified Polymorphic DNA (RAPD)-PCR was performed using 40 decamer primers. Twenty clones were sequenced from each species. Sequence contigs were assembled, annotated, and sequence specific primers were designed. Sequences were analyzed for SNPs, insertion/deletions and restriction sites between A. fabae and A. lentis. A BLASTn search was conducted to determine sequence similarity to known genes. Phylogenetic signal was assessed for each developed SCAR locus.

Results and Discussion- Five primers were polymorphic between the species, resulting in 38 polymorphic amplicons. Direct cloning of RAPD-PCR amplicons resulted in 80 and 95 clones from A. fabae and A. lentis, respectively. A total of 7 intra- and inter-specific polymorphic SCAR markers were developed and characterized demonstrating the usefulness of the technique. BLASTn results of SCAR marker T1 revealed homology to SirH, a trichothecene acetyl transferase gene involved in sirodesmin phytotoxin pathway of Leptosphaeria maculans. These loci will prove useful for diagnostics and population genetics, phylogeographic, and phylogenetic studies to facilitate studies of speciation of these and related taxa.

References