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Location and histopathology of seed-borne bacterial pathogen *Pseudomonas syringae* pv. *pisi* carried by pea seeds

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ABSTRACT

The present study aims to determine the location and histopathology of seed-borne bacterial pathogen *Pseudomonas syringae* pv. *pisi* associated with pea seeds. The pea seeds categorized (asymptomatic, moderately discolored, and shrivelled discolored seeds) and seeds of each category were assayed for the presence of bacterial pathogen *P. syringae* pv. *pisi*. Seed samples carrying high incidence (89.5% and 91.34%) of the bacterial pathogen were used for the microtome sectioning individually. It was observed that the cells of *P. syringae* pv. *pisi* were found in large numbers in radicle and hilum region and space between seed coat and spermoderm in shrivelled discolored seed category while in moderately discolored seed category bacterial cells were less in numbers and found into the inner side of seed coat and endosperm. Bacterial colonization within seed tissue caused necrosis, formation of lytic cavities, and reduction in cell contents.

1. INTRODUCTION

Pea (*Pisum sativum* L.) seeds are used for human consumption and to feed livestock as it is good source of protein, fiber, and vitamins. India is a major dry pea seed producing country with the production of approximately 6 lac tonnes per year [1]. Seed-borne pathogens carried by seeds can cause severe seed deterioration and reduction in yield. *Pseudomonas syringae* pv. *pisi* (Sackett) Young *et al*. is a Gram-negative rod causes bacterial blight disease of pea attacks all parts of the plant and transmitted internally by means of seeds. The bacterial pathogen reduces seed quality and alters the biochemical constitutes of the seeds [2]. The location of a bacterial pathogen in infected seeds depends on the host cultivar, mode of infection, prevailing environmental conditions, and other factors. The colonization of *P. syringae* pv. *pisi* has been observed in embryos of sorghum seeds [3]. Seed contaminated with seed-borne pathogens can cause dissemination of pathogen and disease development. Seed-borne pathogens can be managed effectively if the location of the pathogen can be determined. Hence, the objective of this work was to investigate the location of inoculum within pea seed tissues.

2. MATERIALS AND METHODS

Two infected seed samples (acc. no. Pa-2529 and Pa-2552) carrying high incidence (89.5% and 91.34% on KmB agar media) for bacterial pathogen *P. syringae* pv. *pisi* were selected to study the histopathology and location of the pathogen.

2.1. Dry Seed Examination

Pea seed samples collected from different regions of Rajasthan state were subjected to dry seed examination. Four hundred seeds per samples were taken randomly and examined under naked eyes as well as under stereoscopic binocular microscope (×10-40). On the basis of presence and absence of water-soaked patches, bacterial oozing, brown or black spots, discoloration, shrivelling, etc. seeds were categorized into asymptomatic, moderately discolored, and shrivelled discolored seeds [4,5].

2.2. Microtome Studies

The categorized seeds from the selected naturally infected seed samples were soaked in sterilized distilled water kept in hot air oven at 80°C for 40 min. The selected softened seeds were fixed in 70% alcohol for 48 h in glass vials, dehydrated through tertiary-butyl alcohol series, infiltered, and embedded in paraffin wax. The embedded material was cut into blocks, sectioned at 8-10 µ thickness, deparaffinized, stained within safranin and light green combination, and mounted in DPX [6]. Microtome section were studied under a compound microscope (X-20-100).

3. RESULTS AND DISCUSSIONS

3.1. Dry Seed Examination

In each sample, the seeds were categorized into asymptomatic, moderately discolored, and shrivelled discolored seeds (Fig. 1a - I-III).

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The asymptomatic seeds were characterized as normal round or wrinkled in shape and green in color (**Fig.** 1a - I). Moderately discolored seeds were characterized by water-soaked translucent shining areas to general browning spots (**Fig.** 1a - II). Shrivelled discolored seeds were characterized on the basis of the appearance of dark brown to black spots, shrivelling, and comparatively smaller in size as well as showed split seed coat (**Fig.** 1a - III). Seed-borne bacterial pathogens cause discoloration in seeds and contribute yield losses [7,8]. The percent incidence range of three categories was asymptomatic seeds as 47.25% to 89.5%, moderately discolored seeds as 6.25-41.75% and shrivelled discolored seeds as 3-16% after dry seed examination. After bisecting the seeds, the embryos were also found shrivelled, dark brown in color as compared to asymptomatic seeds. Such seeds yielded growth of bacterial pathogen *P. syringae* pv. *pisi* on incubation.

3.2. Microtome Studies

Seed samples belonging to each category were subjected to microtome studies to find the localization of bacterial pathogen. In earlier studies, the discolored seeds with water-soaked translucent areas on seed surface due to *P. syringae* in sunflower [9] and pea seeds [10] and *Ralstonia solanacearum* in tomato seeds [11] have been reported. The endophytic growth of a rifampicin-marked strain of the seed-borne pathogen *Pseudomonas fuscovaginae* was reported in rice seeds [12]. The ability of bacteria to colonize germinating seeds is an important step for pathogen transmission. Brown and pinkish discolorations by *Xanthomonas campestris* pv. *campestris* in mustard [13] and *X. campestris* pv. *cajani* in arhar have also been reported. Asymptomatic seeds (category I) had bacterial cells (cocci to rod-shaped) in spermoderm in 1 seed out of 10 seeds each in acc. no. Pa-2529 and Pa-2552. In asymptomatic seeds, the bacterial cells found absent in space between spermoderm and cotyledons as well as in embryo region in the two samples studied (Fig. 1b and Table 1). In moderately discolored seeds (category II), the seeds were discolored with browning and water-soaked spots. The bacterial cells colonized the hilum in 2 and 3 seeds and spermoderm in 5 and 3 seeds in acc. no. Pa-2529 and Pa-2552, respectively, out of 10 seeds. The bacterial cells observed in space between spermoderm and cotyledons in 3 seeds in seed belonging to acc. no. Pa-2552 while in embryo region bacterial clumps observed in 6 and 3 seeds in cotyledons and radicle in acc. no. Pa-2529 and Pa-2552, respectively (Table 1). The shrivelled discolored seeds (category III) were distorted, reduced in size with brown to black heavy discoloration as compared to asymptomatic seeds and revealed infection at hilum in 4 and 6 seeds, spermoderm (Fig. 1c) in 10 seeds in each sample, space between spermoderm and cotyledons in 7 and 9 seeds in acc. no. Pa-2529 and Pa-2552, respectively (Table 1). Aggregation and clumps of bacterial cells were also observed at cotyledons in 10 seeds in each sample and radicle in 6 and 7 seeds in acc. no. Pa-2529 and Pa-2552, respectively (Fig. 1d and e and Table 1). The observations revealed that cells of *P. syringae* pv. *pisi* were confined to space between seed coat and spermoderm, radicle and hilum region in shrivelled discolored seeds while in moderately discolored seeds, bacterial cells were observed within the inner side of seed coat and endosperm. The bacterial cells also observed in the micropylar region. Seed coat part of sesame seeds on incubation yielded bacterial colonies of *P. syringae* pv. *sesame* [14]. *Acidovorax citrulli*, the causal agent of bacterial fruit blotch of cucurbits, was observed in the perisperm-endosperm layers and the cotyledons of pistil-inoculated seeds [15]. It was observed that *P. syringae* pv. *pisi* enters pea seeds through the funiculus and micropyle and is usually distributed in the seed coat [16]. The raphe of seeds containing vascular element provides favorable penetration site for *P. syringae* [3]. The bacteria are inter- and intra-cellular in the parenchymatous cells and cause their lysis. The bacterial cavities are small or large and full of slime and bacterial cells. The micropylar

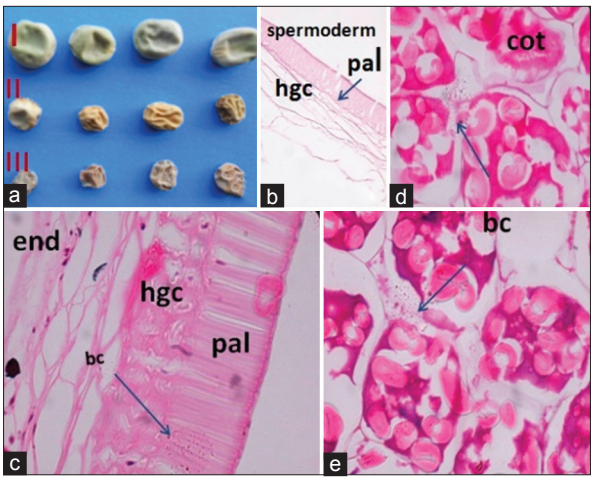

Fig. 1: Location and histopathology of pea seeds naturally infected with *Pseudomonas syringae* pv. *pisi*. A. Seeds categorise : I Asymptomatic, II Moderately discoloured, III Shrievelled discoloured. B. Part of L.S. of seed coat of an asymptomatic seed showing palisade layer and hourglass cells. X 800. C. The cluster of bacterial cells in palisade layer of seed coat of moderately discoloured seed. X 800. D and E. Part of L.S. of shrivelled seed showing clusters of bacterial cells in cotyledonary region. X 800. sc-seed coat, bc- bacterial cells, cot-cotyledon, pal-palisade layer, end-endosperm, hgc-hourglass cells.

Table 1: Location of *P. syringae* pv. *pisi* in a different component layer of pea seeds in categorized seeds in microtome sections (No. of seeds with bacterial cells pv. *pisi*) (10 seeds/category/sample).

Seed categories	Seed components				
	Hilum	Spermoderm	Space between spermoderm and cotyledons	Embryo	
				Cotyledons	Radicle
Seed sample acc. no. Pa-2552					
Asymptomatic seeds	0	1	0	0	0
Moderately discolored seeds	2	5	3	6	3
Shrivelled discolored seeds	4	10	7	10	6
Seed sample acc. no. Pa-2552					
Asymptomatic seeds	1	1	0	0	0
Moderately discolored seeds	3	3	5	4	2
Shrivelled discolored seeds	6	10	9	10	7

P. syringae: Pseudomonas syringae

infection through the funiculus may occur from the bacterial mass present in the pod cavity. Bacterial pathogen *P. syringae* pv. *pisi* has been reported as parenchyma invader and may enter the vessels under field conditions. The bacterium penetrates pods through wounds and spreads in intercellular spaces, forming abundant slime on the inner side of the pod [16]. The bacterial pathogen *P. syringae* pv. *syringae* was found internally located in certified wheat seeds collected from different wheat growing areas of Bangladesh [17]. The bacterial cells colonization was observed in and between the cells of the cotyledonary tissue.

4. CONCLUSIONS

The bacterial pathogen was found aggregated into space between seed coat and spermoderm, inner side of seed coat, endosperm, radicle, and hilum region. The bacterium seems to have penetrated through micropylar region as aggregation of bacterial cells were found near the micropyle region. Bacterial masses were found in and between the cells of counter palisade and parenchymatous tissue. Bacterial colonization caused necrosis, formation of lytic cavities, and reduction in cell contents.

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