Effects of Mixed Pathotypes of *Didymella rabiei* on the Development of Ascochyta Blight on Chickpea

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Abstract

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Ascochyta blight (*Didymella rabiei*) is an economically important disease on chickpea in Syria and other parts of the world. Studies were conducted under plastic-house and field conditions to determine the effects of mixed infection of *D. rabiei* pathotypes on disease development on different chickpea genotypes. The plastic-house results showed that under mixed-genotype infections, disease severity caused by two-genotype mixtures (1+2, 1+3, 1+4, 2+3, 3+4) was not significantly different from the average level of disease caused by the most virulent pathotype in each mixture. Co-infection by Pathotypes-1+2, resulted in higher disease severity compared to Pathotype-2 alone. Co-infection by the four Pathotypes mixture, resulted in lower disease severity compared to Pathotype-4 alone. ILC-194 was the most susceptible genotype, followed by Ghab-1, while ICC-12004 was the most resistant genotype. In field experiments, all disease parameters and percent yield reduction were higher when chickpea genotypes were infected with a mixture of Pathotype-1 and 2 compared to individual inoculations with the two pathotypes. Chickpea variety mixtures did not reduce parameters of disease epidemics. To develop disease epidemics under field conditions, it is better to mix Pathotype-1 and 2 compared with individual inoculations. Further studies are required to learn how pathotype mixtures affect disease epidemics in chickpea-*Ascochyta* pathosystem.

Keywords: Chickpea, Ascochyta blight, *Didymella rabiei,* Competition, Mixed-pathotypes.

Introduction

Chickpea (*Cicer arietinum* L.) is the fourth most important grain legume crops in the world after common bean, soybean and pea (6). Chickpea production covers about 74,000 ha with a total production of 57,351 ton in Syria (6). One of the most important biotic constraints that reduce yield and quality of chickpea worldwide is Ascochyta blight [*Didymella rabiei* (Kovach.) v. Arx)] (= *Mycosphaerella rabiei* Kovach.) (11, 14). *Didymella rabiei* [Anamorph: *Ascochyta rabiei* (Pass.) Lab.] is a haploid, heterothallic ascomycete fungus. Ascochyta blight is reported from 34 countries world-wide. *Didymella rabiei* affects all above-ground parts of chickpea, where stem breakage and pod infection cause high yield and quality losses (15).

Resistance breeding to Ascochyta blight in chickpea is not durable due to high variability of the pathogen populations in different countries. Resistance breakdown is the greatest challenge in chickpea breeding for Ascochyta blight resistance. To develop durable forms of resistance, it is important to monitor changes in the pathogen population structure to predict future resistance breaking populations (22). Resistance breaking *D. rabiei* pathotypes are reported from different countries (3, 19, 21), and as a result some popular chickpea cultivars are withdrawn from production in some countries like Syria. The causes of pathogen variability are mainly attributable with the presence of sexual reproduction and extensive production of few chickpea cultivars. Four pathotypes (1-4) have been reported from Syria where Pathotype-1 is the least virulent and Pathotype-4 is the most virulent one (3, 10, 21).

The advantages of mixtures (multiline cultivars and cultivar mixtures) for disease management has been demonstrated for rusts and powdery mildew of small grain crops like wheat and barley (13). The severity of angular leaf spot (*Phaeoisariopsis griseola*) of common bean was reduced when farmer land races were supplemented with resistant cultivars in Great Lake Region of Africa (16). In potato, tuber yield from resistant and susceptible cultivar mixtures was higher than single genotype stands, and also potato late blight severity was decreased on the susceptible genotype within mixtures (9).

The presence of mixed infections of different isolates of a given pathogen can increase or decrease or has no effect on disease development. Mixed isolates infection of *Mycosphaerella graminicola* on wheat showed low levels of severity and virulence, and in some cases no effect, when compared with single isolate inoculations (18). Miedaner *et al*. (12) showed that the effect of mixed isolates of *Fusarium culmorum* differing in their aggressiveness and mycotoxin production [deoxynivalenol (DON) and nivalenol (NIV)] was lower than isolates inoculated individually on rye genotypes. Selection within pathogen populations can occur rapidly, particularly if there is a considerable difference in fitness between or within genotypes (18). So far, no published results are found on the effects of mixed infections of different pathotypes of *D. rabiei* on disease development on different chickpea genotypes.

The aim of this study was to determine the effects of mixed pathotypes of *D. rabiei* on disease development on different chickpea genotypes differing in resistance levels to Ascochyta blight.

Materials and Methods

Plastic-house Experiment

Four chickpea genotypes, (ICC-12004, highly resistant to Pathotypes 1, 2 , 3, and susceptible to Pathotype-4; FLIP 82-150C (Ghab-3) resistant to Pathotypes-1 and 2 and moderately resistant to Pathotype-3, and susceptible to Pathotype-4; ILC-482 (Ghab-1) resistant to Pathotype-1 and susceptible to Pathotypes-2 ,4, ILC-194 highly susceptible to all pathotypes, were planted in a plastic-house (temp. 22 ± 2°C, 14/10 h photo period) at the International Center for Agricultural Research in the Dry Areas (ICARDA), Syria. Seeds of each genotype were sown in plastic pots (20 cm dia.) with five seeds per pot. The experiment was conducted in randomized complete block design (RCBD) with four replications. The four pathotypes of *D. rabiei* maintained at ICARDA (Legume Pathology Laboratory) were multiplied on chickpea seed extract dextrose agar medium (40 g autoclaved chickpea seeds, 20 g dextrose and 18 g agar in 1 l of sterile distilled water) and incubated for seven days at 20º C (12/12 h light and dark cycle). Seedlings at V3-V4 growth stage (5) were inoculated with spore suspension of $5x10^5$ spores/ml with the four *D. rabiei* pathotypes singly and in mixtures (Pathotype-1+2; 1+3; 1+4; 2+3; 2+4; 3+4; 1+2+3+4). The control pots were sprayed with distilled water. Disease severity was evaluated three weeks after inoculation using a modified 1-9 scoring scale, where 1 = resistant and 9 = highly susceptible (4, 17, 20), and disease severity data were analyzed using GENSTAT 12 Statistical Program. Contrast analysis was performed between individual pathotype inoculations and their mixtures using similar statistical package.

Field Experiments

Seeds of three chickpea genotypes, ILC-194, Ghab-1 and Ghab-3,,, and a mixture of Ghab-3 and ILC-194 (1:1) were sown on December 4, 2008 and January 5, 2010 with a seed rate of 100 kg/ha. The experiment was carried out at Tel Hadya National Agricultural Research Station, Aleppo, Syria. The experiment was laid down in RCBD with three replications. Each plot was 6 m^2 with 10 rows of 2 m long, and 30 cm between rows. To avoid cross-contamination between treatments, wheat was planted between plots as physical barrier. Pathotype-1 and 2 of *D. rabiei* were multiplied for field inoculations following similar procedure described under plastic-house experiment. Chickpea genotypes and variety mixtures were inoculated with spore suspension of $5x10^5$ spores/ml at V3-V4 stages (5). The control plots were sprayed with water.

Disease severity was assessed every week using a modified 1-9 rating scale (4, 17, 20). The area under disease progress curves (AUDPCs) were computed using disease severity data against time using the formula suggested by Shaner and Finney (19).

$$AUDPC = \sum_{i=1}^{n=i} \left[\left(\frac{X_{i+1} + X_i}{2} \right) (t_{i+1} - t_i) \right]$$

Where x_i = the disease severity (%) at i^{th} observation, t_i = time (days after disease occurrence) at the i^{th} observation and n = total number of observations. Apparent infection rate was calculated from severity data using the slope of the regression, to measure disease progresses over time for the different treatments. Plots were harvested at maturity, and yield reduction (YR%) was calculated using the formula: $YR\% = (Y_c - Y_t/Y_c)x100$, Where Y_c is chickpea yield (Kg/ha) in the control, and Y_t is the yield in the treatment (kg/ha). Analyses of variance of disease parameters were done using GENSTAT 12 Statistical Program.

Results

Plastic-house Experiment

Results showed significant differences (P<0.001) among the four pathotypes in affecting chickpea genotypes (Table 1). When single pathotype inoculation was compared with pathotype mixtures, significant differences (P<0.001) were observed on disease severity. Disease severity on Ghab-1 ranged from 5 for the Pathotype-1 to 7.5, where the highest disease severity was recorded when infection was with Pathotype-4 alone and with mixed Pathotypes (4+2) and (4+3). Disease severity from mixtures of the four Pathotypes (6.3) was less than that of Pathotype-4 alone. Disease severity ranged from 3.8 to 6.8 on Ghab-3, where the highest disease severity was recorded from Pathotype-4 and its mixture with Pathotype-1, and the lowest was from Pathotype-1. On the susceptible genotype (ILC-194), the severity ranged from 5.8 for the Pathotype-1 alone to 8.3, whereas the highest severity was caused by the mixture of Pathotypes 3+4, followed by Pathotype-4 alone and its mixture with Pathotype-2 (8.0). On the resistant genotype (ICC-12004), disease severity ranged from 1.3 to 6.0, where the highest severity rating was from infection with Pathotype-4 alone, followed by mixtures of 1+4, 2+4, 3+4 and 1+2+3+4. The lowest severity rating (1.3) was recorded from Pathotype-1.

The mixture of the four pathotypes resulted in lower disease severity on all chickpea genotypes compared with Pathotype-4 alone. In general, Pathotype-4 alone and in mixtures caused the highest disease severity. Mixture of Pathotype (2+3) produced higher disease severity than their individual effect on Ghab-3, ILC-194 and ICC-12004, and low on Ghab-1. By contrast, mixture of Pathotypes 1+3 resulted in similar or lower severity on Ghab-3, ILC-194 and ICC-12004 genotypes, but caused higher disease severity on Ghab-1.

Chickpea genotypes showed significant differences ($P \leq 0.001$) for their reactions to single and mixed pathotype infections (Table 1). The highest mean disease severity (7.3) was observed on the highly susceptible genotype ILC-194, followed by Ghab-1 (6.7). The lowest disease severity (4.1) was recorded on ICC-12004.

The contrast analysis showed significant differences ($P \leq 0.05$) between single and mixed-pathotype infections, the mean of disease severity was higher (6.2) in mixed than in individual (4.3) pathotype infections.

Field Experiments

Disease progress: Slow disease development was observed during the two cropping seasons, mainly on the resistant and moderately resistant genotypes and the mixture of Ghab-3+ ILC-194 (Figure 1). In 2008/09 cropping season, disease progress was slow on all chickpea genotypes inoculated with Pathotype-1 alone, and disease severity was less than a rating of 7 in 60 days after inoculation on the susceptible genotype (Figure 1-A). Disease progressed fast on all genotypes inoculated with Pathotype-2 where the susceptible genotype (ILC-194) was killed in 60 days after inoculation (Figure 1-B). In the case of mixed pathotypes, almost all plants of the highly susceptible genotypes (ILC-194) were killed 40 days after inoculation in 2008/09 season (Fig 1-C). Disease development was slower on all genotypes in 2009/2010 cropping season than in the previous season, where the maximum severity was less than 6 rating on ILC-194 (Figure 1-D, E, F).

Final disease severity: Significant differences ($P \leq 0.001$) were recorded for disease severities among chickpea genotypes and variety mixtures, and the two pathotypes and their mixtures in 2008/09 growing season (Table 2; Fig. 1). The highest disease severity (6.6) were recorded from Pathotype-2 and mixture of Pathotypes 1+2, and the lowest (4.6) was from Pathotype-1. The highest disease severity (8.1) was recorded for the susceptible genotype (ILC-194), followed by mixture of Ghab-3 and ILC-194 (5.9), and Ghab-1 (5.6), and the lowest (4.1) was recorded for Ghab-3. No significant interactions between pathotypes and chickpea genotypes was observed in 2008/09 (Table 2). The highest disease severity (9) was recorded for ILC-194 inoculated with Pathotype-2, followed by pathotypes mixture infections (8.7). The lowest disease severity was observed on Ghab-3 from Pathotype-1 infection (3.3).

In 2009/2010, significant differences ($P \leq 0.001$) in disease severities among chickpea genotypes and variety mixture, and among the two Pathotypes and their mixture were recorded (Table 3; Fig. 1). The highest mean disease severity (4.5) was recorded from mixture of Pathotype-1+2, followed by Pathotype-2 (4.3), and the lowest (3.8) was from Pathotype-1. The highest disease severity (5.1) was recorded on ILC-194, followed by mixture of Ghab-3 and ILC-194 (4.4), then Ghab-1(4), and the lowest (3.1) was recorded on Ghab-3. No significant interactions were observed between pathotypes and genotypes (Table 3). The highest disease severity (5.7) was recorded on ILC-194 for pathotypes mixture, followed by variety mixture and ILC-194 infected with Pathotype-2 (5.0), and the lowest was on Ghab-3 (3.0) for Pathotypes 1 and 2.

Area under disease progress curve: Significant differences ($P \leq 0.001$) were observed among chickpea genotypes and pathotypes in affecting AUDPCs in 2008/09 season (Table 2). The highest mean AUDPC (224.2% days) was recorded from pathotypes mixture, followed by Pathotype-2 (208.3% days), and the lowest (161.7% days) was from Pathotyp-1. The highest mean AUDPC (282.2% days) was recorded on ILC-194, followed by genotype mixture (197.8% days), and Ghab-1 (168.9% days), and the lowest (143.3% days) was on Ghab-3. No significant interactions were observed between cultivars and pathotypes for AUDPC.The highest AUDPC (300% days) was recorded on the highly susceptible genotype (ILC-194) for pathotypes mixture, followed by 290% days for Pathotype-2 on the same genotype, and the lowest (113.3% days) was in plots planted with Ghab-3 and inoculated with Pathotype-1.

Table 1. Mean Ascochyta blight severity (1-9 rating scale) on different chickpea genotypes inoculated with four pathotypes and their mixtures, under plastic-house condition[a].

Pathotypes and their mixtures	Chickpea genotypes				Mean
	Ghab-1	Ghab-3	ILC 194	ICC 12004	
Pathotype 1	5.0 g	3.8 h	5.8 h	1.3 h	3.4
Pathotype 2	6.3 de	4.8 e	6.3 fg	2.3 g	4.9
Pathotype 3	6.5 cd	4.5 ef	7.3 de	3.3 de	5.4
Pathotype 4	7.5 a	6.8 a	8.0 ab	6.0 a	7.1
Pathotype 1+2	6.8 c	4.3 fg	7.3 de	3.5 d	5.4
Pathotype 1+3	6.8 c	4.5 ef	6.5 f	3.0 ef	5.2
Pathotype 1+4	7.3 ab	6.8 a	7.8 bc	5.3 bc	6.8
Pathotype 2+3	6.0 ef	5.3 d	7.5 cd	4.0 d	5.7
Pathotype 2+4	7.5 a	6.3 bc	8.0 ab	5.5 b	6.8
Pathotype 3+4	7.5 a	6.3 bc	8.3 a	5.5 b	6.9
Pathotype 1+2+3+4	6.3 de	6.5 ab	7.8 bc	5.5 b	6.5
Control (water)	1.0	1.0	1.0	1.0	1.0
Mean	6.7	5.4	7.3	4.1	
LSD for Pathotype at 5%			0.399		
LSD for Genotype at 5%			0.231		
LSD for Path. x Geno. at 5%			0.799		

[a]Means followed by the same letter in the same column are not significantly different at $P \leq 0.05$.

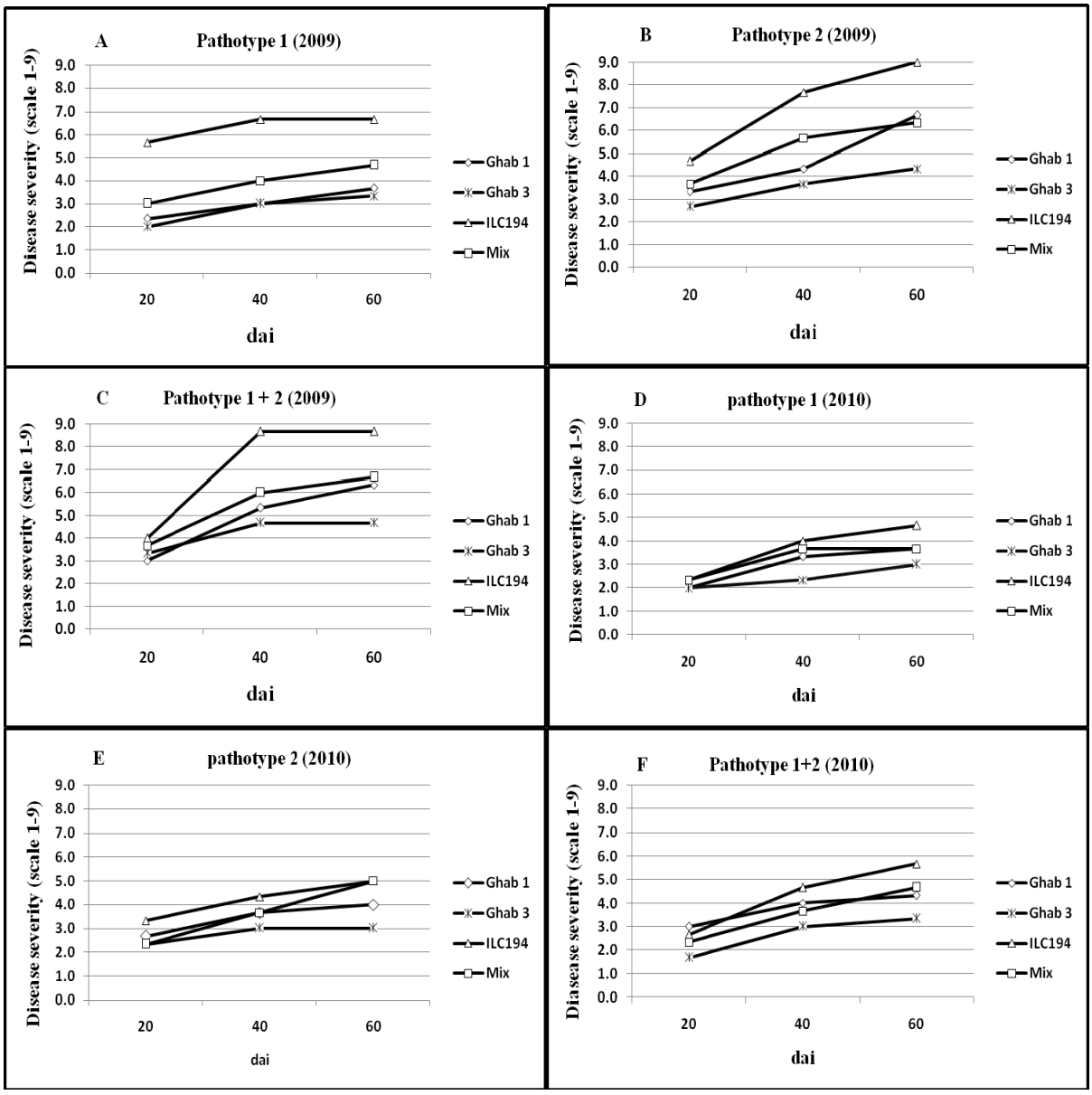

Figure 1. Disease progress curves on four chickpea genotypes inoculated with Pathotype-1 and 2 and their mixture: (A) Pathotype-1; (B) Pathotype-2; (C) Pathotype-1+2 in 2008/09 season; (D) Pathotype-1; (E) Pathotype- 2; (F) Pathotype-1+2 in 2009/2010 season. Mix: Mixture planted with Ghab-3:ILC-194. dai: days after inoculation.

In 2009/2010 season, significant differences ($P \leq 0.001$) were observed among chickpea genotypes and pathotypes in affecting AUDPCs, but not their interactions. The highest AUDPC (145.8% days) was recorded from plots inoculated by pathotype mixture, followed by Pathotype-2 (142.5% days), and the lowest (125.8% days) was recorded from Pathotype-1. The highest mean AUDPC (165.6% days) was recorded on ILC-194, followed by genotypes mixture (141.1% days), and 138.9% days on Ghab-1, and the lowest mean AUDPC (106.7% days) was recorded on Ghab-3. The highest AUDPC (176.7% days) was recorded on ILC-194 for pathotypes mixture, and the lowest (96.7% days) was recorded on Ghab-3 for Pathotype-1 (Table 3).

Apparent infection rates: Significant differences ($P \leq 0.001$) were observed among chickpea genotypes, pathotypes and their interactions, in affecting rate of disease development in both seasons (Tables 2 and 3).

In 2008/09 season, the highest infection rate (0.08 units/days) was recorded for Pathotyp-2 and mixture of Pathotypes-1+2, and the lowest (0.04 units/days) for

Pathotype-1. The highest infection rate (0.09 units/days) was recorded for ILC-194, followed by genotypes mixture (0.08 units/days), and Ghab-1 (0.07 units/days), and the lowest was 0.04 units/days for Ghab-3. The highest infection rate in this season was observed on ILC-194 (0.12 units/day) for pathotype mixture and (0.11 units /day) for Pathotype-2, and the lowest (0.03 units/days) was observed on Ghab-3 for Pathotype-1 and pathotypes mixture, and on Ghab-1 for Pathotype-1 (Table 2).

Table 2. Final disease severity, area under disease progress curves, apparent infection rate and percent yield reduction on chickpea genotypes inoculated with two Pathotypes and their mixtures Pathotype, 2008/09 cropping season, Tel Hadya, Syria[a].

Pathotypes and mixture	Genotypes and mixture	Final disease severity (1-9) [b]	AUDPC[c] (% days)	Apparent infection rate (r)[d]	YR (%)[e]
Pathotype-1	Ghab-1	3.7 fg [(1)]	120.0 jk	0.03 ef	9.4 fghi
	Ghab-3	3.3 gh	113.3 kl	0.03 ef	32.6 e
	ILC-194	6.7 c	256.7 c	0.04 e	70.3 b
	Mixture (Ghab-3+ILC-194)	4.7 e	156.7 ghi	0.04 e	32.6 e
Pathotype-2	Ghab-1	6.7 c	186.7 efg	0.08 c	25.4 ef
	Ghab-3	4.3 ef	143.3 hij	0.04 e	5.1 fghij
	ILC-194	9.0 a	290.0ab	0.11 ab	100.0 a
	Mixture (Ghab-3+ILC-194)	6.3 cd	213.3de	0.07 cd	18.4 efgh
Pathotype-1 +	Ghab-1	6.3 cd	200.0 def	0.08 c	66.9 bc
Pathotype 2	Ghab-3	4.7 e	173.3 fgh	0.03 ef	19.2 efg
	ILC-194	8.7 ab	300.0 a	0.12 a	100.0 a
	Mixture (Ghab-3+ILC-194)	6.7 c	223.3 d	0.08 c	61.0 bcd
LSD for Pathotype at 5%		0.466	15.20	0.012	11.02
LSD for Genotype at 5%		0.538	17.56	0.013	12.72
LSD for Pathotype × Genotype at 5%		0.932	30.41	0.023	22.03

[a] Means followed by the same letter vertically are not significantly different at $P \leq 0.05$.
[b] 1-9 rating scale: where 1 = resistant; and 9 = highly susceptible.
[c] AUDPC :Area under the disease progress curve.
[d] r: Apparent infection rate calculated as slope of the regression.
[e] YR: Percent yield reduction.

Table 3. Mean disease severity, area under disease progress curve, apparent infection rate and percent yield reduction on chickpea genotypes inoculated with two Pathotypes and their mixture, during 2009/2010 cropping season, Tel Hadya, Syria[a].

Pathotypes	Genotypes	Final disease severity (1-9)[b]	AUDPC[c] (% days)	Apparent infection rate (r)[d]	YR (%)[e]
Pathotype-1	Ghab-1	3.7 cdef	123.3 cdefghi	0.04 cd	15.9 defg
	Ghab-3	3.0 efgh	96.7 ijk	0.03 de	3.9 hijkl
	ILC-194	4.7 abc	150.0 abcd	0.06 abc	11.7 fghi
	Mixture (Ghab-3+ILC-194)	3.7 cdef	133.3 cdefgh	0.03 de	11.5 fghij
Pathotype-2	Ghab-1	4.0 bcde	140.0 bcdefg	0.03 de	20.8 cde
	Ghab-3	3.0 efgh	113.3 fghij	0.02 def	22.6 cd
	ILC-194	5.0 ab	170.0 ab	0.04 cd	41.7 a
	Mixture (Ghab-3+ILC-194)	5.0 ab	146.7 abcde	0.07 ab	25.3 c
Pathotype-1 +	Ghab-1	4.3 bcd	153.3 abc	0.03 de	17.2 cdef
Pathotype-2	Ghab-3	3.3 defg	110.0 ghij	0.04 cd	9.5 fghijk
	ILC-194	5.7 a	176.7 a	0.08 a	34.9 ab
	Mixture (Ghab-3+ILC-194)	4.7 abc	143.3 bcdef	0.06 abc	14.5 defgh
LSD for Pathotype at 5%		0.526	15.52	0.013	4.22
LSD for Genotype at 5%		0.608	17.92	0.015	4.87
LSD for Pathotype × Genotype at 5%		1.053	31.04	0.026	8.44

[a] Means followed by the same letter vertically are not significantly different at $P \leq 0.05$.
[b] 1-9 rating scale: where 1 = resistant; and 9 = highly susceptible.
[c] AUDPC :Area under the disease progress curve.
[d] r: Apparent infection rate calculated as slope of the regression.
[e] YR: Percent yield reduction.

In 2009/2010 season, the highest infection rate (0.05 units/days) was recorded for mixture of Pathotypes-1+2, and the lowest (0.04 units/days) for Pathotype-1 and 2. The highest infection rate (0.06 units/days) was recorded for ILC-194, followed by genotypes mixture (0.05 units/days), and Ghab-1 (0.04 units/days), and the lowest was 0.03 units/days on Ghab-3. The highest rate (0.08 units/day) was recorded for ILC-194 for Pathotypes-mixture (1+2), while the lowest (0.02 units/day) was recorded for Ghab-3 infected with Pathotype-2 (Table 3).

Percent yield reduction: Significant differences (*P≤0.001*) were observed among chickpea genotypes and pathogen pathotypes, and their interactions, on percent grain yield reduction in both seasons. The percent yield reduction was higher in 2008/09 than 2009/2010 season (Tables 2 and 3).

In 2008/09 season, the highest percent yield reduction (61.7%) was caused by pathotype mixtures, followed by Pathotype-2 (37.2%), and the lowest (36.2%) for Pathotype-1. The highest yield reduction rate (90.1%) was recorded for ILC-194, followed by the genotypes mixture (37.3%), and Ghab-1(33.9%), and the lowest (18.9%) for Ghab-3. The highest yield reduction (100%) was recorded for the highly susceptible genotype (ILC-194) for the pathotype mixture and Pathotype-2. The lowest yield reduction (5.1%) was recorded for Ghab-3 for Pathotype-2 (Table 2).

In 2009/2010 cropping season, the highest yield reduction (27.6%) was caused by Pathotyp-2, followed by the pathotypes mixture (19%), and the lowest (10.8%) was caused by Pathotype-1. The highest yield reduction rate (29.4%) was recorded for ILC-194, followed by Ghab-1 (18%), and the genotypes mixture (17.1%), and the lowest (12%) was recorded for Ghab-3. The highest yield reduction rate (41.7%) was recorded for ILC-194 infected with Pathotype-2, followed by the same genotype for pathotypes mixture, and the lowest (3.9%) was recorded for Ghab-3 infected with Pathotype-1 (Table 3).

Discussion

In the plastic-house experiment, there were differences in disease severity among single and mixed inoculations of pathotypes on chickpea genotypes. The highest disease severity was caused by Pathotype-4 alone and mixed with other pathotypes. This could be due to high fitness (short incubation and latent period) of Pathotype-4 in affecting chickpea genotypes (*Unpublished data*), and highly competitive over the other pathotypes. Significant reduction in disease severity was recorded on the resistant genotype (ICC-12004) when Pathotype-4 was mixed with other pathotypes. ICC-12004 was resistant to Pathotype-1, 2 and 3, thus the presence of Pathotype-1, 2 or 3 in mixtures did not play a role in affecting ICC-12004 unless Pathotype-4 was present alone or in mixture.

Significant reduction in disease severity was recorded when chickpea genotypes were inoculated with the four pathotype mixtures as compared with Pathotype-4 alone. The reason could probably be due to competition among pathotypes that might have reduced their virulence. The mechanisms by which pathotypes compete with each other are not well understood. It is possible that different pathotypes induce the production of defense proteins at different times in particular cultivars, but yet still successfully infect a given cultivar (24). However, under competition, pathotypes that induce defense proteins earlier than others are better able to overcome this earlier induction would have the competitive advantage, coupled with the subsequent inhibition of other pathotypes (24). Similar results were observed on *Mycosphaerella graminicola* on wheat, *Sclerotinia sclerotiorum* on canola, *Leptosphaeria maculans* on oilseed rape (1, 18), where disease severity was similar or decreased from mixed pathogen pathotypes compared with single-pathotype infections.

Slow blighting was detected more clearly on the resistant than on the susceptible chickpea genotype. This might be due to changes in the genetic structure of pathogen populations that occurred more slowly on resistant than on susceptible cultivars, and already the resistant genotype has resistance genes and induce some pathogenesis related proteins against the pathogen. Also high inoculum pressure generated on the susceptible genotype plays a role in increasing disease severity. Ascochyta blight epidemic spreads in relatively slow manner, especially on resistant genotypes.

Ascochyta blight severity and epidemic parameters were not affected by cultivar mixture. The susceptible genotype (ILC-194) was in equal proportion with the resistant genotype and this caused more inoculum that can affect the other variety in the mixture and caused high disease severity. The resistant genotypes might not act as barrier during splash dispersal of the spores in the mixture (8). In common bean, angular leaf spot severity and rate of disease development were affected when farmer varieties were mixed with 25 and 50% resistant cultivars (16).

Future studies are required to fine tune the appropriate combinations of chickpea genotypes in a mixture to be used as a strategy to manage Ascochyta blight. It is also important to study chickpea intercropped with other crop species to manage Ascochyta blight as seen in common bean chocolate spot management strategy through inter-cropping (7). As opposed to wheat and barley where uniformity is a vital role in advanced food industries, cultivar mixtures as a strategy can be accepted by farmers. After establishing the role of cultivar mixture in managing ascochyta blight of chickpea, the remaining work will be developing genotypes with similar agronomic and quality traits but resistant to different pathotypes of *D. rabiei*. The strategy will also play a vital role in reducing pathogen evolution.

It is interesting to note that competition among pathotypes did not occur in every mixture, but depended on which pathotype(s) were present (18). Inter-genotype competition appeared to be strongly mediated by the host because the competitive ability of *D. rabiei* pathotypes varied differentially among the different chickpea genotypes. This is supported by previous works showing that host genotypes have a strong impact on the dynamics of pathogen populations and host-mediated competition often occurs between pathotypes when they co-exist (23, 25).

The epidemiological parameters like final disease severity, AUDPCs, and apparent infection rate and grain yield reduction rate, were higher in 2008/09 than 2009/2010 cropping season. This result is mainly due to more suitable environment conditions in 2008/09 (lower temperature and higher in rainfall and relative humidity) than in 2009/2010 season.

The plastic-house and field experiments showed that high disease severity was recorded for mixtures of low virulence pathotypes (Pathotypes-1+2) than Pathotype-2 alone. This may be due to absence of competition between the two Pathotypes, or may be that one of these pathotypes induced and increased the aggressiveness of the other. Evaluating of pathotypes and their mixtures under artificial inoculation in relation to disease development under field conditions could be affected by external inoculum sources of isolates with different levels of virulence. Although external sources are not over-ruled, the chances are very low since there are no chickpea fields in the vicinity of the research station where the study was done. It was not possible to study the dynamics of the two pathotypes in the field since no diagnostic markers were available.

Co-infections of different pathotypes with varying mating types under field conditions could facilitate sexual reproduction so that ascospores will be released and act as primary inoculum in the field (2). Mating type (MAT) analysis showed that Pathotypes-2, 3 and 4 were MAT1-1, while Pathotype-1 is MAT1-2 (2).

In conclusion, to develop disease epidemics under field conditions, it is better to mix Pathtype-1 and 2 compared with individual inoculation. Further studies are required to clarify how pathotype mixtures affect disease epidemics in chickpea-*Didymella* pathosystem.

الملخص

عتيق، عمر، أحمد الأحمد، مايكل بابوم، سعيد كمال وماثيو أبنغ. 2012. تأثير الإصابة بخليط من الطرز الممرضة للفطر *Didymella rabiei* في تطور مرض لفحة الأسكوكيتا على الحمص. مجلة وقاية النبات العربية، 30: 266–273.

يعد مرض لفحة الأسكوكيتا المتسبب عن الفطر *Didymella rabiei* من أهم الأمراض التي تحدث خسائر اقتصادية على الحمص في سورية وفي مناطق متعددة من العالم. نفذ هذا البحث في الدفيئة البلاستيكية والحقل لدراسة تأثير الإصابات بخليط من الطرز الممرضة للفطر *D. rabiei* في شدة الإصابة بمرض لفحة الأسكوكيتا. أظهرت نتائج تجربة الدفيئة البلاستيكية بأن شدة الإصابة بالأسكوكيتا الناجمة عن الإعداء بالخلائط، 1+2، 1+3، 1+4، 2+3، 2+4، لم تختلف عن درجة الإصابة بالطراز الممرض الأكثر شراسة ضمن كل خليط. سجلت شدة إصابة أعلى عند استخدام الخليط 1+2 مقارنة مع الطراز الممرض 2 منفرداً. سجلت شدة الإصابة أدنى عند استخدام مزيج من الطرز الممرضة الأربعة معاً مقارنة مع الطراز الممرض 4 منفرداً. كان الطراز ILC-194 أكثر الطرز الوراثية قابلية للإصابة، تلاه غاب 1، بينما كان الطراز ICC-12004 أكثر الطرز الوراثية مقاومة للمرض. أظهرت نتائج التجربة الحقلية بأن جميع مقاييس المرض والنسبة المئوية لانخفاض الإنتاجية كانت أعلى عند استخدام مزيج من كلا الطرازين الممرضين مقارنة مع الإعداء بالطراز الممرض 2 بشكل منفرد. لم يؤثر خليط من كلا طرازي الحمص المقاوم والحساس في خفض وبائية المرض. عند الحاجة للحصول على إصابات وبائية بمرض لفحة الأسكوكيتا في الحقل، من المفضل إجراء الإعداء الاصطناعي بخليط من الطرازين الممرضين 1 و2 بدلاً من استخدامهما بشكل منفرد. من الضروري إجراء دراسات مستقبلية لمعرفة الآلية التي تؤثر فيها خلائط من الطرز الممرضة للفطر *D. rabiei* في الانتشار الوبائي للمرض.

كلمات مفتاحية: لفحة الأسكوكيتا، *Didymella rabiei*، منافسة، خلائط من الطرز الممرضة، حمص.

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