Screening chickpea germplasm for ascochyta blight resistance

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Abstract

The ascochyta blight resistance of 14 chickpea cultivars, 29 imported chickpea lines and 38 local breeding lines to four Australian isolates of Ascochyta rabiei was investigated. Each isolate was tested individually on each chickpea genotype. There were no differences between the four isolates in their pathogenicity. However, there were large differences between chickpea genotypes in the expression of disease symptoms. All of the Australian chickpea cultivars tested were susceptible to A. rabiei. Seven imported lines and three local breeding lines were found to be resistant to A. rabiei. The eight isolates of A. rabiei tested in these studies were all the same mating type, i.e. MAT1-1.

Additional keywords: Ascochyta rabiei, mating types

Introduction

Blight, caused by Ascochyta rabiei (Pass.) Labr., is a devastating foliar disease of chickpea (Cicer arietinum L.) in many countries and has recently been found in commercial chickpea crops in Victoria, Australia (Nasir and Bretag 1997). The pathogen is seedborne, heterothallic with at least two mating types, and persists on host plants and infested crop residues (Kaiser 1997). The disease develops in epiphytotic proportions when the relative humidity is >60% and the temperature is between 10 and 20°C (Singh and Reddy 1990). Seedborne inoculum is important for the survival and dissemination of the ascochyta blight fungus into pathogen-free areas (Kaiser 1997). Necrotic lesions develop on all aerial parts of the plant and cause breaking of stems and death of plant parts above the affected area.

Fungicides such as chlorothalonil are sometimes used to control the disease, but their use is often uneconomical under epiphytotic conditions, because a minimum of four to six sprays can be required (Reddy and Singh 1983). The use of resistant cultivars appears to be the best management option for this disease (Porta-Puglia et al. 1996).

Screening of chickpea germplasm for disease resistance was initiated at the Victorian Institute for Dryland Agriculture, Horsham, Victoria, in order to identify sources of resistance to Victorian isolates of A. rabiei. Resistant lines will be made available to breeders for use in future chickpea breeding programs. In addition, it was important to determine the mating type of the local isolates of A. rabiei.

Methods

Chickpea germplasm Seeds of the chickpea accessions were obtained from the Australian Temperate Field Crops Collection (ATFCC), Horsham, Victoria. There were 14 commercial cultivars (Table 1), 29 imported chickpea accessions purported to be resistant to ascochyta blight (Table 2) and 38 breeding lines from the Australian Chickpea Breeding Program. Information on each
chickpea accession is available from Mr. Allan McIntyre, Curator of ATFCC. The breeding lines were grown in a glasshouse (temperature 20±4°C, relative humidity 70–80%) at Horsham to obtain sufficient quantities of fresh seed for the study.

**Fungal isolates** Eight single-spore isolates of *A. rabiei* (AR1, AR2, AR3, AR4, AR5, AR6, AR7 and AR8) were isolated from a commercial crop of chickpea cv. Desavic grown at Rosebery in the Mallee region of Victoria, Australia (Nasir and Bretag 1997). These isolates have been deposited in the herbaria at the Victorian Institute for Dryland Agriculture, Horsham and at Washington State University, Pullman, United States of America. All of the isolates were included in mating studies, but only isolates AR1, AR2, AR3 and AR4 were used in glasshouse testing of chickpea accessions for disease resistance. Conidia of *A. rabiei* were produced on chickpea-seed-extract agar (CSEA), prepared by boiling 150 g of chickpea seed in 500 mL of deionised water for 30 min, filtering through four layers of cheese cloth and supplementing with 20 g dextrose, 20 g agar and distilled water, to bring the total volume to 1 L. Inoculum was produced by seeding CSEA plates with 0.3 mL of an aqueous spore suspension obtained from a stock culture. Plates were incubated at room temperature (20±2.5°C) for 1 week under continuous illumination provided by a Phillips TLD 36W/08 ‘black light’ (320–420 nm). Conidial suspensions were prepared from 7-day-old CSEA cultures by adding sterile distilled water, rubbing the culture surface gently with a bent glass rod, and filtering through four layers of cheese cloth. The suspensions were adjusted to 1 × 10^5 conidia/mL with the aid of a haemocytometer. Tween 20 (one drop/100 mL) was added to the spore suspension as a wetting agent.

**Cultivation, inoculation and incubation** Six seeds of each accession were sown (2.5 cm deep) into composted pine-bark potting mix in ten plastic pots (10 cm diameter). Seedlings were thinned to five per pot 7 days after sowing. Plants were grown under glasshouse conditions (temperature 20±4°C, relative humidity 70–80%). There were two replicates, with each consisting of one pot of five plants of each accession inoculated with each isolate of *A. rabiei* and an uninoculated control.

As there were too many plants to inoculate in one day, replicate one was inoculated 14 days after sowing and replicate two 16 days after sowing, using the same methods. On each day, five plants (1 pot) of each accession were sprayed with a freshly prepared spore suspension (1 × 10^6 conidia/mL) of each isolate. The pots were rotated during the inoculation procedure to ensure an equal distribution of the spore suspension on the plants. For the uninoculated controls, five additional plants of each genotype were sprayed with sterile distilled water. After inoculation, plants were transferred into a humid chamber where the relative humidity was maintained at 95–100% and the temperature at 18±2°C. Plants were returned to the glasshouse after 48 h in the chamber.

**Disease assessments** The disease reactions of individual plants were assessed 15 days after inoculation on a 1–9 scale, similar to that described by Singh et al. (1981), where 1 = no lesions visible on plant; 3 = a few lesions on leaves but no stem lesions, no damage to plant; 5 = lesions common on leaves, small lesions on stem but causing little damage; 7 = lesions very common on leaves and stem, resulting in the death of some branches; and 9 = lesions profuse on all parts of the plant, stem girdling causing defoliation and the drying of branches (all young shoots killed), sometimes resulting in the death of a plant. The mean of the disease reactions for the five plants in each pot was calculated and these data were analysed with the aid of the statistical package Genstat 5. The data were subjected to analysis of variance and when F values indicated significant differences at a 5% level of probability, Duncan’s multiple–range test was used to separate means into subsets of homogeneous means, at a 5% level of probability.

**Matings** The procedure used to determine the mating type of eight local isolates of *A. rabiei* was similar to that used by Kaiser et al. (1997) with *A. fabae* Speg. and *A. lentis* Vassilievsky. Conidial suspensions (10^6 spores/mL) of each test isolate were mixed separately with analogous spore suspensions of MAT 1-1 and MAT 1-2 (Kaiser et al. 1997). Dried chickpea stem pieces (6 to 8 cm) were autoclaved and immersed for 1 h in the mixed spore suspensions. After 1 h, stem pieces were drained and placed in Petri dishes containing ten filter paper disks moistened with 15 mL of sterile distilled water. The dishes were incubated for 24 h at 20–22°C and then transferred to 10°C for 5 weeks. The Petri dishes were not sealed or placed in an airtight container. After 5 weeks, the stem pieces...