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Studies on the resistance of some Australasian chickpeas (*Cicer arietinum* L.) to *Phytophthora* root rot disease

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Abstract

Phytophthora root rot (PRR) is a disease of chickpea (*Cicer arietinum* L.) caused by the *Phytophthora megasperma* Drechs. f. sp. *medicaginis* (*Pmm*). It is the most serious disease for this crop in Australia because there is no strong resistance exists in current cultivars. Therefore, it is vitally important to identify the new source of resistance for the improvement of chickpea cultivars. A range of chickpea genotypes were evaluated in this study to identify sources of resistance to this disease. Three Australian chickpea varieties (Jimbour, Flipper, and Yorker) were used to assess the pathogenicity of 4 *Pmm* isolates (4019, 4021, 4027, and 4046). The most aggressive pathogenic isolate 4027 and a mixture of two other isolates were selected to assess the resistance of 16 international and Australian chickpea genotypes to PRR. The general score (GS) (0.5 × plant infection rate＋0.5 × plant death rate) was used in this study to indicate the severity of the disease. According to the GS value derived from the most aggressive isolate 4027, 4 genotypes (FLIP97-114C, ICCV 05111, ICCV 98818, and ICCV 96852) were considered resistant with a GS of 3.9 - 4.4, 3 genotypes (Bumper, ICCV 98801, and Yorker) were classified as moderately to resistant with a GS of 4.7 - 4.9, and the rest of them were susceptible with GS scores above 5.8. The large variation in pathogenicity observed for these isolates suggested that the 4 *Pmm* isolates represented different pathogen strains. Significant differences in plant infection rate, plant death rate, and disease development were observed among the chickpea genotypes. These findings indicated that the basis of resistance differed among the chickpea genotypes evaluated. The PRR resistance in chickpea is a multi-gene controlled trait. These resistant genotypes provided different sources of resistance and could be combined in breeding program to produce durable and high level of resistant cultivars.

Keywords: chickpea (*Cicer arietinum* L.); disease resistance; genotype; *phytophthora* root rot; plant death rate; plant infection rate.
Abbreviations: PRR, *phytophthora* root rot; *Pmm*, *Phytophthora megasperma* Drechs. f. sp. *Medicaginis*; PIR, Plant infection rate; PDR, plant death rate; DS, disease score; GS, general score.

Introduction

The production of chickpea *(Cicer arietinum* L.) in Australia is mainly confined to the eastern Australian states of Queensland, New South Wales, and Victoria. And it has increased steadily since its introduction in 1979. *Phytophthora megasperma* Drechs. f. sp. *medicaginis* (*Pmm*) is a soil borne fungus that causes *phytophthora* root rot (PRR) in chickpea. *Phytophthora* produces three kinds of asexual spores, sporangia, zoospores, and chlamydospores. Sporangia can germinate directly to produce hyphae or differentiate to produce 10 - 30 zoospores. Zoospores are aquatic and the production is typically triggered by the flooding both in the field and laboratory. They are the most important route to infect the plant roots, especially when the soil is flooded (Tyler, 2007). In many species, zoospores swim chemotactically toward the compounds released from the roots of the host plants (Tyler, 2002). The zoospores encyst on the root surface from where the hyphae penetrate the root directly from the cyst. Zoospores, as well as sporangia, can also be spread to the upper plant by splashing (Tyler, 2007). Phytopathogens can deliver effector proteins to the cells of the host plant to promote infection. These proteins can be sensed by the plant immune system, leading to the restricted growth of the pathogen (Devergne et al., 1994). They can also display signatures of positive selection and rapid evolution, presumably a consequence of their co-evolutionary arms race with plants (Boutemy et al., 2011). Yield losses caused by PRR were estimated at 50% for individual crop and 20% for a district (Knights et al., 2008). The severe occurrence of PRR and the limited amount of resistant cultivars available has presented the threat to the chickpea industry. Since there is no strong resistant cultivar exists, *Phytophthora* disease is primarily controlled using chemicals (Nene and Reddy, 1987), bacteria (Myatt et al., 1993), and crop rotation (Manning et al., 2000). However, the *Phytophthora* fungus can survive on other legume hosts, such as lucerne and pasture medics, in the soil for up to 3 - 4 years (Manning et al., 2000). The pathogen can also be spread from field to field by water and machinery (Manning et al., 2000) making it difficult to control. Improvement of disease resistance in chickpea cultivars is one of the most effective approaches to minimize the damage caused by this disease. However, genetic variability for disease resistance is critical if cultivars are to be improved through breeding (Irwin et al., 1995). Currently, only partial resistance has been found in some Australian commercial chickpea cultivars (Dale and Irwin, 1991; Knights et al., 2008) and high levels of resistance have not been identified yet (Brinsmead et al., 1985; Dale and Irwin, 1991). Nevertheless, careful monitoring of the pathogenic race spectrum makes it possible to predict the evolution of new races which can, in turn, change the breeding program (Myatt et al., 1993). This approach relies on detailed knowledge of the genetics of host resistance and pathogen virulence (Wolfe and Mcdermott, 1994). In many instances, Australian plant breeders have the advantage of breeding chickpea cultivars resistant to *Pmm* pathogens which have been introduced only recently. This allows them to use overseas experience with respect to

durability of resistant genes and evolution of pathogen races.
In our experiment, a collection of Australian and international chickpea genotypes were inoculated with several Australian *Phytophthora* isolates aimed at comparing the pathogen virulence, glasshouse responses of a range of chickpea genotypes to *Pmm*, and further identifying sources of disease resistance for incorporation into breeding programs. The plant infection rate (PIR), plant death rate (PDR), and disease score (DS) for each genotype were determined. And general score (GS) for each genotype was calculated according to its relative value of PIR and PDR.

Results

Number and mobility of zoospores

Variable numbers of zoospores were liberated from 4 *Pmm* cultures. The densities of zoospores were 0.3×10^3, 13.6×10^3, 1.4×10^3, and 1.0×10^3 spore/mL for isolates 4019, 4021, 4027, and 4046, respectively. Zoospores in the suspension obtained from the isolate 4021 showed much higher mobility compared to that of the other 3 isolates.

Pathogenicity of isolates

The PIRs of 3 chickpea varieties in the presence of different isolates ranged from 1.11% to 77.41 % at 17 d of post inoculation (Table 3) and significant differences existed among isolates (*P<0.0001*) (Table 4). The highest PIR was observed in isolate 4027, which was significantly higher than the isolates 4021 and 4046 at $P = 0.05$ (Table 3). Nine days after inoculation with the 4 isolates, mean PIRs for all three chickpea varieties increased with different tendencies (Fig 1 a). PIRs for isolate 4027 increased more rapidly and were the highest in each investigation. Plants inoculated with the isolate 4019 had lower PIRs than that of 4027, whereas those inoculated with isolate 4046 initially showed a slow increasing on PIRs, but a rapid increasing at later stage with the same tendency as isolates 4027 and 4019. The isolate 4021 did not cause infection on any of the chickpea varieties and was the least pathogenic isolate, whereas 4027 was the most pathogenic and recorded the highest PIRs of 56.7 % ± 16.6, 53.3 % ± 15.0, and 45.6 % ± 9.2 in the three varieties, Flipper, Jimbour, and Yorker, respectively. For the PDR, significant differences (*P<0.0001*) among isolates was also found 17 d after inoculation (Table 4). The highest PDR was recorded from isolate 4027, followed by 4019, 4046, and 4021, respectively (Table 3). Of the 4 isolates, PDRs for 4027 elevated dramatically and reached 98.9 % at 31 d after inoculation; isolates 4019 and 4046 had the similar PDRs; and PDRs for isolate 4021 changed very slowly with an averaged value of 40 % at the end of the observations (Fig 1 b). The average PDRs of the 4 isolates for Flipper, Jimbour, and Yorker were 48.3 % ± 10.2, 40.0 % ± 4.0, and 34.7 % ± 5.9, respectively.
DS was assessed using a 1-9 scale and highly variable among isolates (*P<0.0001*) (Table 4). The highest score (6.4) was observed from isolate 4027, which was significantly higher than that of the other 3 isolates (Table 3). Among the three varieties, Flipper was the most susceptible to PRR with a DS of 5.3, followed by Jimbour (5.1) and Yorker (4.7), respectively.
Overall, isolates 4027 was the most and 4021 was the least pathogenic pathogen, respectively, based on PIR, PDR and DS.

Response of chickpea genotypes to pathogen isolates

Sixteen chickpea genotypes were inoculated with inoculums A and B to investigate resistance to *Pmm* pathogens. Significant differences for PIRs (*P<0.01*) were observed with the inoculum A (Table 5). The lowest PIR appeared in FLIP97-114C (51.3 % ± 7.4) and the highest in ICCV 96853 (92.9 % ± 15.3) (Table 6). Significant differences (*P<0.0001*) were also observed among chickpea genotypes inoculated with the inoculum B (Table 5). The lowest and highest values were 51.5 % ± 0.7 and 100 % ± 0.0 for Bumper and ICCV 96852, respectively. The average PIR for the inoculum B was 83.3 % ± 8.3 compared to the inoculum A at 72.3 % ± 16.9. For most genotypes, with the exception of ICCV 06107, ICCV 96853, ICCV 06108, Howzat, and Bumper, the inoculum B caused higher PIR (Fig 2 a). Overall, the lowest PIR was observed in FLIP97-114C when plants were inoculated with the inoculum A. The PDR for the 16 genotypes to the inoculum A varied significantly (*P<0.0001*) (Table 5). The lowest value (25.4 % ± 15.8) was observed in the genotype ICCV 05111 and the highest (62.2 % ± 1.0) in ICCV 06107 (Table 6). For the inoculum B, the lowest (19.9 % ± 8.2) and highest PDR (48.3 % ± 8.3) were found in FLIP97-114C and ICCV 96852, respectively. The mean PDR for the inoculum A (44.3 % ± 5.1) was higher than that of the B (33.8 % ± 2.5). In contrast to the PIR, high PDRs were observed in most chickpea genotypes while inoculated with the inoculum A, with the exception of ICCV 05111, ICCV 98818, ICCV 96852, and ICCV 98801 (Fig 2 b). The DS was determined as the percentage of infected and/or dead plants relative to the total number of plants in each pot (Liew and Irwin, 1994; Nygaard and Grau, 1989). These values varied among different genotypes (Table 5) and ranged from 4.7 to 6.0 for the inoculum A, and 4.3 to 6.0 for the inoculum B (Table 6). The minimum mean DS (4.3) was recorded in Bumper and the maximum (6.7) in ICCV 06107. Mean DS was 5.6 ± 0.2 for the inoculum A and 5.0 ± 0.1 for the inoculum B. Correlation analysis showed that DS was significantly correlated with PIR and PDR for both inoculum A and B (Table 7). PIR and PDR were also significantly correlated with each other. GS for each genotype was calculated by integrating the values of PIR and PDR at a weight of 50 % each to measure the reaction of the whole plant to pathogen inoculation. GS was significantly correlated with DS ($r = 0.95$, $P < 0.01$). A different order for GS (Table 8) was obtained from inoculums A and B. However, the genotypes Bumper, FLIP97-114C, ICCV 05111, Yorker and Flipper demonstrated the same resistance to *Pmm* as measured by GS regardless of the inoculum.

Discussion

Chickpea is the major pulse crop produced in Australia and the first record of *Pmm* infection on chickpea occurred in Queensland in 1979 (Vock et al., 1980). To date, it is regarded as the most serious disease for chickpea in this country. The chemical control of this disease could cause significant damage to Australian native ecosystems and agricultural industries (Irwin et al., 1995). Since there is no strong resistance existed in chickpea cultivars, identification of new sources of resistance to local *Pmm* pathogen strains becomes very important for chickpea improvement programs. Genotypes ICCV 05111, ICCV 98818, ICCV 96852, ICCV 98801, ICCV 98816, ICCV 06107, ICCV 96853, ICCV 98813, and ICCV 06108 are newly introduced from India. Investigation of their PRR resistance is vitally important to determine the potential use of these germplasm in local crop improvement programs.
Zoospores and oospores are commonly used as inoculum resources to screen plant for resistance to PRR. In this study, *Pmm* isolates derived from diseased chickpea plants were not difficult to culture on V8 juice agar medium as reported by Vock et al (1980) and Dale and Irwin (1990). However, the

Table 1. Chickpea genotypes used in this experiment.

Entry	Genotype	Sources	Entry	Genotype	Sources
1	ICCV 05111	India	9	ICCV 06108	India
2	ICCV 98818	India	10	Jimbour[a]	Australia
3	ICCV 96852	India	11	Howzat	Australia
4	ICCV 98801	India	12	Bumper	Australia
5	ICCV 98816	India	13	Jimbour #1[a]	Australia
6	ICCV 06107	India	14	Flipper	Australia
7	ICCV 96853	India	15	Yorker	Australia
8	ICCV 98813	India	16	FLIP97-114C	Australia

[a] Jimbour and Jimbour #1 are the same variety collected from different sources.

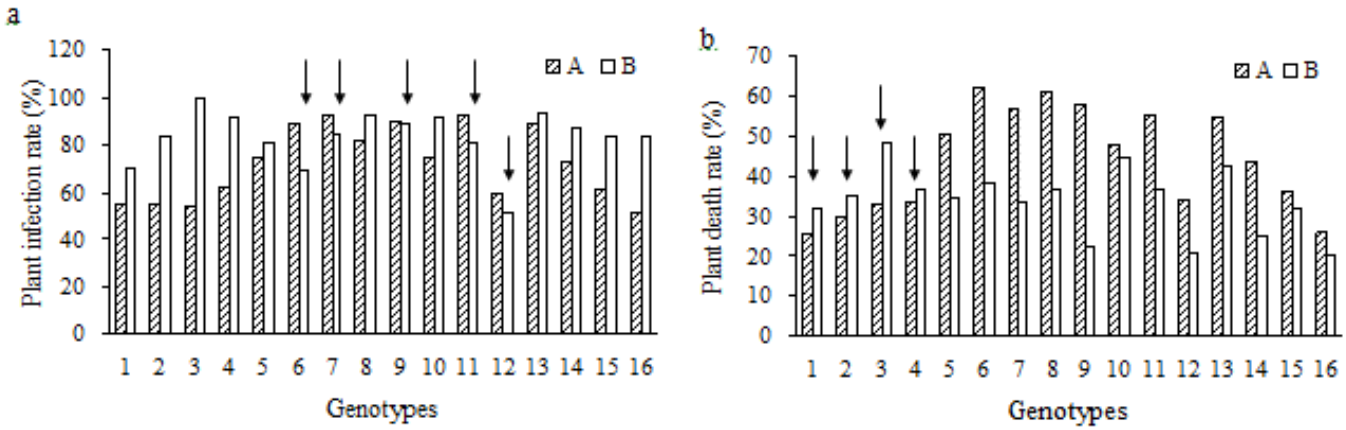

Fig 1. Tendency of plant infection rate (a) and plant death rate (b) for different *Pmm* isolates on 3 chickpea varieties, Flipper, Jimbour, and Yorker.

Table 2. Pathogen isolates being used to inoculate the chickpea plants.

Isolates	Host	Location	Date collected
4019	Chickpea	Gatton QLD	1 April 2004
4021	Chickpea	Gatton QLD	1 April 2004
4027	Chickpea	Gatton QLD	23 May 2001
4046	Chickpea	Livingston NSW	3 Aug 2005

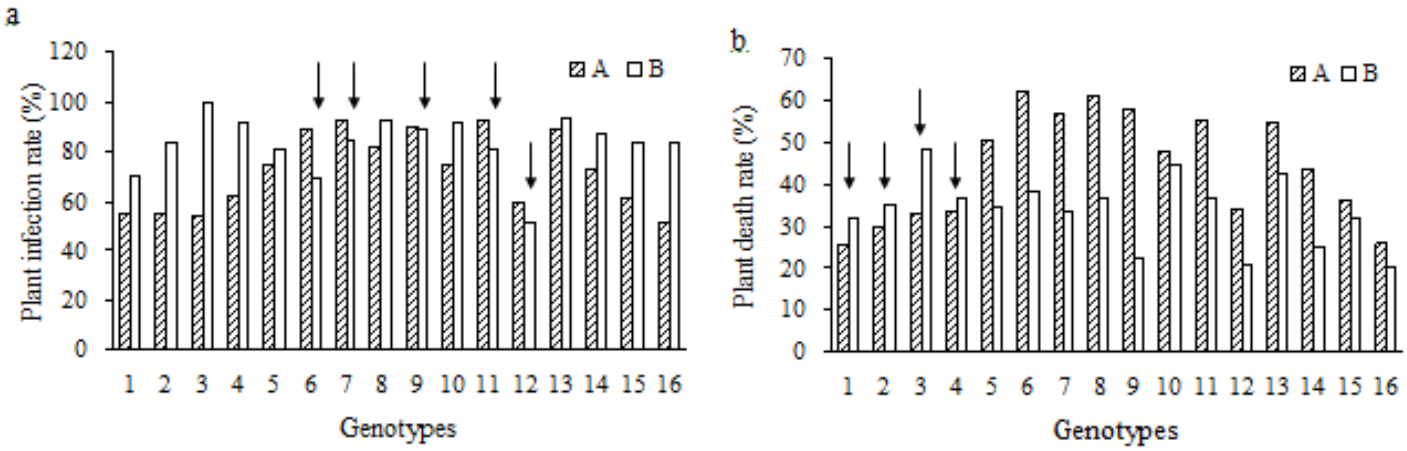

Fig 2. Reactions of different genotypes to *Pmm* isolates: (a) plant infection rate (PIR). "A" is the most aggressive isolate 4027 and "B" is the mixed isolate of less aggressive ones, 4019 and 4046. Arrows indicate genotypes where PIR was high with the single isolate inoculation. (b) plant death rate (PDR). "A" is the most aggressive isolate 4027 and "B" is the mixed isolate of less virulent ones, 4019 and 4046. Arrows indicate genotypes where PDR was high with the mixed isolate inoculation.

production of zoospores was difficult when using rose petal to culture the pathogen from the water soaked soil or roots infected by *Pmm* as described by Dale and Irwin (1990). Our results showed that aerate shaking for 21 hr at 23 °C (Irwin, 1976) might be the best way to produce zoospores from *Pmm* suspension. A reliable glasshouse assay for assessing the resistance levels of chickpea to *Pmm* has been developed using the zoospores as inoculum (Irwin 1976; Dale and Irwin 1990). In this trial, isolate 4021 produced high numbers of zoospores, which was between 9-45 times higher than that of the other 3 isolates, with excellent mobility. However, it produced almost no symptom in the pathogenicity test (Table 3) and so was not used in the second experiment. Although the zoospores from the isolates 4019, 4027, and 4046 had much lower mobility, they showed very strong pathogenicity in two experiments, especially for the isolate 4027 (Table 3, Fig 1). The results indicated that the mobility of the zoospores was not associated with pathogenicity of *Pmm* isolate in this study. It has been reported that the period of exposure to the pathogen under water saturated soil conditions had a marked effect on the expression of resistance to *Pmm* and the number of surviving chickpea seedlings decreased over the time (Brinsmead et al., 1985). Williams and Pascoe (1994) also reported that flooding period influenced the amount of root rot, which was greater in longer flooding treatments. Similar results were obtained from our study. In the genotype screening test, some genotypes did not produce any symptoms at 3 d after inoculation, but most plants were damaged by this disease at 5 d (data not shown). In this investigation, saturated condition was only maintained for 5 d rather than 7 d as described by Dale and Irwin (1990) due to the severe early symptoms. PRR disease was more severe on younger chickpea plants in our study. In the genotype screening assay, plants were at the seedling stage while inoculated at 7 d after sowing. PIRs for the three varieties, Flipper, Yorker, and Jimbour, were 72.6%, 61.7%, and 74.7 %, compared to that of 56.7%, 45.6%, and 53.3 % in the pathogenicity identification test (chickpea plants were inoculated at the elongation stage). Nygaard and Grau (1989) reported that *Pmm* virulence was not affected differentially by the age of lucerne plants at inoculation. The observed differences may be due to different plant materials used in the study (Irwin and Dales, 1982). Our results also demonstrated that chickpea genotypes can be screened at the early seedling stage to save time and labor. The ratio of infected and dead plants increased rapidly with chickpea development (Fig 1). These results were consistent with the previous study (Williams and Pascoe, 1994) which showed that the severity of symptoms became more pronounced with plant development. A mixed isolate inoculum was initially used in this study to screen chickpea genotypes. Compared with the single isolate, it produced higher PIR but lower PDR and DS, indicating the existence of a degree of synergy between the isolates in the mixed inoculum. For most genotypes, the GS values obtained according to the related PIR and PDR values with respect to the mixed isolate were in a narrow range between 5.1 and 5.9 (Table 8). In contrast, a wider GS range was observed while using the single isolate. Besides, the rank of resistance of chickpea genotypes as measured by GS was not consistent. This indicated that a different isolate specific resistance existed in these genotypes and the findings supported previous reports. The Department of Agriculture, NSW, Australia, reported that Howzat was moderately resistant to PRR (Department of Agriculture, 2001) and Jimbour was resistant (Department of Agriculture, 2002). However, different resistance reactions were reported by Hawthorne and co-workers (2006), which demonstrated that Yorker was moderately resistant, Jimbour was intermediate, Howzat was moderately susceptible, Flipper was moderately susceptible to susceptible, and Bumper was very susceptible to PRR. Same results were reported in their late work with the exception of Flipper (moderately susceptible to moderately resistant) (Hawthorne, 2008). Our results on resistance in chickpea genotypes also differed from the above reports. These differences most likely reflected different isolates used (Liew and Irwin, 1994). Further research is required to better characterize the resistance of chickpea to specific pathogen strains. DS was used to score the resistance of chickpea lines to *Pmm* (Knights et al., 2008). In our study, both PIR and PDR were highly variable among the genotypes. But the DS for 16 genotypes distributed in a small range. Therefore, the DS was not effective in differentiating the resistance of different genotypes (Table 6). GS, which incorporates both PIR and PDR values, was introduced to measure the degree of pathogen infection on the chickpea plants. It could distinguish the different resistance among the genotypes (Table 8). According to the GS values derived from the most aggressive isolate 4027 (Table 8), genotypes FLIP97-114C, ICCV 05111, ICCV 98818, and ICCV 96852 were considered moderately resistant, Bumper, ICCV 98801, and Yorker resistant. The other genotypes, including Flipper, Jimbour, ICCV 98816, Jimbour #1, ICCV 98813, Howzat, ICCV 06108, ICCV 96853, and ICCV 06107 with a GS above 5.8, were classified as highly susceptible to PRR. Therefore, genotypes FLIP97-114C, ICCV 05111, ICCV 98818, ICCV 96852, and ICCV 98801 were considered worthy of further investigation using a wider range of *Pmm* isolates and field trials to confirm their responses. The resistant materials should be deployed in tandem with improved management practices (Manning et al., 2000). In order to improve the resistance of the plants and reduce the disease infection, irrigation should be managed to keep soils well drained and routine irrigation schedules modified to minimize the water used for cooling, frost protection, fertilization, and pesticide treatments (Hawthorne et al. 2006). Excess irrigation and periodic flooding should also be avoided (Manning et al., 2000; Cumming, 2010) in that the severity of disease is highly associated with soil conditions, particularly water logging (Cumming, 2010).

Materials and methods

Chickpea genotypes and Pmm isolates

Sixteen chickpea genotypes (Table 1) were obtained from the seed collection at the Plant Breeding Institute, the University of Sydney. Nine were newly introduced overseas germplasm. Four single-spore derived *Pmm* isolates (4019, 4021, 4027, and 4046) were kindly provided by Kevin Moore (DI&I NSW). They were isolated from chickpea growing field at different locations in Australia (Table 2).

Pathogenicity identification of Pmm isolates

In this assay, three chickpea varieties with known reaction (Flipper, moderately susceptible to susceptible; Yorker, moderately resistant; and Jimbour, intermediate) were used to investigate the pathogenicity of different isolates. The variability within treatments increased when zoospores were used as the inoculum source compared to oospores (Dale and Irwin, 1990). Therefore, zoospores were used in this assay as the inoculum source. They were produced from 10-day-old V8 juice agar (V8B; composed of 200 mL V8 juice, 1.5 g $CaCO_3$, 800 mL distilled water, pH 6.5) cultures as described by Irwin (1976). Forty mL of zoospores suspension (10,000 zoospores/mL) was prepared from different initial suspensions

Table 3. Multiple comparison of plant infection rate (PIR), plant death rate (PDR), and disease score (DS) for the pathogenicity identification test.

Isolates	PIR (%)		PDR (%)		DS	
	Mean	s.d.	Mean	s.d.	Mean	s.d.
4019	72.20 ab	3.7	48.9 b	3.4	5.8 b	0.7
4021	1.11 c	1.1	14.4 c	2.5	2.9 d	0.4
4027	**77.41** a	5.5	**65.2** a	5.0	**6.4** a	1.5
4046	56.67 b	6.5	37.8 b	3.2	5.0 c	1.0

All data were collected on 29 Mar 2010, 17 d after inoculation. s.d means standard deviation. Values within a column followed by a different letter are significantly different at $p < 0.05$, according to Duncan's Multiple Range Test. The maximum values are marked as bold.

Table 4. ANOVA of plant infection rate (PIR), plant death rate (PDR), and disease score (DS) for the pathogenicity identification test.

Source of Variation	PIR (%)		PDR (%)		DS	
	F - value	*p - value*	F - value	*p - value*	F - value	*p - value*
Isolates	25.04**	<0.0001	10.20**	<0.0001	11.88**	<0.0001
Genotypes	0.89	0.42	1.39	0.27	0.75	0.48
Interaction	0.51	0.80	0.23	0.96	0.79	0.58

All data were collected on 29 Mar 2010, 17 d after inoculation. F - value means the statistics of the T-test. *p – value* means the level of significance. ** means significant difference at $p < 0.01$.

Table 5. ANOVA of plant infection rate (PIR), plant death rate (PDR), and disease score (DS) for the genotype screening experiment.

Inoculum	PIR (%)		PDR (%)		DS	
	F - value	*p - value*	F - value	*p - value*	F - value	*p - value*
A	4.11**	<0.01	9.92**	<0.0001	4.67**	<0.01
B	4.87**	<0.0001	8.44**	<0.0001	5.49**	<0.0001

All data were collected on 11 Jun 2010, 9 d after inoculation. A stands for the most aggressive isolate, 4027. B stands for the mixture of the least aggressive isolates, 4019 and 4046. F - value means the statistics of the T-test. *p – value* means the level of significance. ** means significant difference at $p < 0.01$.

using autoclaved soil leachate as the diluent to maximize zoospore motility (Harris, 1986). Fifteen seeds of Flipper, Yorker, and Jimbour were coated with 3.6 g/kg thiram before sowing to provide early season control of some common seed- and soil-borne seedling diseases. These plants were sown on Feb 23, 2010 in the plastic pots (25 cm in diameter) in a completely randomized design with 3 replicates and 4 inoculum treatments including one un-inoculated control. They were grown in a glasshouse under 27 °C/ 23 °C (day/night) temperature, 12 hr daylight. Seedlings were thinned to 10 plants per pot at 5 days of sowing. Chickpea seedlings were flooded by keeping the pots inside watertight pots filled to the level of the soil surface with water at 17 d of sowing. Forty mL of zoospore suspension was then poured evenly around the base of the plants. Saturated soil conditions were maintained for 7 d according to the report of Dale and Irwin (1990). PIR, PDR, and DS for each replicate were scored 6 times from 9 d after inoculation at 4-day intervals. DS was assigned using a 1-9 scale (Knights et al., 2008). A plant was considered diseased if it showed wilting and chlorosis, a basal lesion, or both of these symptoms.

Investigation of resistance in different chickpea genotypes

Based on the results of the above study, the most aggressive pathogenic isolate 4027 (termed A) and the mixture of the other 2 pathogenic isolates (4019 and 4046) (termed B) were used to inoculate all 16 chickpea genotypes. The isolate 4021 was not used in this experiment because it did not show any pathogenicity in the first test. Inoculation was conducted using a completely randomized design with 3 replications, following the same methods as described above. This experiment was sown on 26 May 2010. Seven-day-old chickpea seedlings were saturated with water only for 5 days because of the severe early symptom development on chickpea plants. PIR, PDR, and DS for each replicate were scored 6 times from 5 d after inoculation at 4-day intervals. GS for each genotype was calculated according to its relative value of PIR and PDR.

Statistical analysis

The variables PIR and PDR were calculated using the following formulae:

PIR (%) = (Infected plants in each pot / Total plants in each pot) × 100

PDR (%) = (Dead plants in each pot / Total plants in each pot) × 100

GS = PIR × 0.5 + PDR × 0.5

ANOVA procedure in SAS/STAT software version 6 (SAS Institute Inc., 1995) was used to analyse the variation of PIR, PDR, and DS. Duncan's Multiple Range Test was performed to compare the significance of each parameter among different chickpea genotypes. The resistance of the 16 genotypes was ranked based on their GS values after inoculated with the most aggressive and mixed isolates.

Conclusion

The mobility of zoospores was not associated with the pathogenicity in this study. Zoospores obtained from the isolate 4021 exhibited the strongest mobility but the poorest infection rate. The pathogenicity significantly differed among the different pathogen strains. There was no chickpea genotype highly resistant to the PRR, but the variable resistance existed in the observed chickpea genotypes. The good resistance to specific pathogen strain was also observed in some chickpea genotypes, indicating that several different resistance genotypes presented in these germplasm. GS is a good index to measure the degree of pathogen infection on the chickpea plants.

Table 6. Multiple comparison of plant infection rate (PIR), plant death rate (PDR), and disease score (DS) for the genotype screening experiment.

Genotypes	A						B					
	PIR (%)		PDR (%)		DS		PIR (%)		PDR (%)		DS	
	Mean	s.d.	Mean	s.d.	Mean	s.d.	Mean	s.d.	Mean	s.d.	Mean	s.d.
ICCV 05111	55.5 fg	1.4	**25.4** i	15.8	**4.7** f	0.3	70.3 f	4.3	32.1 de	11.3	4.7 c	0.3
ICCV 98818	55.2 fg	0.9	29.5 hi	3.0	5.0 e	0.0	83.3 de	5.8	35.3 bcd	5.3	5.0 b	0.0
ICCV 96852	54.4 fg	27.3	33.2 gh	14.8	5.0 e	1.0	100.0 a	0.0	48.3 a	8.3	6.0 a	0.0
ICCV 98801	62.4 ef	10.8	33.9 gh	9.7	5.3 d	0.3	91.6 ab	7.8	36.6 bcd	12.5	5.0 b	0.0
ICCV 98816	74.5 cd	20.4	50.8 de	4.6	6.0 c	0.0	80.6 e	11.8	34.9 cd	8.4	5.0 b	0.0
ICCV 06107	89.3 ab	1.7	62.2 a	1.0	6.7 a	0.3	69.7 f	0.0	38.6 abcd	1.6	5.0 b	0.0
ICCV 96853	92.9 a	15.3	56.8 abc	3.1	6.0 c	0.0	84.6 cde	18.0	33.9 cd	8.8	5.0 b	0.0
ICCV 98813	82.1 bc	7.9	61.1 ab	1.7	6.3 b	0.3	92.7 ab	5.1	37.1 bcd	8.2	5.0 b	0.0
ICCV 06108	89.5 ab	13.7	57.8 abc	0.6	6.0 c	0.0	88.8 bcd	9.6	22.2 f	3.9	4.7 c	0.3
Jimbour	74.7 cd	9.1	47.8 ef	0.8	6.0 c	0.0	91.4 abc	8.3	44.9 ab	4.4	6.0 a	0.0
Howzat	92.1 a	7.0	55.2 bcd	0.4	6.0 c	0.0	80.5 e	1.8	36.9 bcd	9.5	5.0 b	0.0
Bumper	59.6 fg	8.9	34.1 gh	8.0	5.3 d	0.3	**51.5** g	0.7	20.6 f	1.1	**4.3** d	0.3
Jimbour #1	88.6 ab	10.6	54.5 cd	2.2	6.0 c	0.0	93.3 ab	13.3	42.7 abc	11.3	5.3 b	0.3
Flipper	72.6 de	1.4	43.9 f	2.9	5.3 d	0.3	87.3 bcde	2.5	24.9 ef	3.9	5.0 b	0.0
Yorker	61.7 f	25.7	36.5 g	10.6	5.3 d	0.3	83.6 de	1.1	32.1 de	9.0	5.0 b	0.0
FLIP97-114C	**51.3** g	7.4	25.9 i	2.5	5.0 e	0.0	83.1 de	5.6	**19.9** f	8.2	4.7 c	0.3

All data were collected on 11 Jun 2010, 9 d after inoculation. s.d means standard deviation.Values within a column followed by a different letter are significantly different at $p < 0.05$, according to Duncan's Multiple Range Test. The minimum values are marked as bold.

Table 7. Correlations among plant infection rate (PIR), plant death rate (PDR), and disease score (DS) for the genotype screening experiment.

Index	Isolate A		Isolate B	
	PIR	PDR	PIR	PDR
PDR	0.96**		0.51*	
DS	0.89**	0.96**	0.68*	0.85**

** means significantly correlated at $p=0.01$, * means significantly correlated at $p=0.05$.

Table 8. Sorting of 16 genotypes according to general score (GS) for the single isolate 4027 (Inoculum A) and the mixed isolate (Inoculum B).

Inoculum A	GS^A	Inoculum B	GS^B
FLIP97-114C	3.9	Bumper	3.6
ICCV 05111	4.1	ICCV 05111	5.1
ICCV 98818	4.2	FLIP97-114C	5.2
ICCV 96852	4.4	ICCV 06107	5.4
Bumper	4.7	ICCV 06108	5.6
ICCV 98801	4.8	Flipper	5.6
Yorker	4.9	ICCV 98816	5.8
Flipper	5.8	Yorker	5.8
Jimbour	6.1	Howzat	5.9
ICCV 98816	6.3	ICCV 96853	5.9
Jimbour #1	7.2	ICCV 98818	5.9
ICCV 98813	7.2	ICCV 98801	6.4
Howzat	7.4	ICCV 98813	6.5
ICCV 06108	7.4	Jimbour #1	6.8
ICCV 96853	7.5	Jimbour	6.8
ICCV 06107	7.6	ICCV 96852	7.4

GS = PIR × 0.5 + PDR × 0.5; GS^A and GS^B were obtained according to the related PIR and PDR values with respect to the inoculum A and B. The range of scores indicates the degree of pathogen infection on the chickpea plants. The smaller value means the poorer infection that chickpea genotype had.

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