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Spread of seed-borne *Erwinia rhapontici* in bean, pea and wheat

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Abstract Studies were conducted in the laboratory and greenhouse to determine the distribution of *Erwinia rhapontici* in plants arising from naturally infected seeds of pea or artificially inoculated seeds of bean and wheat, and whether the pathogen is transmitted to the subsequent generation of seeds. Infected seeds were planted in pots of Cornell mix in the greenhouse, and sampled at specified intervals throughout the plant growth cycle (seedling stage, elongation stage, flowering stage, seed formation stage, and maturity). Plating of surface sterilized lateral roots, tap roots, basal stems, mid-stems, apical stems, petioles, pods, and seeds of pea and bean, and of lateral roots, sub-crown internodes, basal stems, mid-stems, apical stems, peduncles, glumes, and seeds of wheat revealed that the bacterial pathogen spread from infected seeds to the lower parts of the plant tissues, but failed to spread further to the seeds produced on these plants. The study concludes that *E. rhapontici* did not establish systemic infection throughout the plants. Possible mechanisms of infection of seeds are discussed.

Keywords Epidemiology · *Erwinia rhapontici* · Impact · Pink seed disease

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Introduction

Pink seed, caused by *Erwinia rhapontici* (Millard) Burkholder, is a seed-borne disease of cereal and pulse crops (Huang et al. 2003b). The disease was first reported on common wheat (*Triticum aestivum* L.) (Howe and Simmonds 1937; Campbell 1958; Roberts 1974; Forster and Bradbury 1990), and later on durum wheat (*Triticum durum* Desf.) (McMullen et al. 1984), pea (*Pisum sativum* L.) (Huang et al. 1990; Schroeder et al. 2002), common bean (*Phaseolus vulgaris* L.) (Huang et al. 2002), chickpea (*Cicer arietinum* L.) (Huang et al. 2003a), and lentil (*Lens culinaris* Medik.) (Huang et al. 2003a). *Erwinia rhapontici* also causes soft rot diseases of horticultural crops such as citrus fruits (*Citrus limon* (L.) Burm., *C. paradisi* M., and *C. sinensis* (L.) Osbeck) (Volcani 1955), garlic (*Allium sativum* L.) (Choi and Han 1989), hyacinth (*Hyacinthus orientalis* L.) (Sellwood and Lelliott 1978; Kokoskova 1992), onion (*Allium cepa* L.) (Ohuchi et al. 1983; Ohuchi 1986),

rhubarb (*Rheum rhaponticum* L.) (Millard 1924; Metcalfe 1940; Letal 1976), tomato (*Lycopersicon lycopersicum* L.) (Volcani 1955; Shaban et al. 1991), and wasabi (*Eutrema wasabi* Maxim.) (Goto and Matsumoto 1986).

Injury of plant tissues is critical in the initiation of infection of plants by *E. rhapontici* (Huang et al. 2003b; Huang and Erickson 2004). After infection of developing seeds, the pathogen multiplies and produces water-soluble pigments called ferrorosamines (Feistner et al. 1983), which accumulate and discolour the seeds. Planting of pea seeds infected by *E. rhapontici* results in reductions in seedling emergence, seedling vigour, seed yield, and seed size (Huang and Erickson 2004). In addition, the marketability of discoloured seeds may be substantially reduced. For example, semolina milled from durum wheat kernels infected with *E. rhapontici* had a pink discolouration, and was deemed unacceptable for use in producing pasta (McMullen et al. 1984).

Although some of the impacts of infection of pulse and cereal crop seeds by *E. rhapontici* have been investigated, the distribution of *E. rhapontici* in plants arising from such seeds has not been determined. The purpose of this study was to determine whether pink seed of pea, bean and wheat can be transmitted from naturally infected or artificially inoculated seeds, to the following generation of seeds.

Materials and methods

Experiments were conducted in the laboratory and greenhouse to determine if *E. rhapontici* from naturally infected seeds of pea and artificially inoculated seeds of bean and wheat were capable of transmitting to the next generation of seeds. Naturally infected seeds of field pea cv. Delta were obtained from a commercial crop grown near Vulcan, Alberta, Canada, that had an outbreak of pink seed following hail damage to the crop. Pea seeds with more than 75% of pink discolouration of seed coat were selected for use in the study. Pea seeds cv. Delta without pink discolouration from the same field were used as the control. In other experiments, healthy seeds of great northern bean cv. US1140 and soft white spring wheat cv. Fielder were artificially inoculated by soaking them for 1 h in a 2×10^7 cfu/ml bacterial suspension of *E. rhapontici*, prepared from 2-day-old cultures grown on potato dextrose agar (PDA) (Difco, Detroit, Michigan, USA) at room temperature (20±2°C). Uninoculated seeds of bean cv. US1140 and wheat cv. Fielder were soaked in sterile water and used as controls. All the seeds of pea, bean and wheat were planted in Cornell mix (Boodley and Sheldrake 1977) in 15-cm diameter plastic pots (4 seeds per pot), a total of 30 pots of each crop were prepared and grown in the greenhouse at 20±5°C under natural light. Plants were watered as required. For each host crop species, the experiment was set up using a complete randomization design with 6 pots (replicates) per treatment. The experiment was run twice.

Samples of each crop from 6 pots (replicates) were collected and tested for presence of *E. rhapontici* at seedling stage, elongation stage, flowering stage, seed formation stage, and maturity. For pea and bean, the plant tissues tested included the lateral roots, tap roots, basal stems, mid-stems, apical stems, petioles, pods, and seeds. For wheat, plant tissues tested included the lateral roots, sub-crown internodes, basal stems, mid-stems, apical stems, peduncles, glumes, and seeds. Each sample with 4 plants was surface sterilized for 60 s in 70% ethanol, air-dried on a paper towel, and plated on PDA in Petri dishes to detect the presence of *E. rhapontici* in the tissues. Plated samples were incubated at room temperature under fluorescent light for 3 days and the presence or absence of *E. rhapontici* was determined by observation of culture characteristics such as the production of a pink pigment (Huang et al. 1990).

Differences between treatments in percent infection of tissues by *E. rhapontici* were analyzed for statistical significance using analysis of variance (ANOVA). Means were separated using Duncan's multiple range tests. Each run of each experiment was analyzed separately, and a combined analysis for all runs of each crop was performed. All statistical analyses were conducted using SAS/STAT® computer software version 8.2 (SAS Institute 1999).

Results

Results of the experiments on peas showed that all seeds naturally infected with *E. rhapontici* germinated and developed into plants. At the seedling stage, *E. rhapontici* was detected in 19% or less of seedlings from infected seeds and the bacteria were

mostly confined to the tap roots, lateral roots and basal stem (Table 1). At the stages of stem elongation, flowering, pod formation and maturity, the frequency of infection of basal stems of peas by *E. rhapontici* was significantly ($P<0.05$) higher than the other tissues, including the tap root, lateral roots, mid-stems and apical stems. At the elongation stage, for example, the frequency of basal stems infected by *E. rhapontici* was 50%, compared to 4% for the lateral roots and mid-stems, and 0% for apical stems (Table 1). Although 42% of basal stems were infected with *E. rhapontici* in samples at seed formation and maturity, the pink-seed pathogen failed to move into pods or seeds. For all controls at the respective growth stages, no pathogen was isolated from any of the pea tissues sampled. All of the control pea seeds were uninfected by *E. rhapontici* (data not shown).

For the experiments conducted on beans, the results showed that all of the artificially infested bean seeds germinated and developed into plants. *Erwinia rhapontici* entered into and spread within the developing bean plants, but was mostly confined to the lower plant parts such as the basal stems, lateral roots and tap roots (Table 2). Basal stems of bean plants had significantly ($P<0.05$) higher frequencies of infection by *E. rhapontici* than other tissues, at all of the plant growth stages tested. For example, the frequency of infection of basal stems at maturity was 65%, compared to 10% for tap roots, 6% for lateral roots, 4% for mid-stems, and 0% for apical stems, petioles, pods, and seeds (Table 2). None of the bean pods or seeds tested positive for the presence of *E. rhapontici*. All of the control bean tissue samples tested negative for presence of *E. rhapontici* at all growth stages (data not shown).

A similar trend was observed in the experiments on wheat. Results demonstrated that artificially inoculated wheat seeds all germinated and developed into plants. The developing plants were often infected with *E. rhapontici*, but the pathogen was only present in the lower parts of the plants such as tap roots, lateral roots, basal stems, and mid-stems, and not in the apical stems, peduncles, glumes, or seeds (Table 3). The frequency of infection by *E. rhapontici* was significantly higher ($P<0.05$) for basal stems than for the other tissues examined, at nearly all plant growth stages. At maturity, the frequency of infection for basal stems was 64%, compared to 26% for subcrown internodes, 19% for lateral roots, 6% for mid-stems, and 0% for apical stems, peduncles, glumes, and seeds (Table 3). None of the wheat tissue controls tested positive for presence of *E. rhapontici*, regardless of plant growth stage (data not shown).

Discussion

This study reveals that seed-borne inoculum of *E. rhapontici* from naturally infected pea seeds and artificially infected bean and wheat seeds failed to

Table 1 Spread of *Erwinia rhapontici* from naturally infected pea seeds to other plant tissues[a] (indoor experiments)

	Infection of pea tissues by *E. rhapontici* (%)				
Tissue	Seedling stage	Elongation stage	Flowering stage	Seed formation stage	Maturity stage
Lateral root	13 b[b]	4 a	4 a	13 ab	17 b
Tap root	15 b	25 b	13 b	21 b	4 a
Basal stem	19 b	50 c	48 c	42 c	42 c
Mid stem	Nd[c]	4 a	17 b	13 ab	4 a
Apical stem	2 a	0 a	8 a	4 a	4 a
Petiole	nd	nd	0 a	4 a	0 a
Pod	nd	nd	nd	0 a	0 a
Seed	nd	nd	nd	nd	0 a
Standard error	1.7	4.2	4.3	4.2	2.9

[a] Plants originated from pea seeds cv. Delta, naturally infected with *E. rhapontici*

[b] Means within each column followed by the same letter are not significantly different (Duncan's multiple range test; $P>0.05$). Data presented are the combined results of two runs

[c] nd=Data not collected (tissue not yet developed)

Table 2 Spread of *Erwinia rhapontici* from artificially infected bean seeds to other plant tissues[a] (indoor experiments)

	Infection of bean tissues by *E. rhapontici* (%)				
Tissue	Seedling stage	Elongation stage	Flowering stage	Seed formation stage	Maturity stage
Lateral root	54 a[b]	31 b	21 b	23 b	6 a
Tap root	48 a	19 a	10 a	11 a	10 a
Basal stem	90 b	77 c	77 c	61 c	65 b
Mid stem	nd[c]	19 a	6 a	10 a	4 a
Apical stem	43 a	13 a	2 a	2 a	0 a
Petiole	nd	nd	13 ab	6 a	0 a
Pod	nd	nd	nd	0 a	0 a
Seed	nd	nd	nd	nd	0 a
Standard error	3.5	3.9	4.1	3.2	2.8

[a] Plants originated from bean seeds cv. US1140, artificially infected by soaking for 1 hour in a 2×10^7 cfu/ml bacterial suspension of *E. rhapontici*

[b] Means within each column followed by the same letter are not significantly different (Duncan's multiple range test; $P>0.05$). Data presented are the combined results of two runs

[c] nd=Data not collected (tissue not yet developed)

establish systemic infection in plant tissues and thereby move into newly produced seeds. Furthermore, the low frequency of infected seedlings and localized distribution of the pathogen in seedlings suggests that the pathogen may occur in the seed coat rather than in the embryo itself. These findings support the notion that *E. rhapontici* is a weak plant pathogen as suggested in previous reports (Huang et al. 2003b; Huang and Erickson 2004; Huang et al. 2007). However, use of infected seeds for planting is still not advisable, since the pathogen has been shown to have serious impacts when the disease occurs in the field, leading to losses in stand establishment, seedling vigour, seed yield, and seed quality (McMullen et al. 1984; Huang and Erickson 2004).

Table 3 Spread of *Erwinia rhapontici* from artificially infected wheat seeds to other plant tissues[a] (indoor experiments)

	Infection of wheat tissues by *E. rhapontici* (%)				
Tissue	Seedling stage	Elongation stage	Flowering stage	Seed formation stage	Maturity stage
Lateral root	27 b[b]	18 ab	24 ab	18 ab	19 b
Subcrown internode	56 c	35 b	48 b	33 b	26 b
Basal stem	65 c	83 c	71 c	56 c	64 c
Mid stem	11 a	8 a	10 a	9 a	6 a
Apical stem	0 a	0 a	0 a	0 a	0 a
Peduncle	nd[c]	nd	0 a	0 a	0 a
Glume	nd	nd	nd	0 a	0 a
Seed	nd	nd	nd	nd	0 a
Standard error	4.3	4.6	3.8	4.0	3.1

[a] Plants originated from wheat seeds cv. Fielder, artificially infected by soaking for 1 hour in a 2×10^7 cfu/ml bacterial suspension of *E. rhapontici*

[b] Means within each column followed by the same letter are not significantly different (Duncan's multiple range test; $P>0.05$). Data presented are the combined results of two runs

[c] nd=Data not collected (tissue not yet developed)

Since transmission from planted seeds to produced seeds does not appear to be a major factor in the dispersal of *E. rhapontici*, other sources of inoculum such as overwintering bacteria on plant debris or soil may be involved (Huang and Erickson 2003). Dissemination and transmission of this pathogen could be by entrance through wounded tissues caused by mechanical injuries due to wind, hail, or movement of irrigation equipment, or due to insect damage as suggested by Huang et al. (2007). Rotem and Palti (1969) highlighted the importance of irrigation in the dispersal and development of plant diseases, and insect transmission of other plant pathogens also has been well documented (Beute and Benson 1979; Purcell 1982). Further studies are required in these areas to elucidate the epidemiology of *E. rhapontici* and thereby provide critical information for formulation of effective control measures for pink seed disease of pulse and cereal crops.

All of the crops affected by pink seed disease such as pea, bean, lentil, chickpea and wheat, are commonly used as food products for humans and livestock. Negative effects on consumer acceptance of products arising from *E. rhapontici*-infected seeds have been observed in durum wheat (McMullen et al. 1984). However, despite the finding that *E. rhapontici* is an opportunistic pathogen with weak pathogenicity, the impact of infection by the pink seed pathogen on the nutritional qualities and palatability of pulse and cereal crop seeds and their derivative products is unknown. Further studies should be conducted to ensure the safety and quality of these valuable food commodities, and to avoid potential negative consequences for consumers and agricultural producers.

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