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First report of *Rhizoctonia solani* AG 2-3 on chickpea in Tunisia

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Summary. Chickpea plants (cv. Béja 1) showing typical symptoms of root and collar rots were collected from the Beja area (Tunisia). Visual diagnostic, isolation and microscopic observation identified the causal organism as *Rhizoctonia solani*. Sequence data of the ITS rDNA region confirmed the species identity and revealed that the anastomosis group of the isolate was AG2-3. Mechanical inoculation of chickpea seedlings resulted in the typical root and collar rot, proving that this isolate is pathogenic on chickpea. This is the first report of *R. solani* AG2-3 causing root and collar rot of chickpea in Tunisia.

Key words: AG2-3, *Cicer arietinum*, collar rot, root rot.

Introduction

Chickpea (*Cicer arietinum* L.) is an important grain legume in Tunisia. With a total acreage of approximately 10,500 ha, this crop has an annual average yield of 941 tons. However, yields fluctuate greatly (Anonymous, 2008) and it is likely that fungal diseases, among other factors, contribute to these fluctuations. To date, more than 50 pathogens have been reported on chickpea from different parts of the world (Bayraktar and Dolar, 2009). However, only a few of them cause serious economic losses, such as Ascochyta blight caused by *Ascochyta rabiei*, Fusarium wilt caused by *Fusarium oxysporum* f. sp. *ciceris*, and root rot caused by number of fungi, including *Rhizoctonia solani* (Bayraktar and Dolar, 2009). This last fungus occasionally causes root and collar rots and gradual suberization of plant tissues. In countries with a large chickpea production another relevant pathogen, *Macrophomina phaseolina* (previously named *Rhizoctonia bataticola*) seems to be the most important cause of seed rot, root rot of seedlings, necrosis of the root collar, and suberization of infected tissues (Dhrub-Sing *et al.,* 1987, Tayars *et al.*, 1988). However, it is clear that *R. solani* too is a major impediment to the establishment of chickpea seedlings, especially in warm soils (Chang *et al.*, 2002).

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Materials and methods

In the spring of 2007, wilting and yellowing symptoms were seen in a chickpea field in the Beja region (north-western Tunisia) sown with cv. Béja1. Almost 70% of the plants in this field were affected. Infected plants were found to have dark-brown lesions on the roots, sometimes extending above the collar region (Figure 1), similar to the symptoms described by Masur *et al.* (2004). Tissues were corky when broken down and the secondary roots were infected.

Four samples each of six wilting and yellowing

plants (roots and collars), scoring 2 to 3 on the 1–4 scale of Tezcan and Yildiz (1991 Yildiz and Doken, 2002), were collected and brought separately to the laboratory for diagnosis. The roots and collars were soaked for 2 min in 1% sodium hypochlorite, followed by 1 min in 70% ethanol, and then rinsed twice in sterilized distilled water. Excess water was removed with sterilized filter paper and isolations were done from the collar region and the roots by transferring necrotic tissue to potato dextrose agar (PDA) and water agar. Plates were incubated at 25°C for 48 hours with a 12 hour day. Isolates were identified on the basis of culture morphology and microscopic characteristics after staining with safranin O according to Sneh *et al.*, (1994).

Results and discussion

Microscopic inspection revealed a buff-colored to dark-brown, regularly septate mycelium with a slight constriction at the septum. Lateral branches arose near the distal septum at an angle of about 45° from the main hypha, with a septum soon after the branch. Sclerotia were irregular in shape, light to dark-brown. More than two nuclei could be seen in the cells close to the tips of young hyphae and the main runner hyphae were less than 7 μm wide. These characters confirmed that the isolates were *R. solani* (Sneh *et al.*, 1994; Champion, 1997).

Figure 1. Collar and root rot symptoms on chick pea plants.

Mycelium was collected from a PDA culture incubated at 25°C for 7 days with a 12-hour day and the DNA was extracted according to Tendulkar *et al*. (2003). The ITS1-5.8S-ITS2 region was amplified with primers ITS1 and ITS4 (White et *al.*, 1992). Amplicons were purified with the Promega purification Kit (Wizard® SV gel and PCR Clean-UpSystem, Madison, WI, USA). The PCR products were directly sequenced in both directions using primers ITS1 and ITS4. A BLAST search of the NCBI GenBank database with the only sequence generated in this work gave 98% similarity to *Rhizoctonia solani* AG2-3. The closest sequence was affiliated to accession number AB054872.1. The sequence was submitted to the GenBank and was given the accession number HM054532.

The pathogenicity of the isolates was tested on chickpea seedlings cv. Béjal. Flasks containing oat (*Avena sativa*) seeds were sterilized by autoclaving at 120°C for 30 min and were then inoculated with one of the identified *Rhizoctonia* isolates grown on PDA. The flasks were incubated at 25°C for 10 days with a 12 hour day and frequently shaken. The inoculum was then used, at a rate of 20 g kg^{-1} of sterilized peat, to inoculate three batches of 30 chickpea seedlings cv. Béja1 sown in six pots (five per pot) each containing 1 kg of sterilized peat. Each batch was repeated three times and disease incidence was recorded every day by assessing the percentage of seedlings that exhibited wilting and yellowing. One week after inoculation, all the inoculated seedlings developed the same symptoms of the disease as were seen in the field from which the diseased samples had originally been collected. No wilting or rot occurred in the controls (Figure 2). One sample per seedling chosen at random from three inoculated seedlings exhibiting symptoms were analysed in the laboratory. The fungus isolated was *R. solani* in all cases. *R. solani* was not detected in any of the control seedlings.

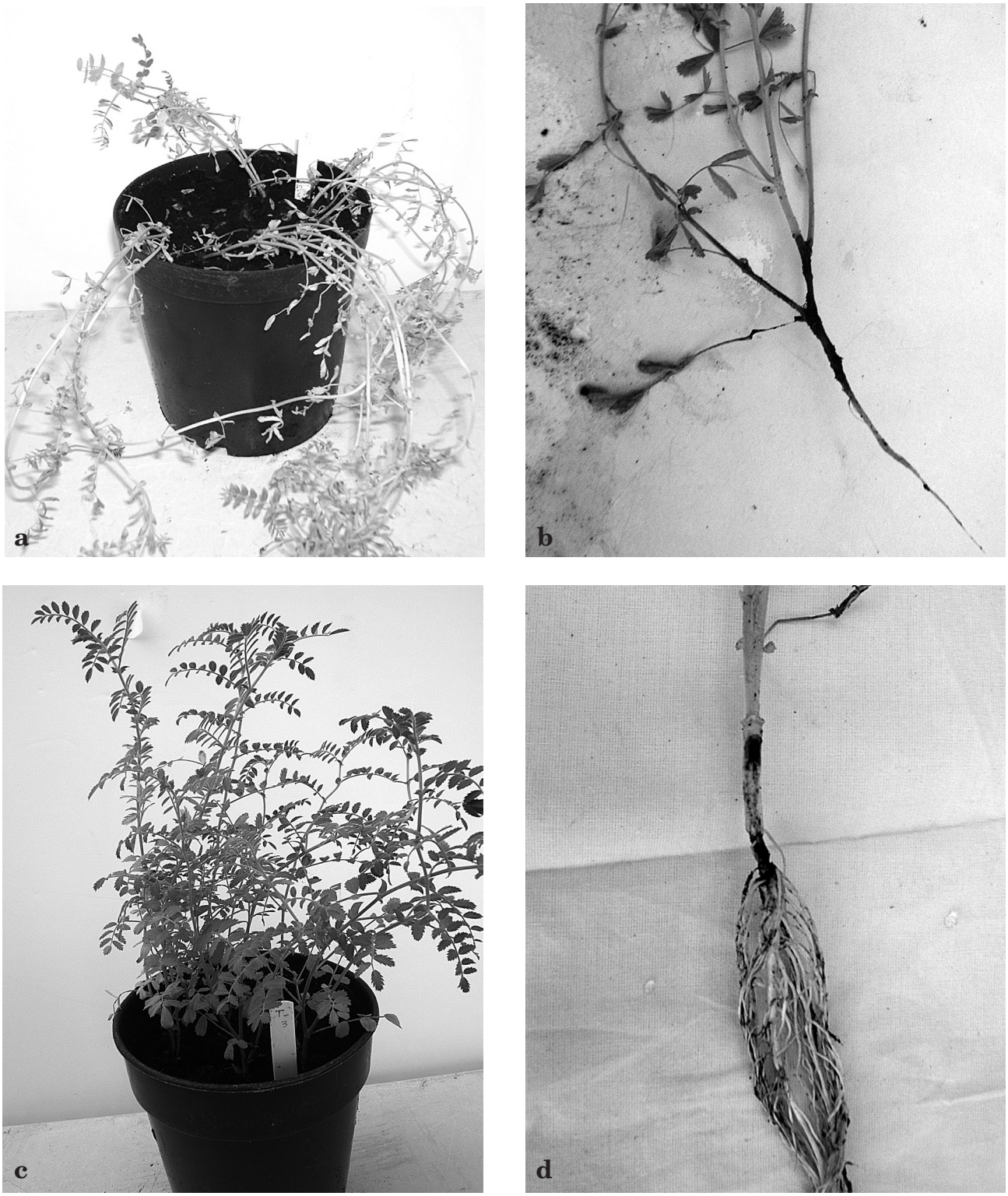

Figure 2. Symptoms caused by inoculating chickpea seedlings with *R. solani*: a and b, inoculated seedlings showing wilting, yellowing and dark brown lesions on the collar region; c and d, non inoculated control.

This is the first report of *R. solani* AG2-3 on chickpea in Tunisia. For a long time it was assumed that the main species of this genus that causes chickpea root rot was *M. phaseolina* (Dhrub-Sing *et al*., 1987, Tayars *et al*., 1988). However, in this case *R. solani* seems to be an important pathogen in its own right in view of the damage that it is causing on chickpea in the Oued Béja station.

Fourteen anastomosis groups of *R. solani* have been described based on hyphal anastomosis behavior, colony morphology, host range, pathogenicity and other characters (Carling *et al*., 1994, Carling *et al.,* 1999, Carling *et al.,* 2002, Toda *et al*., 2004). *R. solani* AG2 is divided into three subgroups (AG2-1, AG2-2 and AG2-3) based on hyphal anastomosis type and thiamine requirements (Toda *et al*., 2004). Three cultural types of AG2-2, designated IIIB, IV and LP, have also been established, based on cultural characteristics, pathogenicity and molecular analysis (Ogoshi, 1987; Sneh *et al*., 1994; Toda *et al*., 2004). Isolates of *R. solani* AG2 infect a number of crop species such as rapeseed (Kataria and Verma, 1992), soybean (Liu and Sinclair, 1991) and species of turf grass (Burpee and Martin, 1992). Further reports have shown that subgroups of AG2 have more specific host preferences. For example, AG 2-3 isolates seem to be pathogenic mainly to soybean.

Although subgroup AG2-3 is reported to be pathogenic to some legumes such as soybean (Naito *et al*., 1995), this subgroup has not been reported on chickpea. The molecular technique used to identify the anastomosis group and subgroups, appears to be reliable, since Salazar *et al*., (1999), using complete DNA sequences of the ITS regions of 31 isolates of *R. solani* AG 2, confirmed the subgroups AG2, AG 2-1, AG 2-2 and AG 2-3**.** Furthermore, a PCR procedure developed by Salazar *et al*. (2000) involving amplification of the 5.8S ribosomal DNA and part of the ITS regions, shows that designed primers in combination with the general fungal primers ITS1F and ITS4B enable the subgroups of AG2 to be distinguished. Due to the considerable yield losses caused by *R. solani* (up to 100% in some winter chickpea fields in the Béja and Bizerte regions) and the rapid expression of the disease, it appears important to look for some means of control, whether chemical or biological, or the breeding of genetic resistance.

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