Asian Journal of Plant Sciences

ISSN 1682-3974

science alert

ANSInet
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Asian Journal of Plant Sciences
Volume 1 Number 2: 193-194, 2002

Study of Antifungal Compounds in the Roots and Stems of Different Chickpea Lines for Resistance to *Fusarium* Wilt

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Abstract: The roots and stems of each of the five advanced chickpea lines produced fungitoxic compounds when extracted in methanol. Three different types of antifungal compounds designated as compound A, B and C were identified through the bioautography on TLC plates. The compound B at R_f value 0.87 was produced in higher amounts in the lines 1004/99, 99115 and 99105 with better wilt resistance. Compounds B and C appears to have no significant role in wilt resistance.

Key words: Antifungal compound, resistance, chickpea, *Fusarium oxysporum* f. sp. *ciceris*

Introduction

Chickpea wilt caused by *Fusarium oxysporum* f. sp. *ciceris* is a major problem in Thal area where, most of the chickpea crop is cultivated. This disease is reported to cause 10% annual yield losses to the crop (Halila *et al*., 1984). The fungus is seed borne as well as soil borne in nature, it is impracticable to control the disease by using fungicides and through crop rotation. Use of resistant varieties is the best way to combat the disease. For this purpose it is necessary to have a full knowledge about the fungus, disease and mode of resistance in host.

Following infection by various biotic agents (e.g., fungi and bacteria) several higher plants rapidly synthesized antibiotic compounds termed as phytoalexins (Ingham, 1972), which are believed to play a significant role in the defense of higher plants against phytopathogenic fungi (Van Etten *et al*., 1982; Mansfield, 1982 and Hahn *et al*., 1985). Kunzuru *et al*. (1996) first recorded Phytoalexins formation by chickpea in 1966. They showed that an antifungal compound cicerin was produced when spore suspension of *Ascochyta rabiei* were incubated in the seed cavities of detached pods. Koster *et al*. (1983) showed that in chickpea and other legumes isoflavones occur mainly as isoflavone 7-O, glucoside, 6-malonoate. Accumulation of such antifungal compounds appears to be an important trait of a resistant plant, (Tani and Mayama, 1982 and Kuc and Rush, 1985). So, much work has been done to identify the antifungal compounds in the stem of chickpea against blight disease, but little information are available about the antifungal compounds produced in the roots of chickpea against wilt disease. Present paper reports the production of antifungal compounds in root of chickpea and the involvement of one compound in wilt resistance.

Materials and Methods

Chickpea material: Chickpea material was kindly provided by Mutation Breeding Division NIAB and Department of Plant Breeding and Genetics, University of Agriculture, Faisalabad.

Pathogen: *Fusarium oxysporum* f. sp. *ciceris* (7952 race 0) was kindly provided by Professor Rafael M. Jimenez Diaz Cordoba, Spain.

Evaluation of resistance: The resistance for wilt disease was evaluated by the following two methods:

Pot Method: The wilt sick soil was prepared (Nene *et al*., 1981) and filled in small plastic pots (4 x 4"). Ten seeds of each check lines were sown in the pots in two replicates. A highly susceptible variety Aug-424 and a resistant variety Paidar 91 were also grown as checks. Pots were placed under 3000 lux fluorescent lights at 30 ± 3°C. The final data was recorded when the susceptible check completely wilted and then percentage wilt incidence in each lines was calculated. The resistance/susceptibility of each line was determined by using a rating scale where 0-10% mortality, highly resistant; 11-20%, resistant; 31-50%, susceptible and 51-100%, highly susceptible (Iqbal *et al*., 1993).

Water culture method: Ten surface sterilized (5 min. in 2.5% sodium hypochlorite) seeds of each of the test lines as well as susceptible check (Aug-424) were sown in autoclaved riverbed sand placed in 15 cm diameter pots. One pot was used for each line. Liquid potato dextrose broth medium (500ml) was prepared and 100ml was placed in 250 ml conical flasks and autoclaved at 15 lb psi and 121°C for 15 minutes. The medium was inoculated with *Fusarium oxysporum* f. sp. *ciceris*, and incubated on a shaker (8 hr each day) at in dark for 14 days. The entire contents of the two flasks were diluted with sterilized distilled water to get approximate spore concentration of 6.5×10^5 spores/ml. Spore suspension (50 ml) was taken in each 50 ml conical flasks. Two weeks old seedlings of the test lines were removed from pots carefully, washed with tap water and then with sterilized distilled water. These seedlings were transplanted into the conical flasks containing the spore suspension in three replicates. The final data was recorded when the susceptible check completely wilted and then percentage wilt incidence in each lines was calculated.

Study of antifungal compounds: Five chickpea lines 99115, 99105, 1004/99, 1018/99 and 5007/99 were sown in plastic pots containing sand: soil (3:1). After 12 days plants were removed from soil, roots were washed thoroughly in tap water and then rinsed in distilled water. A weighed quantity of (1.5 gm) of roots/shoots of each line was taken separately and grinded with the help of pestle and mortar. The material was then extracted in 20 ml of methanol and filtered through Whatman filter paper no. 1. The filtrates were rotary evaporated to dryness and residues were dissolved in 2 ml methanol. A 5 μl of each of the sample was applied to thin layer chromatographic plate (0.5 mm thick silica gel 60 GF254 plates) and developed in solvent system containing chloroform: methanol (9:1). The TLC plates were kept at room temperature for 2 days to remove any solvent. The plates were sprayed with spore suspension of *Cladosporium cucumerinum* (test fungus) in autoclaved Czapek dox medium (Oxoid) and incubated in a humidity chamber at 25°C. After 3 days the plates were removed from the chamber and the R_f values/area of the inhibition zones were calculated.

Results and Discussion

The green house pot screening of five advanced chickpea lines revealed that two lines 1004/99 and 99115 were tolerant and rest of the lines were found susceptible to highly susceptible. In water culture screening the test lines 1004/99, 99115 and 99105 were found better as compared to other two lines. The roots and stems of each of the five advanced chickpea lines produced compounds fungitoxic to *Cladosporium cucumerinum*, as detected by the zones of inhibitions.

Three different types of antifungal compounds designated as compound A, B and C were identified based on their R_f values, which were 0.24. 0.87, 0.95, respectively. Only one inhibition zone (compound B) was yielded by the stems/ roots of the test lines 1004/99 and 99115 at R_f value 0.87. Similarly, the compound B was only produced by the root extract of the line 5007/99 while its stem yielded two compounds B and C. Two inhibitory zones were produced by the methanolic extracts of both of the roots and stems of the test lines 99105, 1018/99. Only the root extract of these two lines produced the compound A, rest of the antifungal compounds were similar as produced by the other lines. Although all the lines produced antifungal compounds but the difference was in their quantity produced by them. The quantity of antifungal compounds was determined by calculating the area of the inhibitory zones produced by these lines. The area of individual zones varied

Table 1: Evaluation of chickpea lines for wilt resistance and for the production of antifungal compounds in their stem and roots

Test lines	Pedigree	Pot	Disease % wilting water culture	Plant parts	No. of inhibition zones	Area of zones (cm^2)	R_f value
1004/99	447x222	20	33	Root	1	3.38	0.87
				Stem	1	1.89	0.87
99115	CM1463-2/94	22	0	Root	1	3.53	0.87
				Stem	1	1.73	0.87
99105	PUSA 329	66	0	Root	2	3.30	0.87
						0.88	0.24
				Stem	2	2.45	0.87
						1.41	0.95
5007/99	1437xCM	90	100	Root	1	2.45	0.87
	72			Stem	2	1.73	0.87
						1.13	.95
1018/99	C27x216	33	66	Root	2	2.26	0.87
						2.07	0.24
				Stem	2	2.65	0.87
						1.13	0.95

greatly and it ranged from 0.88 to 3.53 cm^2 (Table 1). When the data was compared with the level of resistance of different lines it was found that the compound B at R_f value 0.87 was significantly produced in the lines 1004/99, 99115 and 99105 with better wilt resistance. The data showed that compound A and C were not significantly involved in the wilt resistance because the wilt susceptible lines 5007/99 and 1018/99 in sufficient quantity were producing those.

Several other workers have identified the antifungal compounds in chickpea. Barz *et al.* (1990) reported that the accumulation of phenolic in addition to medicarpin and maackiain is increased upon infection i.e., compounds existed in the plants prior to infection increased in the concentration due to the infection stress. Alam and Strange (1995) purified maackain, medicarpin and formononetin from the germinating seeds of chickpea and Farhat *et al.* (1996) identified these compounds in the stem of different chickpea cultivars against *Ascochyta rabiei*.

The present paper reports the production of antifungal compounds in the roots of different chickpea lines and the involvement of one compound B in the wilt resistance.

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