Technique to detect infection by *Fusarium udum* in pigeonpea before symptom appearance*

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A technique, using an electric current has been developed to detect infection in pigeonpea (*Cajanus cajan* (L.) Millsp.) by *Fusarium udum* Butler, prior to the actual appearance of external wilt symptoms. As the prediction validity of this technique is > 94%, this method should be useful in selecting individual plants for crosses in a pigeonpea breeding programme.

Keywords: Pigeonpea; *Fusarium udum* Butler; Disease detection

Wilt of pigeonpea (*Cajanus cajan* (L.) Millsp.) caused by *Fusarium udum* Butler is a serious disease in the Indian subcontinent and eastern Africa (Kannaiyan *et al.*, 1981; Nene, 1980). This soil-borne fungus causes systemic infection in pigeonpea from the seedling to harvesting stages. Diseased plants are diagnosed by the yellowing and wilting of the foliage and by black streaks in the xylem tissue. Plants are usually infected when fairly young but external symptoms of wilt are not pronounced during the early crop growth period, and are more evident at flowering and podding stages (Nene *et al.*, 1980). A need exists to detect plants which are likely to wilt to ensure minimum wastage of effort in making crosses for breeding purposes. Miller-Jones *et al.* (1977) reported detection of infection of small 'cricket-bat' willow (*Salix alba* var. *caerulea* Sm.) by *Erwinia salicis* (Day) Chester before symptom appearance by using an instrument designated model 7950 'Shigometer' (Northeast Electronics Corporation, Concord, New Hampshire). The diseased tissues were distinguished from the healthy ones by their low resistance to pulsed electric current.

Similar work was reported by Caruso *et al.* (1976) on tomatoes to detect pre-symptomatic Fusarium wilt, and by Blanchard and Carter (1980) on Dutch elms to detect *Ceratocystis ulmi* disease before symptom appearance. At ICRISAT an instrument was developed to measure the current flow for detecting the Fusarium wilt fungus infection in pigeonpea plants prior to the appearance of external symptoms; its use helped in producing crosses between healthy individual plants in a breeding programme.

Materials and methods

Miller-Jones *et al.* (1977) measured the electrical resistance in k Ω across the horizontal cross-section of a tree by drilling holes to different depths and embedding electrodes in them. This method is not suitable for plants of smaller stem diameter such as pigeonpea. Hence, two points 25 mm apart, along the length of each plant were marked and the current flow was measured between these points using a special probe described below.

A dial calipers made of non-conducting material was used. Probes were attached, one to the fixed jaw and the other to the movable one (Figs. 1 and 2). Spring-loaded contact points protruded from the

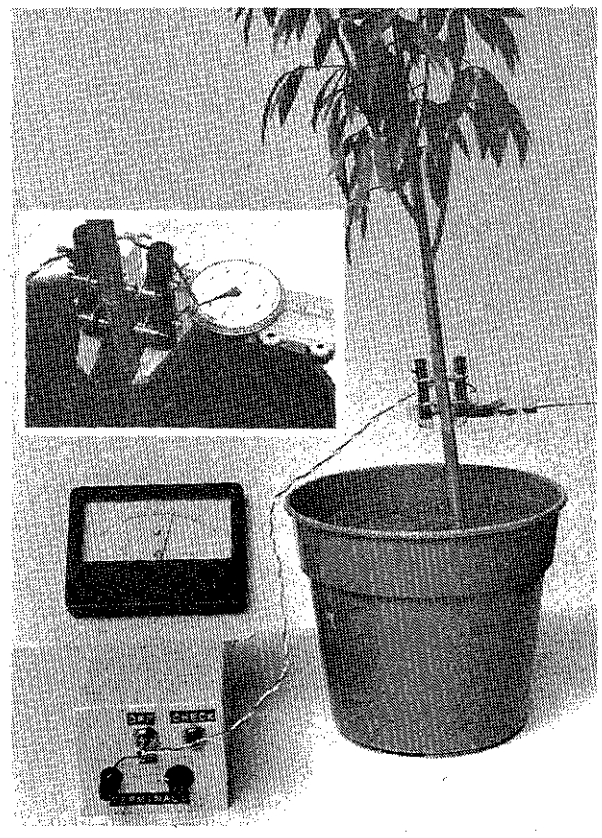

Fig. 1 Experimental set-up for the conductivity and thickness measurements

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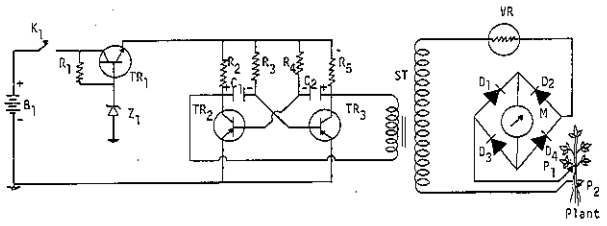

Fig. 2 Schematic diagram of the power source and the pulsed direct current measuring diagram. TR_1, TR_2, TR_3: SL100A transistors; B_1, battery power supply (9 V); K_1, on/off switch; C_1, C_2: capacitors (25 μF, 25 V); R_1, resistor (100 Ω); R_2, R_5: resistor (68 Ω); R_3, R_4: resistor (680 Ω); D_1, D_2, D_3, D_4: diodes (in 4001); M, milliammeter (0–10 mA, direct current); P_1, P_2: probe ends; Z_1, Zener diode (6.2 V); ST, step-up transformer (1:30); VR, variable resistor

edges of the probes, and when the jaws of the calipers were in position to measure the thickness of the plant, the two spring-loaded points were in good contact with the plant tissues at two points along its length ≈ 25 mm apart. Leads were taken from two of these high conducting probes that are diagonally opposite and connected to the output of a miltivibrator and a milliammeter. The multivibrator converts a 9 V direct current source, regulated to 6.2 V by a Zener diode, into a pulsating voltage of 300 V at 150 Hz. Measurements of the diameter of the plant and the current flowing through the plant between the two probe points could be made simultaneously, as needed. The probe tips were cleaned with 95% ethanol before each reading to establish good contacts and to avoid spreading infection from one plant to another.

Experiments were conducted to find out the increase in current flow with the increase in the diameter of the normal healthy plant. Along with the initial current measurements the diameters of all the plants were measured and a regression equation

Table 1 Current conduction values of 10 pigeonpea plants (ICP 10960) which were not suspected for wilt infection and remained healthy. The increase over the initial value for these plants did not go beyond 0.5 mA

Plant number	Number of days after 8 weeks	Thickness of stem (mm)	Current conduction (mA)		
			Measured (a)	Corrected for thickness (b)	Increase over initial value (mA)
1	2	3	4	5	6
1	1	9.8	4.0	4.0	
	3	10.0	4.5	4.3	0.3
	5	10.2	4.5	4.1	0.1
	7	10.4	5.0	4.4	0.4
	12	10.7	5.0	4.1	0.1
2	1	8.3	4.0	4.0	
	3	8.6	4.0	3.7	−0.3
	5	8.9	4.5	3.9	−0.1
	7	9.1	5.0	4.2	0.2
	12	9.6	5.0	3.7	−0.3
3	1	4.7	3.0	3.0	
	3	5.0	3.1	2.9	−0.1
	5	5.6	3.5	2.6	−0.4
	7	5.9	4.0	3.0	0
	12	6.2	4.5	3.0	0
4	1	8.4	3.0	3.0	
	3	8.6	3.0	2.8	−0.2
	5	8.9	3.5	3.0	0
	7	9.2	4.0	3.2	0.2
	12	9.5	4.0	3.0	0
5	1	5.3	3.5	3.5	
	3	5.5	3.5	3.3	−0.2
	5	5.9	3.6	3.0	−0.5
	7	6.1	4.0	3.2	−0.3
	12	6.3	4.5	3.5	0
6	1	1.6	2.0	2.0	
	3	2.1	2.5	2.0	0
	5	2.5	3.0	2.1	0.1
	7	2.7	3.0	1.9	−0.1
	9	2.9	3.5	2.2	0.2
	12	3.2	3.5	1.9	−0.1
7	1	4.0	3.5	3.5	
	3	4.2	3.5	3.3	−0.2
	5	4.4	3.5	3.1	−0.4
	7	4.6	4.0	3.4	−0.1
	9	4.7	4.0	3.3	−0.2
	12	4.9	4.0	3.1	−0.1
8	1	2.4	2.0	2.0	
	3	2.7	2.1	1.8	−0.2
	5	2.9	2.5	2.0	0

Table 1 (contd)

Plant number	Number of days after 8 weeks	Thickness of stem (mm)	Current conduction (mA)		
			Measured (a)	Corrected for thickness (b)	Increase over initial value (mA)
1	2	3	4	5	6
	7	3.1	2.5	1.8	−0.2
	9	3.2	3.0	2.2	0.2
	12	3.4	3.0	2.0	0
9	1	4.5	3.0	3.0	
	3	4.9	3.5	3.1	0.1
	5	5.3	4.0	3.2	0.2
	7	4.5	4.0	3.0	0
	9	5.8	4.0	2.7	−0.3
	12	6.1	5.0	3.4	0.4
10	1	4.6	3.0	3.0	
	3	4.8	3.0	2.8	−0.2
	5	5.2	3.5	2.9	−0.1
	7	5.4	3.5	2.7	−0.3
	9	5.5	3.6	2.7	−0.3
	12	5.8	4.0	2.8	−0.2

Table 2 Current conduction values of 10 pigeonpea plants (ICP 2376) suspected for wilt infection and which wilted subsequently. When the increase over the initial value was > 0.5 mA the plant was put in the 'suspected for wilt' category

Plant number	Number of days after 8 weeks	Thickness of stem (mm)	Current conduction (mA)		
			Measured (a)	Corrected for thickness (b)	Increase over initial value (mA)
1	2	3	4	5	6
1	1	5.8	4.0	4.0	
	3	5.9	4.5	4.4	0.4
	7	6.4	5.0	4.4	0.4
	12	6.9	6.0	4.9	0.9
2	1	7.3	4.0	4.0	
	3	7.4	4.2	4.1	0.1
	7	7.9	5.8	5.2	1.2
3	1	6.8	2.5	2.5	
	3	7.1	3.0	2.7	0.2
	7	7.8	4.5	3.5	1.0
4	1	8.2	3.5	3.5	
	3	8.4	3.5	3.3	−0.2
	5	8.7	5.0	4.5	1.0
5	1	9.4	1.5	1.5	
	3	9.7	2.0	1.7	0.2
	5	10.2	4.5	3.7	2.2
6	1	7.8	3.5	3.5	
	5	8.2	4.5	4.1	0.6
	7	8.4	5.0	4.4	0.9
7	1	7.7	4.0	4.0	
	5	8.2	5.0	4.5	0.5
	7	8.3	5.0	4.4	0.4
	12	8.7	5.8	4.8	0.8
8	1	7.0	4.5	4.5	
	3	7.1	5.0	4.9	0.4
	5	7.3	5.5	5.2	0.7
9	1	5.9	4.0	4.0	
	3	5.9	4.0	4.0	0
	7	6.3	4.4	4.0	0
	9	6.4	5.0	4.5	0.5
	12	6.6	5.8	5.1	1.1
10	1	8.1	3.0	3.0	
	3	8.3	4.0	3.8	0.8
	7	8.9	5.0	4.2	1.2

was developed with the current values in mA and the diameter of the plants in mm. The regression coefficient was 0.95 with $r^2 = 0.81$ (Snedecor & Cochran, 1967). Subsequently, at intervals of 2–4 days, current values only were measured. Utilizing the regression coefficient, the increase in current that would occur due to the increase in the diameter of a healthy plant was predicted and compared with the observed value.

Whenever an increase in the current value was observed in a particular plant, its diameter was measured again. Knowing the increase in diameter, the expected increase in the current caused by the growth of the plant between readings was predicted using the regression coefficient determined as above. This predicted increase in current value, when substracted from the observed current value, gave the increase in current due to reasons other than increase in the diameter of the plant. If the difference was > 0.5 mA, the plant was suspected of being infected by *F. udum*, though there were, as yet, no physical symptoms of wilt. After two weeks these plants began to show typical wilt symptoms such as yellowing, drooping, drying of leaves and the presence of black streaks in the xylem tissue. For further confirmation random samples of these plants were plated on a culture medium and the isolated fungus was identified as *F. udum*.

During two consecutive seasons – 1978 and 1979 – preliminary experiments were conducted under greenhouse conditions in pots with soil containing *F. udum* inoculum. For further confirmation this study was carried out under field conditions during the 1980 rainy season in a uniform '*F. udum* wilt-sick' plot developed in a Vertisol field at ICRISAT. The observations were recorded on 8-week-old, apparently healthy plants of two pigeonpea lines – ICP-2376 (Fusarium-susceptible) and ICP-10960 (Fusarium-resistant).

Results

An increase in electrical conductivity over the predicted value indicated infection by the wilt fungus in apparently health plants. Plants in which the current flow increased by > 0.5 mA, after applying the correction to the current arising from the increase in the diameter of the plant, were included in the 'suspected for wilt' category. Of the 76 plants (48 of ICP-10960 and 28 of ICP-2376) studied, 52 plants (45 of ICP-10960 and 7 of ICP-2376) showed current changes < 0.5 mA and were designated 'free from wilt'; in fact they did not wilt till maturity (Table 1). Seventeen plants (ICP-2376) showed an increase of > 0.5 mA but were looking healthy (Table 2); these were suspected of being infected and they did, in fact, wilt two weeks later. Three plants (ICP-10960) suspected of wilt infection did not wilt. One plant (ICP-2376) which did not show any change in current wilted. Three plants (ICP-2376) died for other reasons. Thus, out of 76 plants studied, the prediction failed for only four plants, giving a prediction validity of $> 94\%$.

Discussion

Miller-Jones *et al.* (1977) used the 'Shigometer' to detect infection by *Erwinia salicis* before symptom appearance. In their experiments, they drilled holes (2.4 mm diameter) into small 'cricket-bat' willow trees to measure the resistance between the probes across the diameter of the tree. In the present study, the instrument measured the current flow across a 25 mm length of the plant without any damage to the plant. The measurement of current flow instead of resistance facilitated the application of a correction factor to the observed current value.

Since the prediction validity was $> 94\%$, and those plants which did not show wide departure from the predicted current values remained healthy up to the harvesting stage, this technique could be utilized in selecting plants for making desired crosses without running the risk of losing these plants due to wilt.

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