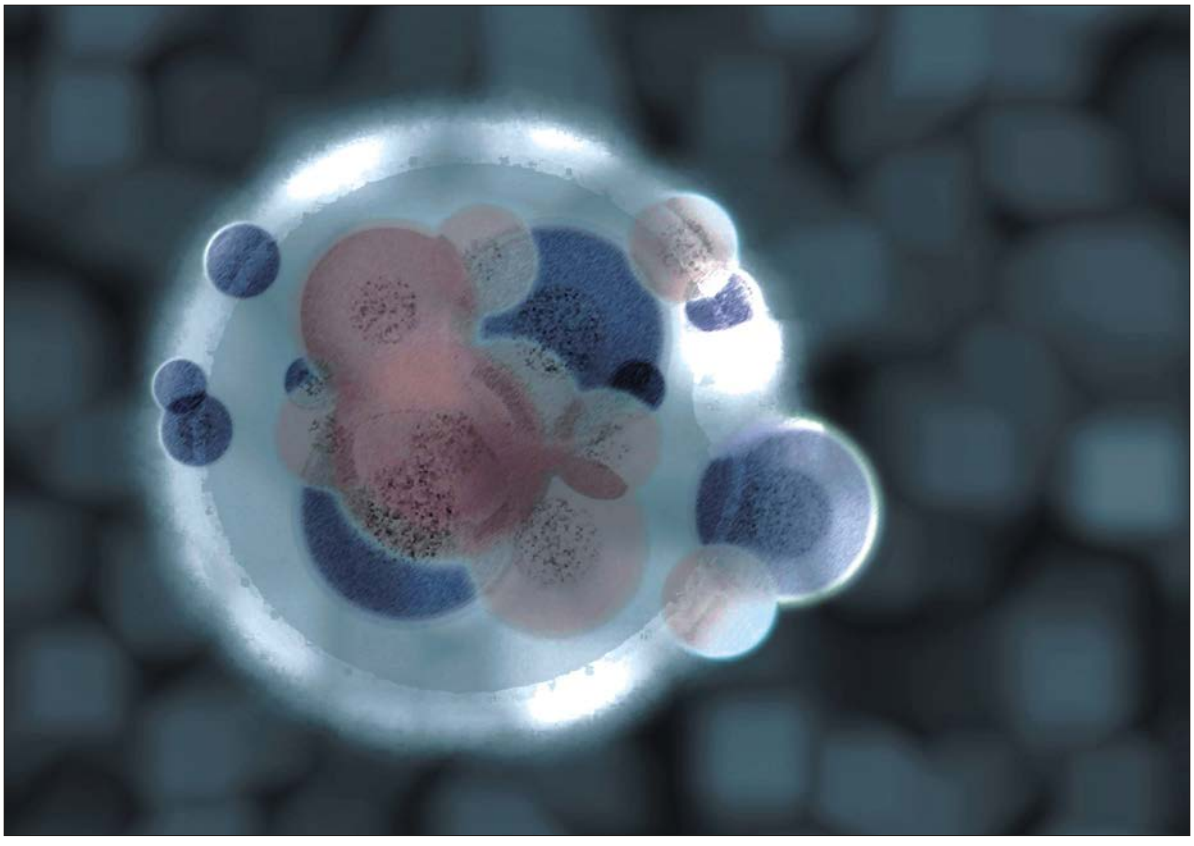

Journal of Biological Sciences

ISSN 1727-3048

science alert

ANSInet
an open access publisher
http://ansinet.com

OnLine Journal of Biological Sciences 1 (5): 384-386, 2001

Mechanism of "Seed to Seedling Infection" by *Ascochyta rabiei* (Pass.) Lab. In Chickpea

Takao Mitsueda, Muhammad Bashir, Zahoor Ahmad and Nabuo Murata
Plant Genetic Resources Institute, National Agricultural Research Centre, Islamabad, Pakistan

Abstract: The mechanism of "seed to seedling infection" of blight disease caused by *Ascochyta rabiei* (Pass.) Lab., was studied while growing naturally infected chickpea seeds and healthy seeds artificially inoculated with pathogen inoculum. The disease symptoms appeared on young seedlings within a week after emerging from soil. The seedling infection was due to contact of growing point to the pathogen inoculum from infected seeds during the process of shoot differentiation under the ground. Based on the observations of disease symptoms on the seedlings from contaminated seeds, it is concluded that contamination of plumule with pathogen inoculum during germination appears to be a major mechanism by which the pathogen transmission occurs on aerial parts of the plants.

Key words: Chickpea, Ascochyta blight, seed, pathogen, infection, transmission

Introduction

Chickpea blight caused by *Ascochyta rabiei* (Pass.) Lab., is one of the most important diseases recorded on chickpea worldwide. Serious losses caused by this disease have been reported (Kaiser, 1992). In Pakistan, 50-70 percent chickpea crop losses were reported during 1979-80 and 1980-81 (Bashir, 1987). The seed-borne nature of *Ascochyta rabiei* has been already established (Dey and Singh, 1994). Many historical facts have shown that the pathogen was introduced with infected seeds into countries or regions that had been previously free of chickpea blight (Kaiser, 1992). As with other seed-borne diseases, chickpea blight poses serious problems not only in farming but also in conserving and exchanging germplasm seeds. Although the organism infecting seeds externally and internally remains active for 5 months or more and cause the disease to seedlings (Dey and Singh, 1994), but the mechanism of infection from contaminated seeds to seedling has not been clarified.

This study was conducted to elucidate the mechanism of occurrence of blight disease in chickpea plants through the seed-borne pathogen to develop an efficient method to control the primary infection by pathogen. In microscopic examination, rather surprising we did not find any sign of migration of the fungus from the lesions on the seeds to the seedlings through the inside of the infected seeds. This study was conducted to explore the mechanism of "seed to seedling infection" by *Ascochyta rabiei* in chickpea seeds.

Materials and Methods

Healthy (visually clean) and blight infected seeds of chickpea cv. Paidar were obtained from Pulses Programme, National Agricultural Research Centre, Islamabad, Pakistan. Healthy seeds were sterilized in 1% sodium hypochlorite solution for 5 min before sowing. Plastic pots (9 cm diameter) were filled with soil autoclaved for 1 hr at 120°C.

Two kinds of inocula were used: naturally infected seeds and healthy seeds artificially inoculated by *Ascochyta rabiei*. For the latter, autoclaved chickpea seeds were inoculated with the pathogen grown on potato dextrose agar media kept in the incubator at 25°C for 3-10 days incubation. These inocula (five pathogen infected seeds) were placed in soil on the top of healthy seeds. The pots were covered with polyethylene bags after planting (Fig.1) to maintain the relative humidity at 90% or more.

To test the possibility for the involvement of pathogen colonized on the seed coat in the contamination of seedling from the same seed as suggested by Maden *et al*. (1975), two types of naturally infected chickpea seeds, one infected proximal and the other distal to cotyledon, were planted after surface sterilization in plastic pots filled with autoclaved soil. In order to test *Ascochyta rabiei* spore suspension as an inoculum potential for seedling infection, the healthy seeds of the same variety were germinated at various growth stages and dipped in spore suspension of the pathogen before sowing in plastic pots filled with autoclaved soil. In all cases, number of blight infected seedlings were counted to compare the treatments.

Results and Discussion

Table 1 showed the result of experiment conducted to clarify the role of seed-borne pathogen causing primary infection to the seedlings. When the infected seeds were placed on the top of the healthy seeds, most of the seedlings coming out of healthy seeds were found blight infected. No infected seedlings were observed when inoculum was not placed on the healthy seeds (control). In pots, where artificially infected seeds were used as inoculum source, the extent of disease occurrence depended on the length of incubation of inoculum from infected seeds. With three days incubation for inoculum production, only 30% seeds were found infected, whereas 100% infection occurred when the incubation time was increased from 5 to 10 days. Apparently the seedlings growing from healthy seeds were infected by their contact with the pathogen inoculum on the infected seeds during the process of germination. In this case, the infection must have occurred by mycelial contact and not by spores because the formation of pycnospores could not be completed in such a short time (3-5 days).

When the naturally infected seeds alone were sown, the disease occurrence depended on the position of the lesions on the seeds. The seeds with lesions close to the cotyledon had low germination, but the emerged seedlings showed high incidence of blight symptoms. On the contrary, the seeds with lesions away from the cotyledon had high germination, but low seedling infection (Table 1). In these cases, where

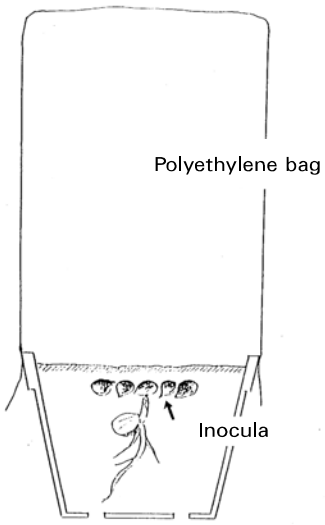

Fig. 1: Method of Inoculation

Fig. 2: Seedlings from diseased seeds (Pathogen in the seed does not migrate to seedling directly

Table 1: Inoculation examination for "Seed to Seedling Infection" by *Ascochyta rabiei*

Inocula	No. of seeds tested	No. of seeds germinated	No. of infected seedlings/percentage
N-1	20	16 (80)	14 (87.5)
A-3	10	10 (100)	3 (30.0)
A-5	10	9 (90)	9 (100)
A-7	10	9 (90)	9 (100)
A-10	10	9 (90)	7 (77.8)
Sing-A	20	8 (40)	4 (50.0)
Sing-B	20	20 (100)	2 (2.0)
Control	20	20 (100)	0 (0)

N-1: Naturally infected seeds were placed on the top of healthy seeds. A-3 to A10: Artificially infected seeds were placed on the top of healthy seeds. The number indicates days of incubation for inoculum production. Sing A, B: Naturally infected seeds were planted. A: Having lesions near the cotyledon. B: Having lesions not near the cotyledon. Control: Healthy seeds only. Figures in parenthesis indicate percentage

the infection occurred from the pathogens on the same seeds, the infection must have occurred still through the contact of seedlings to the pathogen inoculum during their germination process. There were no signs of migration of mycelia or hyphae through the tissue of the infected seeds to the germinating seedlings even when the lesions were located very close to the cotyledon (Fig. 2).

Table 2: Results of inoculation test using spore suspension of *Ascochyta rabiei*

Degree of germination	No. of seeds tested	No. of seeds germinated	No. of infected seedlings
1	10	10 (100)	9 (90)
11	20	20 (100)	20 (100)
111	20	20 (100)	20 (100)
Control	10	10 (100)	0 (0)

1: Only tip of root emerged
11: Immediately before emergence of coleoptile
111: Both of root and coleoptile
Control: Ungerminated seeds were dipped in spore suspension and seeded. Figures in paranthesis indicate percentage

The pattern of symptoms gave a further evidence to the mechanism of "seed to seedling infection" under the ground. Figure 3 showed some typical symptoms on the seedlings. It was observed that disease symptoms were appearing on the peripheral portions of a few leaves and young stalks which grow in parallel (Fig. 3A, B, mark). Presumably, the leaves and the stalks have been closely located in their primordial stage, when they had contacted with pathogen in the soil. It was also observed that most of the symptoms of the disease appeared on young seedlings within a few days (2-3) after emerging from the soil (Fig. 3C-E), although it is believed that incubation period of *Ascochyta rabiei* is 4-6 days at 20°C (Kaiser, 1992). These occurrence of infection, simultaneously offer us another strong evidence that the young seedlings had contact with pathogen inoculum in the process of their differentiation under the ground.

Another experiment was conducted to demonstrate that the germinating seedlings could be infected by *Ascochyta rabiei* pycnospores under the ground. Healthy seeds allowed to germinate in a moist chamber for 2-4 days were dipped in the spore suspension (spore concentration was not taken into consideration at this point) of the pathogen, sown in the pots with autoclaved soil and covered with polyethylene bags. The results are presented in Table 2. Almost all the seedlings inoculated after seed germination, including those inoculated at the stage when only root tips were emerged, were infected. The results of this experiment revealed that spores are mainly infecting the growing seedlings under the ground. Interestingly, no disease symptoms were observed on roots. It is conceivable that chickpea seeds infected and uninfected can take various relative positions in the soil. The cases that may produce the under ground infection on germinating seedling can be classified into three types as illustrated in Fig. 4: Seedlings touching the lesion on the same seed (type-1), seedlings coming out of the lesion-touching occurs very easily (type-11) and seedlings infected by the lesions on other seeds (type-111). Among them, type-111 might be the most common under field conditions. Type-11 must be very rare, in such a case the seeds hardly germinate.

High humidity was essential for the disease development. Symptoms did not appear on the seedlings planted in pots not covered with polyethylene bags. This observation has already been reported (Kaiser, 1992), that serious losses to chickpea crop occurred under wet weather during flowering and fruiting periods. Though the examination of occurrence of the blight disease in seedlings from seeds placed at various topologies as well as through anatomical observations, it is concluded that

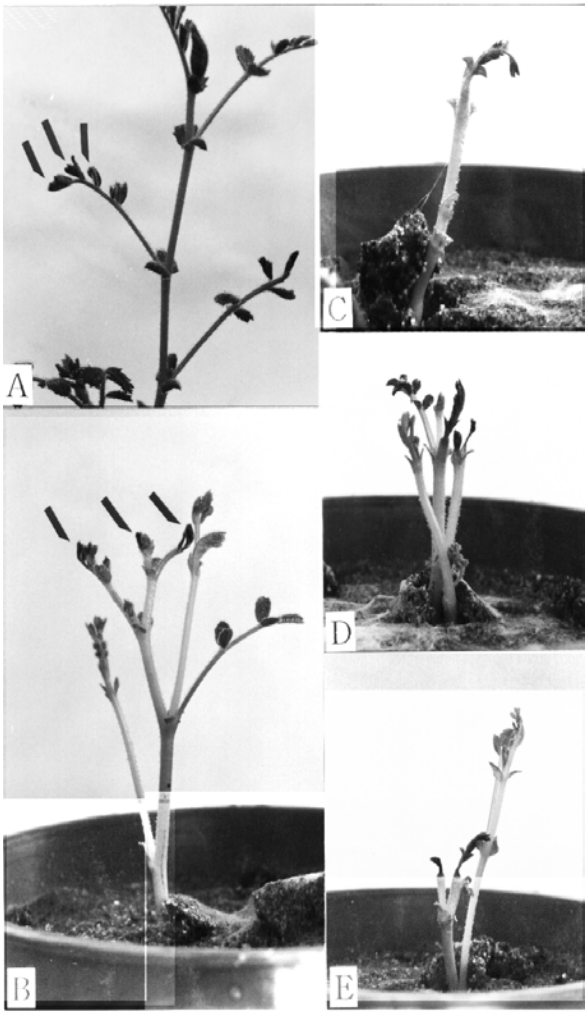

Fig. 3(A-E): Symtoms of primary infection (Arrow marks in A and B show symtoms)

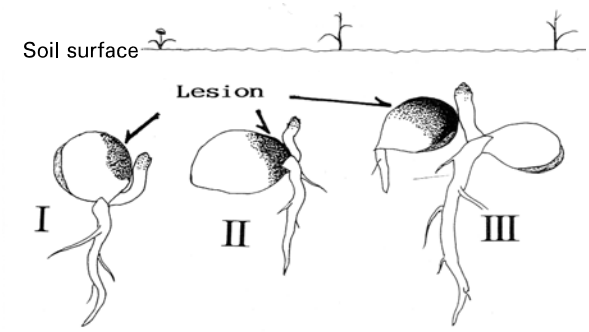

Fig. 4: Mechanism of "Seed to Seedling Infection"

infected seeds are the main source of primary infection of chickpea blight in the field and contamination of plumule with pathogen inoculum (spores or mycelia) during seed germination seems to be major mechanism of "seed to seedling infection". This finding may lead to establish an efficient method to control the disease transmitted through seeds.

References

Bashir, M., 1987. Chickpea improvement in Pakistan with special reference to *Ascochyta blight*. Proceedings of the Meeting of Chickpea Scientists Meet, February 9-12, 1987, ICRISAT., Hyderabad, India.

Dey, S.K. and G. Singh, 1994. Seedborne infection of *Ascochyta rabiei* in chickpea and its transmission to aerial plant parts. Phytoparasitica, 22: 31-37.

Kaiser, W.J., 1992. Epidemiology of Ascochyta Rabiei. In: A Disease Resistance Breeding in Chickpea. Singh, K.B. and M.C. Saxena (Eds.). ICARDA, Syria, pp: 11-134.

Maden, S., D. Singh, S.B. Mathur and P. Neergaard, 1975. Detection and location of seed-borne inoculum of *Ascochyta rabiei* and its transmission in chickpea (*Cicer arietinum*). Seed Sci. Technol., 3: 667-681.