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Incidence and Seed Transmission of *Ralstonia solanacearum* (Smith) in Brinjal (*Solanum melongena* L.) Seeds

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ABSTRACT

Dry seed examination of 97 seed samples of brinjal (*Solanum melongena* L.) collected from 12 districts of Rajasthan; revealed asymptomatic (07.75-97.5%), moderately discoloured (04.50-67.50%) and shrivelled discoloured (03.25-34.75%) seeds. The discolouration varied from brown or black spots on seeds which on incubation yielded the colonies of *Ralstonia solanacearum*. The standard cultural, morphological, biochemical, molecular characterization and pathogenically tests were carried out for identification of the bacterium. The 97 seed samples of 12 districts of Rajasthan revealed 13-100% incidence of the pathogen on TZC agar medium. The seed-borne inoculum caused pre- and post-emergence losses and symptoms of browning of radicle, plumule, necrotic spots with bacterial oozing.

Key words: Brinjal, seed-borne, *Ralstonia solanacearum* (Smith), incidence, disease transmission, TZC agar medium

INTRODUCTION

Bacterial wilt of brinjal caused by *Ralstonia solanacearum* (Smith) (Yabuuchi *et al*., 1995) previously known as *Pseudomonas solanacearum*, has been reported from various part of world (Bradbury, 1986; Richardson, 1990; Balogun and Fawehinmi, 2008; Rakib *et al*., 2011) including India (Sitaramaiah and Sinha, 1983; Chakravarty and Kalita, 2011). The disease is widespread in tropical, subtropical and warm temperate brinjal growing regions of world. In the present study, incidence of the pathogen in seeds grown in Rajasthan state, India and transmission of seed-borne inoculums from seed to plant were studied.

MATERIALS AND METHODS

Identification and incidence of the pathogen in seed samples: Ninety seven seed samples of brinjal collected from 12 districts of Rajasthan (Table 1) were studied by Dry Seed Examination (DSE), incubation on moistened blotters (SBM) (ISTA, 1985) and TZCA (Tetra Zolium Chloride Agar) agar plate method (Kelman, 1954) to find the incidence of *R. solanacearum* in seed samples. Typical bacterial colonies isolated from seeds on Nutrient Agar (NA) medium after 72 h of incubation at 30°C were transferred on TZC agar plate to check the virulence of pathogen. The bacterium from nutrient agar plates were subjected to various tests namely gram's staining, KOH solubility test, levan formation, oxidase test (Kovacs, 1956; Hildebrand and Schroth, 1972), potato soft rot test, arginine dihydrolysis and tobacco hypersensitivity test (LOPAT). For all the tests,

Table 1: Incidence of *Ralstonia solanacearum* in the seeds of brinjal in Rajasthan state, India

District	Total No. of sample	No. of seed samples infected	Incidence on TZCA medium
Jaipur	49	49(03.25-34.25)	49(13-100)
Dausa	4	04(12.25-25.50)	04(30-80)
Tonk	5	05(06.75-14.50)	05(20-90)
Jhunjhunu	5	05(05.25- 13.75)	05(40-100)
Kota	5	05(08.75-19.25)	05(30- 85)
Alwar	2	02(08.25, 10.75)	02(40- 75)
Sikar	6	06(05.25-34.75)	06(40- 85)
Jalor	5	05(06.25-21.75)	05(30-100)
Nagaur	3	03(07.25-18.00)	03(45-80)
Bikaner	8	08(08.75-29.75)	08(20-90)
Jodhpur	2	02(11.25, 17.50)	02(30,70)
Ajmer	3	03(09.25-21.75)	03(50-100)
Total	97	97(03.25-34.75)	97(30-100)

24-48 h old cultures (Lelliott and Stead, 1987) and bacterial suspensions (Kiraly *et al*., 1970) were used. The bacterial isolates identified by various methods were subjected to pathogenicity test (Schaad, 1980) on the host plant and other plant species.

Disease transmission: Two naturally infected seed samples of brinjal (Lab. ac. no. SM-008 and SM-013) carrying 85 and 91% infection of RS on semi-selective medium were selected for transmission studies. The 100 seeds per category per sample were sown on moist blotters (25 seed plate^{-1}) and 1% water agar medium in test tubes (1 seed test tube^{-1}; TTSST) and incubated at 25±2°C for 12/12 h alternating cycles of light and darkness upto 7 and 14 days, respectively. In pot experiment, 100 seeds per category per sample were sown in pots (2 seeds pot^{-1}) and data regarding percent 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 stage of plant growth.

Pathogenicity test: Artificial inoculation of the bacterial isolates was carried out by using techniques of incubation of smothered seed and stab inoculation of seedlings and other parts of the plants. For the pathogenicity test, tomato and chilli plants and seeds were also used along with brinjal (host plant).

RESULTS AND DISCUSSION

Identification of the pathogen and its incidence: Ninety seven seed samples of brinjal collected from 12 districts of Rajasthan revealed asymptomatic (07.75-97.5%), moderately discoloured (04.50-67.50%) and shrivelled discoloured (03.25-34.75%) seeds (Fig. 1a-c). The discolouration varied from dark brown to black or having black spots on their surface and water soaked translucent shining areas (Fig. 1c). Mostly infected seeds showed splitted seed coat. The symptomatic seed, on incubation, yielded the growth of *Ralstonia solanacearum* (Fig. 1f). Similar symptoms on seeds were also reported earlier in tomato (Sharma and Agrawal, 2010) cluster bean (Jain and Agrawal, 2011; Chakravarthy *et al*., 2004a, b) by *Xanthomonas campestris* pv. *campestris* in rape and mustard (Sharma *et al*., 1992).

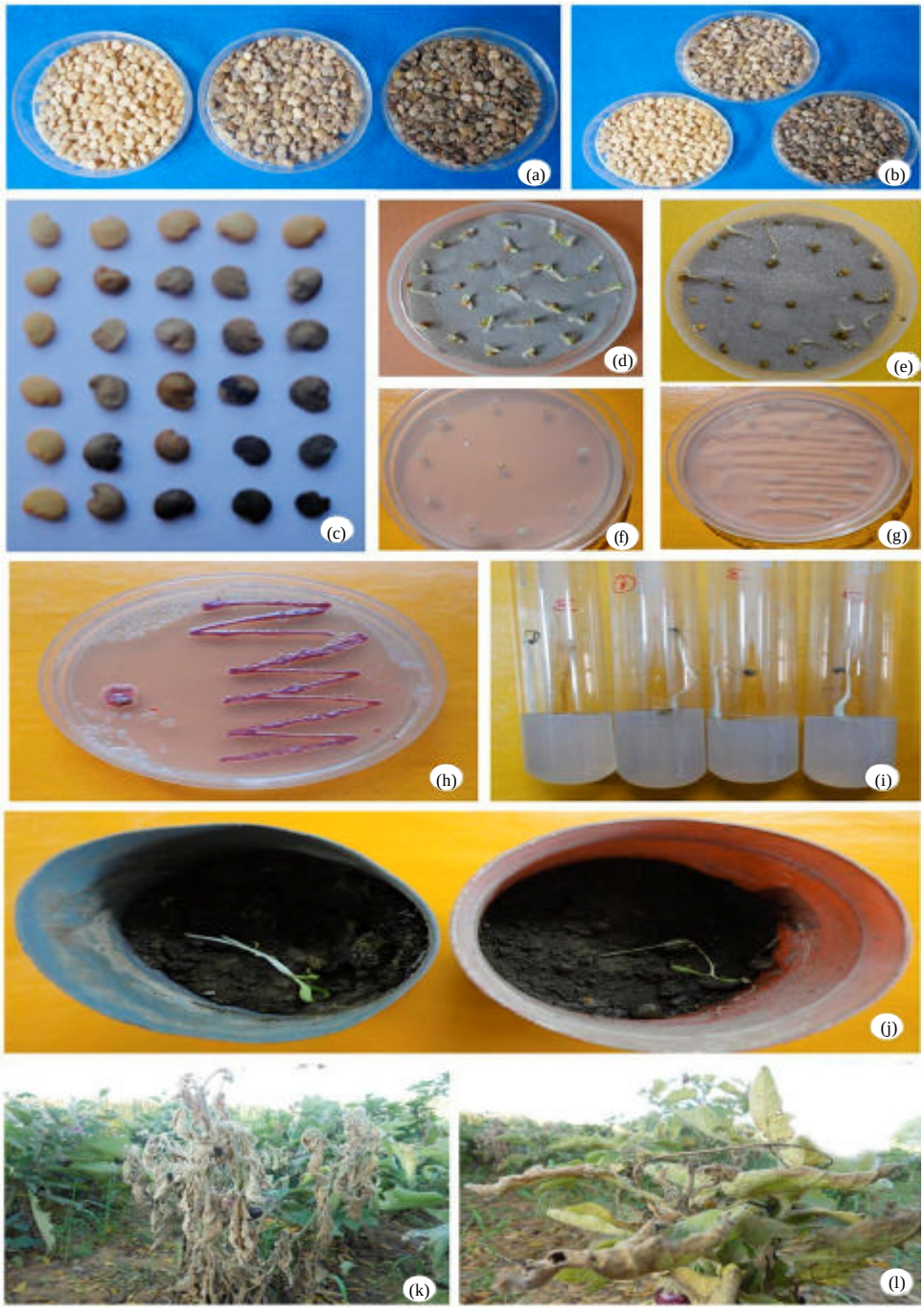

Fig. 1(a-l): Infection of *Ralstonia solanacearum* in brinjal seeds, (a-c) Seed categorization into asymptomatic, moderate discoloured and heavily discoloured, (d-e) Symptoms in standard blotter method, (f-g) Characteristic of white colonies on and around seed on incubation on NA medium, (h) Bacterial colony showing virulent colonies on TZC agar medium, (i) Water agar test tube seedling symptom test (TTSST), (j) Symptoms in pot, (k-l) Symptoms of bacterial wilt causing by *Ralstonia solanacearum* in field

It is a non-fluorescent (Fig. 1g), non-pigmented (or diffusible brown pigment produced) and failure to grow at 40°C. The isolates were gram's negative, KOH solubility test positive, levan negative from sucrose, gelatin hydrolysis weak, Kovac's oxidase test positive, potato soft rot positive, starch and aesculin not hydrolysed, arginine dihydrolase negative. The pathogen shows virulence and studied on TZCA (Kelman, 1954) (Fig. 1h). The bacterial colonies isolated from various seed samples showed virulence and produced pink colour colonies on TZC agar medium and identified to be *R. solanacearum*. 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 pathogen on and around the seeds with incidence range of 13-100% were recorded that showed its wide spread occurrence in Rajasthan state. In pathogenicity test (Host test), after stab inoculation of healthy seedlings at staple stage with the test bacterial cells (108-109 CFU mL^{-1} at 600 nm), necrotic and wilting symptoms were observed. On incubation general browning, rotting of hypocotyle and radicle of other hosts as chilli and tomato.

Disease transmission

Petri plate method: Radicle emergency started after 48 h of incubation and maximum seed germination on 8th day of incubation was 98 and 94% in asymptomatic, 81 and 78% moderate discoloured and 61 and 57% in heavily discoloured seed in samples in SM-008 and SM-013, respectively. The ungerminated seeds showed rotting, browning and oozing of bacterium. The seedling mortality was 1, 4 and 6% in SM-008 and 3, 3 and 4% in SM-013 in the three categories respectively (Fig. 1d-e and 2).

Test Tube Seedling Symptoms Test (TSST): On water agar the seed germination was 83, 78 and 62% in SM-008 and 82, 76 and 59% in SM-013 in the three categories of seeds, respectively on 15th day of incubation. The symptomatic seedlings showed browning of radical and plumule, on cotyledonary leaves which later on showed rotting. The symptomatic seedlings were similar to these as observed in petri plate method. Mortality of seedlings on 15th day was the maximum in heavily discoloured seeds to be 33 and 27% as compared to moderately discoloured seeds (28 and 25%) and asymptomatic seeds (15 and 13%) in SM-008 and SM-013, respectively (Fig. 1i and 2).

Pot experiment: The seed germination started on 10th to 12th day showing in pot experiment continued up to 30 day in symptomatic seeds. After 30 days, the germination was 80, 66, 58% in SM-008 and 79, 68 and 60% in SM-013 in all three categories respectively, falling of plantlets was maximum in heavily discoloured 62 and 57% as compared to moderately (46 and 44%) and asymptomatic seedling (15 and 17%) in SM-008 and SM-013, respectively. The survival of infected plants was 15 and 13 and 8% in the three categories in SM-008 and 19, 12 and 7% in the three categories in SM-013, respectively. The symptoms were recorded up to fruiting stage. Symptomatic plant parts were surface sterilized and plated on NA agar which later yielded colonies of *R. solanacearum* (Fig. 1j and 2). During the field survey similar symptoms were observed (Fig. 1k, l).

Pathogenicity tests: The smoothering of healthy seeds of brinjal with the pure culture of the pathogen, in petri plate method the seedling symptoms test were recorded. The seedling rose,

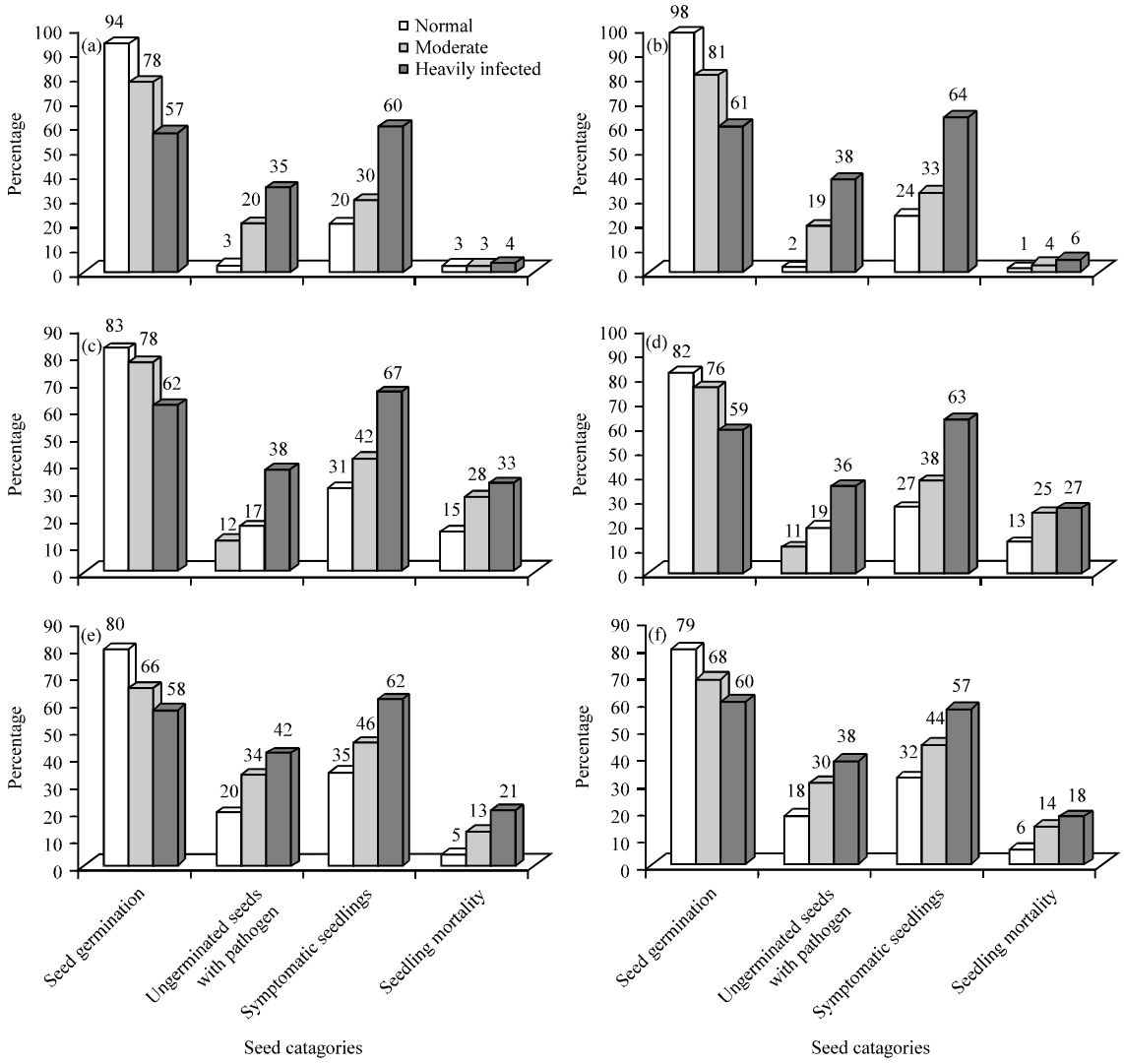

Fig. 2(a-f): Effect of natural seed infection of *Ralstonia solanacearum* in petri plate metod, (a) Sample No. SM-013 and (b) Sample No. SM-008, water agar test tube seedling test, (c) Sample No. SM-0008 and (d) Sample No. SM-013 and pot experiment (e) Sample No. SM-008 and (f) Sample No. SM-013

showed browning in roots followed by rotting and ultimately show mortality. Mortality was found in smoothered seeds in two samples, respectively. The mortality was 85 and 78.25% in petri plate method while 37.8 and 43.25% in water agar test tube seedling symptom test in the two samples, respectively. In case of stab inoculation, seedlings showed browning and rotting of plumule and cotyledonary leaves within 3 days after inoculation. Necrotic brown-sunken lesions with bacterial growth developed on fruits when inoculated (Fig. 1). Occurrence of *X. campestris* pv. *vignaradiata* 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* (108 CFU mL^{-1}) resulted in less germination (49.22%), more mortality (17.25%) and increased time for germination i.e., 18 days (Yadav *et al.*, 2005). The present study revealed

a wide spread heavy occurrence and incidence (13-100%) of the pathogen in seed samples of brinjal grown in as many as 12 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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REFERENCES

Balogun, O.S. and O.A. Fawehinmi, 2008. Influence of seedling age at infection and watering frequency on growth and yield Responses of eggplant to cucumber mosaic virus. Afr. J. General Agric., 4: 195-201.

Bradbury, J.F., 1986. Guide to Plant Pathogenic Bacteria. CAB International Mycological Institute, New York, USA., ISBN-13: 9780000000972, Pages: 332.

Chakravarthy, C.N., M. Krishnappa and B. Thippeswamy, 2004a. Seed-borne nature and transmission of *Xanthomonas axonopodis* pv. *cyamopsidis* in cluster bean (*Cyamopsis tetragonoloba*). J. Mycol. Plant Pathol., 34: 223-227.

Chakravarthy, C.N., M. Krishnappa and B. Thippeswamy, 2004b. Investigation on bacterial blight (*Xanthomonas axonopodis* pv. *cyamopsidis*) of cluster bean [*Cyamopsis tetragonoloba* (L.) Taub.] and *in vitro* control. Indian J. Plant Pathol., 22: 68-74.

Chakravarty, G. and M.C. Kalita, 2011. Comparative evaluation of organic formulations of *Pseudomonas fluorescens* based biopesticides and their application in the management of bacterial wilt of brinjal (*Solanum melongena* L.). Afr. J. Biotechnol., 10: 7174-7182.

Hildebrand, D.C. and M.N. Schroth, 1972. Identification of fluorescent pseudomonas. Proceedings of the 3rd International Conference on Plant Pathogenic Bacteria, April 14-21, 1971, Wageningen, The Netherlands, pp: 281-287.

ISTA, 1985. International rules for seed testing. Seed Sci. Technol., 4: 1-177.

Jain, R. and K. Agrawal, 2011. Incidence and seed transmission of *Xanthomonas axonopodis* pv. *cyamopsidis* in cluster bean. J. Agric. Technol., 7: 197-205.

Kelman, A., 1954. The relationship of pathogenicity of *Pseudomonas solanacearum* to colony appearance on a tetrazolium medium. Phytopathology, 44: 693-695.

Kiraly, Z., Z. Klement, F. Solymosy and J. Voros, 1970. Methods in Plant Pathology. Academia Kiado, Budapest, Hungary, Pages: 509.

Kovacs, N., 1956. Identification of *Pseudomonas pyocyanea* by the oxidase reaction. Nature, 178: 703-703.

Lelliott, R.A. and D.E. Stead, 1987. Methods for the Diagnosis of Bacterial Diseases of Plants. In: Methods in Plant Pathology, Vol. 2, Preece, T.F. (Ed.). Blackwell Scientific Publications, Palo Alto, USA., pp: 65-78.

Parashar, R.D. and D.D.K. Sharma, 1984. Detection of *Xanthomonas campestris* pv. *Cyamopsidis* in guar seed lots. Indian Phytopathol., 37: 353-355.

Rakib, A., A.A. Mustafa, A. Adhab and A.H. Kareem, 2011. Antiviral activity of vitorg, 2-nitromethyl phenol and Thuja extract against Eggplant Blister Mottled Virus (EBMV). Afr. J. Microbiol. Res., 5: 3555-3558.

Richardson, M.J., 1990. An Annotated List of Seed-Borne Diseases. 4th Edn., International Seed Testing Association, Zurich, Switzerland, ISBN-13: 9783906549187, Pages: 345.

Schaad, N.W., 1980. Laboratory Guide for Identification of Plant Pathogenic Bacteria. 2nd Edn., American Phytopathological Society, Saint Paul, MN., USA., ISBN-13: 9780890540282, Pages: 72.

Sharma, D.K. and K. Agrawal, 2010. Incidence and histopathology of *Ralstonia solanacearum* in tomato seeds. J. Mycol. Plant Pathol., 40: 115-119.

Sharma, J., K. Agrawal and D. Singh, 1992. Detection of *Xanthomonas campestris* pv. *campestris* (Pammel) Dowson infection in rape and mustard seeds. Seed Res., 20: 128-133.

Sitaramaiah, K. and S.K. Sinha, 1983. Relative efficacy of some selected antibiotics on bacterial wilt (*Pseudomonas solanacearum* race 3) of brinjal. Indian J. Mycol. Plant Pathol., 13: 277-281.

Soni, P.S. and B.S. Thind, 1991. Detection of *Xanthomonas campestris* pv. vignaeradiatae from green gram seeds and *X. campestris* pv. *vignicola* (Burkh) Dye from cowpea seeds with the help of bacteriophages. Plant Dis. Res., 6: 6-11.

Yabuuchi, E., Y. Kosako, I. Yano, H. Hotta and Y. Nishiuchi, 1995. Transfer of two *Burkholderia* and an *Alcaligenes* species to *Ralstonia* gen. nov.: Proposal of Ralstonia pickettii (Ralston, Palleroni and Douderoff 1973) comb. nov., *Ralstonia solanacearum* (Smith 1896) comb. nov. and *Ralstonia eutropha* (Davis 1969) comb. nov. Microbiol. Immunol., 39: 897-904.

Yadav, S.C., R. Nath and R.K. Yadav, 2005. Occurrence of bacterial blight (*Xanthomonas axonopodis* pv. *cyamopsidis*) on cluster bean. Proceedings of the 2nd Global Conference on Plant Health-Global Wealth, November 25-29, 2005, Udaipur, India, pp: 46.