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Full Length Research Paper

Seed to plant transmission of *Xanthomonas campestris* pv. *vignicola* isolates in cowpea

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Seed transmission of *Xanthomonas campestris* pv. *vignicola* was investigated to ascertain the importance of seed as a primary source of inoculum for bacterial blight disease in cowpea. The study was carried out using seeds of five cowpea varieties (TVx 12349, IT86D-721, IT82D-889, Ife Brown and TVx 3236) artificially inoculated with three bacterial isolates (Ikenne, Kano and Ibadan), and seeds harvested from infected plants. Results showed that seed to plant transmission caused 6 – 24% post-emergence seedling mortality and 26 – 49% incidence of blight in plants raised from infected seeds. These results support seed transmission of *X. campestris* pv. *vignicola* in cowpea and suggest that the distant spread of bacterial blight on cowpea may also be due to seed transmission.

Key words: Bacterial blight, *Vigna unguiculata,* cowpea, virulence, seed transmission.

INTRODUCTION

Four bacterial diseases have been documented to attack cowpea. Two of them are of economic importance. They are: bacterial blight (induced by *Xanthomonas campestris* pv. *vignicola* [Burkholder] Dye) and bacterial pustule (induced by *X. campestris* pv. *vignaeunguiculatae*) (Rachie, 1985; Emechebe and Florini, 1997; Shoaga, 1998). Bacterial blight is the most widespread disease of cowpea, having been reported from all regions of the world in which cowpea is cultivated (Emechebe and Florini, 1997). Bacterial pustule on the other hand, has only been reported mainly in Africa (Emechebe and Florini, 1997). The other bacterial diseases of cowpea are bacterial wilt (induced by *Pseudomonas syringae* pv. *solanacearum*) and halo blight (induced by *P. syringae* pv. *tabaci*) (Emechebe and Florini, 1997). Both were reported in Brazil and are of minor economic importance to cowpea production (Emechebe and Florini, 1997).

Bacterial blight of cowpea was first reported in Perkins, Oklahoma in 1931 and Texas in 1942 (Preston, 1949). The pathogen was isolated from cowpea seed and was named *Xanthomonas vignicola* Burkh in 1944 (Preston, 1949). The bacterium was subsequently reported from many countries in Africa and India (Patel, 1981; Allen, 1991). A checklist by Allen (1980) showed that bacterial blight was first recorded in Tanzania in 1964 although Kaiser and Ramos (1979) considered their work to be the first in East Africa. Williams (1975) first recorded the disease in Nigeria.

This disease has been reported to induce yield loss of 26.4% (1975) and 18.1% (1976) in "Ife Brown", 23.6% (1975) and 19.2% (1976) in "New Era" (Ekpo, 1978). In areas where cowpea is commercially grown, yield depression due to *X. campestris* pv. *vignicola* may be as high as 71% in pod, 68% in seed and 53% in fodder (Okechukwu et al., 2000).

It has been observed that as cowpea is introduced into new areas where it has not been previously grown, bacterial blight rapidly becomes established, resulting in serious losses. The incidence of bacterial blight in new production areas suggests that infected seed may be the primary source of inoculum that initiates infection in the field. This study was conducted to determine the degree of seed to plant, and plant to seed transmission of *X. campestris* pv. *vignicola* in cowpea.

MATERIALS AND METHODS

Seeds of Ife brown, TVx 12349, IT86D-721, IT82D-889 and TVx 3236 obtained from the cowpea breeding unit of the International Institute of Tropical Agriculture (IITA), Ibadan, were used for the study. Preliminary screening of the seeds in seed health test by direct plating on nutrient agar gave 100% seed germination and

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Table 1. Seedling emergence (%) in 5 cowpea varieties grown from seeds artificially inoculated with *X. campestris* pv. *vignicola* at Ibadan in 2000.

	X. campestris pv. vignicola Isolates						
Varieties	**Control**	**Ibadan**	**Ikenne**	**Kano**			
	Emergence	**Emergence**	**Reduction (%)**	**Emergence**	**Reduction (%)**	**Emergence**	**Reduction (%)**
IT86D-721	75.0 abc	68.0 a-e	9.3	65.5 a-e	12.7	63.3 a-e	15.6
TVx 3236	90.0 a	78.2 abc	13.3	71.7 a-d	20.4	88.3 a	1.9
TVx 12349	80.0 ab	65.5 a-e	18.2	65.0 a-e	18.8	53.3 b-e	33.3
IT82D-889	67.5 a-e	58.3 b-e	13.6	43.3 e	35.8	45.0 de	33.3
Ife brown	70.0 a-e	51.7 c-e	26.2	54.6 b-e	22.1	45.0 de	35.7

Means followed by the same letters are not significantly different using DMRT at P ≤ 0.05.

indicated complete freedom from infection by *X. campestris* pv. *vignicola*. Bacterial isolates from Kano, Ikenne and Ibadan were used for the study. They were isolated from Ife brown showing typical blight symptoms.

Seed to plant transmission *of X. campestris* pv. *vignicola* from artificially inoculated seeds. Bacterial suspension (10^8 colony forming units (CFU)/ml) was prepared in sterile distilled water from 24 h old nutrient agar cultures for each of the bacterial isolates. Fifty seeds of each cowpea variety were artificially inoculated by soaking for 24 h in each of the bacterial suspensions. Control seeds were similarly soaked in sterile distilled water. Inoculated and control seeds were separately sown on 10 April, in heat-sterilized top soil contained in 25 cm-diameter plastic pots at the rate of 10 seeds per pot. The experimental design was a split plot (5 varieties and 3 isolates plus control) with 4 replications. The pots were placed in IITA screen house with daylight regime 11 – $12^1/_2$ h at 25 – 28°C and relative humidity of about 70 - 90%. Soil was kept moist by sprinkling every other day with tap water. Seedling emergence was determined 7 days after planting and percentage post-emergence damping-off was determined 14 days after seedling emergence using the formula:

$$y - x \Big/ y \times \frac{100}{1}$$

Where, y = total number of plants after thinning; x = number of living plants at assessment time.

Data on disease incidence were obtained after the first appearance of disease symptoms. There were four disease assessments performed at weekly intervals. The first rating started 26 days after planting. Disease incidence was obtained by calculating the proportion of plants per replicate that was infected to the total number of plants present. At maturity all pods per replicate were harvested, and manually threshed.

Seed to plant transmission from naturally infected seeds

Seeds harvested from infected plants and those from uninfected plants (control) were separately planted in 25 cm-diameter pots containing sterile soil in the screenhouse, to evaluate seed to plant transmission of *X. campestris* pv. *vignicola* in natural infection. The experimental design was a split plot (5 varieties and 3 isolates plus control) with 4 replications. Seeds were sown at the rate of 10 per pot and 5 pots per treatment combination (variety/isolate). The five pots per treatment combination were considered as a replicate. Data collected included percentage seedling emergence, post-emergence damping-off, and disease incidence as described above.

RESULTS

Seed to plant transmission of bacterial blight in cowpea grown from artificially inoculated seeds

Seedling emergence

The effect of artificial inoculation of cowpea seeds with *X. campestris* pv. *vignicola* on percentage seedling emergence is shown in Table 1. Though the control plants had higher percentages of seedling emergence, none of the inoculated varieties had a significantly ($P \leq 0.05$) lower percentage emergence compared to their respective controls. In general, percentage seedling emergence varied from 63 to 68% in IT86D-721, from 72 to 88% in TVx 3236, from 53 to 65% in TVx 12349, from 43 to 58% in IT82D-889, and from 45 to 55% in Ife brown. Across the varieties, the reductions in seedling emergence associated with Kano, Ikenne, and Ibadan isolates were 24, 22 and 16%, respectively.

Post-emergence mortality

The effects of seed to plant transmission of bacterial isolates on post-emergence seedling mortality of cowpea grown from seeds artificially inoculated with *X. campestris* pv. *vignicola* are shown in Table 2. Significant ($P \leq 0.05$) post-emergence mortality was recorded in all variety/isolate combinations. The seedling mortality percentages in IT82D-889/Kano isolate (22.8%), IT82D-889/Ibadan isolate (18.5%) and Ife brown/Kano isolate (15.9%) were comparable and significantly higher than those of other variety/isolate combinations (2.9 - 12.0%).

Blight incidence

Table 3 shows the disease incidence in five cowpea varieties raised from seeds artificially inoculated with *X. campestris* pv. *vignicola* isolates and the control at four assessment periods. The disease incidence on the first

Table 2. Post-emergence mortality (%) in five cowpea varieties grown from seeds artificially inoculated with *X. campestris* pv. *vignicola* at Ibadan in 2000.

Varieties	Bacterial isolates			
	Ibadan	Ikenne	Kano	Control
IT86D-721	7.5 b	6.6 b	12.0 b	0.0 c
TVx 3236	7.9 b	5.8 b	7.9 b	0.0 c
TVx 12349	7.5 b	2.9 b	9.6 b	0.0 c
IT82D-889	18.5 a	10.0 b	22.8 a	0.0 c
Ife brown	12.0 b	8.8 b	15.9 a	0.0 c

Means followed by the same letters are not significantly different using DMRT at P ≤ 0.0.

Table 3. Bacterial blight incidence (%) in five cowpea varieties grown from seeds artificially inoculated with three *X. campestris* pv. *vignicola* isolates over a period of four weeks in the screenhouse at Ibadan in 2000.

Variety	Isolate	Assessment periods							
		First		Second		Third		Fourth	
IT86D-721	Ibadan	5.0	a	28.8	a	57.5	a	81.3	b
	Ikenne	0.0	a	26.4	a	52.7	a	79.6	b
	Kano	0.0	a	28.1	a	56.3	a	79.2	b
	Control	0.0	a	0.0	b	0.0	b	0.0	c
TVx 3236	Ibadan	0.0	a	30.0	a	60.0	a	75.0	b
	Ikenne	0.0	a	27.1	a	54.2	a	77.1	b
	Kano	0.0	a	30.0	a	60.0	a	75.0	b
	Control	0.0	a	0.0	b	0.0	b	0.0	c
TVx 12349	Ibadan	1.7	a	28.8	a	57.5	a	79.2	b
	Ikenne	0.0	a	29.6	a	59.2	a	77.1	b
	Kano	0.0	a	30.8	a	61.7	a	83.3	b
	Control	0.0	a	0.0	b	0.0	b	0.0	c
IT82D-889	Ibadan	5.0	a	30.6	a	61.3	a	100.0	a
	Ikenne	0.0	a	30.0	a	60.0	a	100.0	a
	Kano	7.5	a	32.1	a	64.2	a	100.0	a
	Control	0.0	a	0.0	b	0.0	b	0.0	c
Ife brown	Ibadan	5.0	a	30.8	a	61.7	a	81.3	b
	Ikenne	0.0	a	28.3	a	56.7	a	83.3	b
	Kano	4.1	a	32.7	a	65.5	a	84.1	b
	Control	0.0	a	0.0	b	0.0	b	0.0	c

Means followed by the same letters are not significantly different using DMRT at P ≤ 0.05.

week of assessment (26 days after planting) ranged from 0 to 8% with no significant difference (P≤0.05) among the inoculated varieties. Among the inoculated treatments, the highest incidence was recorded in IT82D-889/Kano combination (8%). By the second assessment period, there was a significant difference (P≤0.05) between the control plants and the inoculated varieties (Table 3). While the control plants had no incidence of disease, the inoculated plants had disease incidence ranging from 26% in IT86D-721/Ikenne isolate combination to 33% in Ife brown/Kano isolate combination. There was, however, no significant difference (P≤0.05) among all the variety/ isolate combinations. This trend was the same for the third week of assessment, except that the disease incidence in inoculated varieties was higher and ranged from 53% in IT86D-721/Ikenne isolate combination to 65% in Ife brown/Kano isolate combination. On the fourth assessment period, IT82D-889 inoculated with Ibadan, Ikenne and Kano isolates had 100% disease incidence; the other variety/isolate combinations had a disease incidence that ranged from 75 to 84%. In general, disease incidence in plants derived from inoculated

Table 4. Seedling emergence (%) in five cowpea varieties grown from seeds harvested from infected plants at Ibadan in 2000.

Varieties	Ibadan	Ikenne	Kano	Control
IT86D-721	73.3 ab	80.0 a	66.7 b	100.0 a
TVx 3236	75.0 a	86.7 a	73.3 ab	100.0 a
TVx 12349	73.3 ab	80.0 a	80.0 a	100.0 a
IT82D-889	80.0 a	70.0 ab	80.0 a	100.0 a
Ife brown	80.0 a	80.0 a	80.0 a	100.0 a

Means followed by the same letters are not significantly different using DMRT at P ≤ 0.05.

Table 5. Post-emergence seedling mortality (%) in five cowpea varieties grown from seeds harvested from infected and uninfected plants at Ibadan in 2000.

Varieties	Bacterial isolate			
	Ibadan	Ikenne	Kano	Control
IT86D-721	7.5 b	9.6 b	10.0 b	0.0 c
TVx 3236	7.1 b	5.8 b	7.9 b	0.0 c
TVx 12349	6.7 b	5.8 b	9.6 b	0.0 c
IT82D-889	20.8 a	11.7 b	23.8 a	0.0 c
Ife brown	10.0 b	8.8 b	17.9 a	0.0 c

Means followed by the same letters are not significantly different using DMRT at P ≤ 0.05.

Table 6. Bacterial blight incidence (%) in five cowpea varieties grown from seeds harvested from infected plants at Ibadan in 2000.

Varieties	Ibadan	Ikenne	Kano	Control
IT86D-721	28.8 c	26.4 c	42.2 ab	0.0 d
TVx 3236	45.0 ab	40.6 ab	45.0 ab	0.0 d
TVx 12349	38.4 b	39.4 ab	46.3 ab	0.0 d
IT82D-889	46.8 ab	45.0 ab	49.4 a	0.0 d
Ife brown	42.1 ab	37.5 b	40.6 ab	0.0 d

Means followed by the same letters are not significantly different using DMRT at P ≤ 0.05.

seeds increased with time while plants from uninoculated seeds remained blight-free.

Seed to plant transmission of bacterial blight in cowpea grown from seeds harvested from infected plants

Seedling emergence

Percentage seedling emergence in cowpea varieties grown from seeds harvested from infected plants is shown in Table 4. In varieties infected with Ibadan isolateof *X. campestris* pv. *vignicola* seedling emergence varied between 73.3 and 80.0% compared to 100% recorded for seeds harvested from uninfected control plants. Seed-borne Ikenne and Kano bacterial isolates were also associated with reduced seedling emergence ranging from 70.0 to 80.0% and 66.7 to 80.0%, respectively. Overall, there was no significant reduction in seedling emergence regardless of the isolate/variety combination except in seed infection of IT86D-721 with Kano isolate.

Post-emergence seedling mortality

Seed to plant transmission of bacterial blight is presented in Table 5. Significant ($P \leq 0.05$) post-emergence mortality was recorded in all variety/isolate combinations. The seedling mortality percentages in IT82D-889/Ibadan isolate (20.8%), IT82D-889/Kano isolate (23.8%) and Ife brown/Kano isolate (17.9%) were comparable and significantly higher.

Blight incidence

Incidence of bacterial blight in plants grown from seeds harvested from plants infected with *X. campestris* pv.

vignicola is presented in Table 6. Bacterial blight was not observed in plants grown from seeds harvested from uninfected plants, but plants derived from seeds harvested from infected plants developed characteristic blight symptoms.

All the three bacterial isolates were transmitted from seed to plant in all tested varieties. However, the degree of seed to plant transmission varied significantly ($P \leq 0.05$) with variety/isolate combinations with a range of 26.4 – 49.4% blight incidence across the varieties and isolates. The highest blight incidence was recorded for IT82D-889/Kano isolate combination while the lowest incidence was observed in IT86D-721/Ikenne isolate combination than those of other variety/isolate combinations (5.8 - 10.0%).

DISCUSSION

The varieties varied in their response to artificial infection with bacterial isolates. The blight incidence arising from seed-borne infection varied from 39.6% in the least compatible Ikenne isolate/TVx 3236 combination to 50% in the most compatible Kano isolate/IT82D-889 combination. The differential disease reactions to the isolates implies the existence of pathogenic variation in isolates of *X. campestris* pv. *vignicola* as earlier reported (Jainkittvong et al., 1989; Shoaga, 1998). The variations observed in pathogenicity of the isolates may be due to their origin, Kano isolate being introduced from the arid/semi-arid, and Ikenne isolate from the humid forest, to Ibadan in the derived savanna agroecology. Also, variations in the molecular components of isolates of *X. campestris* pv. *vignicola* from cowpea leaves collected from various geographic areas may affect isolate/seed compatibility and the degree of each seed to progeny transmission of each bacterial isolate (Verdier et al., 1998).

Bacterial transmission in seeds harvested from infected plants caused 6-24% post-emergence seedling mortality and 26-49% blight incidence. The percentage seed-transmitted blight incidence varied among the different varieties. This variation may be due to the different levels of infected seeds in the seedlot harvested. Such variation in the percentage of infected seedlings ranging from 15 to 28.3% has been reported (Shoaga, 1998). In a similar work on rice, Veena et al. (1996) reported variation in the percentage of rice seedlings infected with *X. oryzae* pv. *oryzae*.

This study has shown the importance of seed-borne inoculum of *X. campestris* pv. *vignicola* in disease initiation. Even though seed-borne inoculum is considered insignificant in causing bacterial blight in areas where the disease has already been established (Veena et al., 1996), infected seeds are important means of dispersal of the pathogen to disease-free areas. Such transmission will lead to scattered foci of infection that are an ideal condition for an outbreak of an epidemic (Mathur et al., 1988; Veena et al., 1996; Shoaga, 1998). The use of pathogen-free seeds for planting will help avoid all the deleterious effects of seedling mortality, foliar blight and stem canker in surviving plants, the cumulative effect of which may lead to yield losses.

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