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Estimation of protein in two cultivars of cabbage infected with black rot bacterium *Xanthomonas campestris* pv. *campestris*

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Abstract- A significant vegetable crop that is grown all around the world is cabbage. There have been reports of black rot disease in cabbage from many parts of the world, which is caused by bacterial infection. It was initially recorded in India by Patwardhan during the Bombay Presidency. Subsequently, it was discovered to be prevalent in multiple West Bengali regions as well as Pune. Large-scale cultivation of this vegetable crop is practiced in and around Ranchi, where the significant cultivars "Pride of India" and "Express" are frequently planted. The black rot bacterium "*Xanthomonas campestris* pv. *campestris*" frequently attacks both CVS. The pathogenesis and resistance of host plants are significantly influenced by protein. involvement of the host-bacterium relationship Numerous researchers have previously documented the presence of proteins and their accumulations and reductions in the host tissue of various plants. The host's susceptibility and resistance responses are inextricably linked to the active protein components of the host tissue after infection. In the extract of seedling tissue infected with bacteria, the protein content of the cultivar "Pride of India" was significantly lower than that of healthy seedlings starting at 24 hours and continuing for up to 96 hours. On the other hand, the bacterium-inoculated seedlings of cv. "Express" showed a notable rise in protein content compared to the healthy ones for the first 72 hours, but then a significant decline in protein content over the healthy ones for the next 96 hours. Compared to cv. "Express," a greater quantity of protein was found in the healthy tissue extract of the seedlings of cv. "Pride of India" starting at 72 hours and continuing until 72 hours. Nevertheless, after 96 hours of incubation, the seedling of the later cultivar, i.e., Express, had slightly less protein than a healthy one. According to experimental data, the cabbage cultivars "Pride of India" and "Express" exhibit varying degrees of susceptibility to the black rot bacteria "*Xanthomonas campestris* pv. *campestris*," with cultivar "Pride of India" showing the highest degree of symptom manifestation.

Key words: Cabbage, Pride of India, Express, Protein, *Xanthomonas campestris* pv. *campestris*, black rot disease

INTRODUCTION

Cabbage is an important vegetable crop which is cultivated all around the world. Occurrence of black rot disease of cabbage due to bacterial infection has been reported from Australia, Ceylon, several countries of Europe, India, Japan, Russia, South Africa, and USA.[1] In India, it was first reported in the then Bombay Presidency by Patwardhan (1928)[2]. Later on, it was found to be endemic in Pune[3] and several areas of West Bengal[4]. This vegetable crop is cultivated in large scale in and around Ranchi where two important cultivars "Pride of India" and "Express" are commonly grown. Both cvs. are often attacked by black rot bacterium "*Xanthomonas campestris* pv. *campestris*".

Protein has an important role to play in pathogenesis and resistance of host plants. In a part review, these aspects

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have been hinted by Prasad (1989)[5], (1990)[6]. Participation of host bacterium interaction proteins and their accretions and diminutions in host tissue of different plants were reported by several workers in the past.[7-12] Resistant and suscept responses of host are intrinsically related to post-inoculative activated protein components of the host tissue. Even specific proteins of resistant plant tissue do agglutinate avirulent and or virulent strains of several pathogenic bacteria.

MATERIAL & METHODS

Isolation of the bacterium from diseased cabbage leaves. An isolate was prepared from the infected leaves collected from fields. A surface sterilised inoculation chamber was used for performing isolation experiment. The infected leaves of the cabbage having black veins, a prominent disease symptom, were surface sterilized by washing with 0.01% of mercuric chloride solution twice and later by repeated washing with sterilized distilled water. Infected portion of this leaf was transferred with the help of sterilized inoculation needle into autoclave test-tubes and Petri dishes containing GYCA medium.[13] The test - tubes and Petri dishes were placed for 24 h at 22°C. A yellowish substances come out around the inoculated leaves in culture medium. This growth was inoculated in a fresh culture tube media and repeated reinoculation was done to get the isolated culture.

Cabbage seeds of two cvs. "Pride of India" and "Express" were inoculated with isolated bacterial culture and incubated at 22°C for 24 to 168 h. one gram of seeds or seedlings were placed immediately in 150 ml conical flask containing boiling 80% ethyl alcohol and boiled for 100 min. Approximately 10 ml alcohol was used for boiling seeds and seedling tissue. The extract was cooled in a pan of cold water. The tissues were crushed thoroughly in a blender for 10 min. the homogenised tissue was filtered through two layers of cheese cloth.

The ground tissue was re-extracted for 3 min. using 3 ml of hot 80% alcohol. The extract was cooled and again passed through cheese cloth.[14] Both the extracts were then filtered through Whatman No - 41 filter paper. The alcohol insoluble extract contained protein along with many other macro-molecules. For complete alcohol soluble molecules, the alcohol insoluble residue was taken up in about 10 ml of hot ethanol and centrifuged for 20 min at 2000 g and supernatant was discarded.

The protein present in tissue was separated by precipitation other interfering substances prior to their estimation. For this the pellet was suspended in 5 ml of 5% trichloroacetic acid (TCA) at low temperature for about 25 min. in ice bath. 1 ml of aliquot was taken in a centrifuge tube and 1 ml. of 10% TCA at 0°C was added into it to precipitate the proteins. It was allowed to stand for 15 min. in the ice-bath, the was centrifuged and supernatant was discarded. The process was repeated twice. The pellet was re-extracted once with absolute ethanol and twice with hot ethanol ether mixture (3:1) by discarding supernatants every time after centrifugation. This pellet contained the protein and nucleic acid.

The protein content was determined by colorimetric method introduced by Lowry *et al.* (1951)[15]. The protein samples were suspended in 1 ml of 1 N NAOH at 100°C. This was kept as such for 5 min. 5 ml of alkaline copper reagent was added to the sample and the mixture was allowed to stand at room temperature for 15 min.

One drop of Folin-ciocalteu reagent was added to the sample and the contents of the tube were mixed rapidly. The mixture was allowed for 30 min. The absorbance was measured at 750 nm after 15 min. (A standard, using bovine serum albumen was prepared).

RESULT & DISCUSSION

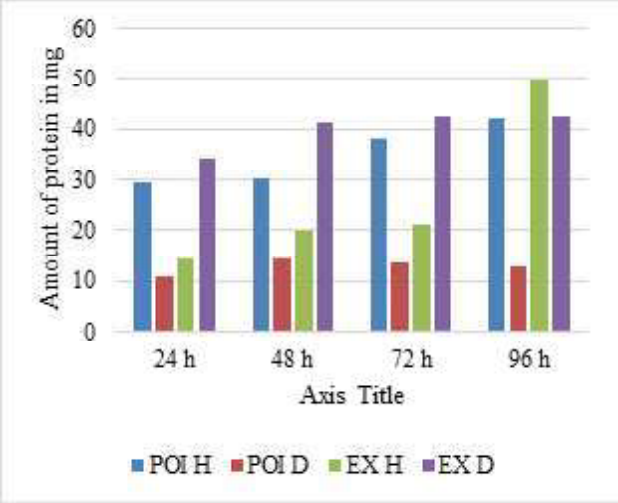
The seedling out of healthy and *Xanthomonas campestris* pv. *campestris*- inoculated seeds were assessed for their protein content in their tissue extracts for the duration of 24-96 h of incubation. Three replicants were taken and average amount of protein (in mg) was estimated. The consolidated results are presented in table-1 and comparative values in plate-1. As is evident from the table 1 and plate-1 in cv. "Pride of India" from 24 h onwards and up to 96 h in bacterium infected seedling tissue extract, there was a marked diminution in protein content over that of healthy ones. In contrast to this the bacterium inoculated seedlings of cv. "Express" there is remarkable increase in the amount of protein over that of healthy ones up to 72 h but at 96 h there was substantial decrease in amount of protein over healthy ones. Comparatively in healthy tissue extract of seedlings of cv. "Pride of India" larger amount of protein was present from 72 h onwards and up to 72 h than cv. "Express". However, in the seedling of latter cv. i.e. Express the amount of protein during 96 h of incubation was marginal less than healthy one. During this period

degree of symptoms appeared in both cultivars were morphologically noticed. Rate of infection was more in "Pride of India" than cv. "Express".

Table 1- Amount of total protein (in mg) in 24 h – 96 h healthy and *Xanthomonas campestris* pv. *campestris* inoculated seedlings of cabbage cultivars "Pride of India" (POI) and "Express" (EX).

Hours of Incubation	Cabbage Cultivars	Condition	Optical Density	Total Protein
24 h	POI	H	0.49	29.50
	POI	D	0.31	10.83
	EX	H	0.33	14.58
	EX	D	0.35	34.00
48 h	POI	H	0.52	30.33
	POI	D	0.34	14.26
	EX	H	0.40	19.80
	EX	D	0.65	41.50
72 h	POI	H	0.59	38.00
	POI	D	0.34	13.60
	EX	H	0.42	21.30
	EX	D	0.65	42.38
96 h	POI	H	0.62	42.10
	POI	D	0.34	13.00
	EX	H	0.70	50.00
	EX	D	0.67	42.38

Plate 1- A comparative peresentation of amount of protein in 24h-96h healthy and *Xanthomonas campestris* pv. *campestris* inoculated seedlings of cabbage cultivars POI and EX



Curiously, it was observed that in cv. 'Express" the amount of protein was more in cabbage seedlings infected with "*Xanthomonas campestris* pv. campestris" over the healthy one, during the pathogenesis of up to 72 h. This seems that new proteins were developed to resist the pathogens in the beginning, so the amount was more than healthy counterparts. And after 72 h, the amount of protein marginally increased in cabbage the seedlings of healthy Express cultivar over diseased one. However, in "Pride of India" the amount of protein in healthy seedlings was more from 24 h onwards 96 h over the bacterium inoculated seedlings. It appears that the proteins which was present in healthy seedlings were used up by pathogen, indicates the gradual diminution in protein amount in infected condition of seedlings. cabbage cultivar "Pride of India" is susceptible to the black rot bacterium "*Xanthomonas campestris* pv. *campestris*" and cultivar "Express" is moderately susceptible to the same pathogen.

To secure a compatible interaction, pathogen effectors target plant factors encoded by susceptibility (S) genes to manipulate host processes to their advantage. Suppression of defenses, nutrient acquisition, and transport of bacterial proteins in the host cell are some of the processes, pathogens use to cause disease.[16] Although S genes are exploited by pathogens to promote disease, their mutation can lead to durable, recessively inherited, and potentially broad-spectrum resistance in plants.[17]

On the basis experimental observation, it can be concluded that cabbage cultivar "Pride of India" is susceptible to the black rot bacterium "*Xanthomonas campestris* pv. *campestris*" and cultivar "Express" is moderately resistant to the same pathogen.

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