A Rapid Method for Screening and Chemical Control of Seed Rot and Collar Rot in chickpea.

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Abstract

A rapid and reliable greenhouse method was developed to screen chickpeas for resistance to collar rot caused by *Sclerotium rolfsii* Sacc. Groundnut shells were superior to sorghum straw, sand-maize meal, and potato dextrose broth in supporting the growth and formation of sclerotia of the pathogen. Soil infested with sclerotial inoculum developed on groundnut shells was used to study the reactions of 12 chickpea cultivars to the collar rot pathogen. There were significant differences in their susceptibility to seed rot, damping off, and collar rot. Seed treatment with Rizolex$^{(R)}$ or a mixture of Thiram$^{(R)}$ + Rizolex$^{(R)}$ was effective in controlling seed rot and collar rot.

Introduction

Collar rot and damping off caused by *Sclerotium rolfsii* Sacc. are important diseases of chickpea (*Cicer arietinum* L.) in wetsoils. The fungus is soilborne, has a very wide host range, and is widely distributed in warm climates (Punja, 1985). The disease is severe in fields where chickpea is sown after paddy, causing seedling mortality and reducing the plant population considerably. Effective chemical seed treatment and growing of resistant chickpea cultivars are economically viable means of reducing losses due to collar rot in farmers' fields. Various greenhouse techniques to screen chickpeas for resistance to collar rot have been reported (Gurha and Singh, 1982; Sugha *et al.*, 1991). In these techniques, seedlings are inoculated with the fungal sclerotia or seeds of test genotypes are sown in soil infested with the pathogen. In the present study, we compared different media for multiplication and production of sclerotia of *S. rolfsii*. Using the medium that best supported sclerotial production, inoculum was produced, incorporated in soil, and tested for its efficacy to screen chickpeas for resistance to seed rot and collar rot. The effectiveness of different fungicides for seed treatment to control the disease was also studied.

Materials and Methods

Selection of Medium

A single sclerotial culture of *S. rolfsii* isolated from a diseased chickpea plant and maintained on potato dextrose agar was used in this study. Four non-synthetic media were compared for the production of sclerotia with the following compositions; 100 g dried sorghum straw, cut into 2-3 cm pieces, soaked in water for 2 h and autoclaved; 100 g groundnut shells in small pieces (0.5-1 cm) soaked in water for 2 h and autoclaved; 100 g sand-maizemeal medium (10 g maize meal, 90 g fine sand, 20 mL water and autoclaved); and 100 mL potato dextrose broth (200 g peeled potatoes, 20 g dextrose, 1000 mL distilled water and autoclaved).

The flasks containing media (100 g or 100 mL) were each inoculated with a single sclerotium. The flasks were incubated at 25^{0}C for 20 days. The contents were mixed in 1 L water, stirred for 30 seconds on a magnetic stirrer, and allowed to settle. The contents were then poured through a sieve (0.6 mm

mesh), and sclerotia and debris were washed into a petri dish and sclerotia counted (Rodriguez-Kabana *et al.*, 1974). For each medium, three replicate samples were included and the assay was repeated.

Screening method

The potting medium was prepared by mixing groundnut shells inoculum with autoclaved soil (medium Vertisol, pH 7) at the rate of 100 g inoculum per 4 kg soil. Metal trays (70 x 30 x 16 cm) were filled with infested soil. Twelve chickpea cultivars reported susceptible/resistant (Gurha and Singh, 1982; Gurha and Dubey, 1983) were sown in the infested soil. Ten seeds were sown of each cultivar, and the cultivars were completely randomized in three replications. Observations were recorded on germination, damping-off (10 days after sowing, DAS) and collar rot (40 DAS). The temperature in the greenhouse ranged from 28-30^{0}C. The experiment was repeated with reproducible results. The data were subjected to statistical analysis.

Seed Treatment

Effect of seed treatment with Thiram 75 WP$^{(R)}$, Captan 75 WP$^{(R)}$, Rizolex 50 WP$^{(R)}$ (tolchlophos methyl), and Thiram 75 WP$^{(R)}$ + Rizolex 50 WP$^{(R)}$ (1:1w/w) was tested on emergence of chickpea, using soil infested with *S. rolfsii* in the greenhouse. The fungicide dosage was 3 g of commercial formulation per kg of seed. Fungicide slurries were prepared by mixing each fungicide in 5 mL of water in 250 mL flasks. One hundred grams of seed was added to each flask, agitated for 2 min, and air-dried. Fungicide-treated and untreated seeds of two chickpea cultivars, Annigeri (*desi*) and ICCV 6 (*kabuli*) were sown in infested soil in metal trays in three replications. The percentage figures were converted to arcsine values before statistical analysis. The experiment was repeated to confirm the results.

Results and Discussion

Screening Method

Groundnut shells were superior in supporting the growth and formation of sclerotia (11145 sclerotia/100 g medium). If groundnut shells are not available, sorghum straw could be used for multiplication of inoculum (Table 1). Groundnut shells and sorghum straw are readily available, and the medium preparation is easy and economical. Undecomposed tissues in the medium added to soil also support the formation of sclerotia. Sclerotia are capable of initiating infection and serve as the primary source of inoculum for initiation of disease in the field (Punja and Grogan, 1981). Temperatures in the range of 28-30^{0}C and high humidity (moist soil surface) are favorable for disease development (Shew *et al.*, 1987). Sugha *et al.* (1991) described a technique in which inoculum was placed at the collar region of the seedling, but this was found time-consuming when large plant populations were to be screened. Gurha and Singh (1982) used inoculum multiplied on sand-maize meal medium to infest the soil. However, information on the number of sclerotia produced was not provided. In the present study, inoculum multiplied on groundnut shells was superior to sand-maize meal medium for multiplication of the fungus.

Table 1. Production of sclerotia of *Sclerotium rolfsii* on different media after 20 days of incubation at 25^{0}C

Medium	No. of sclerotia/100g/ mL medium
Groundnut shells	11145
Sorghum straw	5054
Potato dextrose broth	1873
Sand-maize meal medium	1665
SE ±	285.7
CV %	10.0

Emergence of chickpea was reduced significantly in soil artificially infested with *S. rolfsii* (Table 2). JG 74, ICC 1023, and ICC 5063 showed resistance to seed rot. *Kabuli* chickpea cultivars ICCV 2 and ICCV 6 were susceptible to seed rot and showed low seedling emergence. Emergence in noninfested soil ranged from 86.6% in ICCV 6 to 100% in BG 212, H 208, ICC 1023, ICC 5063 and ICC 6161. Data on post-emergence damping-off (sudden death within 10 DAS) and collar rot were collected separately. The incidence of post-emergence damping-off was low (20%) in BG 212, JG 74, H 208, K 850, Annigeri, and ICC 5105, and nil in ICC 1023 and ICC 5063. Seedling emergence was also high in the latter two cultivars (Table 2). Collar rot incidence was high (>25%) in all the cultivars, but there were differences among the cultivars in mortality; JG 74, ICC 1023, and ICC 5063 had <30% collar rot and <20% damping-off incidence.

Gurha and Dubey (1983) reported H 208, K 850, ICC 1023, ICC 5105, and ICC 6161 as tolerant to *S. rolfsii* (<20% mortality). However, no mention was made of seedling emergence. In the present study JG 74, ICC 1023, and ICC 5063 showed resistance to seed rot and low incidence of damping-off and collar rot. Though no cultivar was highly resistant to *S. rolfsii*, there were differences in their susceptibility to seed rot and damping-off. Partial resistance (low mortality) to *S. rolfsii* is expressed by several genotypes in repeated screening under severe disease pressure, suggesting that genotypes are stable in disease suppression and that this could be enhanced by selection in field trials (M.P. Haware, unpublished).

Seed Treatment

Pre-emergence damping-off and seed rot were significantly reduced when seed was treated

Table 2. Reaction of 12 chickpea cultivars to seed rot, damping-off, and collar rot in soil artificially infested with *Sclerotium rolfsii*

Genotype	Infested soil			Non-infestred soil
	Emergence (%)	Damping-off (%)	Collar rot (%)	Emergence (%)
BG-212	76.6	13.1	39.3	100.0
JG-74	96.6	13.7	24.1	96.6
H-208	86.6	11.5	46.3	100.0
K-850	56.6	17.7	53.3	96.6
Annigeri	73.3	13.6	63.7	96.6
ICCV 2	50.0	40.0	60.0	96.6
ICCV 6	43.3	46.6	53.3	86.6
ICC 1023	96.6	0.0	24.1	100.0
ICC 5063	96.6	0.0	27.8	100.0
ICC 5105	60.0	16.6	77.8	90.0
ICC 6161	70.0	47.6	52.4	100.0
ICC 4988	80.0	37.5	29.2	96.67
SE ±	2.82	1.92	3.64	2.30
CV %	6.6	15.5	13.7	4.1

Chickpea genotypes showed no mortality in non-infested soil.

Table 3. Effect of fungicides on emergence and collar rot (*Sclerotium rolfsii*) incidence of chickpea in greenhouse experiment

Cultivar	Captan 75 WP		Rizolex 50 WP		Thiram 75 WP		Thirum + Rizolex 1:1 w/w		Untreated control	
	1	2	1	2	1	2	1	2	1	2
Annigeri	80.0 (65.3)	100.0 (90.0)	92.5 (77.4)	32.4 (34.6)	58.3 (49.9)	100.0 (90.0)	91.7 (73.5)	45.4 (42.4)	38.3 (38.0)	100.0 (90.0)
ICCV 6	97.5 (82.5)	95.6 (82.9)	93.3 (76.3)	33.2 (35.1)	65.8 (54.4)	100.0 (90.0)	84.2 (67.0)	40.1 (39.2)	48.3 (44.3)	100.0 (90.0)

	Cultivar		Fungicides		Cultivar x Fungicide	
	1	2	1	2	1	2
SE ±	(0.8)	(1.468)	(3.52)	(1.912)	(4.52)	(2.829)
CV %	(2.2)	(3.7)	-	-	(13.7)	(6.7)

1 = Emergence (%), 2 = Collar rot (%).

Figures in parentheses are arcsine transformed values.

with Captan(R), Rizolex(R) or Thiram(R) + Rizolex(R). However, collar rot (up to 40 DAS) incidence was significantly reduced when seeds of Annigeri and ICCV 6 were treated with Rizolex(R) alone or in combination with Thiram(R). The mixture was more effective than the individual fungicides. Untreated (control) and captan and thiram treated seed had 100% mortality within 40 days. It seems that the protection effects of Rizolex(R) on chickpea seeds are longer lasting than in other fungicides (Table 3).

These studies provide convincing evidence of the role of *S. rolfsii* in seed rot and low emergence of chickpea in fields infested with the fungus. White-seeded chickpeas (*kabuli*) were affected most in seed emergence. It was also observed that the age of the seedlings had a profound effect on their susceptibility to *S. rolfsii* in the greenhouse trial. Seedlings developed resistance to the pathogen after 40 days and only sporadic death of plants was observed. Therefore, seedlings should be observed for up to 40 DAS.

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