Haware, M. P. and Kannaiyan, J. (1992), *Seed Sci. & Technol.*, **20**, 597–601

Seed transmission of *Fusarium udum* in pigeonpea and its control by seed-treatment fungicides

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(Accepted July 1992)

Summary

The pigeonpea wilt pathogen, *Fusarium udum*, was transmitted by infected seeds of five wilt susceptible pigeonpea cultivars collected from diseased plants. The fungus was not isolated from the wilt susceptible cultivars, ICP 2376 and ICP 8518. Seed treatment with a mixture of benomyl and thiram completely eradicated the pathogen.

Introduction

Wilt of pigeonpea (*Cajanus cajan* (L.) Millsp.) caused by *Fusarium udum* Butler has been reported from India and several other countries (Nene, Sheila and Sharma, 1989). It is a serious disease problem in the Indian subcontinent and eastern Africa (Kannaiyan, Nene, Reddy, Ryan and Raju, 1984). Seed transmission of the wilt fungus has been reported by some workers (Shukla and Bhargava, 1976; Jeswani and Gemawat, 1981). On the contrary, Mohanty (1946) reported that *Fusarium udum* was not carried within the seed. The available evidence on the internal seed-borne nature of *Fusarium udum* is not conclusive. Since we at ICRISAT are involved in considerable international exchange of pigeonpea seed, we decided to investigate the internal seed-borne nature of the wilt fungus and control of the seed-borne phase with seed-treatment fungicides.

Materials and methods

Collection of seed

Seeds of seven wilt susceptible cultivars (ICP 2376, ICP 8518, ICP 1903, ICPX 78148, BDN-1, C-11 and Hy 3C) grown in a wilt sick plot in 1983-84 and 1985-86 at ICRISAT Center, Patancheru, India were collected from plants that wilted prior to maturity and from plants that did not wilt. To avoid contamination during harvesting and threshing, seed was removed from dried pods of individual healthy and wilted plants by hand and air-dried at room temperature. The seed was bulked into 14 lots (seed from healthy and wilted plants of the seven cultivars) and stored in paper bags at 5°C.

Testing procedures

In all the experiments, unless stated otherwise, (i) 400 seeds of each cultivar were used and (ii) seed was surface-sterilized by dipping for two minutes in a 2.5% solution of sodium hypochlorite. The blotter test was used to measure seed germination (ISTA, 1966). Seed was placed in plastic Petri dishes on top of three layers of moistened, sterilised blotters and incubated at 25 °C for one week. For detecting *Fusarium udum* in seed, Nash's medium was used (Nash and Snyder 1962). Nash's medium consists of 1.5% peptone, 0.1% KH_2PO_4; 0.5% $MgSO_4$; $7H_2O$; 2% agar and 0.75% pentachloronitrobenzene. The medium was adjusted to pH 6, autoclaved and 300 mg of streptomycin sulphate and 250 mg of neomycin sulphate were added to a litre of medium prior to pouring into Petri dishes. The surface-sterilized seeds were plated on this medium (10 seed/plate) and incubated at 25 °C for one week in a cycle of 12 hours of near-UV light followed by 12 hours of darkness. The number of seeds germinated on blotter and number of colonies of *Fusarium udum* on Nash's medium were recorded.

A disease transmission test was also conducted to confirm the transmission of *Fusarium udum* by infected seed. The surface-sterilized seeds were sown in autoclaved, (121 °C for 2 hours) fine riverbed sand in 15 cm diameter plastic pots in a greenhouse. Germination was recorded 15 days after planting and seedlings were checked for wilt symptoms up to 60 days. Isolations were attempted from wilted plants.

Pathogenicity tests

Isolations were made on potato dextrose agar (PDA) from plants showing wilting in the disease transmission test. To determine pathogenicity of culture isolates, 10 day-old healthy pigeonpea seedlings of ICP 2376 were root dipped in conidial suspensions of the isolates and transplanted into autoclaved riverbed sand in 15 cm pots. To eliminate the chances of seed-borne infection, the seedlings for the pathogenicity test were raised in sterile sand from surface-sterilized seed collected from healthy plants.

Seed treatment with fungicides

Fungicide formulations used were Benlate 50 W.P.® (50% *benomyl*), Bavistin W. P.® (50% *carbendazim*), Tecto-60® (60% *thiabendazole*), and Thiride® (75% *thiram*). The fungicidal mixtures tried were 1 : 1 in proportion. Unless stated otherwise the fungicidal dosage was 2.5 g of the commercial formulation per kg of seed.

Results

Detection of the pathogen on medium

Fusarium udum colonies were mostly rosy in colour with felted aerial mycelium. The macroconidia were 'hook shaped' which enabled easy identification of colonies. *Fusarium udum* was present in the seeds of five susceptible pigeonpea cultivars; viz., ICP 1903, PCPX 78148, BDN-1 (tested over two years) and C-11 and Hy 3C (tested in 85/86 only). It was not isolated from two other wilt susceptible cultivars ICP 2376

Table 1. Detection of *Fusarium udum* in seeds of 7 healthy and wilted pigeonpea cultivars.

Cultivar	Percentage seed infection on Nash's medium[1]				Seedling disease transmission (percentage wilt)[2]			
	A		B		A		B	
	83 84	85–86	83–84	85 86	83 84	85–86	83 84	85 86
ICP-2376*	NT	0	NT	0	NT	0	NT	0
ICP-8518*	NT	0	NT	0	NT	0	NT	0
ICP-1903	4.0	2.75	0	0	2.2	2.0	0	0
ICPX 78148	4.0	2.50	0	0	4.5	2.2	0	0
BDN-1	4.5	3.75	0	0	3.0	3.7	0	0
C-11*	NT	2.0	NT	0	NT	1.5	NT	0
Hy 3C*	NT	2.0	NT	0	NT	1.5	NT	0

A = Seeds from wilted plants.
B = Seeds from healthy plants.
NT = Not tested.
[1,2] = No. of seeds tested were 400 for each cultivar.
* Note that the data were obtained for the year 85–86 only.

and ICP 8518 which were tested in one year only (Table 1). These results confirm the seed borne nature of the pigeonpea wilt pathogen. The pathogen was also not isolated from seeds of healthy plants. The pathogenicity of *Fusarium udum* isolates from seed was confirmed.

Wilt in a disease transmission test

Wilt occurred in those plants of five cultivars which showed seed infection on selective medium. Wilt was not observed in seedlings of ICP 2376 and 8518. Likewise wilt was absent in the seedlings grown from seeds collected from healthy plants of seven cultivars (Table 1). These results confirm the seed transmission of the fungus in a medium

Table 2. Effect of fungicidal seed treatment on seed-borne *Fusarium udum*.

Fungicide	Application rate (g a.i./kg)	Infection detected on Nash's medium[1] (%)	
		BDN 1	Hy 3C
Benomyl	1.25	2	5
Carbendazim	1.25	3	2
Thiabendazole	1.50	4	3
Thiram	1.875	4	7
Benomyl + Thiram	0.625 + 0.9375	0	0
Carbendazim + Thiram	0.625 + 0.9375	2	1
Check		12	17

[1] = No. of seeds tested were 400 per fungicide per cultivar.

Table 3. Effect of benomyl + thiram[1] (1 : 1) seed treatment on seed-borne *Fusarium udum*

Cultivar	Percentage germination		Percentage infection			
			Nash's medium		In sand[3]	
	A	B	A	B	A	B
BDN-1	87.5	89.0	13.0	0	3	0
Hy 3C	96.25	95.5	19.25	0	4.25	0

[1] Fungicide at 2.5 g/kg of commercial formulation applied as wet seed treatment.
[2] Blotter test.
[3] Plants were observed for wilting up to 60 days after sowing.
A Untreated seeds.
B Fungicide treated seeds.
No. of seeds tested for a cultivar were 400 in each test.

of riverbed sand. *Fusarium udum* was isolated from wilted seedlings and its pathogenicity was confirmed.

Seed treatments with fungicides

Fusarium udum was not recovered from seed treated with the mixture of benomyl and thiram on Nash's medium (Table 2). Benomyl or thiram alone and thiabendazole or carbendazim did not eradicate the pathogen from the seed. Benomyl + thiram (1 : 1) in a commercial formulation (1.25 g benomyl + 1.25 g thiram/kg seed) completely eradicated *Fusarium udum* from infected seed of BDN-1 and Hy 3C. The effectiveness of the fungicide mixture was again confirmed in a laboratory and glasshouse test, where seedlings were raised from fungicide-treated and untreated seed of two cultivars. Wilting was not observed in seedlings grown from fungicide treated seeds (Table 3). No adverse effect on seed germination from seed treatment was detected.

Discussion

The results demonstrated that *Fusarium udum*, the pigeonpea wilt pathogen, infected pigeonpea seeds. A proportion of seeds collected from wilted plants bore the fungus but seeds from healthy plants did not. This was confirmed both on the selective agar medium and in disease transmission tests in riverbed sand in the glasshouse.

The possibility of the seed transmission of *Fusarium udum* was pointed out by Jeswani and Gemawat (1981) and Shukla and Bhargava (1976). However they failed to obtain clear evidence of internal seed transmission of *Fusarium udum*. It was not clear from their papers if the seeds they used for those tests were obtained from wilted plants. *Fusarium udum* can be isolated as a surface contaminant from pigeonpea seed. Likewise the name of the cultivar from which the seed was obtained was not mentioned. It is important to know the cultivar, because there may be a clear difference between cultivars with regard to seed infection of the developing seed (Table 1). In

limited tests the pathogen did not infect the seeds of two susceptible cultivars of pigeonpea. Other susceptible cultivars allowed the pathogen to establish in the seed before wilting.

Infected seed may be the primary means of spreading *Fusarium udum* over long distance and into new areas where it could become a primary source of inoculum for disease development. Fungicidal treatment of seed may be an important method of preventing or delaying the introduction of *Fusarium udum* in wilt-free pigeonpea growing areas. A mixture of benomyl and thiram effectively eradicated seedborne *Fusarium udum* and therefore international movement of pigeonpea seed needed for germplasm exchange should not be affected adversely, provided treated seeds are used.

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