NOTAS CIENTÍFICAS

A comparison of methods for the detection of *Ascochyta rabiei* in chickpea seeds

Francisco José Sautua[1], Santiago Agustín Casey[1], Raúl Lorenzo Zapata[1], María Mercedes Scandiani[2], Marcelo Aníbal Carmona[1]

[1]Universidad de Buenos Aires, Facultad de Agronomía, Cátedra de Fitopatología, Av. San Martin 4453, C1417DSE, Ciudad Autónoma de Buenos Aires, Argentina. [2]Centro de Referencia de Micología (CEREMIC), Facultad de Ciencias Bioquímicas y Farmacéuticas, Universidad Nacional de Rosario, Suipacha 531, S2002LRK, Rosario, Argentina.
Autor para correspondencia: Marcelo Aníbal Carmona (carmonam@agro.uba.ar)
Data de chegada: 16/09/2017. Aceito para publicação em: 04/02/2019.

10.1590/0100-5405/185396

ABSTRACT

Sautua, F.J.; Casey, S.A.; Zapata, R.L.; Scandiani, M.M.; Carmona, M.A. A comparison of methods for the detection of Ascochyta rabiei in chickpea seeds. ***Summa Phytopathologica***, v.45, n.2, p.197-199, 2019.

Seed health is one of the most important factors affecting the quality of chickpea (*Cicer arietinum*) seeds. The present study aimed to compare and identify the best incubation methods for detecting *Ascochyta rabiei* associated with chickpea seeds. Four protocols were compared for their sensitivity in detecting *A. rabiei*: T1) Incubation on paper substrate or filter paper method (blotter test) without surface disinfection, T2) Blotter test through the water restriction technique, T3) PDA plate test, and T4) MEA plate test. Four independent chickpea seed lots, naturally infected with *A. rabiei*, were sampled from Córdoba Province and other four were sampled from Buenos Aires Province, Argentina. Each treatment was applied to a total of 400 seeds from each locality for the methods to be comparable. T2 and T3 were statistically more sensitive in detecting *A. rabiei*-infected seeds from Córdoba. Only these two treatments were repeated for seeds from Buenos Aires Province, and T3 proved to be more sensitive; thus, it is recommended for routine sanitary analysis of chickpea seeds.

Keywords: Ascochyta blight, *Cicer arietinum*, Standard blotter test, PDA plate test

RESUMO

Sautua, F.J.; Casey, S.A.; Zapata, R.L.; Scandiani, M.M.; Carmona, M.A. Comparação de métodos para detecção de *Ascochyta rabiei* em sementes de grão-de-bico. ***Summa Phytopathologica,*** v.45, n.2, p.197-199, 2019.

A saúde das sementes é um dos fatores mais importantes que afetam a qualidade das sementes de grão-de-bico (*Cicer arietinum*). O presente estudo tem como objetivo comparar e identificar melhores métodos de incubação para a detecção de *Ascochyta rabiei* associada a sementes de grão-de-bico. Quatro protocolos foram comparados em sua sensibilidade para detectar *A. rabiei*: T1) Incubação em substrato de papel ou método do papel de filtro ("blotter test") sem desinfecção superficial, T2) Botter test através da técnica de restrição de água, T3) Teste de placa com PDA e T4) Teste de placa com MEA. Quatro lotes de sementes de grão-de-bico independentes, naturalmente infectados com *A. rabiei*, foram amostrados na província de Córdoba e quatro na província de Buenos Aires, Argentina. No total, cada tratamento foi aplicado a um total de 400 sementes de cada localidade, para que os métodos sejam comparáveis. O T2 e T3 foram estatisticamente mais sensíveis para detectar sementes de Córdoba infectadas com *A. rabiei*. Apenas estes dois tratamentos foram repetidos com sementes da província de Buenos Aires, onde o T3 se mostrou mais sensível, por isso é recomendado para as rotinas de análise sanitária das sementes de grão-de-bico.

Palavras chave: Mancha de ascochyta, *Cicer arietinum*, "Standard blotter test", Teste de placa com PDA

Ascochyta blight (AB) is the most frequent and damaging disease affecting chickpea (*Cicer arietinum*) worldwide. Under conditions suitable for disease development, AB can cause extensive crop losses (up to 100%) in most regions of the world where the crop is commonly grown (7). It is caused by *Ascochyta rabiei* [Pass.] Labrousse, teleomorph *Didymella rabiei* (Kovacheski) (Syn. *Mycosphaerella rabiei* Kovacheski), a fungus that selectively attacks chickpea (2). In Argentina, *A. rabiei* was first reported in 2011 (9). This pathogen is believed to have entered Argentina through seed, as occurred in other countries such as Australia (1993), Iran (1968), Canada (1974) and the United States (1983) (10). A lot of chickpea seeds with 1‰ grains infected with *A. rabiei* leads to a 400 primary outbreaks of the disease in the implanted culture (3). Therefore, to avoid AB in the fields, the batch of seeds used for planting must be ensured to be healthy (4). Growers should make certain that their seed comes from fields and areas that are free of AB. Unfortunately, a seed that looks healthy may be infected with low levels of *A. rabiei*. This is why the method for analyzing the health of the used seed is extremely important. The objective of the present study was to compare different incubation methods for the detection of *A. rabiei* associated with chickpea seeds. Four protocols of pathological seed analysis were tested to compare their efficiency for *A. rabiei* detection. The experiments were carried out at the Plant Pathology Laboratory of the School of Agronomy of the University of Buenos Aires. The four treatments consisted in: T1) Standard blotter test (SBT): seeds without surface disinfection were plated on sterilized water-soaked filter papers in plastic trays and were incubated at 21°C+/-

Table 1. Incidence (%) of *Ascochyta rabiei* in Chañarito variety chickpea seeds. Abbreviations: PDA = potato dextrose agar; MEA = malt extract agar.

	Experiment repetition			
Seed lots from Córdoba Province	1	2	3	4
T1 Standard blotter test	4 a	2 a	4 a	4 a
T2 Blotter test through the water restriction technique	20 b	30 b	16 b	22 b
T3 PDA plate test	19 b	28 b	18 b	24 b
T4 MEA plate test	3.13 a	1.25 a	1.88 a	1.88 a
Seed lots from Buenos Aires Province	1	2	3	4
T2 Blotter test through the water restriction technique	11 a	14 a	16 a	15 a
T3 PDA plate test	28 b	19 b	18 a	21 b

Within a column, values followed by different letters are significantly different at 5% level of probability.

1°C for 7 days under a 12h light:dark cycle. After incubation, the seeds were observed under a stereomicroscope (60x) for pycnidia and under an optical microscope (400x) for conidia confirmation (5). T2) Blotter test through the water restriction technique (BTWRT): seeds were surface-disinfected with 1% sodium hypochlorite (NaOCl) solution for 1-2 minutes; then, the seeds were rinsed 2 times with demineralized sterile water for one minute each and finally allowed to dry on sterile filter paper. The solution was drained and seeds were air-dried in the biological cabinet for 30 minutes. The remainder of the procedure, incubation and detection of *A. rabiei* were performed as described for treatment T1 (5). T3) PDA plate test: seeds were surface-disinfected with 0.1% sodium hypochlorite (NaOCl) solution for 1-2 minutes; then, they were rinsed with demineralized sterile water for one minute and finally allowed to dry on sterile filter paper. Dried seeds were plated on potato dextrose agar (PDA) poured in 9-cm glass Petri dishes (5). PDA was prepared according to the traditional recipe (200g peeled and sliced potatoes, 20g dextrose, 17g Agar, 1000cc distilled water) (1). Each plate carried 18 ml of the culture medium. The remainder of the procedure, incubation and detection of *A. rabiei* were performed as described for treatments T1 and T2. T4) MEA plate test: this treatment was the same as T3 but, instead of PDA, malt extract agar (MEA) was used as the culture medium. MEA was prepared according to the traditional recipe (20g/l Agar, 20g/l Malt Extract Agar, 1000cc distilled water) (5). Eight independent chickpea seed lots naturally infected with *A. rabiei* were randomly sampled: four from the center of Córdoba Province and four from Coronel Pringles, in Buenos Aires Province, Argentina. All seed lots corresponded to "Chañarito" chickpea variety. In a first experiment, the four treatments were applied to seed samples from Córdoba. Afterwards, in a second experiment, treatments T2 and T3 were applied to seed samples from Buenos Aires. For protocols T1 and T2 (blotter tests), 4 replicates were performed and the experimental unit consisted of a plastic tray with dimensions of 16x20x5cm, containing 25 seeds each. On the other hand, for protocols T3 and T4 (agar plate assays), 10 replicates were performed and the experimental unit consisted of a 90mm-diameter glass Petri dish containing 10 seeds each. Experiments were repeated 4 times (4 seed lots, respectively). In each experiment, each treatment was applied to 400 seeds, for the methods to be comparable. Treatments were arranged in a completely randomized block (RCB) design and the incubator shelves served as blocks. The evaluated response variable was the incidence, defined as the percentage of infected seeds in a sample. Infection was defined as the presence of symptoms and signs (pycnidia and conidia) characteristic of AB caused by *A. rabiei* (4). One-way analysis of variance (ANOVA) was used to analyze the results, and means were compared according to Tukey's test ($\alpha = 5\%$).

The prevalence of *A. rabiei* was 100%, that is, the presence of *A. rabiei* was detected in 100% seed samples from Córdoba (first experiment). T2 and T3 plate tests were significantly different from T1 and T4 plate tests, since the former two tests were statistically more sensitive in detecting *A. rabiei*-infected seeds (Table 1). No statistically significant differences were found between these two treatments. Visual sporulation of the fungus (pycnidia) on the seed was generally heavier in both methods. On the other hand, T1 and T4 plate tests were less sensitive and efficient in detecting *A. rabiei* infections. According to the results of the first experiment, T2 and T3 plate tests were chosen to be tested in the seed samples from Buenos Aires. In this second round of experiments, T3 plate test was statistically more sensitive in detecting *A. rabiei* infections than T2 for three of four replicates of the experiment (Table 1). According to the obtained results, T2 and T3 plate tests should be used for the diagnosis of *A. rabiei* infections in chickpea seeds. Between these two methods, the PDA plate test proved to be more sensitive; thus, it is recommended for routine sanitary analysis of chickpea seeds. The PDA plate test has been shown to be efficient for the study of *A. rabiei* resistance to fungicides (6) and *A. pisi* seed infections (8). Nevertheless, additional studies are needed to establish the accuracy of PDA medium in seed samples of different varieties and with different levels of *A. rabiei* infection.

ACKNOWLEDGMENTS

We thank Ing. Adrian Poletti for providing samples of chickpea seeds.

REFERENCES

1. Andrews, W.H.; Hammack, T.S. **Bacteriological analytical manual**. 8th ed. Washington, D.C.: US Food and Drug Administration FDA, 1998. Available on: < https://www.fda.gov/food/foodscienceresearch/laboratorymethods/ucm2006949.htm >. Accessed on: July 17, 2017.
2. Bayaa, B.; Chen, W. Ascochyta Blight of Chickpea. In: Chen, W.; Sharma, H.C.; Muehlbauer, F.J. (Ed.). **Compendium of chickpea and lentil diseases and pests**. St. Paul: APS Press, p.34-40, 2011.
3. del Moral de la Vega, J. **Programa sanitario para el control de la rabia del garbanzo [Didymenlla rabiei (Kovachevski) v. Arx)]**. Madrid: Ministerio de Agricultura, Pesca y Alimentación, 1995. 188p.
4. Harveson, R.M.; Markell, S.G.; Goswami, R.; Urrea, C.A.; Burrows, M.E.; Dugan, F.; Chen, W.; Skoglund, L.G. Ascochyta blight of chickpeas. **Plant Health Progress**, St. Paul, v. 12, n. 1, p.1-9, 2011.
5. Mathur, S.B.; Kongsdal, O. **Common Laboratory Seed Health Testing Methods for Detecting Fungi**. 1st ed. Zürich: ISTA, 2003. 425p.
6. Owati, A.S.; Agindotan, B.; Pasche, J.S.; Burrows, M. The Detection and

Characterization of QoI-Resistant Didymella rabiei Causing Ascochyta Blight of Chickpea in Montana. **Frontiers in Plant Science**, Lausanne, v.8, p.1165, 2017.

7. Pande, S.; Sharma, M.; Gaur, P.M; Tripathi, S.; Kaur, L.; Basandrai, A.; Khan, T.; Gowda, C.L.L.; Siddique, K.H.M. Development of screening techniques and identification of new sources of resistance to Ascochyta blight disease of chickpea. **Australasian Plant Pathology**, Basel, v.40, p.149-156, 2011.
8. Sivachandra Kumar, N.T.; Banniza, S. Assessment of the Effect of Seed Infection with Ascochyta pisi on Pea in Western Canada. **Frontiers in Plant Science**, Lausanne, v.8, p.933, 2017.
9. Viotti, G.; Carmona, M.; Scandiani, M.; Formento, N.; Luque, A. First Report of *Ascochyta rabiei* Causing Ascochyta Blight of Chickpea in Argentina. **Plant Disease**, St. Paul, v.96, n.9, p.1375, 2012.
10. Viotti, G.; Carmona, M.; Scandiani, M.; Formento, N.; Luque, A. La "rabia" o tizón del garbanzo en Argentina: una enfermedad destructiva. **Análisis de Semillas**, Rosario, v.6, n.21, p.22-25, 2012.