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Biological control of *Fusarium* wilt of pigeonpea *Cajanus cajan* (L.) Millsp with chitinolytic *Alcaligenes xylosoxydans*

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Alcaligenes xylosoxydans protected pigeonpea from *Fusarium* wilt in a pot experiment and field trials. When seeds of pigeonpea (*C. cajan*) were treated with *A. xylosoxydans* and sown in soil infested with *Fusarium*, the incidence of wilt was reduced by 43.5% and resulted in 58% higher grain yield. The antifungal activity of *A. xylosoxydans* was based on chitinase production and was comparable in efficacy to commercial antifungal agents such as benlate, monitor WP, thiram and bavistin.

Keywords: *Alcaligenes xylosoxydans*, Biocontrol, Biological control, Chitinase, *Fusarium udum*, Pigeonpea

Pigeonpea [*Cajanus cajan* (L.) Millsp.] is one of the important legumes grown predominantly in the semi-arid tropics of Indian sub-continent. The crop has a great significance in Indian agriculture because of its multiple use as food, feed, fodder, fuel and its role in sustaining agricultural productivity. In India, pigeonpea is grown on 3816 ha, contributes 2876 thousand tonnes of grain annually. *Fusarium* wilt, caused by *Fusarium udum* Butler, is a major disease of pigeonpea throughout the world. It has been reported in Bangladesh, Ghana, India, Indonesia, Kenya, Malawi, Mauritius, Myanmar (Burma), Nepal, Nevis, Tanzania, Thailand, Trinidad, Tobago, Uganda and Venezuela. Annual loss due to wilt in India and eastern Africa is estimated to be around 71 and 5 million US dollars, respectively[1]. In spite of cultivation of wilt-resistant cultivars of pigeonpea (GT 100 and BDN 2), wilt incidences upto 30% are reported from pigeonpea-growing regions of Gujarat. At present, fungicides such as thiram, bavistin and benomyl are generally used for controlling *Fusarium* wilt. Due to pesticide-related hazards, the need for adopting biological methods for the control of plant diseases have been emphasized[2-3]. Biological control is a potential alternative to chemical control, and currently, there are a number of fungal biocontrol products commercially available in the market[4]. Some examples of microbial biocontrol agents are *Trichoderma harzianum* for *Alternaria brassicola*[5], *Coniothyrium minitans* for *Sclerotinia sclerotiorum*[4], *Paenibacillus* sp. 300 for *Pythium ultimum*[6], *Pseudomonas aureofaciens* AB254 for *Botrytis cinerea*[7] and *Pseudomonas fluorescens Pf*7-14 for *Rhizoctonia solani*[8].

The present investigation deals with the control of wilt of pigeonpea by using *Alcaligenes xylosoxydans*, which produces high level of chitinase that is responsible for breakage and destruction of chitin containing cell wall of *Fusarium udum*, thus controlling growth of plant pathogenic infection of pigeonpea. This is perhaps the first report on the use of chitinolytic *A. xylosoxydans* for biocontrol of a fungal plant pathogen.

Organism, culture conditions and media used—*Alcaligenes xylosoxydans* IMI No. 385022, used in this investigation, was isolated from seafood industrial waste and identified by CAB International, Surrey, UK. The culture conditions for growth were same as described earlier[9].

Effect of A. xylosoxydans, benlate, and monitor WP on control of infection in Cajanus cajan caused by F. udum in pot experiments—Seeds of *C. cajan* were surface-sterilized by washing with sterile distilled water, followed by treating with 0.1% $HgCl_2$ in 0.05*N* HCl for 3 min, and washing again with sterile distilled water thrice. Ten seeds were placed in eight sterile petriplates under aseptic conditions as indicated in Table 1. In each system, the volume was finally made

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Table 1 — Protocol for the determination of biocontrol potential of *A. xylosoxydans*, benlate and monitor WP

Plates	*A. xylosoxydans* (10^8cfu/ml)	Benlate (mg)	Monitor WP (mg)	*F. udum* (10^6 spores/ml)	Distilled water (ml)
A	—	—	—	—	20
B	—	—	—	5	15
C	5	—	—	—	15
D	—	3	—	—	20
E	—	—	3	—	20
F	5	—	—	5	10
G	—	3	—	5	15
H	—	—	3	5	15

to 20 ml with sterile distilled water. After five days, the radicle and plumule lengths were measured and the per cent germination was determined.

Eight pots were filled with 500 g of sterilized soil. The same eight treatments were used by transferring the germinated seeds from the petriplates into the pots. Plant growth was monitored for one month after which the shoot length, root length and the number of leaves were recorded. The plantlets were dried in an oven at 55° ± 2°C to constant weight.

Effect of A. xylosoxydans, benlate, monitor WP, thiram and bavistin on the control of infection in C. cajan caused by F. udum in field trials — The field trials were conducted in a rain irrigated and well maintained *Fusarium* wilt sick plot at Gujarat Agricultural University, Model Farm, Vadodara during the monsoon season of 2001-2002. The seed treatment included *A. xylosoxydans* (10^8 cfu/ml) 100 ml/kg, benlate at 3 g/kg, monitor WP (*Trichoderma* spp. based formulation) at 4 g/kg, thiram at 3 g/kg and bavistin at 2 g/kg.

Prior to sowing, the seeds of a wilt susceptible cultivar of pigeonpea (T-15-15) were treated with biofungicides or chemical fungicides as described and were sown at inter and intra row spacings of 120 and 20 cm, respectively. Untreated seeds were also sown as the control. The experiment was carried out in a randomized block design with four replications.

The percentage wilt incidence (PWI) was calculated according to the formula:

$$PWI = (\text{Number of diseased plants/Total number of plants}) \times 100$$

The data for per cent of plants with wilt were transformed into arc sin values and statistically analyzed. Grain yield and yield components, namely, number of pods, number of branches, height, and fresh and dry plant weight were recorded. All yield attribute data were statistically analyzed (Indigenous software of Department of Statistics, B.A. College of Agriculture, Anand, India).

Section staining — The sections of pigeonpea shoot were stained according to Willey[10].

Fungal infections in plants were controlled by using chemical/or biological fungicides or introduction of chitinase gene into plants. Genetically transformed strain of *Trichoderma virens* constitutively over-expressing chitinase gene exhibited significantly enhanced biocontrol potential against cotton seedling diseases incited by *R. solani* as compared to the wild type strain[11]. Superior biocontrol activity of chitinase overproducing strain *Pseudomonas stutzeri* YPL-M26 against *F. solani* has also been reported[12]. Enhanced resistance against fungal pathogens has been reported in transgenic plants with chitinase gene in a number of plants such as strawberry[13], cucumber[14], elite indica rice[15], wheat[16] and japonica rice[17]. Apart from introducing chitinase gene in plants, chitinase-producing microorganisms have also been employed to control fungal plant pathogens. *P. fluorescens* was effective in reducing *F. moniliforme* infection in five different cultivars of sorghum[18]. When cotton seedlings were inoculated with strains of *Bacillus cereus*, *B. subtilis* and *B. pumilus*, the disease incidence by *R. solani* was reduced in the greenhouse by 51, 46 and 56%, respectively[19]. *Stenotrophomonas maltophilia* strain C3 reduced 80% foliar necrosis (brown patch disease) caused by *R. solani* in tall fescue[20]. Development of *Fusarium* wilt symptoms was prevented in pigeonpea on treatment with the antagonist *Bacillus brevis* in pot and field[3]. Both *A. xylosoxydans* and its culture filtrate inhibited *Fusarium* sp. to the extent of 44 and 74%, *in vitro* respectively[9]. Seeds of *C. cajan* infected with *F. udum* resulted in complete inhibition of germination, indicating the virulence of the pathogen (Table 2).

When the seeds were treated with *A. xylosoxydans*, monitor WP and benlate, disease incidence against *Fusarium udum* was reduced by 100, 73 and 100%, respectively. The biofungicide potential of *A. xylosoxydans* in conferring protection against *Fusarium* infection was comparable to commercially available fungicides. In wilt-infested plots, when *C. cajan* seeds were sown without any treatment, 85% wilt incidence was recorded. Wilt incidence was reduced to 43.5% when seeds were treated with *A. xylosoxydans*. Treatment of pigeonpea seeds with benlate, thiram, bavistin and monitor WP reduced the wilt incidence by 46, 39.25, 33 and 41%, respectively (Table 3). Grain yield with seeds treated with *A. xylosoxydans*, benlate, thiram, bavistin and monitor WP was 503, 537, 458, 377 and 475 kg/ha, respectively; while the untreated control was 295 kg/ha. Microscopic studies of transverse section of shoot of infected and healthy plants showed the presence of fungal mycelia in the former, not in the latter (Fig. 1).

A. xylosoxydans offered a significant protection (43.5%) to infection by *F. udum* in the field trials. Chemical fungicides, benlate, thiram, bavistin as well as the biofungicide monitor WP also gave good protection. The pods per plant and grain yield enhanced due to treatment with *A. xylosoxydans* and the chemical and biological fungicides as compared to untreated control. Thus, chitinolytic strain of

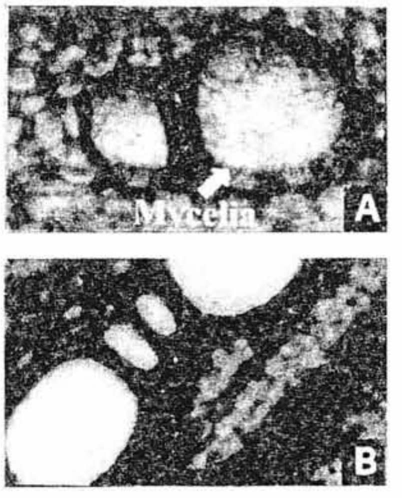

Fig. 1—Transverse section of *F. udum* (A) infected; and (B) uninfected pigeonpea shoot (400 x magnification)

Table 2—Effect of *A. xylosoxydans*, benlate and monitor WP on the growth of *C. cajan* infected with *F. udum* in pots

Treatment	Germination (%)	Root length (cm)	Shoot length (cm)	Average dry wt (g)
Control (No treatment)	80	4.3 ± 1.5	19.0 ± 3.0	0.4937
F. udum	00	—	—	—
A. xylosoxydans	80	2.9 ± 1.9	21 ± 3.0	0.4985
Benlate	80	3.9 ± 2.7	20.3 ± 4.2	0.5677
Monitor WP	60	5.7 ± 2.4	13.3 ± 7.5	0.2705
A. xylosoxydans+ *F. udum*	80	6.1 ± 0.9	14.6 ± 6.9	0.3126
Benlate + *F. udum*	80	2.5 ± 0.5	21.9 ± 1.9	0.5250
Monitor WP + *F. udum*	50	2.5 ± 0.9	10.4 ± 2.1	0.2455

Table 3—Comparative effect of *A. xylosoxydans*, chemical and biological fungicides on the control of *Fusarium* wilt in *C. cajan* (T-15-15) seeds under field trials

Treatment	% Wilt[a,b]	Dry weight of biomass (kg/ha)	Pods/plant	Height (cm)	Grain yield (kg/ha)
Benlate	38.47 (38.75)	2771	203	205	537
A. xylosoxydans	39.90 (41.25)	2583	213	206.5	503
Monitor WP	41.38 (43.75)	2531	217	199	475
Thiram	42.40 (45.50)	2370	215	201	458
Bavistin	45.99 (51.75)	2562	209	201	377
Untreated control	67.31 (84.75)	2567	194	204	295
SEm ±	1.63	63	8.28	4.61	19.70
CD @ 5 %	4.90	191	[f]NS	NS	59.37
CV %	7.08	8.94	7.95	4.54	9.06

[a]Arc sin transformed values [b] Figures in parentheses are original values
[f]NS: Not significant

A. xylosoxydans was comparable in efficacy of controlling infection and increasing yields with chemical fungicides and biocontrol agent.

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References

1 Reddy M V, Raju T N, Sharma S B, Nene Y L & McDonald D, Handbook of Pigeonpea diseases, information Bulletin No. 42 (International Crop Research Institute for the Semi-Arid Tropics, Patancheru, Andhra Pradesh) 1993.

2 Dhar V, Seven decades of research on sterility mosaic of pigeonpea, *Indian J Pulses Res*, 13 (2000).

3 Bapat S & Shah A K, Biological control of fusarial wilt of pigeonpea by *Bacillus brevis*, *Can J Microbiol*, 46 (2000) 125.

4 de Vrije T, Antoine N, Buitelaar R M, Bruckner S, Dissevelt M, Durand A, Gerlagh M, Jones EE, Luth P, Oostra J, Ravenberg W J, Renaud R, Rinzema A, Weber F J & Whipps J M, The fungal biocontrol agent *Coniothyrium minitans*: production by solid-state fermentation, application and marketing, *Appl Microbiol Biotechnol*, 56 (2001) 58.

5 Mora A & Earle E D, Combination of *Trichoderma harzianum* endochitinase and a membrane-affecting fungicide on control of *Alternaria* leaf spot in transgenic broccoli plants, *Appl Microbiol Biotechnol*, 55 (2001) 306.

6 Singh P P, Shin Y C, Park C S & Chung Y R, Biological control of *Fusarium* wilt of cucumber by chitinolytic bacteria, *Phytopathology*, 89 (1999) 92.

7 Mathre D E, Cook R J & Callan N W, From discovery to use: Traversing the world of commercializing biocontrol agents for plant disease control, *Plant Dis*, 83 (1999) 972.

8 Krishnamurthy K & Gnanamanickam S S, Biological control of rice blast by *Pseudomonas fluorescens* strain *Pf7*-14: evaluation of marker gene and formulations, *Biol Control*, 13 (1998) 158.

9 Vaidya R J, Shah I M, Vyas P R & Chhatpar H S, Production of chitinase and its optimization from a novel isolate *Alcaligenes xylosoxydans*: potential in antifungal biocontrol, *World J Microbiol Biotechnol*, 17 (2001) 691.

10 Willey RL, *Microtechniques—A laboratory guide* (The Macmillan Co., New York) 1971, 1.

11 Baek J M, Howell C R & Kenerley C M, The role of an extracellular chitinase from *Trichoderma virens* Gv29-8 in the biocontrol of *Rhizoctonia solani*, *Curr Genet*, 35 (1999) 41.

12 Lim H S, Kim Y S & Kim S D, *Pseudomonas stutzeri* YPL-1 genetic transformation and antifungal mechanism against *Fusarium solani*, an agent of plant root rot, *Appl Environ Microbiol*, 57 (1991) 510.

13 Asao H, Nishizawa Y, Arai S, Sato T, Hirai M, Yoshida K, Shinmyo A & Hibi T, Enhanced resistance against a fungal pathogen *Sphaerotheca humuli* in transgenic strawberry expressing a rice chitinase gene, *Plant Biotechnol*, 14 (1997) 145.

14 Tabei Y, Kitade S, Nishizawa Y, Kikuchi N, Kayano T, Hibi T & Akutsu K,. Transgenic cucumber plants harboring a rice chitinase gene exhibit enhanced resistance to gray mold (*Botrytis cinerea*), *Plant Cell Rep*, 17 (1998) 159.

15 Datta K, Tu J, Oliva N, Ona I, Velazhahan R, Mew T W, Muthukrishnan S & Datta S K, Enhanced resistance to sheath blight by constitutive expression of infection-related rice chitinase in transgenic elite indica rice cultivars, *Plant Sci*, 160 (2001) 405.

16 Chen W P, Chen P D, Liu D J, Kynast R, Friebe B, Velazhahan R, Muthukrishnan S & Gill B S, Development of wheat scab symptoms is delayed in transgenic wheat plants that constitutively express a rice thaumatin-like protein gene, *Theor Appl Genet*, 99 (1999) 755.

17 Nishizawa Y, Nishio Z, Nakazono K, Soma M, Nakajima E, Ugaki M & Hibi T, Enhanced resistance to blast (*Magnoporthe grisea*) in transgenic japonica rice by constitutive expression of rice chitinase, *Theor Appl Genet*, 99 (1999) 383.

18 Raju N S, Niranjana S R, Janardhana G R, Prakash H S, Shetty H S & Mathur S B, Improvement of seed quality and field emergence of *Fusarium moniliforme* infected sorghum seeds using biological agents, *J Sci Food Agri*, 79 (1999) 206.

19 Pleban S, Ingel F & Chet I, Control of *Rhizoctonia solani* and *Sclerotium rolfsii* in the greenhouse using endophytic *Bacillus* spp., *Eur J Plant Pathol*, 101 (1995) 665.

20 Giesler L J & Yuen G Y, Evaluation of *Stenotrophomonas maltophilia* strain C3 for biocontrol of brown patch disease, *Crop Protect*, 17 (1998) 509.