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INCIDENCE AND TRANSMISSION OF *XANTHOMONAS AXONOPODIS* PV. *VESICATORIA* (DOIDGE) DYE IN CHILLI (*CAPSICUM* SPP.) SEEDS GROWN IN RAJASTHAN STATE

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Dry seed examination of 103 samples of chilli (*Capsicum* sp.) collected from 16 districts of Rajasthan revealed asymptomatic (06.25-94.25%), moderately discoloured (01.75-42%) and shrivelled-discoloured (01.25-27.75%) seeds in 103, 101 and 100 seed samples, respectively. The discolorations varied from cream to purple-brown spots, water-soaked translucent areas which yielded colonies of *Xanthomonas axonopodis* pv. *vesicatoria* on incubation. The standard cultural, biochemical and pathogenecity tests were carried out for identification of the bacterium. The isolates were gram's negative, KOH solubility test positive, levan negative, lipase activity positive, Kovac's oxidase negative or weak, nitrates were not denitrified or reduced but catalase positive, starch hydrolyzing, gelatin liquefying, hypersensitivity on tobacco leaves after infiltration. Out of 103seed samples, 85 (82.52%) seed samples of 16 districts of Rajasthan revealed 10-100% incidence of the pathogen on Tween-80 medium. The seed-borne inoculum caused pre- and post- emergence losses and symptoms of browning of radicle, splitting of plumule; necrotic spots with bacterial oozing and bright in cotyledonary leaves.

Keywords: Chilli seeds; Incidence; Seed-borne; Transmission; *Xanthomonas axonopodis* pv. *Vesicatoria* (Doidge) Dye.

Introduction

Chilli used as spice and vegetable crop globally. The crop is affected by a number of fungal, bacterial and viral diseases which reduces the quality and nutritive value of crop. Bacterial leaf spot (BLS) disease of sweet pepper and tomato is reported from several countries of eastern and southern Africa, USA, Ethiopia, Kenya, Malawi, Mozambique and South Africa[1,2]. The diseases found universally at relatively warm and moist conditions caused by *Xanthomonas axonopodis* pv. *vesicatora* (Doidge) Dye (syn: *Xanthomonas campestris* pv *vesicatoria*) (XAV). The bacterium is a gram-negative, rod-shaped which attack on all the aerial part of plant and produce symptoms[3-5]. XAV suspected from various infected plant parts was confirmed by isolation on Tween B[6,7]. In Rajasthan, the disease caused 7.5 to 16.6 per cent loss in the yield of fruits[8] at required

temperature range of 22-34°C with high humidity for maximum infection[9]. In USA, when epidemics begin early in the crop (before flowering) losses in marketable fruits may be more than 50%[10]. It was suggested the handling of plants to reduce the wounding and prevent the mechanical transmission of the pathogen. The pathogen transmit through field equipments and by workers or through wound, stomata and more lesions occurred on plant wounded by wind-blown sand or when leaves got rubbed against each other in stormy wind [10,11].

Materials and Methods

Identification and Incidence of the pathogen in seed samples:

A total of 103 seed samples of chilli (*Capsicum* spp.) collected from 16 districts of Rajasthan were subjected to dry seed examination, standard blotter method (incubation on moistened blotters)[12] and Tween-80 agar[13,14] to find the incidence of *Xanthomonas axonopodis* pv. *vesicatoria* in chilli seed samples. The bacterial cultures were maintained on Nutrient agar medium. The pure typical bacterial colonies isolated from seeds were incubate at 30^{o}C for 72 hrs[15] on YDC agar medium were re-transfer to YDC agar plates to obtain pure cultures. Such pure cultures were subjected to various tests namely gram's staining, KOH solubility test, levan formation, oxidase test [16,17], potato soft rot test, nitrate reductase test[18], arginine dihydrolysis, gelatin hydrolysis test, hypersensitivity test in tobacco and pathogenecity tests[13] for the identification of the bacterial species. For all the tests 24-48 hrs old cultures[13] and bacterial suspensions[19] were used. The bacterial isolates identified by various methods as described above were subjected to pathogenecity tests[20] on the host plant and other plant species. In the present study, incidence of pathogen in seed samples of chilli from Rajasthan state, India and transmission of seed-borne inoculums from seed to plant were studied. The bacterial identification studied by available detailed by various scientists[14, 20-26] .

Disease transmission:

Two naturally infected seed samples of chilli (Lab. ac. nos. Ca-1227 and Ca-1234) carrying 95% and 100% infection of *Xanthomonas axonopodis* pv. *vesicatoria* on semi selective medium 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 (5seeds/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:

An artificial inoculation 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:

A total of 103 seed samples of 16 districts revealed asymptomatic (06.25-94.25%), moderately discoloured (01.75-42%) and heavily discoloured (01.25-27.75%) seeds in 103, 101 and 100 seed samples, respectively (Fig.1A,B; Table 1). The discolourations varied from cream to purple brown spots and water-soaked translucent shining areas. The symptomatic seeds on incubation

yielded the growth of *Xanthomonas axonopodis* pv. *vesicatoria* (XAV) (Fig.1C,G). Heavily discoloured seeds on bisecting found distorted embryo and cotyledons yielded the pathogen. Similar symptoms on seeds were also reported earlier in tomato[27], chilli[28], brinjal[29,30] cluster bean[31-33], in rape and mustard[34] and in pigeon pea[35-36,51].

Table 1. Incidence of *Xanthomonas axonopodis* pv. *vesicatoria* in seeds of chilli in Rajasthan State, India.

S. No	**Name of Districts**	**No. of seed samples**	**Incidence in seed samples infected**	**TWEEN-80**
1.	AJMER	2	2(14.75, 21.75)	2(70, 100)
2.	ALWAR	4	4(12.25–20.50)	3(70-80)
3.	BHARATPUR	7	7(09.75-14.50)	7(50-80)
4.	BIKANER	2	2(05.25, 07.75)	2(60, 60)
5.	BUNDI	1	1(17.5)	1(90)
6.	CHURU	1	1(13.25)	1(60)
7.	DAUSA	7	7(02.25–18.25)	6(40-100)
8.	HANUMANGARH	5	5(08.75–14.75)	3(80-80)
9.	JAIPUR	42	40(01.25–27.25)	32(10-100)
10.	JODHPUR	6	6(02.25–21.75)	5(50-100)
11.	KARUALI	2	2(11.25, 17.5)	2(50, 50)
12.	KOTA	2	2(08.25, 10.75)	2(50, 80)
13.	SIKAR	2	2(09.27, 12.75)	2(100, 100)
14.	SAWAI MADHOPUR	7	7(06.75–27.75)	6(30-100)
15.	TONK	3	2(09.75–18.00)	3(70-100)
16.	UDAIPUR	10	10(07.00–18.75)	8(30-100)
	TOTAL	**103**	**100(01.25–27.75)**	**85 (10-100)**

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 of *Xanthomonas axonopodis pv. vesicatoria.* The incidence was studied on Tween-80 and YDC agar medium. On Tween-80 medium XAV appears as circular, raised, yellow colonies surrounded by zone of whitecrystals of calcium salt of fatty acids released from tween by lipolytic enzymes[6,37]. The isolates were gram's negative, KOH solubility test positive, levan negative, lipase activity positive (Fig.1C-G), Kovac's oxidase negative or weak, nitrates were not denitrified or reduced but catalase positive, starch hydrolyzing, gelatin

Fig.1. Infection of *Xanthomonas axonopodis* pv. *vesicatoria* in chilli, (A) Seeds categorization into asymptomatic (left), moderately discoloured (middle) and heavily discoloured (right) showing degree of discolorations, (B) degree of discolouration, asymptomatic (upper most) to moderately discoloured (lower row) and heavily discoloured (lower last two rows) showing degree of discolorations, (C & D) Characteristic off white, shiny, raised with undulated margin of colonies on and around seeds and seedlings on incubation on NA agar medium, (E) Characteristic yellow or off white, mucoid, shiny and raised colonies on YDC agar medium, (F) hypersensitivity reaction (HR Reaction) on tobacco leaf showing positive reaction, (G) Bacterial isolates showing positive lipase activity on Tween-80 medium on incubation on and around seeds.

hydrolyzing, arginine variable, HR test positive, 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 hrs. Pale-cream to variable shades of yellow coloured bacterial colonies of XAV on and around the seeds in 85 (84.52%) samples from 16 districts of Rajasthan with an incidence range of 10-100% were recorded which suggests its wide spread occurrence in Rajasthan state (Table1). It may further build up the inoculum of the pathogen in the fields. The heavy incidence (≥20%) of the pathogen was recorded in samples belonging to all the districts of Rajasthan. Higher incidence (1-90%) of *Xanthomonas campestris* pv. *campestris* was recorded by YDC agar plate method in cluster bean seeds grown in districts of Karnataka[32].

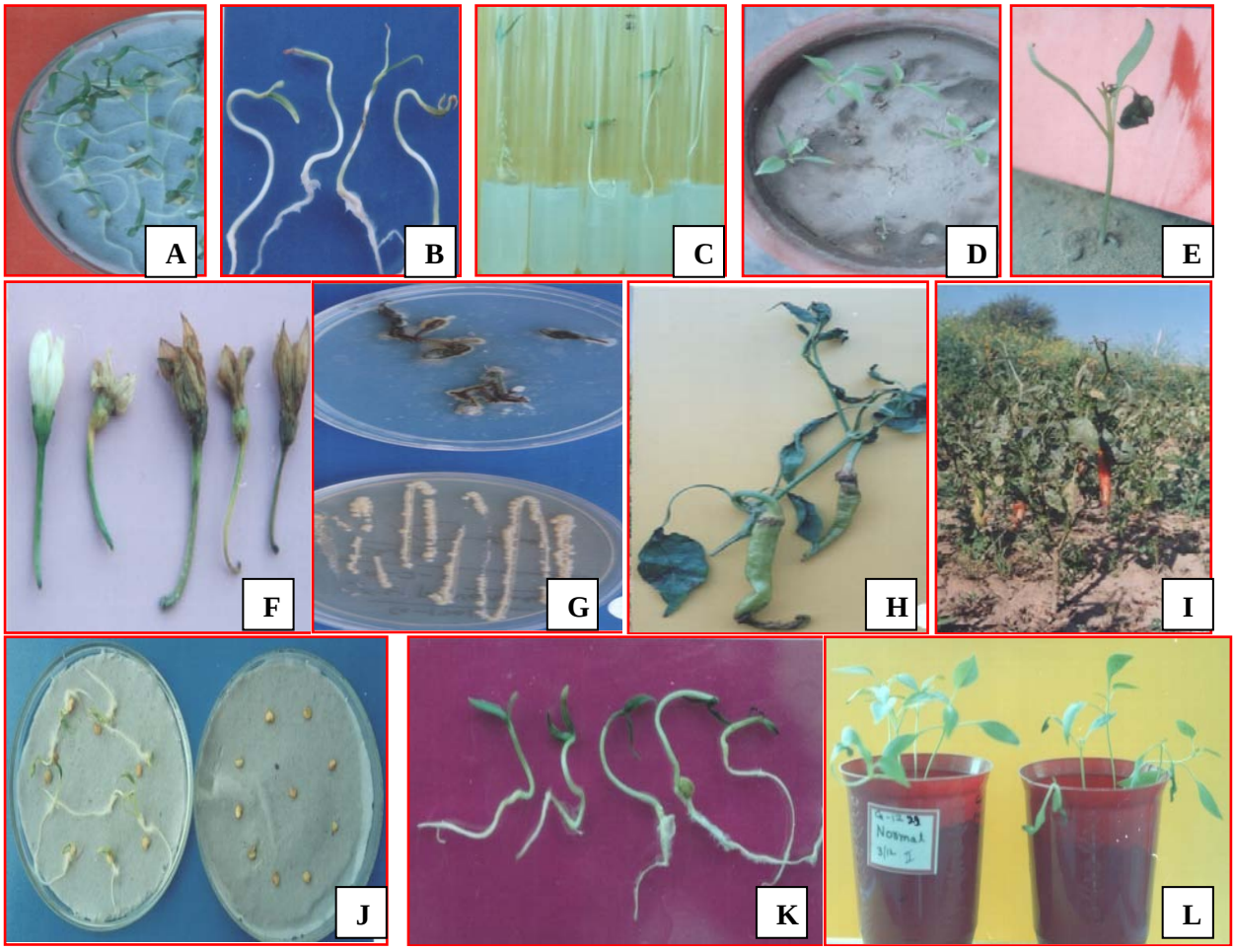

Fig. 2. Infection of *Xanthomonas axonopodis* pv. *vesicatoria* in chilli (A&B) seeds in Petri plate method, (C) water agar test tube seedling symptom test, (D & E) pot experiment showing loss of seed germination and blight of leaves,(F & G) infected floral buds and bacterial colony on and around it on NA medium, (H)infected fruits attached on twig showing the blight symptoms (I) infected plant in the field with symptoms; pathogenicity test (J)smothering of seeds, (K) stab inoculation of staple seedlings (8 days) (L) stab inoculation and infilteration of young seedlings (4 leaves stage).

In pathogenicity test (Host test), after stab inoculation of healthy seedlings at staple stage with the test bacterial cells, symptoms of blight were observed followed by general browning, rotting of hypocotyle and radicle. Similar symptoms were reported in cluster bean, cowpea, brinjal, tomato and black gram.

Disease transmission:

Radicle emergence started after 48 hrs of incubation. The maximum seed germination on 8th day of incubation was 94 and 90% in asymptomatic, 84 and 88 % in moderately discoloured and 62 and 74% in heavily discoloured seeds of ac. nos. Ca-1227 and Ca-1234 respectively (Fig. 2A, B). 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.2C). The symptoms initiated as browning and puffing of radicle and plumule which later showed rotting. The heavily infected seedlings showed mortality 02.25, 04.61 and 36.33% in sample Ca-1227 and 03.11, 12.61, and 65.33% in Ca-1234 in all the three categories, respectively (Fig.3H1-H4).

On 15th day of incubation, in water agar the seed germination was 93, 53 and 22% in ac. no. Ca-1227 and 98, 56 and 18% in ac. no. Ca-1234 in the three categories of seeds respectively (Fig. 2C). The symptomatic seedlings showed browning of radicle and plumule and blighted lesions on cotyledonary leaves which later on showed rotting and bacterial oozing (Fig. 2B). 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 81 and 87% as compared to moderately discoloured seeds (58 and 55%) and asymptomatic seeds (00 and 15%) in both the samples respectively (Fig.3H1-H4; Fig. 2D,E). Similar results were observation in sunflower by *Pseudomonas syringae*[38], brinjal by *Ralstonia solanacearum*[53], tomato by *Ralstonia solanacearum*[27] and chilli[28], soybean, crucifer seeds by *Xanthomonas campestris* pv. *campestris*[34] and pigeon pea by *Xanthomonas campestris* pv. *Cajani*[35,36].

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 74, 40 and 23% in ac. no. Ca-1227 and 90, 54 and 34% in Ca-1234 in all the three seed categories, respectively. The disease first appeared as small spots which coalesced, enlarged and developed into 'V' shaped sign at margin of lamina to irregular blighted areas in leaves (Fig. 2D,E). The survival of infected plants was 54.74, 32.66 and 30.33% and 82.88, 66.33 and 34.72% in both the samples in all the three categories, respectively. The symptoms were recorded up to fruiting stage (Fig.2 F-I). Symptomatic plant parts were surface sterilized and plated on YDC and Tween-80 agar medium, which later yielded colonies of XAV. Seeds obtained from plants after pot experiment were also categorized into asymptomatic and discoloured seeds.

Pathogenicity tests:

On smothering of healthy seeds of chilli with the pure culture of the pathogen in Petri plate method and test tube seedling symptom test, the seedlings showed browning and puffing at radicle followed by rotting and ultimately mortality. Mortality in smoothered seeds in two samples was 62.57% and 49.85% in Petri plate method (Fig. 2J,K) while 36.33% and 45.50% in test tube seedling symptom test respectively. Stab inoculated seedlings showed browning and rotting of plumule and cotyledonary leaves within 3 days after inoculation (Fig. 2L). Inoculated leaves also exhibited

yellowing and necrotic lesions, which started towards tip of leaves to mid rib. Necrotic brown-sunken lesions with bacterial growth developed on fruits after inoculation. Occurrence of *X. campestris* pv. *vignaradiata* has been recorded in artificial inoculated pods[39]. Parashar and Sharma (1984)[40] recorded 83.3% seed infection after artificial infection. The higher concentration of *X. axonopodis* pv. *cyamopsidis* (conc.108cfu/ml) resulted in less germination (49.22%), more mortality (17.25%) and increased time for germination *i.e.* 18 days[41]. The present study revealed a wide spread heavy occurrence and incidence (10-100%) of the pathogen in seed samples of chilli grown in as many as 16 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.

When epidemics begin early in the crop (before flowering) losses in marketable fruits may be more than 50%[10]. The pathogen was found seed-borne (10-15%) also subsists on infected plant debris, weeds and volunteer tomato plants[42]. The incidence was less than 5% persisted from one season to next in crop debris or on weed hosts[43].

In the present study which is based on the data from moistened blotters (SBM), water agar test tube seedling symptom tests, pot experiments and pathogenicity test. The observations clearly showed that the seed infection of XAV in chilli gave poor germination with pre-and post emergence mortality. The failure of seed germination and the incidence of seedling mortality were correlated with the degree of discolouration of the seeds. The seedlings obtained from asymptomatic seeds were more vigorous and had low incidence of pathogen including mild symptoms as compared to discoloured seeds.

The pathogen caused typical disease symptoms of small and large brown leaf spots which later on coalased and showed complete brightening of leaves. In the present study, the infected plants resulted in development of small berries with brown necrotic spots. The infected berries showed brown to black discoloured seeds with browning of placenta and fruit wall.

It was found in histopathological study that the bacteria mainly penetrated through funiculus in seed and through stomata in cotyledonary leaves. In soybean it is found that *Psudomonas syringae* pv. *glycinea* penetrated through stomata and multiplied in intercellular spaces of mesophyll[44,52].

The pathogenic tests conducted by artificial inoculated of seeds showed similar phytopathological effects and disease symptoms as found in naturally infected seeds in the various tests. The root-shoot transition region and apical shoot of seedlings were found to be more susceptible at cotoledonary leave stage. In *Phaseolous vulgaris* and cotton on inoculation of seeds by *X. c.* pv. *phaseoli* also found a significant reduction in seed germination[45,46].

The inoculated leaf at 6-leaf stage of plant produced localised symptoms as brown spots with or without chlorotic halo and streak on the transition zone. The open flowers, after inoculation showed rotting whereas the bud dried and fall off. The inoculated leaves showed heavy bacterial growth in the mesophyll cells of cotyledonary leaves. Reddy, Ahmed and Verma (1986)[47] studied that *Xanthomonas campestris* either killed the seeds before germination or seedling soon died after emergence or caused a progressive wilt of older plant in *Cicer arietinum.*

In this study, the initial symptoms of brown necrotic spots on cotyledonary leaves

and brown spots on hypocotyl and transition zone of seedlings. Due to severe infection the drooping of leaves was observed that showed defoliation. It is also reported that bacterial spots developed on seedlings and mature plants showed severe defoliation due to heavy infection [2,43,48].

Shukla and Gupta (2004)[49] suggested that interaction between relative humidity and temperature revealed that maximum severity (50.3%) was observed at 28°C under 100% relative humidity while minimum (13.2%) at 20°C with 80% relative humidity in case of tomato due to *X. c.* pv. *vesicatoria*. The higher relative humidity predisposed the plants to bacterial spot disease[9,50]. The disease severity is also influenced by age of plant, maximum (65%) when the plants were 55 days old and minimum (16%) at 25 days old[9,49]. The bacterium was found to penetrates through stomata and then proliferated in inter cellular spaces of mesophyll cells in this study. It is found that 84 hrs leaf wetness favours as results duration of the rapid multiplication and colonization of *X. c* pv. *vesicatoria* in the inter cellular fluid under constant water-soaked conditions[49].

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