Role of sexual reproduction in the aggressiveness of *Didymella rabiei* affecting chickpea

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Abstract

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Chickpea production is affected by Ascochyta blight disease in many countries and popular cultivars are put out of production due to the evolution of highly aggressive pathogen population. Sexual reproduction is believed to play an important role in the evolution of aggressive population in *Didymella rabiei*. Three crosses were made using four parental isolates (AR01 as MAT1-2 and AR02, AR03 and AR04 as MAT1-1) with varying levels of aggressiveness collected from Syria. Twenty randomly selected progenies per cross together with their respective parents were tested on susceptible (ILC-263) and resistant (ICC-12004) chickpea genotypes for their aggressiveness. Moreover, the mating type frequency was determined using multiplex Mating type markers. Latent period and disease severity were used to measure isolate aggressiveness in the progenies. The mean latent period ranged from 6-12 days in all progenies. The progenies showed significant differences on their aggressiveness in the three crosses. High level of aggressiveness of the progenies was generated from AR01 by AR04 crosses. The frequency of the two mating types was almost equal in all crosses. Our findings showed that sexual reproduction can create progenies that can adapt to resistant cultivars and could affect the effectiveness of commonly used fungicides to manage Ascochyta blight.

Keywords: Chickpea, *Didymella rabiei*, mating type, progenies.

Introduction

Chickpea (*Cicer arietinum* L.) is a major cool-season food legume crop in many parts of the world. The crop is produced for local consumption and export markets as well as in rotation to improve soil fertility benefiting succeeding cereal crops.

Chickpea (Desi and Kabuli types) is mainly planted in spring but in some countries like Syria winter planting is adopted where seed yield can be increased from 18-32% over traditional spring production (Mazid *et al.,* 2013). Chickpea production is affected by many diseases, insect pests, viruses and parasitic nematodes in many countries (Li *et al.,* 2015).

Ascochyta blight (*Didymella rabiei* Kovatsch.) is the major biotic factor contributing for low yield and quality gaps in chickpea in many countries (Pande *et al.,* 2005). Disease epidemics can be initiated from infected seeds, infected debris, and air borne ascospores produced from the teleomorph (Shtienberg *et al.,* 2000). The anamorph (*Ascochyta rabiei* (Pass.) Lab.) is characterized by the presence of dark brown, spherical to pear-shaped pycnidia containing conidia on the aerial parts of chickpea plants. The teleomorph (*D. rabiei*) is characterized by dark brown to black pseudothecia on the overwintering chickpea debris (Kanouni *et al.,* 2011).

The existence of two mating types of *D. rabiei* was reported in Syria, Canada, Turkey, Iran, Tunisia, Spain, and USA (Ali *et al.,* 2012; Armstrong *et al.,* 2001; Atik *et al.,* 2011; Azizpour & Rouhrazi, 2017; Bayraktar *et al.,* 2007; Kaiser & Küsmenoglu, 1997; Navas-Cortes *et al.,* 1998;

Peever *et al.,* 2004; Rhaiem *et al.,* 2007). In most countries, Mating Type 1 is predominating in the pathogen populations. Breeding for host plant resistance and integrated disease management are considered as effective strategies to combat Ascochyta blight in many countries (Gan *et al.,* 2006; Sharma & Ghosh, 2016). The mode of Ascochyta blight resistance in chickpea is reported to be controlled by major (Labdi *et al.,* 2013) and minor genes (Muehlbauer & Chen, 2007; Rubiales & Fondevilla, 2012; Sharma & Ghosh, 2016). The most popular cultivar ILC- 3279 in west Asia released for winter sowing showed partial resistance to *D. rabiei* populations in Syria (Singh & Reddy, 1993). Ascochyta blight resistant chickpea cultivars are reported to lose their resistance in some countries due to appearance of highly virulent/aggressive races of *D. rabiei* populations (Atik *et al.,* 2013; Chen *et al.,* 2004; Imtiaz *et al.,* 2011; Mehmood *et al.,* 2017; Reddy & Kabbabeh, 1985; Vail & Banniza, 2008).

Limited studies have been made to determine the driving forces leading to the development of new virulent/ aggressive Ascochyta populations affecting cool-season food legumes. Studies on the inheritance of virulence on some *Ascochyta* spp. affecting food legumes showed that sexual reproduction could contribute in the generation of more aggressive progenies that could threaten resistant cultivars (Ahmed & Morrall, 1998; Beata & Pang, 2003; Peever *et al.,* 2012). The objective of this study was to determine if sexual reproduction plays a role in creating aggressive in *D. rabiei* population progenies by using progenies obtained from crosses between fungal isolates

with varying level of aggressiveness and comparing the effect of these progenies on chickpea genotypes with varying levels of Ascochyta blight resistance.

Materials and Methods

Crossing

Four isolates of *D. rabiei* with varying levels of aggressiveness (AR01, AR02, AR03 and AR04) collected from Syria were used in the crossing (Imtiaz *et al.,* 2011; Udupa *et al.,* 1998). The isolates were used to screen kabuli chickpea breeding lines and accessions for Ascochyta blight resistance at the International Center for Agricultural Research in the Dry Areas (ICARDA). The parent isolates were classified as MAT1-1 (AR02, AR03 and AR04) and MAT1-2 (AR01) by Atik *et al* (2011). The parents were grown on chickpea dextrose agar (CDA: 4% chickpea flour; 2% dextrose and 2% agar in one liter of distilled water) for one week inside a culture room at 21-23°C under 16/8 h light and darkness. Three crosses namely Cross-1 (AR01 X AR02); Cross-2 (AR01 X AR03) and Cross-3 (AR01 X AR04) were made using sterilized chickpea stem inoculation technique (Trapero-Casas & Kaiser, 1992).

Sterilized stem pieces from healthy chickpea plants were inoculated with 7×10^5 spores.ml^{-1} in suspension of the crosses and parent progenies. Stem pieces (6-8 cm long) were soaked in spore suspensions for 1 h, drained and placed in glass Petri dishes containing 10 filter papers moistened with sterile distilled water. After 7-9 weeks under darkness and 10°C inside incubator, small parts of stem pieces were checked microscopically for the presence of pseudothecia , the stem pieces were then air-dried at 21-23°C in a Laminar Flow hood and ascospores were discharged from inoculated stem pieces by placing pieces of stem (2.5 cm long) on a water agar block placed on the inner surface of a Petri dish which was inverted over a bottom dish that contained water agar. Ascospores discharged downwards onto the surface of the plate were incubated at 21 to 23°C for 24 h to observe their germination. Twenty randomly selected single ascospores from each cross were transferred to CDA and incubated for one week at 22-23°C under similar light regime mentioned earlier. Each progeny was kept inside 1.5 ml Eppendorf tube containing sterilized distilled water at -20°C until further use.

Seedling inoculation

Seeds of two chickpea genotypes, namely, ILC-263 (Kabuli) susceptible to the four parent isolates and ICC-12004 (Desi) resistant to AR01, AR02 and AR03 but susceptible to AR04 (Imtiaz *et al.,* 2011; Udupa *et al.,* 1998) were surface sterilized using 2% NaOCl, dried on filter paper under laminar flow and planted in plastic pots (12 cm diameter) filled with sterilized soil mixture. Four seeds/pot of each chickpea genotype were planted and kept in the glasshouse (18-20°C, 16/8 h photoperiod). The aggressiveness of progenies and parent isolates of each cross was separately tested on the two chickpea genotypes in randomized complete block design with three replications (one pot with four seedlings as one replication) in a glasshouse at the

Lebanese Agricultural Research Institute (LARI), Tal Amara, Lebanon in 2014.

At 4-6 leaf stages, chickpea seedlings were inoculated with spore suspension (5×10^5 ml^{-1}) of the F$_1$ progenies and their respective parents until runoff using 0.25 liter hand sprayers. Inoculated seedlings were covered with polyethylene sheets for 72 h under glasshouse conditions at 18-20°C, and after removing the cover, seedlings were kept under high humidity in the glasshouse (more than 85%) maintained by using controlled mist irrigation system. Latent period (LP) was recorded as the interval (days) between inoculation and first appearance of pycnidia on infected leaves and/or stem of the seedlings. Disease severity was rated 15 days after inoculation using 1-9 rating scale where 1= healthy plant, no disease; 2 = lesions present, but small and inconspicuous; 3= lesions easily seen, but plants are mostly green; 4= severe lesions clearly visible, stem infection is clear; 5= lesions girdle stems, most leaves show lesions; 6= plants collapse, tips die back; 7= plants dying, but at least green leaves present; 8= nearly all plants dead but still with a green stem; and 9= dead plants (Chen *et al.,* 2004).

Mating type of *D. rabiei* progenies

The mating type frequency of 57 progenies from the three crosses was assessed using MAT specific markers (Barve *et al.,* 2003). Four discs (5 mm diameter) of each progeny and parent progenies were inoculated into 250 ml flasks containing 50 ml of potato dextrose broth medium. After 4-6 days of incubation on rotary shaker (50 rpm and 20°C), mycelia were harvested from the flasks by vacuum filtration method using two layers of sterilized cheese cloth, lyophilized for five days and stored at -30°C. Approximately 50mg of the lyophilized mycelium was transferred to microfuge tubes and re-lyophilized for an additional day. Mycelium of each isolate was grinded to a fine powder by liquid nitrogen using ceramic mortars (Chongo *et al.,* 2004). Total fungal genomic DNA was extracted from all progenies and parental progenies using the hexadecyltrimethyl -ammonium bromide (CTAB) extraction buffer (1.4 M NaCl, 0.1 M Tris-HCl pH 8.0, 20mM EDTA, 0.2 % β-mercaptoethanol) using the method of Atik *et al*. (2011). The powdered lyophilized mycelia were dissolved in 1.2 ml of CTAB and transferred to 2 ml Eppendorf tubes and placed in water bath at 65°C for 60 min. After removing Eppendorf tubes from the water bath, 600 µl of chloroform-isoamyl alcohol (24:1 v/v) were added and tubes were hand shaken gently for 15 min and centrifuged for 20 min at 13000 rpm. The upper part of the liquid was transferred to another Eppendorf tube and 700 µl of cool isopropanol were added and shaken gently for 2 min then it was kept in the freezer for 10 min, centrifuged for 15 min at 13000 rpm and the supernatant was discarded. Finally, the DNA pellet was washed twice with 1 ml of 70% ethanol, allowed to be air dried for 3 hours and dissolved in 1× TE buffer (10 mM Tris Cl, PH 8.0, 1mM EDTA). The quantity and quality of the extracted DNA were assessed by running 1 µl of the DNA on 1% of agarose gel, stained with ethidium bromide and photographed under UV illumination.

Mating types were determined using multiplex MAT-specific PCR with three primers: MAT1-1 specific primer

Sp21 (5'-ACAGTGAGCCTGCACAGTTC-3'), MAT1-2 specific primer Tail 5 (5'-CGCTATTTTATCCAAGACACACC-3') and flanking region-specific primer Com1 (5'-GCATGCCATATCGCCAGT-3') were combined in equal concentrations in a single multiplex PCR (Barve *et al.,* 2003). Reactions were made up of 12 ng DNA template, 400 nM of each primer (Tail5, Com1andSP21), 2.5 µl 10× TAE buffer, 1.5 mM MgCl$_2$, 25 mM dNTPs each, 1 U *Taq* DNA polymerase (Invitrogen Life Technologies) and were adjusted to a volume of 25 µl with sterile, double-deionized water. Amplifications were performed with a PTC-200 Peltier Thermal Cycler (MJ Research, Waltham, MA) with an initial denaturation step of 95°C for 3 min followed by 44 cycles of 95°C, for 30 s, 60°C for 30 s and 72°C for 1 min for each cycle, with a final extension of 72°C for 5 min. Amplified products were separated by electrophoresis on a 1.5% of agarose gel stained with ethidium bromide and photographed under UV illumination.

Data analyses

Latent period (LP) and disease severity (mean of four plants/pot) were analyzed using the residual (restricted) maximum likelihood (REML) method in linear mixed model (Garrett *et al.,* 2004) using Genstate Software (16th edition). In the model, chickpea genotypes and pathogen progenies were assigned as fixed and replications as random factors. Least square differences were calculated using the standard errors of the differences.

Results

Pseudothecia were observed 7-9 weeks after incubation from all crosses. The frequency of the two mating types in the three crosses was almost equal (Table1). However, there were more progenies with MAT1-2 in the cross of AR01 X AR03 than the other two crosses (Figure 1).

Table 1. Segregation of MAT1-1, MAT1-2 in crossed progeny using MAT specific primers.

Cross	MAT1-1	MAT1-2	Ratio
AR01xAR02	10	10	10:10
AR01xAR03	8	10	8:10
AR01xAR04	11	8	11:8

Figure 1. Multiplex polymerase chain reaction for MAT genes of 18 progenies and two parents from AR01 (MAT1-2= 470bp) X AR03 (MAT1-1=700bp) cross.

In the aggressiveness studies, all progenies of the three crosses and parent progenies caused infections on both chickpea genotypes. In Cross-1, significant differences were not observed among progenies for latent period (LP). The duration for the formation of pycnidia ranged from 7-12 days among progenies infecting the two chickpea genotypes. Six progenies (five on ICC-12004 and one on ILC-263) did not produce pycnidia under the prevailing environmental conditions in the glasshouse (Figure 2). Some progenies showed shorter LP than their parents on the two chickpea genotypes.

Significant differences (P < 0.001) were observed among progenies on their aggressiveness on the two chickpea genotypes. Most progenies caused low mean disease severity (< 4 rating) on genotype ICC-12004 and 12 progenies caused higher disease severity on ILC-263 than AR01 parental progeny (Table 2). The mean severity ranged from 1.5 to 5.9 and the highest mean disease severity was caused by AR02.

Table 2. Aggressiveness of progenies of AR01 X AR02 cross as measured by disease severity on two chickpea genotypes.

Progenies and parents	Disease severity		
	ICC-12004	ILC-263	Mean
AR01	2.2	3.9	3.0
AR02	4.8	7.0	5.9
AR01XAR02-01	2.2	4.5	3.3
AR01XAR02-02	1.8	3.6	2.7
AR01XAR02-03	1.8	2.0	1.9
AR01XAR02-04	1.4	2.6	2.0
AR01XAR02-05	2.5	5.0	3.8
AR01XAR02-06	2.7	4.7	3.7
AR01XAR02-07	1.4	1.6	1.5
AR01XAR02-08	1.9	3.0	2.4
AR01XAR02-09	1.8	2.3	2.1
AR01XAR02-10	1.7	4.5	3.1
AR01XAR02-11	2.8	4.9	3.9
AR01XAR02-12	1.8	4.9	3.3
AR01XAR02-13	3.0	5.9	4.4
AR01XAR02-14	2.4	2.9	2.7
AR01XAR02-15	1.9	6.4	4.2
AR01XAR02-16	2.7	3.7	3.2
AR01XAR02-17	2.8	5.2	4.0
AR01XAR02-18	2.7	4.6	3.6
AR01XAR02-19	2.6	4.5	3.5
AR01XAR02-20	2.4	5.0	3.7
Mean	2.3	4.2	

Standard error of the difference for progenies= 0.628
LSD (0.01 df= 21) for progenies= 2.831 X 0.63=1.78

In cross-2, significant differences (P < 0.001) were observed among progenies for LP (Figure 3). The duration for the formation of pycnidia range was 7-12 days and all progenies showed long LP on genotype ICC-12004. Some progenies formed pycnidia in shorter duration than parent progenies. For disease severity, the interaction between chickpea genotypes and progenies for disease severity was significant (P < 0.03). The mean disease severity ranged

from 1.8 to 4 rating indicating low levels of progenies aggressiveness. All progenies caused low disease severity (< 3 rating) on genotype ICC-12004 and four progenies caused > 5 rating on genotype ILC-263 (Table 3).

In cross-3, all progenies produced pycnidia on both chickpea genotypes except one progeny did not produce pycnidia on genotype ICC-12004, and significant differences were observed among progenies for LP. The duration of pycnidia formation range was 6-12 days (Figure 4). The interaction between chickpea genotypes by progenies for disease severity was highly significant (P < 0.001). The mean

disease severity ranged from 3.5 to 6.3 rating and progenies showed low disease severity on ICC-12004 (< 4 rating) but high disease severity on ILC-263, and one progeny caused higher disease severity on ILC-263 than AR04 parental progeny (Table 4).

The correlations of LP and aggressiveness of progenies (as measured by disease severity) were negative and significant. The correlations ranged from medium (Cross-1, r = -0.3, P < 0.01; Cross-2, r =-0.4 P < 0.001) to high (Cross-3, r =-0.04, P < 0.001).
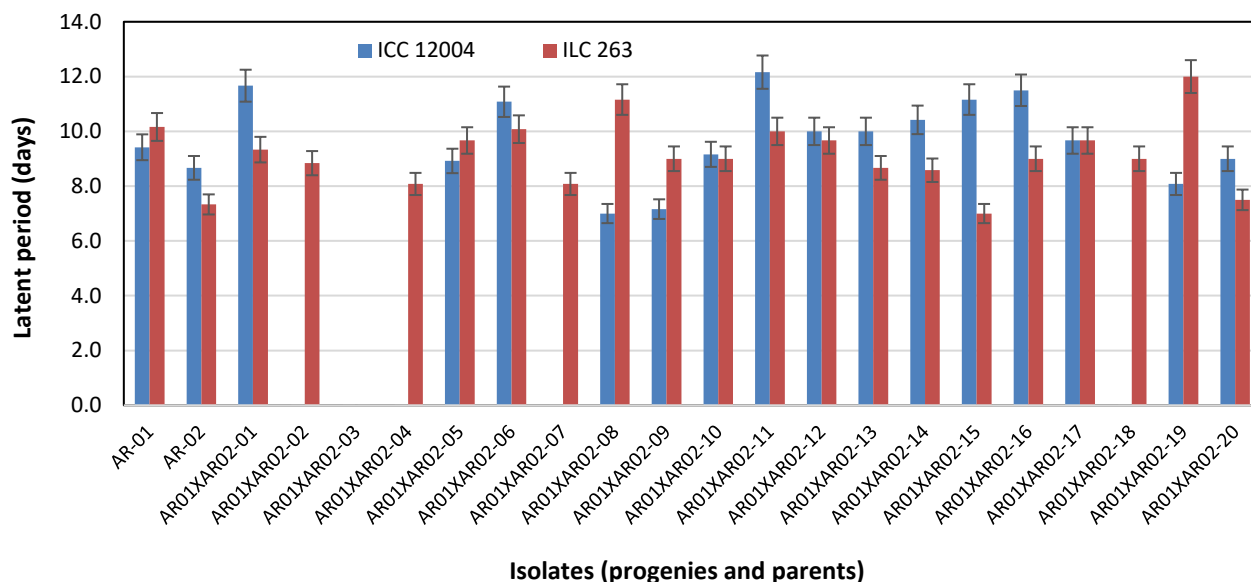

Figure 2. Duration of pycnidia formation by progenies (AR01 X AR02 cross) inoculated on two chickpea genotypes. Bars represent standard errors.
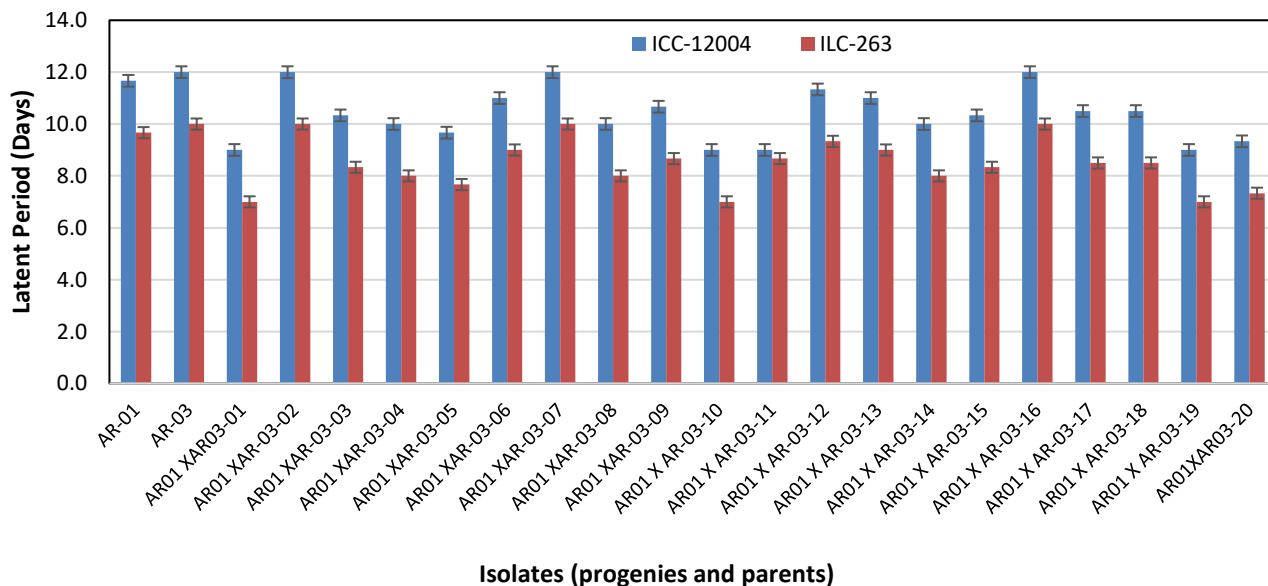

Figure 3. Duration of pycnidia formation by progenies (AR01 X AR03 cross) inoculated on two chickpea genotypes. Bars represent standard errors.
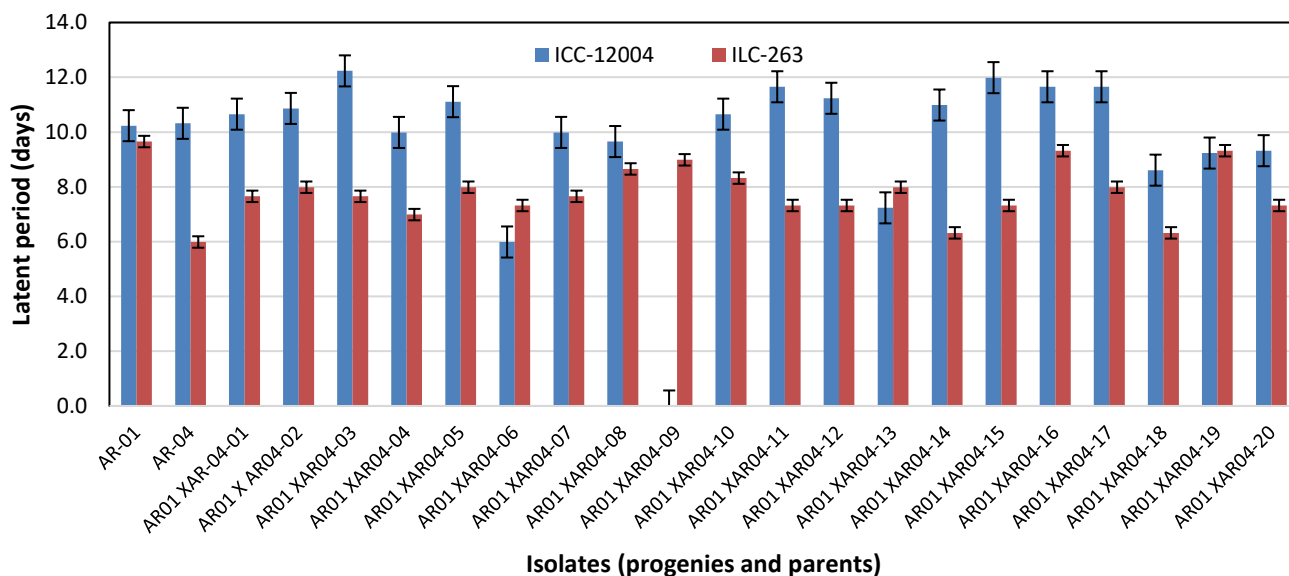
Figure 4. Duration of pycnidia formation by progenies (AR01 X AR04 cross) inoculated on two chickpea genotypes. Bars represent standard errors.

Table 3. Aggressiveness of progenies of AR01 X AR03 crosses as measured by disease severity on two chickpea genotypes.

Progenies and parents	Disease severity		
	ICC-12004	ILC-263	Mean
AR01	1.5	3.8	2.7
AR03	2.2	4.0	3.1
AR01 XAR03-01	1.6	2.6	2.1
AR01 XAR03-02	1.6	3.1	2.3
AR01 XAR03-03	1.8	4.6	3.2
AR01 XAR03-04	1.7	5.1	3.4
AR01 XAR03-05	1.4	3.9	2.7
AR01 XAR03-06	2.1	5.8	4.0
AR01 XAR03-07	2.1	2.5	2.3
AR01 XAR03-08	1.9	5.3	3.6
AR01 XAR03-09	1.2	2.4	1.8
AR01 XAR03-10	1.8	4.0	2.9
AR01 XAR03-11	1.5	2.8	2.2
AR01 XAR03-12	1.6	3.3	2.5
AR01 XAR03-13	1.3	3.3	2.3
AR01 XAR03-14	2.4	4.9	3.6
AR01 XAR03-15	1.8	5.2	3.5
AR01 XAR03-16	1.4	3.6	2.5
AR01 XAR03-17	2.0	3.8	2.9
AR01 XAR03-18	1.5	2.9	2.2
AR01 XAR03-19	2.1	3.9	3.0
AR01 XAR03-20	1.8	3.7	2.8
Mean	1.7	3.8	

Standard error of differences for genotype by isolate interaction = 0.6392
LSD (0.01 df= 21) for genotype X isolate interaction= 1.81

Table 4. Aggressiveness of progenies of AR01 X AR04 crosses as measured by disease severity on two chickpea genotypes.

Progenies and parents	Disease severity		
	ICC-12004	ILC-263	Mean
AR01	1.8	5.6	4.3
AR04	4.3	8.2	6.3
AR01 XAR04-01	2.3	9.0	5.6
AR01 X AR04-02	2.4	6.3	4.4
AR01 XAR04-03	2.1	6.4	4.3
AR01 XAR04-04	2.6	6.4	4.5
AR01 XAR04-05	2.3	6.3	4.3
AR01 XAR04-06	2.0	6.2	4.1
AR01 XAR04-07	2.9	7.6	5.3
AR01 XAR04-08	2.7	7.0	4.8
AR01 XAR04-09	1.8	5.4	3.6
AR01 XAR04-10	3.2	6.1	4.7
AR01 XAR04-11	3.9	7.2	5.5
AR01 XAR04-12	1.3	6.5	3.9
AR01 XAR04-13	1.6	5.4	3.5
AR01 XAR04-14	2.4	7.3	4.8
AR01 XAR04-15	2.0	6.1	4.1
AR01 XAR04-16	2.9	5.7	4.3
AR01 XAR04-17	2.8	6.8	4.8
AR01 XAR04-18	2.3	7.9	5.1
AR01 XAR04-19	1.6	6.3	3.9
AR01 XAR04-20	2.4	5.3	3.9
Mean	2.4	6.6	

Standard error of differences (progenies)= 0.5198
LSD (0.01 df= 21) for progenies= 1.47

Discussion

Ascochyta blight is a high risk biotic yield limiting factor in many traditional and non-traditional chickpea producing countries (Chen *et al.*, 2017; Rubiales & Fondevilla, 2012; Viotti *et al.*, 2012). Pathogens with sexual and asexual reproduction are considered as high risk (Heitman, 2015; McDonald & Linde, 2002) and *D. rabiei* can be classified under this category since it can cause complete crop failure.

The major threat of resistant breeding is the evolution of virulent/aggressive *D. rabiei* populations in many countries. In our study the range in duration for the formation of pycnidia was similar to all progenies and parents in the three crosses. Some progenies did not produce pycnidia and the reasons are not known. Early formation of pycnidia is critical to the pathogen fitness and also for creating epidemics under favorable conditions compared to progenies with long LP. Some progenies showed high aggressiveness on the susceptible genotype than the resistant ILC-12004. The formation of progenies with high virulence can be potential threat for resistant cultivars and also make fungicides less effective (Chang *et al.*, 2007; Wise *et al.*, 2008). Similar result showed that progenies of a weakly (progenies from Idaho, USA) and highly virulent (progenies northern Syria) *D. rabiei* progenies showed high disease severity on the susceptible cv. Spanish White than the moderately resistant cv. Dwelley (Peever *et al.*, 2012).

The identification of the two mating types in almost equal frequency is similar to those reported from field populations of *D. rabiei* leading to random mating that can create high genetic diversity in the population. Published work showed that MAT1-1 is more dominant than MAT1-2 in *D. rabiei* populations (Ali *et al.*, 2012; Atik *et al.*, 2011; Bayraktar *et al.*, 2007; Kaiser & Küsmenoglu, 1997; Navas-Cortes *et al.*, 1998; Nourollahi *et al.*, 2011; Rhaiem *et al.*, 2007). However, MAT1-2 was dominant over MAT1-1 in North Dakota population (Ali *et al.*, 2012). Some studies showed that extensive production of resistant chickpea (Leo *et al.*, 2015; Mehmood *et al.*, 2017; Peever *et al.*, 2004) and lentil (Davidson *et al.*, 2016) cultivars brought the appearances of aggressive pathogen populations *Didymella* spp affecting the two crops.

Conservation cropping where chickpea straw is left in the field will play a key role in generating new aggressive progenies through sexual reproduction in countries where the two mating types exist and environmental conditions are conducive for sexual reproduction. The progenies can affect resistant cultivars and make commonly used fungicides to manage Ascochyta blight less effective.

In conclusion, sexual reproduction through the mating of low by high aggressive parents in *D. rabiei* can create progenies with more aggressiveness and short LP that are critical to create disease epidemics that lead to high yield and quality losses in chickpea. Moreover, the response of the progenies to commonly used fungicides against Ascochyta blight and the role of mating type gene in pathogen isolate fitness should be studied.

Acknowledgements

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الملخص

عطار، باسم، سعيد أحمد، مقدس كايم، إيليا الشويري، هند غنام وعلاء حموية. 2020. تأثير التكاثر الجنسي على شراسة الفطر *Didymella rabiei* المسبب لمرض لفحة الحمص. مجلة وقاية النبات العربية، 38(1): 17−24.

يؤثر مرض لفحة الأسكوكايتا سلبياً في إنتاجية محصول الحمص في العديد من البلدان، ومن الممكن للأصناف المقاومة المعتمدة أن تخرج من الاستخدام بسبب ظهور مجتمعات من هذا الممرض أكثر شراسة. من المحتمل أن يؤدي التكاثر الجنسي للفطر *Didymella rabiei* دوراً أساسياً في نشوء مجتمعات أكثر شراسة. تم تنفيذ ثلاثة تهجينات باستخدام أربعة عزلات من الفطر (أربعة طرز) مجموعة من سورية؛ الطراز الممرض الأول AR01 وهو من النمط التزاوجي الثاني MAT1-2 مع بقية الطرز الممرضة الثاني والثالث والرابع AR02، AR03 و AR04، على التوالي وهي جميعاً من النمط التزاوجي الأول MAT1-1 حيث تختلف هذه الطرز الممرضة الأربعة فيما بينها بدرجة الشراسة. تم اختيار عشوائي لـ 20 نسلاً من كل تهجين واختبرت شراسة هذه الأنسال مقارنة مع آبائها (بحسب كل تهجين) على طرازي الحمص: ILC-263 الحساس للممرض و ICC-12004 المقاوم له، كما تم تحديد تردد الأنماط التزاوجية في الأنسال الناتجة باستخدام التفاعل التسلسلي للبوليميراز المتعدد. تم قياس شراسة الأنسال الناتجة عن التهجينات بوساطة فترة الكمون (Latent period) وشدة المرض. تراوحت فترة الكمون بين 6−12 يوماً في جميع الأنسال وكانت هناك فروق معنوية في شراسة الأنسال المتحصل عليها من التهجينات الثلاثة. تم الحصول على أعلى مستوى من الشراسة في الأنسال المتحصل عليها من التهجين بين الطرازين الممرضين الأول AR01 والرابع AR04. كانت نسبة تردد النمطين التزاوجيين الأول والثاني متساوية تقريباً في جميع التهجينات. أظهرت هذه الدراسة أنه من الممكن للتكاثر الجنسي عند هذا الفطر أن ينتج سلالات جديدة قد تكون أكثر شراسةً على أصناف الحمص المعتمدة كأصناف مقاومة للمرض، وقد تكون هذه السلالات أقل تأثراً بالمبيدات المستخدمة لمكافحة المرض.

كلمات مفتاحية: *Didymella rabiei*، النمط التزاوجي، الأنسال، الحمص.

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