Biological Control of Botrytis Gray Mold of Chickpea

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Botrytis gray mold (BGM) is an important disease of chickpea in northern India, Bangladesh, Nepal, and Pakistan. Its occurrence was also noted in Myanmar in Jan 1993. A high level of resistance to the disease has not yet been found in chickpea germplasm. Chemical control alone is not cost efficient and repeated application of fungicides results in development of resistance. Use of biological agents is rapidly emerging as an effective substitute for chemical control. The present investigation was aimed at identifying potential biocontrol agents for *Botrytis cinerea* Pers. ex Fr. in chickpea.

Screening of antagonists

In vitro. Several fungi isolated from chickpea rhizosphere were co-inoculated with the BGM pathogen, *Botrytis cinerea* on potato dextrose agar (PDA) in petri dishes and incubated at 25°±1°C. Observations on the ability of the fungal antagonists to overgrow the test pathogen were recorded over time. The isolates were grouped according to the time taken to completely overgrow the test pathogen. Five isolates of *Trichoderma* (nos. 1, 15, 23, 26, and 39) were the most effective in overgrowing the *Botrytis cinerea* colony in dual culture. These isolates were then screened in a growth room for their ability to suppress gray mold of chickpea.

Screening in growth room. For screening of antagonists and for other experiments in a growth room, the technique developed for screening for resistance to BGM was followed. Botrytis gray mold-susceptible cultivar H 208 was used throughout the investigations. Seedlings were raised in plastic trays (38 × 27 × 7 cm^3) containing sand:vermiculite (4:1) for 10 d in a greenhouse. Seedlings were shifted to a growth room before inoculation. The temperature was maintained at 24°±2°C. Relative humidity was maintained at 95-100% for the first 3 d and for the next 7 d leaf wetness was maintained by intermittent spraying of water from a knapsack sprayer. *B. cinerea* was multiplied on potato dextrose broth for 10 d at 25°±1°C. The fungal mat along with spores was harvested and macerated in a wearing blender for 2 min and filtered through two layers of muslin cloth. The spore concentration was adjusted to 3 × 10^5 mL^{-1}. The inoculum fragments were sprayed on the seedlings until run-off (approximately 50 mL $tray^{-1}$) and allowed to dry for 2-3 h before increasing the humidity with a humidifier

Table 1. Biological control of botrytis gray mold of chickpea in a growth room with *Trichoderma* (T 15) isolate, ICRISAT Center.

	Day and disease rating[1]					
Treatment	3	5	7	9	11	Mean
Td 10^7 spores mL^{-1} + CMC[2]	1.75	2.75	4.00	4.25	4.75	3.50
Td 10^7 spores mL^{-1}	1.75	3.00	4.00	4.50	5.25	3.70
Td 10^8 spores mL^{-1} + CMC	1.75	2.00	3.50	3.75	4.25	3.05
Td 10^8 spores mL^{-1}	2.75	3.00	3.75	4.25	5.00	3.75
Td 10^7 spores mL^{-1} (broth) + CMC	1.25	1.50	2.50	3.25	4.25	2.55
Td 10^7 spores mL^{-1} (broth)	1.75	2.25	3.25	3.75	4.50	3.10
CMC	6.00	6.50	8.25	8.50	8.50	7.55
Control (*Botrytis*-inoculated seedlings)	6.25	7.00	8.50	8.75	8.75	7.85
Mean	2.91	3.50	4.72	5.13	5.66	
	Treatments		Days	Treatment × days		
CD (5%)	0.47		0.30	0.89		
CV (%)	13.50					

1. Mean of four replications.
2. CMC = Carboxy methyl cellulose (0.5%).

defensor. The symptoms appeared within 48 h after inoculation. Disease was scored on a 1-9 point scale.

The selected isolates of *Trichoderma* (nos. 1, 15, 23, 26, and 39) were multiplied on PDA in petri dishes for 7 d. The spores were harvested using a camel-hair brush and their concentration in sterilized water was adjusted to 10^8 conidia mL^{-1}. The spore suspension was sprayed on seedlings 1 d before *Botrytis* inoculation with a hand sprayer. The disease rating was recorded 10 d after *Botrytis* inoculation. Seedlings sprayed with only *Botrytis* served as control. Of the five isolates of *Trichoderma* tested, one (no. 15) was effective in reducing BGM incidence in chickpea. This isolate, designated as *Trichoderma* (T 15) was used for further study.

Biological control

Trichoderma (T 15) was further evaluated at two spore concentrations (10^7 and 10^8 spores mL^{-1}, harvested from PDA petri dishes) with or without carboxy methyl cellulose (CMC, 0.5% water suspension). In another treatment, T 15 was multiplied on potato dextrose broth for 7 d, churned, and the spore concentration was adjusted to 10^7 mL^{-1} (the suspension also contained mycelial fragments). This was applied alone or with CMC. *Trichoderma* was applied to seedlings 1 d before *Botrytis* inoculation, and observations recorded from the third day onward every alternate day. All the treatments with T 15 gave significant control of the disease (Table 1). T 15 spore suspension from broth culture (10^7 conidia mL^{-1} along with mycelial fragments) applied with 0.5% CMC gave maximum control. In subsequent experiments, this combination used as a standard method of *Trichoderma* application gave similar results.

During the 1993/94 postrainy season, field trials will be conducted at the Govind Ballabh Pant University of Agriculture and Technology (GBPUAT), Pantnagar, to evaluate the efficacy of *Trichoderma* to control BGM of chickpea.

Evaluation of Chickpea Lines for Resistance to Ascochyta Blight

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Blight caused by *Ascochyta rabiei* (Pass.) Lab. is a major disease of chickpea in Sri Ganganagar district of Rajasthan state in India. Since 1986/87, chickpea entries received from ICRISAT are being screened against blight. This paper reports the performance of entries during a 5-year period (1986/87 to 1990/91) at the Agricultural Research Station, Sri Ganganagar.

Every year, the entries in the nursery were sown in Nov in a randomized-block design in two replications. Chickpea lines were sown in single-row plots 5 m in length with susceptible cultivar Pb 7 as infector row after every two test rows. The interrow and plant-to-plant spacing were 30 and 10 cm, respectively. Susceptible cv H 208 was also sown as border around the field to provide an additional inoculum.

Artificial epiphytotics of blight were created by spraying a fungal spore suspension (50 000 mL^{-1}) at 40-50 d crop stage followed by a light field irrigation by the border strip method the next morning. Three more inoculum sprays were given at 10-d intervals. Leaf wetness in the disease nursery was maintained by frequent spraying of water between 0900 and 1700 using a knapsack sprayer.

Disease severity was recorded three times during the growing season on a 1-9 scale (Singh et al. 1981). Disease was scored at seedling stage and two subsequent observations were taken at flowering and podding/maturity stages.

Of 73 chickpea entries screened during the 5-year period, none was highly resistant (rating 1 or 2). Three entries, however, were resistant (rating 3) in 2 years of testing. These were: ILC 72, ILC 3279, and ICCX 790151-2P-1H-1H-2H-1H-1HWR-BH.

Thirteen entries were moderately resistant (rating 4). Of these, ICC 1069 and ILC 3864 showed consistent reaction in 4 years of testing. The entries which showed a rating of 4 consistently for 3 years of testing were ICC 3932, ICC 6373, ILC 200, ILC 202, ICCL 86447, FLIP 83-47C, MCK 54, and for 2 years of testing were ICCX 800859-BPN-BPN-BPN-BPN-3BPN-1HWR-BH, ICCX 810457-3H-1H-1H-1HWR-BH.

Reference

Singh, K.B., Hawtin, G.C., Nene, Y.L., and **Reddy, M.V.** 1981. Resistance in chickpea to Ascochyta blight. Plant Disease 65:586-587.