

## Effect of elevated temperature and CO<sub>2</sub> on quorum sensing mediated virulence in soft rot causing *Pectobacterium carotovorum* pv. *carotovorum*

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### ABSTRACT

The global climate is changing and in the last one century 0.7°C has already risen and as well for CO<sub>2</sub> also, the same scenario came out. In this climate change condition the pathogen may behave differently for signal molecule (Acyl homoserine lactone) mediated communication and it essential for a successful host invasion and survival of the pathogen in the nature. The present experiment was conducted under controlled environmental condition to establish the effect of elevated temperature and CO<sub>2</sub> towards signal molecules mediated Disease Severity Index, virulence factor production with six different treatment combinations of temperature and CO<sub>2</sub>. *Pectobacterium carotovorum* pv. *carotovorum*, a soft rot causing plant pathogen produce high levels of extracellular enzymes (cellulase) only after sensing the quorum or minimal amount of cell density by Acyl homoserine lactone mediated cross communication. The result showed significant effect for all the treatment combination. In comparison between T<sub>2</sub>C<sub>1</sub> (24°C + 380 ppm CO<sub>2</sub>) and T<sub>2</sub>C<sub>2</sub> (26°C + 600 ppm CO<sub>2</sub>), the disease progress was more rapid (27.1 mm on 9<sup>th</sup> day) in case of elevated CO<sub>2</sub> conditions. The highest disease severity index (73.18%) was found in T<sub>3</sub>C<sub>2</sub> (28°C + 600 ppm CO<sub>2</sub>) in the phytotron, whereas, maximum disease severity index (78.92%) was found at 33°C in the laboratory and minimum (23.19%) was found at 37°C. The maximum cellulose (4 cm diameter holo zone) was noticed in T<sub>3</sub>C<sub>2</sub> (28°C + 600 ppm CO<sub>2</sub>). Therefore, at elevated temperature in combination with elevated CO<sub>2</sub> there was more disease severity in case of soft rot in tomato.

**Key words:** Disease severity index, cellulase, soft rot, quorum sensing.

### INTRODUCTION

Quorum sensing (QS) is one kind of cell-to-cell communication where several gram-negative bacteria synthesize *N*-acyl homoserine lactone (*N*-HSL) signal molecules which are essential for recognizing and responding signals (Fuqua *et al.*, 5). Such regulatory systems operate to allow bacteria to sense cell density and to synchronize the functions of the entire population. In *Pectobacterium carotovorum* species, QS regulation is involved in pectinolytic, cellulase and protease activities and plant pathogenesis (Pe'rombelon *et al.*, 12). *Pectobacterium carotovorum* is mesophilic pathogen prefer higher temperature (25-35°C) for pathogenesis. In the context of climate change not only the pathogen will adapt but plant also may induce resistance mechanism. Plant pathogen interaction is a complex system, where a huge number of factors interact simultaneously. Climate change parameters like elevated CO<sub>2</sub> and temperature will change the plant architecture, plant internal system, microclimate and thus risks of infection (Burdon *et al.*, 1). Besides, increased CO<sub>2</sub> can result in physiological changes to the host plant that can

increase host resistance to pathogens (Coakley *et al.*, 4). The rise in atmospheric temperature causes detrimental effect on growth, yield and quality of rice crop by affecting its phenology, physiology and yield ponent (Sheehy *et al.*, 16; Peng *et al.*, 12). Abiotic stress such as heat and drought may contribute to plant susceptibility to pathogen or it may induce general defense pathways, which increase resistance. Therefore, there may be possibility to adopt such mechanism to avoid the degradation of quorum sensing signal molecule by communication of pathogens with each other. Hence, to evaluate the effect of elevated CO<sub>2</sub> and temperature on QS-mediated virulence in tomato plant, the present investigation was performed under controlled environmental condition (Phytotron) with elevated CO<sub>2</sub> and elevated temperature treatments combinations.

### MATERIALS AND METHODS

Tomato cv. Pusa Ruby was grown under controlled environmental growth conditions (National Phytotron Facility, IARI, New Delhi) to study the effect of climate change variables on bacterial pathogenesis of soft rot in tomato in different treatments of elevated temperature and CO<sub>2</sub>. Tomato plants were grown

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under six different treatments, viz. T<sub>1</sub>C<sub>1</sub>: 24°C + 380 ppm CO<sub>2</sub> (control), T<sub>2</sub>C<sub>1</sub>: 26°C + 380 ppm CO<sub>2</sub>, T<sub>2</sub>C<sub>2</sub>: 26°C + 600 ppm CO<sub>2</sub>, T<sub>3</sub>C<sub>1</sub>: 28°C + 380 ppm CO<sub>2</sub>, T<sub>3</sub>C<sub>2</sub>: 28°C + 600 ppm CO<sub>2</sub>, T<sub>4</sub>C<sub>1</sub>: 30°C + 380 ppm CO<sub>2</sub>. All the agronomic practices were followed till fruiting stage.

Soft rot infested tomato fruits were collected from the research farm of Division of Vegetable Science, IARI, New Delhi. The isolation of pure colony of bacteria was done by following the procedure given by Śledź *et al.* (17) with little modification where six different pure colonies were isolated. During morphological, biochemical and molecular characterization isolate one (IS 1) was conformed as *P. carotovorum* pv. *carotovorum* (*Pcc*). Inoculum of *Pcc* was made at density of 10<sup>7</sup> CFU/ml to inoculate the fruit by fruit stabbing method. In each plant single fruit was inoculated. The symptom of the disease appeared at 3-5 days after inoculation depending on the treatment and environmental condition. The infested fruits were scored (Fig. 1) and finally Disease Severity Index (DSI) was calculated by following the formula given by Sezing *et al.* (15).

$$\text{Disease Severity Index (X)} = \frac{\sum (a \times c)}{(Z \times N)} \times 100$$

Where, X = Disease Severity Index; a = scale value c= number of fruits in each scale value Z = the highest scale value N = total number of fruits/ plants evaluated (Mitchell *et al.*, 10).

To evaluate the effects of elevated temperature on quorum sensing mediate pathogenesis, green mature fruits (separated from plants) of tomato was inoculated and incubated aseptically under seven different temperatures, viz., 24, 26, 28, 30, 33, 35 and 37°C conditions for 10 days in the laboratory. Progress of disease was measured every day after inoculation. Scoring and disease severity was calculated as described earlier. For enzyme

assay the procedure given by Kasanna *et al.* (8) was followed. Infested fruits (75% infested) were collected from phytotron as well as from laboratory from each treatment. Diseased fruits were smashed in a mortar and pestle (pre-sterilized with 70% ethyl alcohol) and the soup was filtered through 0.2 µm Gelman filtration unit. Liquid broth (LB) agar was supplemented with 0.4% carboxy methyl cellulose (CMC) for cellulase assay. Extracted soup (10 µl) from the diseased tomato of each treatments were poured in a well made by number 2 cork borer in the centre of the CMC plate. The plates were allowed 5 min. for absorption in to the well and then incubated at 28°C for 36 h. The plates were then flooded with a solution containing (2 g KI and 1 g iodine) in 300 ml distilled water.

## RESULTS AND DISCUSSION

The Disease progress curve of soft rot under six different CO<sub>2</sub> and temperature conditions in phytotron condition is presented in Fig.1. The result showed that among the six different treatments, maximum disease progress (45 mm diameter from the inoculation point) was noticed in T<sub>3</sub>C<sub>2</sub> treatment (28°C + 600 ppm CO<sub>2</sub>), whereas, minimum progress was found in T<sub>1</sub>C<sub>1</sub> (24°C + 380 ppm CO<sub>2</sub>), which was comparable to complete disease abortion. The data for comparative study of different treatment for disease progress at different days was given in Table 1. In comparison between T<sub>2</sub>C<sub>1</sub> (24°C + 380 ppm CO<sub>2</sub>) and T<sub>2</sub>C<sub>2</sub> (26°C + 600 ppm CO<sub>2</sub>), it was clear that disease progress was more rapid (27.1 mm on 9<sup>th</sup> day) in case of elevated CO<sub>2</sub> condition. Similar results were found in T<sub>3</sub>C<sub>1</sub> (28°C + 380 ppm CO<sub>2</sub>) and T<sub>3</sub>C<sub>2</sub> (28°C + 600 ppm CO<sub>2</sub>) though the cumulative progress was higher (45.3 mm on 9<sup>th</sup> day) in case of high temperature and elevated CO<sub>2</sub> condition.

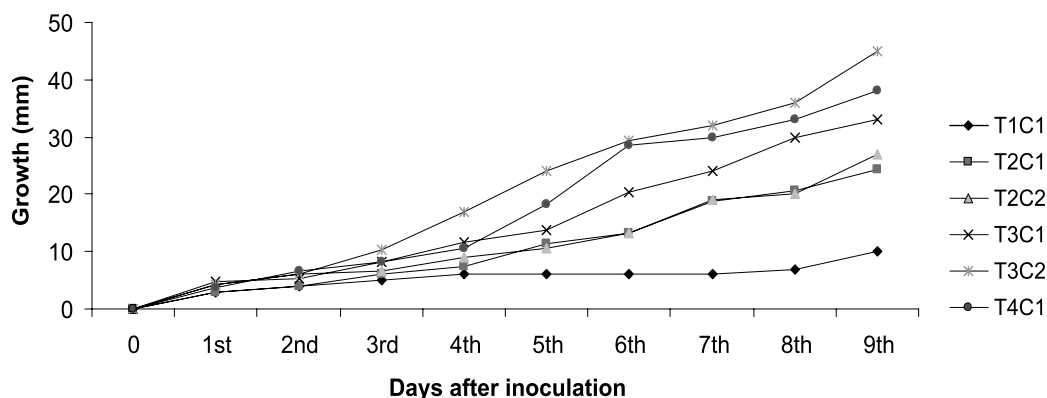
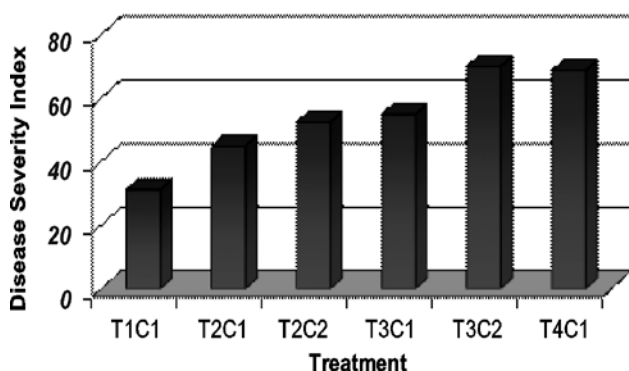


Fig. 1. Disease progress curve in tomato under elevated temperature and CO<sub>2</sub> in phytotron.

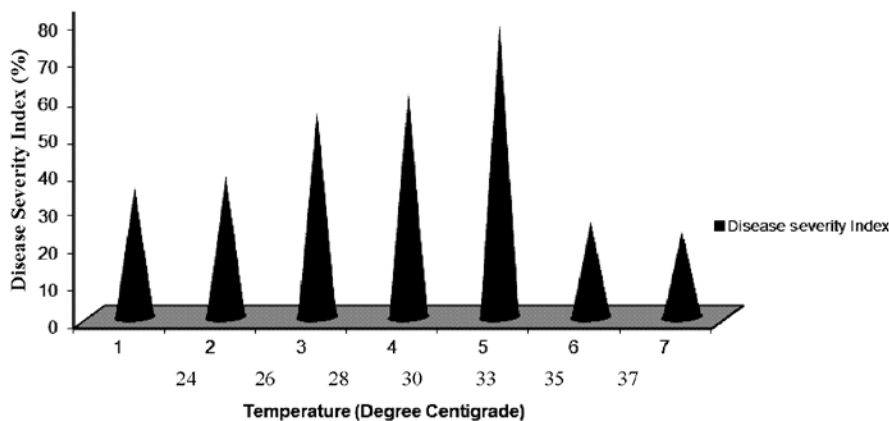
**Table 1.** Effect of elevated temperature and CO<sub>2</sub> on soft rot disease progress in phytotron.

| Treatment                     | Soft rot disease progress (mm) at different days |      |       |       |       |       |      |      |       |
|-------------------------------|--|------|-------|-------|-------|-------|------|------|-------|
|                               | 1  | 2    | 3     | 4     | 5     | 6     | 7    | 8    | 9     |
| T <sub>2</sub> C <sub>1</sub> | 3  | 4    | 6     | 7.35  | 11.33 | 13.34 | 15   | 17.1 | 24.33 |
| T <sub>2</sub> C <sub>2</sub> | 4.33   | 6    | 6.66  | 9     | 10.66 | 13.33 | 19   | 20.3 | 27.1  |
| T <sub>3</sub> C <sub>1</sub> | 4.66   | 5.33 | 8.33  | 11.65 | 13.66 | 20.36 | 24   | 30.2 | 33.21 |
| T <sub>3</sub> C <sub>2</sub> | 4.33   | 6    | 10.32 | 17    | 24    | 29.32 | 32   | 36.4 | 45.30 |
| CD at 5%                      | 0.41   | 0.56 | 0.14  | 0.34  | 0.72  | 0.91  | 0.74 | 0.58 | 0.52  |

In case of Disease Severity Index (DSI) in the phytotron all the treatments except T<sub>4</sub>C<sub>1</sub> (30°C + 380 ppm CO<sub>2</sub>) were significantly different where maximum (73.18%) was found in T<sub>3</sub>C<sub>2</sub> (28°C + 600 ppm CO<sub>2</sub>) and minimum (31.12%) was found in case of T<sub>1</sub>C<sub>1</sub> (24°C + 380 ppm CO<sub>2</sub>) was noticed (Fig. 2). The treatment T<sub>4</sub>C<sub>1</sub> and T<sub>3</sub>C<sub>2</sub> was statistically found at par. There was variable DSI for all the treatments which were statistically different in laboratory condition



**Fig. 2.** Effect of elevated temperature and CO<sub>2</sub> on Disease Severity Index in tomato grown under six different regimes.

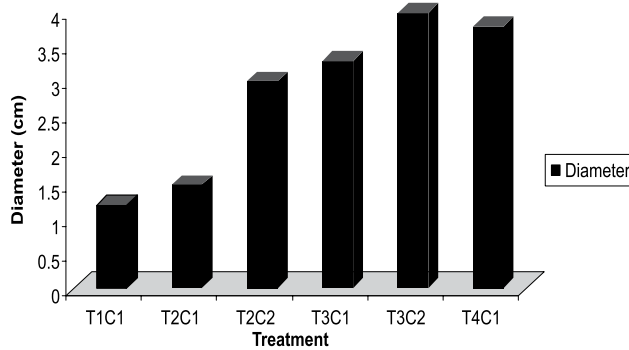


**Fig. 3.** Effects of temperature on Disease Severity Index under controlled conditions.

(Fig. 3). The graph clearly indicates that, maximum DSI (78.92%) was found at 33°C, whereas, the minimum (23.19 %) at 37°C.

Environmental factors strongly regulate the quorum sensing signal molecules and hence cellulase (virulence factor) production by *Pectobacterium carotovorum* pv. *carotovorum*. The effect of temperature and CO<sub>2</sub> on extracellular enzyme on quorum sensing mediated cellulase production is presented in Fig. 4. Cellulase production was found highest (4 cm dia halo zone) in case of T<sub>3</sub>C<sub>2</sub> (28°C + 600 ppm CO<sub>2</sub>) followed by T<sub>4</sub>C<sub>1</sub> (30°C + 380 ppm CO<sub>2</sub>), which was very clear from the halo zone formed surrounding the inoculation well. The minimum cellulase was found in case of T<sub>1</sub>C<sub>1</sub> (26°C + 380 ppm CO<sub>2</sub>). This result clearly showed the highest disease severity in case of T<sub>4</sub>C<sub>1</sub> (30°C + 380 ppm CO<sub>2</sub>).

Interaction of the pathogens in the plant system is more complex than the simulated laboratory based study their behavior. Elevated CO<sub>2</sub> levels tend to result in changed plant structure. At multiple scales, plant organs may increase in size like increased leaf area, increased leaf thickness, higher number of leaves, higher total leaf area per plant and stems and branches with greater diameter (Pritchard, 14). However, interactions with other changing climatic

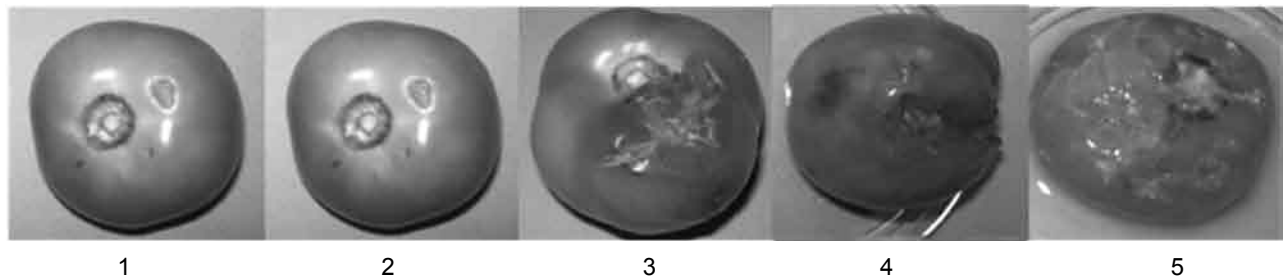


**Fig. 4.** Effect of temperature and CO<sub>2</sub> on extracellular enzyme cellulase (virulence factor) production after inoculation in tomato.

variables may complicate the effects of elevated CO<sub>2</sub> on plant pathogenesis. From our study, after artificial inoculation, the DSI was found faster in case of elevated CO<sub>2</sub> and elevated temperature combined treatment. This may be due to the fact that when high-temperature stress is exacerbated, plant response may be similar to those induced by water stress, with symptoms including wilting, leaf burn, leaf folding, abscission and physiological responses including changes in RNA metabolism, protein synthesis, enzymes, isoenzymes and plant growth hormones. These changes will certainly nullify the positive effects of the CO<sub>2</sub> fertilization in the plant system and will affect susceptibility to pathogens, though the wide range of changes may make an interaction which is difficult to predict (Christiansen *et al.*, 2). Hence, probably under elevated CO<sub>2</sub> and elevated temperature combination, the tomato plant became more susceptible for soft rot infestation and thereby T<sub>3</sub>C<sub>2</sub> (28°C + 600 ppm CO<sub>2</sub>) showed maximum disease severity under phytotron conditions. Nagaraja *et al.* (11) also reported that the disease severity was more by *F. mangiferae* under *in vitro* conditions with increasing temperature upto 40°C.

Under laboratory conditions, DSI was found maximum upto 33°C and at 35 and 37°C, the pathogenesis was almost absence because of blockage of the quorum sensing or cross talk by degradation of the signal molecules. Hasegawa *et al.* (7) also reported similar findings that at elevated temperatures (34 to 35°C) acyl homoserine lactone (AHL) production was reduced. Hence, for inducing the virulent gene, the concentration of oxo hydroxy homoserine lactone (OHHL) was below the quorum concentration at the elevated temperature, which was not sufficient to induce extracellular enzyme producing gene. The lack of extracellular pectate lyase (PEL) production at elevated temperatures (31.2°C) by *Pcc* has been documented previously (Lanham *et al.*, 9). From the present study maximum cellulase in T<sub>3</sub>C<sub>2</sub> (28°C + 6000 ppm CO<sub>2</sub>) resulted in maximum disease severity. Chakraborty *et al.* (3) found an increase in disease severity for six biotrophic fungi during study of the effects of CO<sub>2</sub> on plant disease.

In a study of plant disease in tallgrass prairie, Mitchell *et al.* (10) found that elevated CO<sub>2</sub> increased the pathogen load of C3 grasses, perhaps due to increased leaf longevity and photosynthetic rate. Garret *et al.* (6) reported that global climate change will affect plant disease in concert with other global change phenomena. Therefore, under controlled environmental conditions soft rot disease was found more in the elevated CO<sub>2</sub> and temperature conditions upto a certain limit. The production of extracellular enzyme was higher at elevated CO<sub>2</sub> and temperature that induce the severity of disease symptom. Finally, increase of the temperature beyond the critical temperature may suppress the disease due to blocked or interrupted cross communication among the pathogens. Therefore, in this climate change context with increased CO<sub>2</sub> and temperature the soft rot disease in tomato will be a devastating disease.



**Fig. 5.** Disease scoring 0 to 4 scale, where 0 = intact fruit, no lesion or soft rot, 1 = 1/4 of the fruit has lesions or soft rotting, 2 = 2/4 of the fruit has lesions or soft rotting, 3 = 3/4 of the fruit has lesions or soft rotting, and 4 = the entire fruit is covered with lesions or soft rotting.

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