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Incidence and seed transmission of *Xanthomonas axonopodis* pv. *cyamopsidis* in cluster bean

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Dry seed examination of 149 samples of cluster bean [*Cyamopsis tetragonoloba* L. (Taub.)] collected from 25 districts of Rajasthan revealed asymptomatic (0.5-94.5%), moderately discoloured (2.75-97.5%) and shrivelled-discoloured (0.25-100%) seeds in 132, 147 and 149 seed samples, respectively. The discolorations varied from cream to purple-brown spots and water-soaked translucent areas which yielded colonies of *Xanthomonas axonopodis* pv. *cyamopsidis* on incubation. The standard cultural, biochemical and pathogenecity tests were carried out for identification of the bacterium. 142 (95.30%) seed samples of 25 districts of Rajasthan revealed 7.5-100% incidence of the pathogen on Yeast dextrose calcium carbonate agar medium. The seed-borne inoculum caused pre- and post- imergence losses and symptoms of browning of radicle, splitting of plumule, necrotic spots with bacterial oozing and bright in cotyledonary leaves.

Key words: Cluster bean, seeds, *Xanthomonas axonopodis* pv. c*yamopsidis* incidence, transmission

Introduction

Occurrence of bacterial blight and leaf spot of cluster bean caused by *Xanthonas axonopodis* pv. *cyamopsidis* (XAC) has been reported from United States (Orellana *et al.*, 1965), Arizona (Mihail and Alcorn, 1985), Madison (Undersander *et al.,* 1991) and Brazil (Almeida *et al.,*1992). In India, the disease has been reported from the states of Rajasthan (Patel *et al.,* 1953), Haryana (Gandhi and Chand, 1985) and Karnataka (Patel and Patel, 1958; Chakravarthy *et al.,* 2004a). In the present study incidence of the pathogen in seeds grown in Rajasthan state, India and transmission of seed borne inoculum from seed to plant were studied.

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Materials and methods

Identification and Incidence of the pathogen in seed samples

One hundred forty nine seed samples of cluster bean collected from 25 districts of Rajasthan were studied by dry seed examination, incubation on moistened blotters (Anonymous, 1985) and YDC agar plate method to find the Incidence of *X. axonopodis* pv. *cyamopsidis* in seed samples. After 72 h of incubation at 30^{o}C (Schaad and Kendrick, 1975) typical bacterial colonies from seeds raised on YDC agar plate were transferred to YDC agar medium plates to obtain pure cultures which were subjected to various tests namely gram's staining, KOH solubility test, levan formation, oxidase test (Kovac's, 1956; Hildbrand and Scroth, 1972), potato soft rot test, nitrate reductase test (Fahy and Persley, 1983), arginine dihydrolysis, gelatin hydrolysis test, hypersensitivity test in tobacco and pathogenecity tests (Lelliot and Stead, 1987) for the identification of the bacterial species. For all the tests 24-48 h old cultures (Lelliot and Stead, 1987) and bacterial suspensions (Kiraley *et al.,* 1970) were used. The bacterial isolates identified by various methods as described above were subjected to pathogenecity tests (Schaad, 1980) on the host plant and other plant species.

Disease transmission

Two naturally infected seed samples of cluster bean (lab. ac. nos. Ct-818 and Ct-825) carrying 80% and 89% infection of *X. axonopodis* pv. *cyamopsidis* were selected for transmission studies. 100 seeds per category per sample were sown on moist blotters (10 seeds/plate) and 1% water agar medium in test tubes (1seed/test tube) and incubated at 25±2^{o}C for 12/12 h alternating cycles of light and darkness up to 7 days and 14 days respectively. In pot experiment, 100 seeds per category per sample were sown in pots (5 seeds/pot) and data on per cent seed germination, ungerminated seeds associated with the pathogen (bacterial colonies), seedling symptoms and mortality were recorded. Isolation of the pathogen was carried out from the infected plant parts at different stages of plant growth.

Pathogenecity test

Artificial inoculations of the bacterial isolates was carried out by techniques of incubation of smoothered seeds and stab inoculation of seedlings and other parts of the plants.

Results and discussion

Identification of the pathogen and its incidence

149 seed samples of 25 districts revealed asymptomatic (0.5-91%), moderately discoloured (2.75-97.5%) and shrivelled-discoloured (0.25-100%) seeds in 132, 147 and 149 seed samples (Fig. 1a). The discolourations varied from cream to purple brown spots and water-soaked translucent shining areas. Mostly infected seeds showed splitted seed coat. The symptomatic seeds on incubation yielded the growth of *X. axonopodis* pv. *cyamopsidis* (*XAC*) (Fig.1b). Similarly symptoms on seeds caused by *XAC* have been reported earlier in cluster bean (Chakravarthy *et al.,* 2004a,b), by *X. campestris* pv. *campestris* in rape and mustard (Sharma *et al.,* 1992) and by *X. c.* pv. *cajani* in pigeon pea (Sharma *et al.,* 2001, 2002).

The bacterial colonies isolated from various seed samples were produced convex to domed, circular, entire, yellow, mucoid, shiny and raised colonies on YDC agar medium and identified to be *X. axonopodis* pv. *cyamopsidis*. The isolates were gram's negative, KOH solubility test positive, levan negative, lipase activity positive (Fig.1c), oxidase negative, starch hydrolyzing (Fig. 1d), gelatin hydrolyzing, arginine variable, did not reduce nitrate and no rotting of potato tissue occurred. The pathogen induced positive hypersensitivity reaction on tobacco leaves after infiltration. The turgidity of leaves was lost within 6-10 h followed by local necrosis and desiccation of affected leaf tissues after 36 h.

Pale-cream to variable shades of yellow coloured bacterial colonies of *X. axonopodis* pv. *cyamopsidis* on and around the seeds in 142 (95.30%) seed samples from 25 districts of Rajasthan with an the incidence range of 7.5-100% were recorded which suggests its wide spread occurrence in Rajasthan state. It may further build up the inoculum of the pathogen in the fields. The heavy incidence (≥25%) of the pathogen was recorded in samples belonging to all the districts of Rajasthan excluding Sirohi. Higher incidence (1-90%) of *XAC* has also been recorded by YDC agar plate method in cluster been seeds grown in districts of Karnataka (Chakravarthy *et al.,* 2004a). In pathogenecity test (Host test), after stab inoculation of healthy seedlings at staple stage with the test bacterial cells (10^8-10^9 cfu/ml at 600 nm), symptoms of blight were observed followed by general browning and rotting of hypocotyle and radicle in cluster bean, cowpea and black gram.

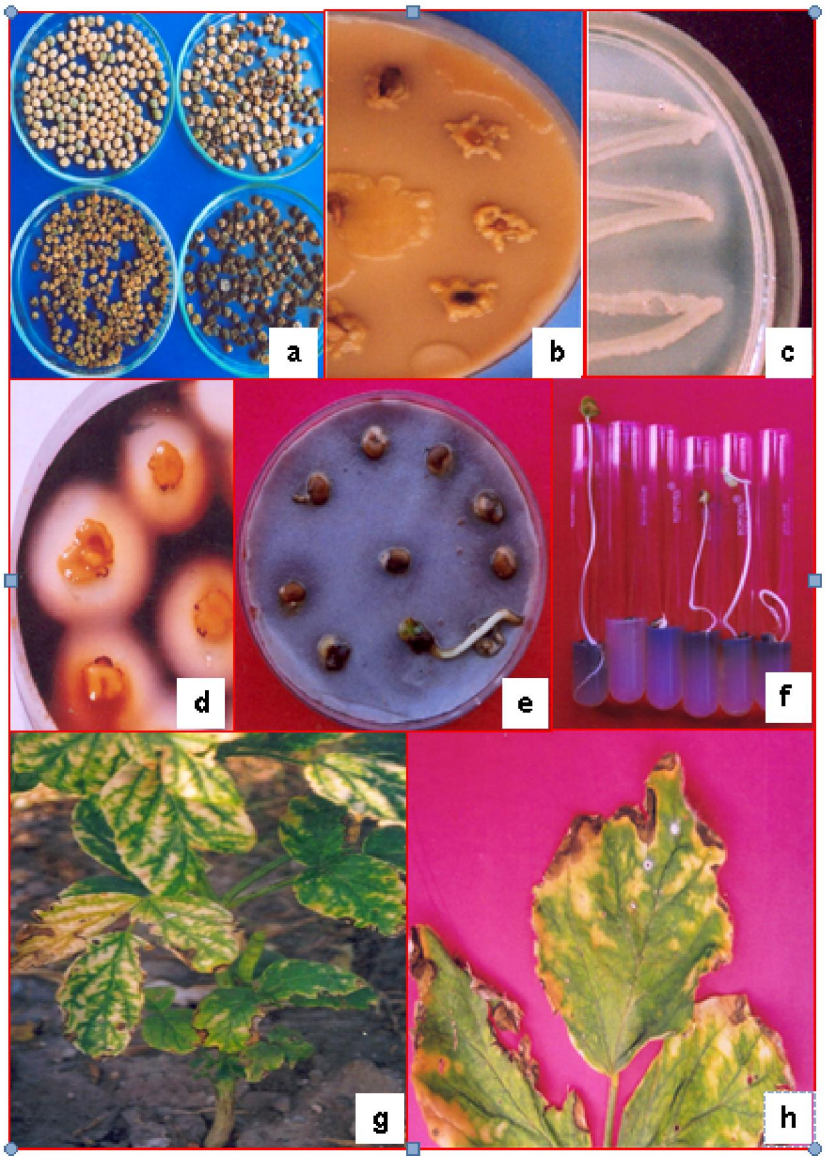


Fig. 1. Infection of *Xanthomonas axonopodis* pv. *cyamopsidis* in cluster bean, (a) Seeds categorization into asymptomatic (upper left), moderately discoloured (upper right) and heavily discoloured (lower plates) showing degree of discolorations, (b) Characteristic yellow, mucoid, shiny and raised colonies on and around seeds on incubation on YDC agar medium,(c) Bacterial isolates showing positive lipase activity on Tween 80 medium on incubation, (d) Bacterial isolate showing hydrolysis of starch on modified SX medium,E, F. Seeds in Petri plate method, (e) and water agar test tube seedling symptom test, (f) showing loss of seed germination, bacterial oozing on and around ungerminated seeds and browning and rotting of seedlings, Symptoms of bacterial leaf blight in pot experiment (g), blighted leaf (h).

Disease transmission

Radicle emergence started after 48 h of incubation. The maximum seed germination on 8th day of incubation was 95 and 91% in asymptomatic, 83 and 90 % in moderately discoloured and 75 and 41% in heavily discoloured seeds of ac. nos. Ct-818 and Ct-825, respectively. The ungerminated seeds showed rotting with heavy pale-cream to yellow oozing of the bacterium on and around the seeds and browning and rotting of seedlings (Fig. 1e). The symptoms initiated as browning and puffing of radicle and plumule which later showed rotting. The heavily infected seedlings showed mortality 0, 3.61 and 29.33% in ac. no. Ct-818 and 2.19, 11.11 and 74.19% in ac. no. Ct-825 in the three categories, respectively.

On water agar the seed germination was 97, 88 and 83% in ac. no. Ct-818 and 96, 92 and 48% in ac. no. Ct-825 in the three categories of seeds respectively on 15th day of incubation. The symptomatic seedlings showed browning of radicle and plumule and blighted lesions on cotyledonary leaves which later on showed rotting and bacterial oozing (Fig. 1f).

The symptomatic seedlings were similar to those as observed in Petri plate method. Mortality of seedlings on 15th day was the maximum in heavily discoloured seeds to be 44.57 and 31.25% as compared to moderately discoloured seeds (6.81 and 19.56%) and asymptomatic seeds (0 and 6.25%) in ac. nos. Ct-818 and Ct-825 respectively (Fig. 2A).

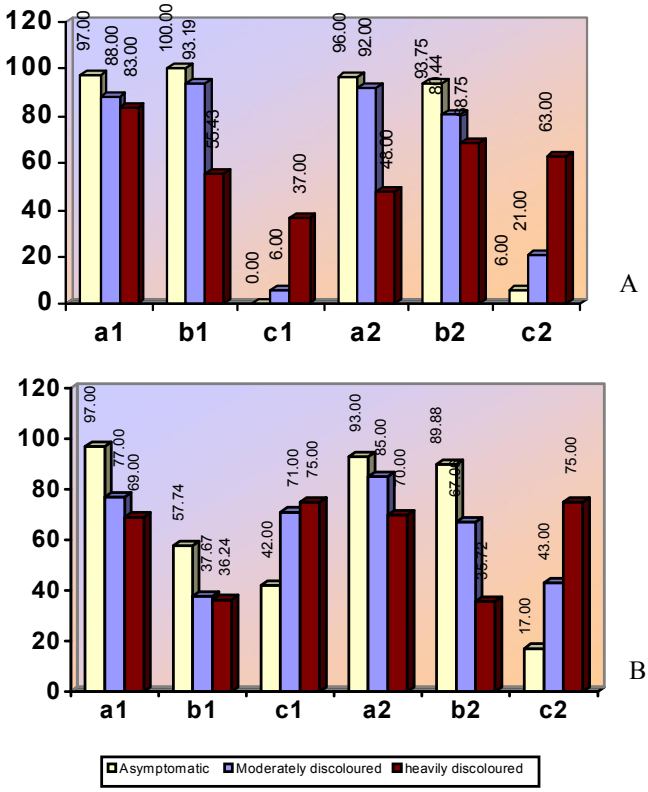
The seed germination started on 10 day of sowing in the pot experiment and continued up to 45 days in symptomatic seeds. After 30 days the germination was 97, 77 and 69% in ac. no. Ct-818 and 93, 85 and 70% in ac. no. Ct-825 in the three seed categories, respectively. The disease first appeared as small spots which coalesced, enlarged and developed into 'V' shaped to irregular blighted areas in leaves (Fig. 1g, h). The survival of infected plants was 57.74, 37.67 and 36.24% and 89.88, 67.06 and 35.72% in ac. nos. Ct-818 and Ct-825 in the three categories, respectively.

The symptoms were recorded up to fruiting stage. Symptomatic plant parts were surface sterilized and plated on YDC agar, which later yielded colonies of *X. axonopodis* pv. *cyamopsidis*. Seeds obtained from plants after pot experiment were also categorized into asymptomatic and discoloured seeds (Fig. 2B).

Table 1. Incidence of *Xanthomonas axonopodis* pv. *cyamopsidis* in seeds of cluster bean in Rajasthan State, India.

No.	Districts	No. of seed samples	No. of seed samples infected
1	Ajmer	3	3(22.5-33.5)
2	Alwar	4	4(17.5-44.5)
3	Bharatpur	3	3(56.5-78)
4	Bhilwara	3	3(12.5-99)
5	Bikaner	6	6(43.5-92.5)
6	Bundi	4	4(32.5-44.5)
7	Churu	3	3(10-62.5)
8	Dausa	6	5(29.5-63.5)
9	Dholpur	1	1(39)
10	Dungarpur	1	1(38)
11	Hanumangarh	6	6(34-91.5)
12	Jaipur	26	26(32.5-97.5)
13	Jalore	2	2(52.5, 84)
14	Jhunjhunu	10	9(33.5-74.5)
15	Jodhpur	8	8(43-95)
16	Karauli	1	1(89)
17	Kota	5	5(27.5-100)
18	Nagaur	6	6(28.5-100)
19	Pali	18	14(7.5-83.5)
20	Sawai Madhopur	4	4(23.5-73)
21	Sikar	8	8(11.5-81.5)
22	Sirohi	1	1(19.5)
23	Sri Ganganagar	11	10(19-91.5)
24	Tonk	7	7(15.5-94)
25	Udaipur	2	2(44, 44.5)
	Total	149	142 (7.5-100)

*The figure in parenthesis is per cent incidence range of the pathogen.



a_1 a_2 Seed germination, b_1 b_2 Seedling survival, c_1 c_2 Total loss in ample ac. nos. Ct- 818 (a1, b1, c1) and Ct- 825 (a2, b2, c2).

Fig. 2. Effect of natural seed infection of *X. axonopodis* pv. *cyamopsidis* in water agar test tube seedling test (A) and in pot experiment (B) in two samples.

Pathogenecity tests

On smothering of healthy seeds of cluster bean with the pure culture of the pathogen, in Petri plate method and water agar test tube seedling symptom test, the seedlings raised, showed browning and puffing at radicle followed by rotting and ultimately mortality. Mortality was found in smoothered seeds in two samples respectively. The mortality was 65.57% and 52.85% in Petri plate method while 33.87% and 43.07% in water agar test tube seedling symptom test in the two samples, respectively. Stab inoculated seedlings showed browning and rotting of plumule and cotyledonary leaves within 3 days after inoculation. Inoculated leaves also exhibited yellowing and necrotic lesions, which started from tip of leaves. Necrotic brown-sunken lesions with bacterial growth developed on fruits after inoculation. Occurrence of *X. campestris* pv. *vignaeradiata* has been recorded in artificial inoculated pods (Soni and Thind,

1991). Parashar and Sharma (1984) recorded 83.3% seed infection after artificial infection. The higher concentration of *X. axonopodis* pv. *cyamopsidis* (conc.10^8cfu/ml) resulted in less germination (49.22%), more mortality (17.25%) and increased time for germination *i.e.* 18 days (Yadav and Nath, 2005). *XAC found* to be the most destructive pathogen causing bacterial blight reduces quality of cluster bean and cause yield loss (Gupta, 1978; Gandhi and Chand, 1985; Chakravathy *et al.,* 2004 a,b).

The present study revealed a wide spread heavy occurrence (95.30%) and incidence (7.5-100%) of the pathogen in seed samples of cluster bean grown in as many as 25 districts of Rajasthan State, India. The seed borne inoculum was found to play a major role in its transmission and diseases development from seed to the growing crop.

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