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REGULAR ARTICLE

PATHOGENICITY AND HOST RANGE OF *XANTHOMONAS CAMPESTRIS* PV. *CAMPESTRIS* – INCITANT OF BLACK ROT OF CRUCIFERS

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SUMMARY

Black rot caused by *Xanthomonas campestris* pv. *campestris* (Pammel) Dowson is a serious disease of cabbage in Jammu and Kashmir. Amongst different inoculation methods tested, 'Vein inoculation' method gave quicker symptom expression and highest lesion progression followed by 'Hydathode inoculation' and 'Clip inoculation' methods, respectively. Spray inoculation for stomatal penetration of the pathogen failed to express any symptoms. Host range studies under conditions of artificial inoculation revealed that the bacterium infected almost all the crucifer crops cultivated in Kashmir, besides some cruciferous weeds but failed to infect any of the non-cruciferous plant species tested.

Key words: Black rot, cabbage, host range, pathogenicity, *Xanthomonas campestris* pv. *campestris*

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1. Introduction

It is often very difficult to reproduce a disease artificially while attempting to prove pathogenicity of its suspected pathogen. Besides providing an environment favourable for the disease development after inoculation of the pathogen, it is essential to adopt an inoculation technique, which is easy to perform and gives good results. Various methods and techniques, based on mode of penetration of pathogens are therefore, developed from time to time to test their pathogenicity. Similarly, knowledge of the host spectrum of a pathogen is of immense value as it helps to earmark various cultivated plants endangered by its infection. These studies also help to understand the mode of survival of a pathogen, particularly during the off-season, when its main host is absent. Black rot is a major disease of cabbage in Kashmir valley where it has been reported to prevail with an incidence and intensity of 26.7–63.3 and 5.8-32.7%, respectively (Bhat and Masoodi, 1999). Although some work on its management in Kashmir has already been conducted (Bhat and Masoodi, 2000 ;Bhat, *et al.*, 2000) but no work has so far been done to study the danger posed by the disease to other vegetable crops cultivated in the valley. Present studies were therefore taken up to compare different techniques for inoculation of *Xanthomonas campestris* pv. *campestris* into cabbage for inciting black rot disease and using the most efficient technique for inoculation of other plant species to study the host range of the pathogen.

2. Materials and Methods

Four techniques used for inoculating 48 h old culture of *X. campestris* pv. *campestris* into 40 days old cabbage (*Brassica oleracia* var. *capitata* L.) cv. Golden Acre seedlings, raised in 25 cm earthen pots containing sterile soil, were compared for their efficiency and quick symptom expression. The techniques used were:

Hydathode inoculation

Potted seedlings were watered in the evening and covered under mist chambers, which were lifted in the morning and a

bacterial suspension (cfu $1x10^9$) atomized gently on the leaves without dislodging the guttation droplets. The plants were left open till afternoon when the contaminated droplets retracted into the leaves through hydathodes.

Stomatal inoculation

Molten wax was applied to the leaf margins of potted seedlings to block the hydathodes. Bacterial suspension was sprayed on under surface of the leaves of potted seedlings as in case of hydathode inoculation. Wax from the margins was removed 24 h after inoculation.

Vein inoculation method

Entomological pins mounted on four inch long cylindrical wooden rod with their heads embedded in lac were used to gently prick through the drops of bacterial suspension placed on surface of the leaves ensuring injury of veins and veinlets without cutting through them.

Clip inoculation method

Scissors were sterilized, cooled and dipped in a heavy bacterial suspension and immediately used to make a 4mm incision into the lamina at the apex and symmetrically 1cm to each side of the apex.

The seedlings inoculated by each of the methods were again watered and covered under mist chambers. Plants inoculated with sterile distilled water in each case were maintained as control. Observations for expression of black rot symptoms were recorded after 7 days of inoculation, alternately for one month. Average lesion progression in mm in terms of necrosis proximal to the inoculation site of leaves was recorded as per the method described by Robinson and Callow (1986).

In order to study the host range of the pathogen, different plant species of various families were also raised in 25 cm earthen pots and inoculated by 'hydathode' and 'Vein inoculation' methods. Observations on expression of characteristic black rot symptoms and days taken to express first symptoms were recorded daily upto 21 days.

3. Results and Disscussion

Data on symptom expression, black rot incidence and intensity (Table 1) reveals that the pathogen could successfully cause infection of the susceptible host by with varied incidence and intensity and some variation in symptom expression when inoculation was conducted by three inoculation techniques *viz.,* hydathode inoculation, Vein inoculation and clip inoculation methods. All the inoculated plants were infected (100% incidence) when inoculation was conducted by pricking veins and veinlets giving 52.0% disease intensity with broader lesions spreading radially from the point of inoculation having most prominent blackening of veins and veinlets. An incidence and intensity of 90.0 and 42.0%, respectively was recorded in case of hydathode inoculation method producing chlorotic V-shaped lesions with black veinlets advancing from leaf margins towards the centre of the leaf. Lesions were narrow and restricted in case of clip inoculation method wherein a disease incidence of 85% and intensity of 32% was recorded. It is evident from the data presented in table 2 (Fig. 1)that lesion progression was highest (95 mm) after 29 days of inoculation in case of 'Vein inoculation' method followed by hydathode and clip inoculation methods giving average lesion progression of 77 and 40 mm, respectively after 29 days. These results are substantiated by the findings of Staub and Williams (1972) and Bandyopadhyay and Chattopadhyay (1985), who have reported quicker symptom expression in case of vein inoculation than hydathode inoculation method. Clip inoculation method could not prove as efficient as the other two methods during the present investigation, which may probably be due to lack of a liquid path for bacterium as provided by inoculum droplets used in case of 'Vein inoculation' method and guttation droplets formed on hydathodes before 'hydathode inoculation'.

Fig. 1. Lesion progression (mm) in black rot of cabbage by employing different techniques of inoculation

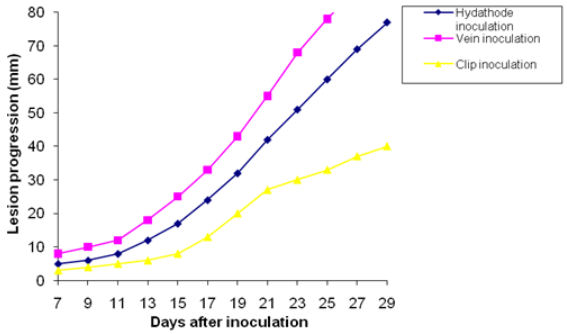

Table 1. Effect of different inoculation methods on symptom expression, incidence and intensity of black rot of cabbage

S. No.	Inoculation method	Disease incidence	Black rot intensity	Symptoms
1	Hydathode inoculation	90	42	Chlorotic V-shaped lesions on margins with black veins and veinlets, progressing towards centre of the leaf and turned necrotic in later phase.
2	Stomatal inoculation	-	-	There was no symptom development indicating that the pathogen does not penetrate through stomata.
3	Vein inoculation	100	52	Chlorotic lesions with network of black veins and veinlets, followed by necrosis of effected tissue, spreading outwards from the point of inoculation.
4	Clip inoculation	85	32	Narrow V-shaped lesions progressing downwards from the clipped leaf apex having net work of dark veins and veinlets.

Table 2. Lesion progression of black rot of cabbage by employing different methods of inoculation

Days after inoculation	Average lesion progression (mm)			
	Hydathode inoculation	Stomatal inoculation	Vein inoculation	Clip inoculation
07	05	-	08	03
09	06	-	10	04
11	08	-	12	05
13	12	-	18	06
15	17	-	25	08
17	24	-	33	13
19	32	-	43	20
21	42	-	55	27
23	51	-	68	30
25	60	-	78	33
27	69	-	87	37
29	77	-	95	40

Table 3. Host range of *Xanthomonas campestris* pv. C*ampestris* under conditions of artificial inoculation

Family	Common Name	Botanical Name	Infectivity
	Cabbage	*Brassica oleracia* var. *capitata*	+
	Cauliflower	*Brassica oleracia* var. *botrytis*	+
	Knol khol	*Brassica oleracia* var.*caulorapa*	+
Brassicaceae	Kale	*Brassica oleracia* var. *acephala*	+
(Crucifers)	Turnip	*Brassica rapa*	+
	Mustard	*Brassica campestris*	+
	Radish	*Raphanus sativus*	+
	Shepherd's purse	*Capsella bursa-pastoris*	+
	Wild crucifer weed	*Rorippa indica*	+
	Tomato	*Lycopersicon esculentum*	-
Solanaceae	Brinjal	*Solanum melongena*	-
	Potato	*Solanum tuberosum*	-
	Chilli	*Capsicum annum*	-
Malvaceae	Okra	*Hibiscus esculentus*	-
	Bottle gourd	*Laginaria siceraria*	-
	Pumpkin	*Cucurbita moschata*	-
Cucurbitaceae	Muskmelon	*Cucumis melo*	-
	Watermelon	*Citrullus lanatus*	-
	Squash	*Cucurbita maxima*	-
	Cucumber	*Cucumis sativa*	-
Umbliferae	Carrot	*Dacus carota*	-
	Beans	*Phaseolus vulgaris*	-
	Peas	*Pisum sativum*	-
Leguminoceae	Green gram	*Phaseolus aureus*	-
	Cow pea	*Vigna radiata*	-
	Soyabean	*Glycine max*	-
	Maize	*Zea mays*	-
Graminaceae	Rice	*Oryza sativa*	-
	Wheat	*Triticum aestivum*	-
	Oats	*Avena sativa*	-

+ Positive reaction, - no reaction

Spray inoculation on under surface of leaves after plugging the hydathodes by molten wax did not produce any symptoms indicating that the bacterium fails to penetrate through stomata. Although Bhide (1949) reported that two out of eight plants spray inoculated with bacterial suspension of *X. campestris* pv. *campestris* containing 0.25%

gelatin and 0.03 or 0.05% sodium oleate produced leaf spot type symptoms but Cook, *et al.* (1952) reported that evidence on stomatal penetration of this bacterium has never been observed. Hydathodes and wounds have been reported as the only natural mode of penetration of this pathogen (Shaw and Kado, 1988).

The results of the artificial inoculation of plants with *X. campestris* pv. *campestris* (Table 3) show that out of 28 plant species of seven different families tested, it successfully infected cabbage *(Brassica oleracia* var. *capitata* L.), cauliflower (*Brassica oleracia* var. *botrytis*), knol khol (*Brassica oleracia* var.*caulorapa*), kale (*Brassica oleracia* var. *acephala*), turnip (*Brassica rapa*), mustard (*Brassica campestris*), radish (*Raphanus sativus*), all cultivated cruciferous crops and the wild crucifers shepherd's purse (*Capsella bursa-pastoris*) and wild crucifer weed (*Rorippa indica*). Symptoms in all of these plants were expressed in four or five days. The bacterium failed to infect any of the non-cruciferous plants tested. These findings reveal that the bacterium is a potential threat to all of the cruciferous crops cultivated and the inoculum can survive on wild crucifers also. Therefore, the management strategy of the disease should be framed keeping in view this broad host spectrum of the pathogen. Seebold *et al.*(2008) have also reported that *X. campestris* pv. *campestris* infects many wild and cultivated crucifers including shepherd's purse (*Capsella bursa-pastoris*) and have recommended removal of crucifer weeds for control of the disease.

4. Conclusion

Amongst different inoculation methods tested, 'Vein inoculation' method gave quicker symptom expression and highest lesion progression in causing black rot of cabbage. Host range studies revealed that the bacterium infected almost all the crucifer crops cultivated in Kashmir, besides some cruciferous weeds but failed to infect any of the non-cruciferous plant species tested.

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