



## Comparative physiological and histological investigations on resistant and susceptible pomegranate genotypes infected with *Xanthomonas axonopodis* pv. *punicae*

Priya BT<sup>1,2,3\*</sup>, Murthy BNS<sup>3</sup> & Laxman RH<sup>3</sup>

<sup>1</sup>Dr. YSR Horticulture University, Venkataramannagudem, Andhra Pradesh, India

<sup>2</sup>College of Horticulture, University of Horticultural Sciences, Bengaluru, Karnataka, India

<sup>3</sup>ICAR-Indian Institute of Horticultural Research, Bengaluru, Karnataka, India

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*Xanthomonas axonopodis* pv. *punicae* (*Xap*) is a bacterial pathogen wreaking havoc in pomegranate cultivation. It causes bacterial blight disease dwindling yield and making fruit unfit for consumption. Physiological and histological investigations during host-pathogen interaction are prerequisite to assess the onset of defense mechanism in plants. Therefore, we tried to compare the pomegranate resistant (IC 318734) and highly susceptible (Ruby) genotypes challenged with *Xap*. The bacterial suspension containing *Xap* cells of 0.3 OD<sub>600</sub> (~10<sup>6</sup> to 10<sup>7</sup> CFU mL<sup>-1</sup>) was used for challenge inoculation. Uniformly grown resistant and highly susceptible plants were selected, the surface of leaves was pricked and spray-inoculated with bacterial suspension using native strain IHR1 (NCBI Gen Bank ID: KT 222897). Simultaneously, the control plants were also sprayed with only distilled water and observed. A total of three replications with five plants per replication were maintained and evaluated under completely randomized design. Physiological investigations were recorded using Portable photosynthesis system (LCpro+, ADC BioScientific limited, UK) for one cycle of disease progression viz., 0, 1, 5, 10, 15 and 20 days after bacterial spray inoculation (DAI). Significant changes in gas exchange parameters were witnessed on pathogen inoculation. Higher reduction in mean percent change of photosynthetic and transpiration rate, instantaneous water use efficiency, internal CO<sub>2</sub> content, stomatal conductance and relative water content were noticed in highly susceptible genotype than resistant one. On contrary, an increased percent mean change of intrinsic water use efficiency, carboxylation capacity and lignin was documented in resistant genotype. Relative injury caused due to bacterial infection was found high in highly susceptible genotype than resistant one. Histological investigations in highly susceptible and resistant genotype were studied on 20<sup>th</sup> day of *Xap* inoculation using Scanning Electron Microscopy. Highly susceptible genotype exhibited maximum deformed cells, tissues and other visible abnormalities upon *Xap* inoculation. Thus, this study forms a basis for effective disease management and breeding programmes in pomegranate.

**Keywords:** Bacterial blight, Biotic stress, Carboxylation capacity, Internal CO<sub>2</sub> content, Lignin, Photosynthetic rate, Plant-pathogen interaction, Relative injury, Relative water content, Stomatal conductance, Transpiration rate, Water use efficiency

*Xanthomonas axonopodis* pv. *punicae* (*Xap*) is a highly evolved bacterial pathogen that spreads through rain droplets carried by wind and one among the numerous pathovars of phytopathogenic xanthomonads wreaking havoc in pomegranate cultivation<sup>1</sup>. The area under pomegranate has increased from 2.34 (2017-18) to 2.75 lakh ha (2019-20) with a corresponding production of 28.45 to 32.56 lakh tonnes in India<sup>2</sup>. However, the proportional increase in production was not witnessed due to several abiotic and biotic stresses. *Xap* causes bacterial blight disease in pomegranate accounting for

about 80% of production loss in India and poses a potential threat to pomegranate growing areas across the world<sup>3</sup>. An array of symptoms is caused by *Xap* in pomegranate and primarily it enters through stomata or other natural openings of the plants. Further, it can also enter through injuries caused during intercultural operations. Disease progress and severity is observed high till twenty days of pathogen inoculation and there after remained static/decline<sup>4</sup>. The invasion of the pathogen is identified by the signatures of small and irregular water soaked spots on leaves, fruits and mature branches. Scattered spots on leaves remain translucent against the light; gradually these spots may coalesce to form bigger patches and leaves wither off on severe infections. Brown to black

\*Correspondence:  
E-Mail: tpriyahort@gmail.com

lesions with crevices develops on fruits, often burst open exhibiting aril due to bacterial infection, making fruits unmarketable. Initially, discolorations around the nodes on stems and in advance stages, the onset of cracks and breakdown of branches are noticed<sup>5</sup>. Containment of disease spread involves the spraying of expensive chemicals and several cultural operations, which inclines the cost of cultivation<sup>6,7</sup>.

Diseases in plants generally involve three interactive components: host, pathogen and environmental factors. The relationship between these factors and disease can best be visualized as a triangle, which causes several changes in plant morphology. Alteration of plant growth on pathogen infection can be speculated through physiological and histological investigations, which is prerequisite to understand the compatible and incompatible interaction between the host and pathogen at the cellular level. Further, information on physiological and histological background on pathogen development and host resistance reactions against bacterial blight are lacking. Furthermore, profound insight of disease resistance reaction in pomegranate genotypes against bacterial blight would enable better understanding of defense mechanism and thereby effective disease management and breeding program in pomegranate<sup>8-10</sup>.

In the present investigation, we have made an attempt to compare the resistant and susceptible genotypes of pomegranate with respect to their histopathological and physiological changes upon inoculation with *Xap*. In addition, bacterial blight development and the occurrence of scrupulously discernible resistance reactions in resistant and susceptible genotypes of pomegranate are also examined.

### Materials and Methods

The experiment was conducted at the Indian Institute of Horticultural Research, Bengaluru, India under controlled condition. The minimum and maximum temperatures ranged from 17.4-20.8°C and 30.4-31.2°C, respectively with a mean relative humidity (RH) of 59.63% during the experimentation period. The resistant (IC 318734) and highly susceptible genotype (Ruby) were selected from the previous research work for the current study<sup>11</sup>. The one year old plants propagated through hard wood cuttings were utilized for physiological and

histological investigations. The bacterial suspension containing *Xap* cells grown in LB broth incubated at 25±3°C for 72 h was harvested, re-suspended in sterile distilled water and prepared the inoculum of 0.3 OD<sub>600</sub>(~10<sup>6</sup> to 10<sup>7</sup>CFU mL<sup>-1</sup>) for challenge inoculation. The uniformly grown resistant and highly susceptible plants were selected for imposing treatments. The surface of leaves was pricked and spray inoculated with bacterial suspension, native strain IHR1 (NCBI Gen Bank ID: KT 222897). Correspondingly, control plants were also sprayed with only distilled water and observed. After inoculation, plants were covered with polythene sheet and initially kept under humid conditions to induce disease development. Three replications with five plants per replication were maintained and evaluated under a completely randomized design.

### Physiological investigations

The control and diseased plants of resistant (IC 318734) and highly susceptible (Ruby) genotype of pomegranate were evaluated for one disease cycle period *viz.*, 0, 1, 5, 10, 15 and 20 days after spray inoculation (DAI) using Portable photosynthesis system (LCpro+, ADC BioScientific limited, UK)<sup>4,12</sup>

### Gas exchange parameters

Photosynthetic rate ( $P_N$ ), transpiration rate ( $E$ ), stomatal conductance ( $g_s$ ), internal leaf CO<sub>2</sub> ( $C_i$ ) and internal leaf temperature were measured<sup>13</sup>. A minimum of three measurements were taken during 09:30 to 11:30 h from fully mature leaves of morphological identical plants of resistant and highly susceptible genotype at various stages *viz.*, 0, 1, 5, 10, 15 and 20 days of pathogen inoculation. The mean CO<sub>2</sub> concentration during the measurement was 388 μmolmol<sup>-1</sup>. Instantaneous water use efficiency ( $WUE$ ) was calculated using photosynthetic rate and transpiration rate ( $P_N/E$ ), Intrinsic water use efficiency ( $WUE_i$ ) was calculated from photosynthetic rate and stomatal conductance ( $P_N/g_s$ ) and Carboxylation capacity ( $CE$ ) was calculated from photosynthetic rate and internal leaf CO<sub>2</sub> ( $P_N/C_i$ ). Further, the same leaves were used for the estimation of relative water content and electrolyte leakage percentage.

### Relative water content

The relative water content ( $RWC$ ) of leaves was measured according to Smart & Bingham<sup>14</sup>. After sampling, a fresh weight of ten leaf discs of size 10 mm diameter was taken immediately and then immersed in distilled water for 7 h at ambient

temperature. The leaves were then blotted dry and weighed prior to oven drying at 70°C for 48 h. The leaf relative water content was calculated using the formula,  $RWC (\%) = [(FW-DW)/(TW-DW)] \times 100$ , where FW, DW and TW are fresh, dry and turgid weights of the tissue, respectively<sup>13</sup>.

#### Relative injury (RI)

The cell membrane stability was estimated as percent relative injury<sup>15</sup>. Leaf discs of 10 mm diameter were cleaned and collected in two sets of test tubes containing 1.0 mL distilled water. One set was incubated at 25°C while the other at 50°C for 15 min, mixed thoroughly, added 15 mL distilled water following elevated temperature exposure and initial electrical conductivity using a conductivity meter (SYSTRONICS; India) was measured and referred as C1 and T1, respectively. Later, the tubes were incubated at 10°C for 18 h, autoclaved (121°C, 1.2 kgcm<sup>-2</sup>) for 15 min, immediately cooled to 25°C and final electrical conductivity was measured and referred as C2 and T2, respectively. The percent relative injury (RI) was calculated using the formula  $Relative\ Injury\ (\%) = \{1 - [1 - (T1/T2)] / [1 - (C1/C2)]\} * 100$

#### Lignin

The lignin was determined by Klason method<sup>16</sup>. The samples were dried completely in the oven and ground to a fine powder. 100 mg of sample was weighed in a small beaker and added 1.0 mL of 72 per cent H<sub>2</sub>SO<sub>4</sub>. The mixture was placed in a water bath at 30±0.5°C and stirred frequently for one hour. It was then diluted and transferred quantitatively to a 125 mL Erlenmeyer flask, using 28 mL of water for each 1.0 mL of acid. Secondary hydrolysis was done in an autoclave at 120°C for one hour. The hot solution was filtered using glass wool and the Klason lignin residue was washed with hot water to remove the acid. The crucibles containing the samples were then dried to constant weight at 105°C and weighed. Lignin is expressed as a percentage of the original sample in ash.

#### Statistical analysis

Data was analyzed using software SPSS package (SPSS Inc. Version 16.0) for all sets of data and means were compared using Duncan multiple comparison test at P=0.01.

#### Histological investigations

Here, the samples were collected from control and diseased plants of resistant (IC 318734) and highly susceptible (Ruby) genotype of pomegranate on 20<sup>th</sup>

day after inoculation (DAI) and subjected to Scanning Electron Microscopy (SEM) observation for comparative study. Here, five biological samples were analyzed to obtain uniform results.

#### Scanning Electron Microscopy (SEM) of plant surface

Plant tissues were initially prepared following fresh-hydrated method<sup>17</sup> for further environmental SEM examination. The microphotographs were captured at IISC-Indian Institute of Science, Bengaluru using SEM (XL-30 Environmental SEM, 30 kV, Tungsten source, SE and BSE imaging with EDXS). Here, the plant tissues (leaf and stem) were cut into pieces of 3-10 mm in size. The cut face of one selected piece was sealed with glue (conductive carbon glue) and mounted on SEM stubs with a conductive adhesive tape. The sample holder of SEM was cooled with ice close to 0°C before insertion of the sample into the SEM to slow down the desiccation. Further, the images of various plant cells were captured in healthy and diseased plant tissues of both the resistant and highly susceptible genotype for comparative study.

#### Epicuticular wax content on leaf

Leaf area of 1cm<sup>2</sup> was measured and immersed in 15 mL of chloroform for 15 s at room temperature (32±2°C). The extract was evaporated to dryness in a water bath at 70°C and then, 5 mL acidic K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub> was added and heated in a boiling water bath for 30 min. After cooling, 5 mL distilled water was added and measured using UV-VIS spectrophotometer at 590 nm. The concentration of epicuticular wax was calculated from a standard curve prepared using polyethylene glycol (PEG 6000)<sup>18</sup>.

## Result

### Physiological changes due to *Xanthomonas axonopodis* pv. *punicae* infection

#### Gas exchange parameters

Here, photosynthetic rate (PN), transpiration rate (E), stomatal conductance (gs), internal leaf CO<sub>2</sub> (Ci), internal leaf temperature, instantaneous water use efficiency (WUE), intrinsic water use efficiency (WUEi) and carboxylation capacity (CE) were estimated and significant changes were evinced on progress of the disease in both susceptible and resistant genotype (Tables 1 and 2)

#### Photosynthetic (PN) and Transpiration rate (E)

Higher reduction in mean percent change of photosynthetic and transpiration rate was noticed in

Table 1 — Changes in plant physiology viz., photosynthetic rate, transpiration rate, instantaneous WUE and intrinsic WUE on *Xap* inoculation

Genotype	Days after inoculation	Photosynthetic rate ( $\mu\text{mol}/\text{m}^2/\text{s}$ )			Transpiration rate ( $\text{mmol}/\text{m}^2/\text{s}$ )			Instantaneous WUE ( $\mu\text{molCO}_2/\text{mmol}/\text{H}_2\text{O}$ )			Intrinsic WUE ( $\mu\text{molCO}_2/\text{mmol}/\text{H}_2\text{O}$ )		
		Un-treated	Treated	% change	Un-treated	Treated	% change	Un-treated	Treated	% change	Un-treated	Treated	% change
Ruby (Highly Susceptible)	0	10.22 <sup>bc</sup>	10.22 <sup>bcd</sup>	0.00	6.15 <sup>bc</sup>	6.15 <sup>abc</sup>	0.00	1.66 <sup>c</sup>	1.66 <sup>c</sup>	0.00	60.07 <sup>a</sup>	60.07 <sup>abc</sup>	0.00
	1	15.98 <sup>a</sup>	13.93 <sup>a</sup>	-12.83	4.15 <sup>cd</sup>	3.81 <sup>bcd</sup>	-8.19	3.85 <sup>a</sup>	3.67 <sup>a</sup>	-4.68	57.19 <sup>a</sup>	54.29 <sup>abc</sup>	-5.07
	5	14.80 <sup>ab</sup>	8.66 <sup>b</sup>	-41.49	4.46 <sup>cd</sup>	3.81 <sup>bcd</sup>	-14.57	3.40 <sup>ab</sup>	2.27 <sup>bc</sup>	-33.24	61.02 <sup>a</sup>	57.88 <sup>abc</sup>	-5.15
	10	15.52 <sup>ab</sup>	13.11 <sup>ab</sup>	-15.53	8.28 <sup>a</sup>	7.11 <sup>a</sup>	-14.13	1.88 <sup>c</sup>	1.84 <sup>bc</sup>	-2.13	51.28 <sup>ab</sup>	73.31 <sup>a</sup>	+42.96
	15	13.57 <sup>abc</sup>	11.70 <sup>abcd</sup>	-13.78	6.12 <sup>bc</sup>	5.36 <sup>d</sup>	-12.42	2.22 <sup>c</sup>	2.19 <sup>bc</sup>	-1.35	46.72 <sup>ab</sup>	52.87 <sup>abc</sup>	+13.16
	20	13.27 <sup>abc</sup>	12.36 <sup>abc</sup>	-6.86	5.55 <sup>bcd</sup>	5.21 <sup>cd</sup>	-6.13	2.43 <sup>c</sup>	2.40 <sup>bc</sup>	-1.23	52.35 <sup>ab</sup>	61.62 <sup>bc</sup>	+17.71
	Mean	13.89	11.66	-16.05	5.78	5.24	-9.34	2.57	2.34	-8.94	54.77	60.00	+9.54
IC 318734 (Resistant)	0	9.09 <sup>c</sup>	9.09 <sup>cd</sup>	0.00	3.60 <sup>d</sup>	3.60 <sup>d</sup>	0.00	2.52 <sup>bc</sup>	2.52 <sup>b</sup>	0.00	44.35 <sup>ab</sup>	44.35 <sup>bc</sup>	0.00
	1	8.86 <sup>c</sup>	8.74 <sup>d</sup>	-1.35	3.63 <sup>d</sup>	3.60 <sup>d</sup>	-0.83	2.44 <sup>c</sup>	2.43 <sup>bc</sup>	-0.41	38.45 <sup>ab</sup>	41.30 <sup>c</sup>	+7.41
	5	10.44 <sup>bc</sup>	9.51 <sup>cd</sup>	-8.91	5.35 <sup>bcd</sup>	5.04 <sup>cd</sup>	-5.79	1.93 <sup>c</sup>	1.89 <sup>bc</sup>	-2.07	45.57 <sup>ab</sup>	63.67 <sup>ab</sup>	+39.72
	10	14.83 <sup>ab</sup>	14.27 <sup>a</sup>	-3.78	6.18 <sup>bc</sup>	6.01 <sup>abc</sup>	-2.75	2.42 <sup>c</sup>	2.40 <sup>bc</sup>	-0.83	25.57 <sup>b</sup>	40.50 <sup>c</sup>	+58.39
	15	13.02 <sup>abc</sup>	12.86 <sup>ab</sup>	-1.23	5.18 <sup>bcd</sup>	5.11 <sup>cd</sup>	-1.35	2.57 <sup>bc</sup>	2.57 <sup>b</sup>	-0.39	40.79 <sup>ab</sup>	53.18 <sup>abc</sup>	+30.38
	20	12.23 <sup>abc</sup>	11.97 <sup>abcd</sup>	-2.13	7.09 <sup>ab</sup>	7.01 <sup>ab</sup>	-1.13	1.74 <sup>c</sup>	1.73 <sup>c</sup>	-0.57	45.13 <sup>ab</sup>	47.89 <sup>bc</sup>	+6.12
	Mean	11.41	11.07	-2.97	5.17	5.06	-2.12	2.27	2.25	-0.88	39.97	48.48	+21.29
SEm $\pm$	-	1.66	1.07	-	0.68	0.56	-	0.30	0.25	-	9.14	6.89	-
CD (0.01%)	-	6.56	4.23	-	2.68	2.21	-	1.18	0.98	-	36.15	27.25	-

[SEm, Standard Error of the Mean; CD (0.01%), Critical Difference at 1% levels; Treated, Pathogen Inoculated; Untreated, Pathogen Un-inoculated]

Table 2 — Changes in internal CO<sub>2</sub>, carboxylation capacity and stomatal conductance on *Xap* inoculation

Genotype	Days after inoculation	Internal CO <sub>2</sub> (ppm)			Carboxylation capacity			Stomatal conductance ( $\text{mmol}/\text{m}^2/\text{s}$ )		
		Un-treated	Treated	% change	Un-treated	Treated	% change	Un-treated	Treated	% Change
Ruby (Highly Susceptible)	0	247.67 <sup>de</sup>	247.67 <sup>cd</sup>	0.00	0.041 <sup>abc</sup>	0.041 <sup>bcd</sup>	0.00	0.17 <sup>d</sup>	0.17 <sup>bc</sup>	0.00
	1	288.33 <sup>abc</sup>	246.00 <sup>cd</sup>	-14.68	0.055 <sup>a</sup>	0.057 <sup>a</sup>	+3.64	0.28 <sup>abcd</sup>	0.26 <sup>b</sup>	-7.14
	5	315.33 <sup>a</sup>	244.33 <sup>de</sup>	-22.52	0.047 <sup>abc</sup>	0.035 <sup>cd</sup>	-25.55	0.24 <sup>bcd</sup>	0.15 <sup>c</sup>	-37.50
	10	302.00 <sup>ab</sup>	249.00 <sup>bc</sup>	-17.55	0.051 <sup>ab</sup>	0.053 <sup>ab</sup>	+3.92	0.30 <sup>bc</sup>	0.18 <sup>bc</sup>	-40.00
	15	262.33 <sup>cde</sup>	224.67 <sup>cb</sup>	-14.36	0.052 <sup>ab</sup>	0.051 <sup>ab</sup>	-1.92	0.33 <sup>b</sup>	0.22 <sup>bc</sup>	-33.33
	20	229.00 <sup>c</sup>	217.67 <sup>e</sup>	-4.95	0.058 <sup>a</sup>	0.057 <sup>a</sup>	-1.72	0.25 <sup>bcd</sup>	0.20 <sup>bc</sup>	-20.00
	Mean	274.11	238.22	-13.09	0.051	0.049	-3.91	0.26	0.19	-26.90
IC 318734 (Resistant)	0	269.51 <sup>bcd</sup>	269.51 <sup>ab</sup>	0.00	0.034 <sup>bc</sup>	0.034 <sup>d</sup>	0.00	0.21 <sup>cd</sup>	0.21 <sup>bc</sup>	0.00
	1	312.67 <sup>a</sup>	277.00 <sup>a</sup>	-11.41	0.028 <sup>c</sup>	0.032 <sup>d</sup>	+14.29	0.24 <sup>bcd</sup>	0.22 <sup>bc</sup>	-8.33
	5	291.33 <sup>abc</sup>	233.67 <sup>cde</sup>	-19.79	0.036 <sup>abc</sup>	0.041 <sup>bcd</sup>	+13.89	0.23 <sup>bcd</sup>	0.15 <sup>c</sup>	-34.78
	10	297.67 <sup>abc</sup>	271.00 <sup>a</sup>	-8.96	0.050 <sup>ab</sup>	0.053 <sup>ab</sup>	+6.00	0.58 <sup>a</sup>	0.36 <sup>a</sup>	-37.98
	15	251.33 <sup>de</sup>	238.33 <sup>cde</sup>	-5.17	0.052 <sup>ab</sup>	0.050 <sup>ab</sup>	+2.04	0.32 <sup>bc</sup>	0.25 <sup>b</sup>	-21.88
	20	249.67 <sup>de</sup>	246.67 <sup>cd</sup>	-1.20	0.049 <sup>ab</sup>	0.054 <sup>abc</sup>	+3.84	0.27 <sup>bcd</sup>	0.25 <sup>b</sup>	-7.41
	Mean	278.69	256.03	-8.13	0.041	0.044	+7.31	0.31	0.24	-22.50
SEm $\pm$	-	11.74	7.42	-	0.01	0.04	-	0.04	0.03	-
CD (0.01%)	-	46.43	29.34	-	0.04	0.01	-	0.15	0.11	-

[SEm, Standard Error of the Mean; CD (0.01%), Critical Difference at 1% levels; Treated' Pathogen Inoculated; Untreated, Pathogen Un-inoculated]

susceptible genotype (-16.05, -9.34%) than resistant one (-2.97, -2.12%) (Fig. 1). Infected/treated plants showed the highest *PN* of 14.27  $\mu\text{molm}^{-2}\text{s}^{-1}$  on 10<sup>th</sup> DAI in resistant genotype compared to other treatments. Whereas, lowest recorded in susceptible genotype on 5<sup>th</sup> DAI (8.66  $\mu\text{molm}^{-2}\text{s}^{-1}$ ). Results showed decreased transpiration rate of challenged plants compared to control plants. It also revealed that the maximum *E* (7.11  $\mu\text{molm}^{-2}\text{s}^{-1}$ ) was recorded on 10<sup>th</sup> DAI in susceptible genotype and was found on

par with 20<sup>th</sup> DAI (7.01  $\mu\text{molm}^{-2}\text{s}^{-1}$ ) in the resistant genotype. Resistant genotype maintained the highest transpiration rate even on 20<sup>th</sup> DAI, as the infection progressed (Table 1).

#### Instantaneous WUE (WUE) and Intrinsic WUE (WUE<sub>i</sub>)

Decreased percent mean change of water use efficiency (WUE) was recorded with susceptible genotype (-8.94%) than resistant one (-0.88%). On contrary, an increased percent mean change of WUE<sub>i</sub> was documented in resistant genotype (+21.29%)

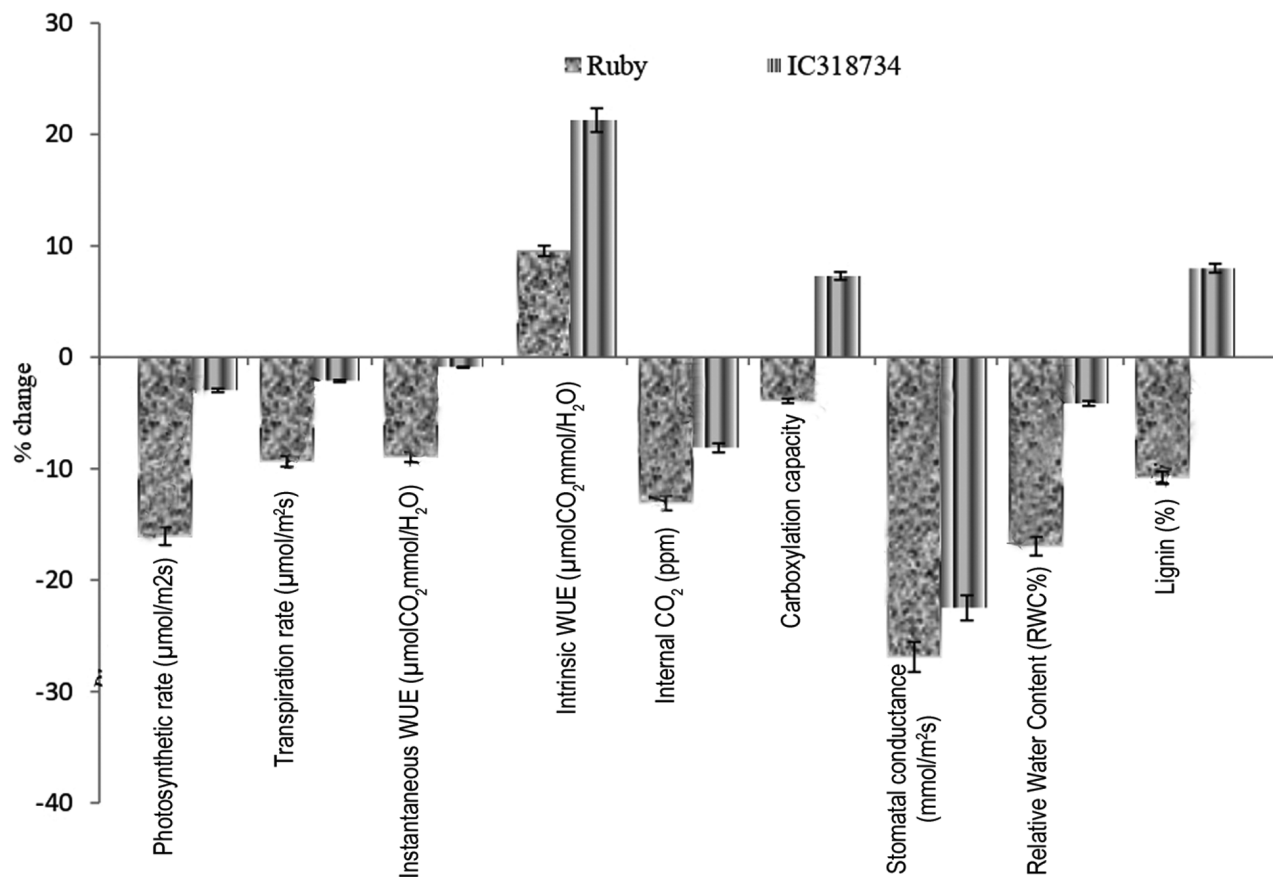


Fig. 1 — Physiological variations observed in highly susceptible (Ruby) and resistant (IC 318734) genotypes upon *Xap* inoculation.

compared to susceptible one (+9.54%) (Fig. 1). Significantly highest *WUE* recorded with susceptible genotype on 1<sup>st</sup> DAI ( $3.67 \mu\text{mol mmol}^{-1}$ ) followed by 15<sup>th</sup> DAI ( $2.57 \mu\text{mol mmol}^{-1}$ ) in resistant genotype while lowest recorded with treated control in susceptible genotype ( $1.66 \mu\text{mol mmol}^{-1}$ ). A similar trend was followed with un-inoculated plants of resistant and susceptible genotype. However, the maximum percentage reduction in *WUE* recorded in susceptible genotype (-33.24%) on 5<sup>th</sup> DAI over its control while on the same day, reduction percentage was minimum in resistant genotype (-2.07%) (Table 1). Highest *WUE<sub>i</sub>* recorded with the susceptible genotype on 10<sup>th</sup> DAI ( $73.31 \mu\text{mol mmol}^{-1}$ ) and was on par with all the treatments except 1<sup>st</sup> and 10<sup>th</sup> DAI in resistant genotype, which recorded the lowest *WUE<sub>i</sub>* compared to other treatments. The resistant genotype recorded an increased mean percentage of *WUE<sub>i</sub>* over its control (+58.39 %) when compared to the susceptible genotype (+42.96%) on 10<sup>th</sup> DAI (Table 1).

#### *Internal CO<sub>2</sub> content (C<sub>i</sub>)*

It is conspicuous from the Table 2 that pathogen infected plants showed lower *C<sub>i</sub>* than control plants. On the other hand, the highest *C<sub>i</sub>* (277.00 ppm) recorded on 1<sup>st</sup> DAI followed by 10<sup>th</sup> DAI in the resistant genotype and both the treatments were on par with each other. The lowest content of *C<sub>i</sub>* (217.67 ppm) was recorded with susceptible genotype on 20<sup>th</sup> DAI (Table 2). Decreased mean percent change recorded in susceptible genotype (-13.09%) compared to resistant genotype (-8.13%) (Fig. 1).

#### *Carboxylation capacity (C<sub>E</sub>)*

Decreased percent mean change was observed with susceptible genotype (-3.91%) while increased percent mean change was recorded in resistant genotype (+7.31%) (Fig. 1). *C<sub>E</sub>* was found increased on 20<sup>th</sup> DAI than the initial day (0<sup>th</sup> DAI) in both resistant and susceptible genotypes (Table 2).

#### *Stomatal conductance (g<sub>s</sub>)*

A reduction in stomatal conductance was noticed on pathogen attack when compared to control.

Stomatal conductance was highest in resistant genotype ( $0.36 \text{ mmol m}^{-2} \text{ s}^{-1}$ ) on 10<sup>th</sup> DAI and found significant over other treatments while lowest recorded in susceptible and resistant genotypes on 5<sup>th</sup> DAI ( $0.15 \text{ mmol m}^{-2} \text{ s}^{-1}$ ) (Table 2). Further, a high reduction in percent mean change of stomatal conductance was noticed in susceptible (-26.90%) than resistant (-22.50%) genotype (Fig. 1).

#### Relative water content (RWC)

It was noticed that there were no significant differences between the genotypes on various days in control plants. RWC % was found to decrease on progress of the disease in both susceptible and resistant genotypes. However, the resistant genotype recorded the highest RWC % even during exposure period of biotic stress compared to the susceptible genotype. Throughout infection, the overall percent reduction mean change of RWC over its control was high in susceptible (-16.69%) compared to resistant genotype (-4.14%) (Table 3 and Fig. 1).

#### Relative injury (RI)

No significant difference was observed between the genotypes on various days in control plants. RI % recorded high in susceptible genotype than resistant genotype on all the days of pathogen infection (Fig. 1). In resistant genotype, RI % was high on 15<sup>th</sup> day (43.75%) after inoculation and later, it was found to reduce gradually. Whereas on the same day (15<sup>th</sup> DAI), RI % in susceptible genotype was very high (61.76%) and found to increase further on progress of the disease (Table 3).

#### Lignin content

Decreased percent mean change was recorded in susceptible (-10.80%) genotype over its control while increased percent mean change was recorded in resistant one (+8.00%) (Fig.1). Lignin content of control plants was found high in susceptible genotype than resistant one. Paradoxically, pathogen-treated plants exhibited significantly high lignin content in resistant genotype than susceptible ones. Similarly, it was found that lignin content showed an increased trend initially and later decreased in both the genotypes. However, the lignin content recorded highest on 10<sup>th</sup> DAI in resistant genotypes (16.55%) with 15.73% increase over its control (Table 3).

#### Histological changes due to *Xanthomonas axonopodis* pv. *punicae* infection

##### Dorsal and ventral side of leaf

It was observed that the parenchyma cells on dorsal side of the leaf were healthy and arranged precisely in untreated controls of resistant and susceptible genotype. Plants when challenged with pathogen exhibited distorted structures of parenchyma cells in both the genotypes. However, maximum dent in parenchyma cells was noticed in susceptible genotype compared to resistant genotype on 20<sup>th</sup> DAI (Fig. 2A). Similarly on ventral side of the leaf, after pathogen inoculation, it was observed that the bacterial growth was high in susceptible genotypes and further expanded blocking the stomata and stomatal pores on 20<sup>th</sup> DAI when compared to resistant genotype (Fig. 2B).

Table 3 — Changes in relative water content, relative injury and lignin content on *Xap* inoculation

Genotype	Days after inoculation	Relative Water Content (RWC %)			Lignin (%)			Relative injury (RI %)	
		Un-treated	Treated	% Change	Un-treated	Treated	% Change	Un-treated	Treated
Ruby (Highly Susceptible)	0	69.38 <sup>a</sup>	69.25 <sup>ab</sup>	-0.19	15.57 <sup>a</sup>	15.57 <sup>b</sup>	0.00	5.52 <sup>a</sup>	5.84 <sup>f</sup>
	1	69.45 <sup>a</sup>	64.11 <sup>d</sup>	-7.69	15.62 <sup>a</sup>	15.54 <sup>b</sup>	-0.51	5.33 <sup>a</sup>	30.04 <sup>d</sup>
	5	69.22 <sup>a</sup>	46.67 <sup>f</sup>	-32.58	15.80 <sup>a</sup>	13.20 <sup>de</sup>	-16.46	5.61 <sup>a</sup>	49.95 <sup>b</sup>
	10	69.38 <sup>a</sup>	48.28 <sup>fc</sup>	-30.41	15.82 <sup>a</sup>	12.39 <sup>e</sup>	-21.68	5.51 <sup>a</sup>	61.76 <sup>a</sup>
	15	69.33 <sup>a</sup>	58.33 <sup>e</sup>	-15.87	15.55 <sup>a</sup>	13.22 <sup>de</sup>	-14.98	5.23 <sup>a</sup>	61.76 <sup>a</sup>
	20	69.85 <sup>a</sup>	59.26 <sup>e</sup>	-15.16	15.50 <sup>a</sup>	13.79 <sup>d</sup>	-11.03	5.48 <sup>a</sup>	62.45 <sup>a</sup>
	Mean	69.43	57.65	-16.96	15.64	13.95	-10.80	5.44	45.30
IC 318734 (Resistant)	0	71.26 <sup>a</sup>	71.13 <sup>a</sup>	-0.18	13.90 <sup>bc</sup>	13.90 <sup>d</sup>	0.00	5.28 <sup>a</sup>	5.42 <sup>f</sup>
	1	71.23 <sup>a</sup>	69.54 <sup>a</sup>	-2.37	14.00 <sup>bc</sup>	14.96 <sup>bc</sup>	+6.86	5.85 <sup>a</sup>	16.1 <sup>e</sup>
	5	71.28 <sup>a</sup>	64.76 <sup>ab</sup>	-9.15	14.04 <sup>bc</sup>	15.41 <sup>b</sup>	+9.76	5.46 <sup>a</sup>	31.49 <sup>d</sup>
	10	71.26 <sup>a</sup>	66.67 <sup>cd</sup>	-6.44	14.30 <sup>bc</sup>	16.55 <sup>a</sup>	+15.73	5.71 <sup>a</sup>	43.71 <sup>c</sup>
	15	71.23 <sup>a</sup>	68.25 <sup>bcd</sup>	-4.18	13.60 <sup>b</sup>	14.90 <sup>bc</sup>	+9.56	5.86 <sup>a</sup>	43.75 <sup>c</sup>
	20	71.29 <sup>a</sup>	69.45 <sup>abc</sup>	-2.58	13.30 <sup>c</sup>	14.13 <sup>cd</sup>	+6.24	5.12 <sup>a</sup>	40.75 <sup>c</sup>
	Mean	71.25	68.30	-4.14	13.85	14.97	+8.00	5.55	30.20
SEm±	-	1.87	1.19	-	0.26	0.32	-	0.34	1.38
CD (0.01%)	-	7.39	4.70	-	1.02	1.26	-	1.34	5.45

[SEm, Standard Error of the Mean; CD (0.01%), Critical Difference at 1% levels; Treated, Pathogen Inoculated; Untreated, Pathogen Un-inoculated]

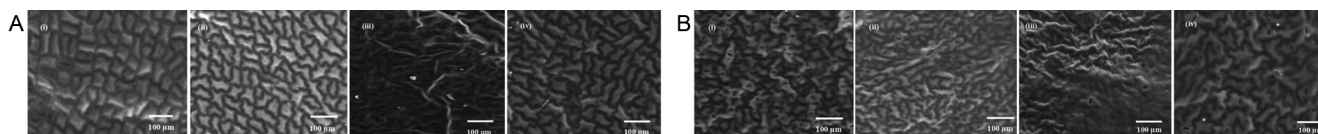


Fig. 2 — SEM photographs showing the parenchyma cells on (A) dorsal side; and (B) ventral side of leaf before and after *Xap* inoculation in pomegranate genotypes at magnification of 1000X. [(i): Healthy susceptible (Ruby); (ii): Healthy resistant (IC 318734); (iii): Infected susceptible (Ruby) & (iv): Infected resistant (IC 318734) genotype]

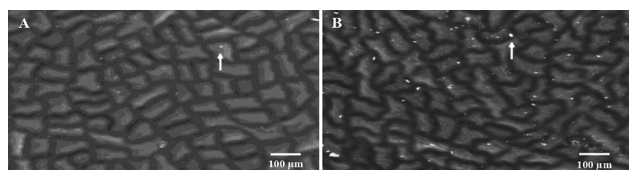


Fig. 3 — SEM images of epicuticular wax crystals on dorsal side of leaf at magnification of 1000X in genotypes of pomegranate. (A) Highly susceptible (Ruby); and (B) Resistant (IC 318734) genotype

#### *Epicuticular wax crystals on leaf*

The SEM analysis showed that epicuticular wax crystals were present on the dorsal side of pomegranate leaves. However, it was noticed that a higher degree of crystalline wax was present and found pronounced in resistant genotype (IC 318734) than susceptible genotype (Ruby) (Fig. 3). IC 318734, found to contain high wax content ( $548.12 \mu\text{g dm}^{-2}$ ) compared to the Ruby, which is susceptible ( $433.75 \mu\text{g dm}^{-2}$ ) genotype. The results also showed that both the genotypes significantly vary with each other as depicted from Fig. 4.

#### *Trichomes on the stem*

Variation in density of trichomes was witnessed in genotypes. It was observed that a higher density of trichomes was present in resistant than susceptible genotype (Fig. 5).

#### *Cross-sectional view of stem*

The healthy and diseased stems were compared and found that all the cells of tissue were intact in the healthy plant whereas in diseased tissue, cells were seen disintegrated. Further, after inoculation of a pathogen, the cells of the pith (P), primary xylem (PX), secondary xylem (SX), phloem (PH), periderm (PE), vascular cambium (VC), cork (C) and resin duct (RD) in susceptible genotype were found more deformed and disorganized compared to resistant genotype (Fig. 6A). The closer view of the stem using SEM also suggested that pith (P), proto-xylem (PX), meta-xylem (MX) and medullary ray (MR) were seen prominent in control/untreated plants of both the genotypes. Further as mentioned earlier, the plants

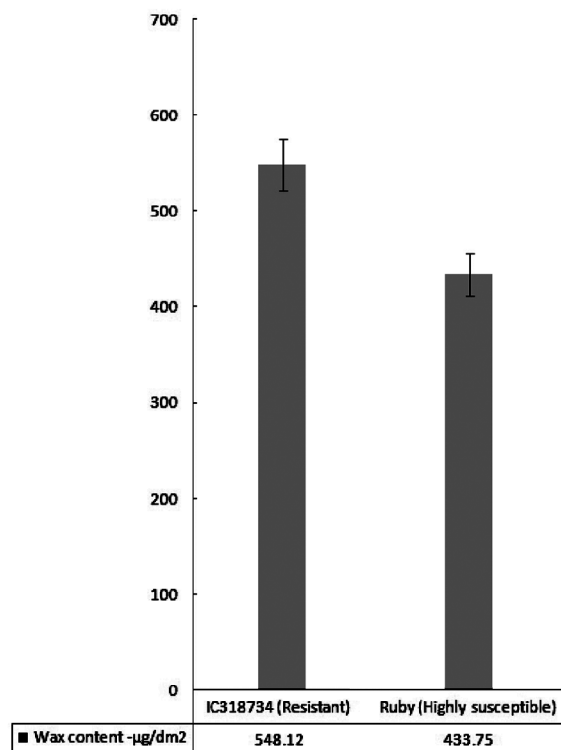


Fig. 4 — Wax content ( $\mu\text{g}/\text{dm}^2$ ) in highly susceptible (Ruby) and resistant (IC 318734) genotypes

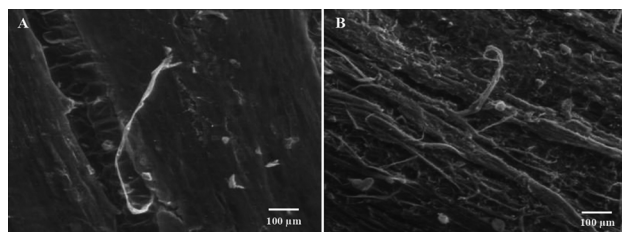


Fig. 5 — SEM image view of trichomes on stem at magnification of 1000X in pomegranate genotypes. (A) Highly susceptible (Ruby); and (B) Resistant (IC 318734) genotype

exposed to pathogen exhibited disintegration of tissues in both the genotypes. However, the susceptible genotype succumbed to more damage than the resistant genotype (Fig. 6B).

## Discussion

Pathogens employ different strategies to invade a plant that causes stress. Production of secondary



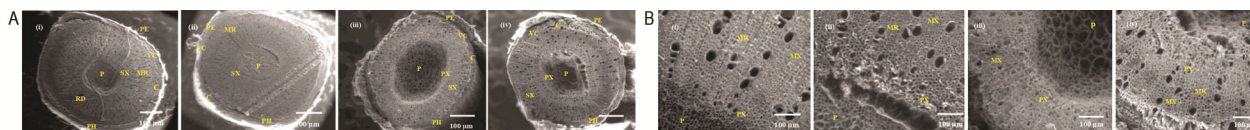


Fig. 6 — SEM images showing the cross-sectional view of stem before and after *Xap* inoculation in pomegranate genotypes at magnification of (A) 150X; and (B) 500X. [(i): Healthy susceptible (Ruby); (ii): Healthy resistant (IC 318734); (iii): Infected susceptible (Ruby) & (iv): Infected resistant (IC 318734) genotype. In (A): P, Pith; PX, Primary xylem; SX, Secondary xylem; PH, Phloem; PE, Periderm; VC, Vascular cambium; C, Cork; RD, Resin Duct; and in (B): P, Pith; PX, Primary xylem; MX, Meta-xylem; MR, Medullary Ray]

metabolites and alteration in plant taxonomy is witnessed as signature of defense mechanism in plants to maintain physiological homeostasis<sup>19</sup>. In the present investigation, a significant decrease in photosynthesis, transpiration and stomatal conductance was noticed on pathogen infection. Reduction in photosynthetic and transpiration rate was evinced till 10 days of bacterial inoculation and later found to increase (Table 1 & 2). Morphological and metabolic alterations on pathogenesis might be the cause for reduction in physiological activities. Pathogenesis also associated with hypertrophy of chlorenchyma, reduction of air spaces, or obstruction of conductive tissue and stomata during initial days of inoculation. Further, the decrease in photosynthesis causes simultaneously increased demand for assimilates very often, which leads to a transition of source tissue into sink tissue during plant–pathogen interactions. This transition of source to sink is found evident with the decrease in concentration of primary metabolites present in genotypes on pathogen infection.

The reduction in photosynthesis is also attributed to the reduction in internal carbon. The reduction in CO<sub>2</sub> induces sequential activities like Rubisco deactivation, stomatal closure and reduction in transpiration rate. Higher intrinsic *WUE* might plausibly indicate the potentiality of genotype to sustain the biotic stress<sup>20</sup>. Decrease in *RWC* was noticed in pathogen infected plants<sup>21</sup>. The high percentage decrease of *RWC* over its control was observed in susceptible genotype than resistant one (Table 3). Further, physiological status of resistant genotype was found to restrain after 20 days of bacterial inoculation in comparison to susceptible genotype. Inbuilt resistance developed during pathogenesis abstain the growth of pathogen on progression of infection in resistant genotype<sup>22</sup>. Thus, increased physiological components were recorded with incompatible interaction than compatible one<sup>23</sup>. Similar results were reported in tobacco<sup>24</sup> and

pepper<sup>25</sup> and specified that reduction in relative water content is an indication of water transport in the soil-plant-atmosphere and thereby respiratory systems of the plants would adversely get affected<sup>25</sup>.

The relative injury is an indication of cellular damage and cell membrane stability in plants. In the present study, a high percentage of relative injury was recorded with treated susceptible genotype (Table 3). Higher relative injury characterizes the percentage decrease in performance of the genotype under stress relative to the non-stress conditions<sup>26</sup>. Electrolyte leakage is an indication of stress response in intact plant cells. This phenomenon is widely used as a test for the stress-induced injury of plant tissues and ‘a measure’ of plant stress tolerance<sup>27</sup>. The relative injury recorded high in susceptible genotypes implying more ion leakage on infection than resistant one, suggesting stress-induced K<sup>+</sup> efflux inhibits energy-consuming ‘anabolic’ reactions and stimulates ‘energy-releasing’ catabolic processes. Thereby, ceases growth and ‘redirects’ the energy flow to adaptation and healing needs. This could be a critical step in plant cell adaptation to any stress factor but when exceeds beyond threshold level affects cell membrane stability, which is witnessed in the present study during compatible interaction of plant and pathogen<sup>28</sup> (Table 3). Further, the obtained results are in conformity with the results of Blatt *et al.*<sup>29</sup> in plant-pathogen interaction.

Pathogenesis is a complex process involving the capability of pathogens on interaction with microclimate causes infection of a host plant. The cell wall associated immune responses are suppressed by certain effectors of *Xap*, causing bacterial blight in pomegranate<sup>30,31</sup>. The potential host plant activates an array of defense mechanisms to resist the development of disease. Besides, it is a primary challenge even for the pathogen to breach the physical barrier of the host cell. It is worth mentioning that microscopy is a valuable and essential tool for monitoring pathogen invasion. Therefore, histological



studies of the host-pathogen interaction could help identify events that occur during the pathogenesis and ultimately leads to a better understanding of the resistance mechanism<sup>32</sup>. It was observed from the study that maximum cell deformation occurred in susceptible genotype than resistant genotype. Destruction of parenchymatous cells was visualized maximum in susceptible genotype under SEM (Fig. 2A). Parenchyma cells are reported as a source of defense mechanism that restricts pathogen growth by the compartmentalization processes<sup>33</sup>. Resistance and susceptibility can thus be predicted based on the genotype and its response to the pathogen. Pathogen-derived factors are known to perturb stomata, which are an important apparatus of plant that regulates gas exchange and improves *WUE* (Fig. 2B). Further, variation in disease severity level may be attributed to stomatal opening or closing during pathogen attack<sup>8</sup>. Therefore, alteration in stomata impacts the regular functioning of plants, which causes detrimental effects to crop yield<sup>34</sup>.

Waxes are an integral part of the plant defense system that impedes the spread of pathogens<sup>35</sup>. The SEM analysis showed epicuticular waxes on leaves of pomegranate plants act as a barrier for pathogen ingress. The crystalline waxes were less pronounced in susceptible genotype than resistant one. Similarly, quantitative differences in the wax deposition were also observed in this experiment, where resistant genotype recorded higher than susceptible genotype (Figs 3 & 4). The wax content and its abundance were known to affect resistance to pathogens and was stated that surface epicuticular waxes could impede the entry of pathogens by providing a physical barrier to penetration<sup>36</sup>. Similar observations were made in cassava against bacterial blight<sup>37</sup>. Further, genotype with higher wax content on the abaxial leaf surface is non-wettable and seems unlikely as the route of entry for *Xanthomonas axonopodis* pv. *punicae*. Similar work was described in cassava against *Xanthomonas axonopodis* pv. *manihotis*<sup>38</sup>. Trichome density on the stem was high in resistant genotype than susceptible one (Fig. 5). The presence of dense trichomes signifies the protection against several biotic and abiotic stresses and retains water contents<sup>39</sup>. Wax and trichomes prevent excess water loss and potentially improve *WUE* in various crops<sup>40</sup>. Higher density of trichomes comprehends more *WUE* as reported by Galdon *et al*<sup>41</sup> in tomato crop.

Furthermore, cell and tissue deformation on pathogen infection was seen, which involved pith

enlargement, under development of vascular bundles and cambium aberration in susceptible genotype than resistant one (Fig. 6A & B). Generally, bacterial infection disrupts the vascular bundles and spreads the diseases in plants<sup>42</sup>. Lignin content was recorded high in resistant genotype than susceptible (Table 3). Lignification is a mechanism for disease resistance in plants and during defense responses, lignin or lignin-like phenolic compound accumulation was found to occur in a variety of plant-microbe interactions. The events of cell wall lignification by a distinct layer of phloem and cortex parenchyma cells and suberin lamellae deposition in response to the pathogen, also suggested as part of an intrinsic defense response<sup>43</sup>. Callose and lignin are components of cell wall, which have been implicated both in growth processes and defense responses<sup>44,45</sup>. Lignin also plays important role in plant protection, which seems to result in the production of pectin-derived immune elicitors, inducing defense responses<sup>46</sup>. Thus, observations specified that manipulation of cell wall components could cause adaptive changes or modifications of other components, as part of defense mechanism in plants<sup>47</sup>. Therefore, modulation in physiological and histological components is essential for plant sustainability during plant-pathogen interaction.

## Conclusion

*Xanthomonas axonopodis* pv. *punicae* causing bacterial blight in pomegranate is a major constraint in many pomegranate growing countries. A systematic study of plant-pathogenic interaction unravels underlying defense mechanism in susceptible and resistant genotypes of pomegranate. In present investigation, physiological changes *viz.*, high reduction in percent mean change of photosynthetic and transpiration rate, instantaneous water use efficiency, internal CO<sub>2</sub> content, carboxylation capacity, stomatal conductance, relative water and lignin content was noticed in susceptible genotype upon *Xap* infection. Likewise, histological changes *viz.*, maximum dent in parenchyma cells, blockage in stomatal pores, widened pith, blockage in xylem and phloem cells were observed in susceptible genotype on progress of *Xap* infection. Therefore, these changes contributed to maximum extent of damage in highly susceptible genotype (Ruby) in comparison to resistant (IC 318734) genotype. Comparative analysis thus suggests that alteration in physiology and histology of pomegranate varied greatly in susceptible and resistant genotypes at structural and cellular level

during blight pathogenesis. The study also prompted that the *Xap*-pomegranate interactions during incompatible response would lead to the compromised plant defense mechanism by suppressing relative injury caused through an array of events. Observations from this study would be useful for effective disease management and various breeding program in pomegranate.

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### Conflict of interest

Authors declare no competing interests.

### References

- Sharma V, Midha S, Ranjan M, Pinnaka AK & Patil PB, Genome sequence of *Xanthomonas axonopodis* pv. *punicae* Strain LMG 859. *J Bacteriol*, vol? (2012) 2395.
- Anonymous, Agricultural statistics, Ministry of agriculture and farmers welfare, Govt. of India, (2020) 36.
- Mondal KK & Mani C, ERIC-PCR-generated genomic fingerprints and their relationship with pathogenic variability of *Xanthomonas campestris* pv. *punicae*, the incident of bacterial blight of pomegranate. *Curr Microbiol*, 59 (2009) 616.
- Sharma J, Sharma KK, Kumar A, Mondal K, Thalor S, Maity A, Gharate R, Chinchure S & Jadhav VT, Pomegranate bacterial blight: symptomatology and rapid inoculation technique for *Xanthomonas axonopodis* pv. *punicae*. *J Plant Pathol*, 99 (2017) 109.
- Singh NV, Abburi VL, Ramajayam D, Kumar R, Chandra R, Sharma KK, Sharma J, Dhinesh Babu K, Pal RK, Mundewadikar DM, Saminathan T, Cantrell R, Nimmakayala P & Reddy UK, Genetic diversity and association mapping of bacterial blight and other horticulturally important traits with microsatellite markers in pomegranate from India. *Mol Genet Genom*, 290 (2015) 1393.
- Jadhav VT & Sharma KK, Integrated management of disease in pomegranate. *ISHS Acta Horticulturae ISPMMF*, 890 (2009) 467.
- Lalithya KA, Manjunatha G, Raju B, Kulkarni MS & Lokesh V, Plant growth regulators and signal molecules enhance resistance against bacterial blight disease of pomegranate. *Phytopathology*, 165 (2017) 727.
- Kumar P, Dashyal MS, Doddaraju P, Methi BS & Girigowda M, Differential gene responses in different varieties of pomegranate during the pathogenesis of *Xanthomonas axonopodis* pv. *punicae*. *3 Biotech*, 11 (2021) 180.
- Doddaraju P, Kumar P, Gunnaiah R, Gowda AA, Lokesh V, Pujer P & Manjunatha G, Reliable and early diagnosis of bacterial blight in pomegranate caused by *Xanthomonas axonopodis* pv. *punicae* using sensitive PCR techniques. *Sci Rep*, 9 (2019) 10097.
- Singh NV, Parashuram S, Sharma J, Potlannagari RS, Karuppannan DB, Pal RK, Patil P, Mundewadikar DM, Sangure VR, Arun PVPS, Mutha NVR, Kumar B, Tripathi A, Peddamma SK, Kothandaraman H, Yellaboina S, Banghel DS & Reddy UK, Comparative transcriptome profiling of pomegranate genotypes having resistance and susceptible reaction to *Xanthomonas axonopodis* pv. *punicae*. *Saudi J Biol Sci*, 27 (2020) 3514.
- Priya BT, Murthy BNS, Gopalakrishnan C, Artal RB & Jagannath S, Identification of new resistant sources for bacterial blight in pomegranate. *Eur J Plant Pathol*, 146 (2016) 609.
- Mathur S, Sharma MP & Jajoo A, Improved photosynthetic efficacy of Maize (*Zea mays*) plants with Arbuscular Mycorrhizal Fungi (AMF) under high temperature stress. *J. Photochem. Photobiol. B, Biol.*, 180 (2018) 149.
- Zrig A, Tounekti T, AbdElgawad H, Hamouda F & Khemira H, Influence of light intensity and salinity on growth and antioxidant machinery of *Thymus vulgaris* L. *Indian J Exp Biol*, 58 (2020) 323.
- Smart R & Bingham G, Rapid estimates of relative water content. *Plant Physiol*, 53 (1974) 258.
- Wang CH, Yeh DM & Sheu CS, Heat tolerance and flowering-heat-delay sensitivity in relation to cell membrane thermostability in chrysanthemum. *J Am Soc Hort Sci*, 133 (2008) 754.
- Fagerstedt KV, Saranpaa P, Tapanila T, Immanen J, Serra JAA & Nieminen K, Determining the composition of lignins in different tissues of silver birch. *Plants*, 4 (2015) 183.
- Ensikat HJ, Ditsche-Kuru P & Barthlott W, Scanning electron microscopy of plant surfaces: simple but sophisticated methods for preparation and examination. In: *Microscopy: science, technology, applications and education*, (Ed. Mendez-vilas & Diaz; FORMATEX Microscopy series 4, Spain), 2010, 248.
- Riedel M, Eichner A & Jetter R, Slippery surfaces of carnivorous plants: composition of epicuticular wax crystals in *Nepenthes alata* Blanco pitchers. *Planta*, 218 (2003) 87.
- Silva M, Jifon JL, Santos CM, Jadoski CJ & Silva J, Photosynthesis capacity and water use efficiency in sugarcane genotypes subject to water deficit during early growth phase. *Braz Arch Biol Technol*, 56 (2013) 1.
- Lemoine R, Camera SL, Atanassova R, Dedaldechamp F, Allario T, Pourtau N, Bonnemain JL, Laloi M, Thevenot PC, Maurousset L, Faucher M, Girousse C, Lemonnier JP & Durand M, Source to sink transport of sugar and regulation by environmental factors. *Front Plant Sci*, 4 (2013) 1.
- Ghanbary E, Fathizadeh O, Pazhouhan I, Zarafshar M, Tabari M, Jafania S, Parad GA & Bader MKF, Drought and pathogen effects on survival, leaf physiology, oxidative damage, and defense in two middle eastern oak species. *Forests*, 12 (2021) 247.
- Martinez HEP, Maia JTLS, Ventrella MC, Milagres CC, Cecon PR, Clemente JM & Garbin CZ, Leaf and stem anatomy of cherry tomato under calcium and magnesium deficiencies. *Braz Arch Biol Technol*, 63 (2020) 1
- Wang M, Sun Y, Sun G, Liu X, Zhai L, Shen Q & Guo S, Water balance altered in cucumber plants infected with *Fusarium oxysporum* f. sp. *cucumerinum*. *Sci Rep*, 5 (2015) 7722.
- Synkova H, Semoradova S, Schnablova R, Muller K, Pospisilova J, Ryslava H, Malbeck, J & Cerovska N, Effects

- of biotic stress caused by Potato virus Y on photosynthesis in transgenic and control *Nicotiana tabacum* L. *Plant Sci*, 171 (2006) 607.
- 25 Pazarlar S, Gumus M & Ozteki NGB, The effects of Tobacco Mosaic Virus infections on growth and physiological parameters in some pepper varieties (*Capsicum annum* L.). *Not Bot Horti Agrobo Cluj Napoca*, 41 (2013) 427.
  - 26 Nijabat A, Bolton A, Rehman M, Shah A, Hussain R, Naveed NH, Ali A & Simon P, Cell membrane stability and relative cell injury in response to heat stress during early and late seedling stages of diverse carrot (*Daucus carota* L.) Germplasm. *Hort Sci*, 55 (2020) 1446
  - 27 Lee B & Zhu JK, Phenotypic analysis of Arabidopsis mutants: electrolyte leakage after freezing stress. In: *Cold Spring Harbor Protocol*, (Ed. Weigel and Glazebrook, CHSL press, USA), 2010, 4970
  - 28 Demidchik V, Straltsova D, Sergey S, Medvedev GA & Pozhvanov A, Stress-induced electrolyte leakage: the role of K<sup>+</sup>-permeable channels and involvement in programmed cell death and metabolic adjustment. *J Exp Bot*, 65 (2014) 1259.
  - 29 Blatt MR, Grabov A, Brearley J, Hammond K & Jones JDG, K<sup>+</sup> channels of Cf-9-transgenic tobacco guard cells as targets for *Cladosporium fulvum* Avr9. *Plant J*, 19 (1999) 453.
  - 30 Mondal KK, Emerging phyto-bacterial diseases in India: research status and challenges. In: *Perspectives of Plant Pathology in genomic era*, (Eds. P Chowdappa, P Sharma, D Singh & AK Misra; India Phytopathological Society, New Delhi), 2016, 167.
  - 31 Mondal KK, Dialogue between Xop-T3SS effectors of *Xanthomonas oryzae* pv. *oryzae* and rice during bacterial blight pathogenesis. *Indian Phytopathol*, 70 (2017) 269.
  - 32 Sillero JC & Rubiales D, Histological characterization of resistance to *Uromyces viciae fabae* in faba bean. *Phytopathology*, 92 (2002) 294.
  - 33 Ouellette G, Mohamed C & Simard M, Histopathology of *Fusarium* wilt of staghorn sumac (*Rhus typhina*) caused by *Fusarium oxysporum* f. sp. *callistephi* Race 3. III. host cell and tissue reactions. *Phytoprotection*, 87 (2006) 17.
  - 34 Marcell LM & Beattie AG, Effect of leaf surface waxes on leaf colonization by *Pantoea agglomerans* and *Clavibacter michiganensis*. *Mol Plant Microbe Interact*, 15 (2002) 1236.
  - 35 Wang X, Kong L, Zhi P & Chang C, Update on cuticular wax biosynthesis and its roles in plant disease resistance. *Int J Mol Sci*, 21 (2020) 5514.
  - 36 Lewandowska M, Keyl A & Feussner I, Wax biosynthesis in response to danger: its regulation upon abiotic and biotic stress. *New Phytol*, 227 (2020) 698
  - 37 Zinsou V, Wydra K, Ahohuendo B & Schreiber L, Leaf waxes of cassava (*Manihot esculenta* Crantz) in relation to ecozone and resistance to *Xanthomonas blight*. *Euphytica*, 149 (2006) 189.
  - 38 Cooper RM, Kemp B, Day R, Gomez-Vasquez R & Beeing JR, Pathogenicity and resistance in *Xanthomonas blight* of cassava. In: *Proceedings of 10<sup>th</sup> International Conference on plant pathogenic bacteria* (Ed. DeBoerS; Kluwer academic publishers, Netherlands), (2001) 319.
  - 39 Karabourniotis G, Liakopoulos G, Nikolopoulos D & Bresta P, Protective and defensive roles of non-glandular trichomes against multiple stresses: structure–function coordination. *J For Res*, 31 (2020) 1.
  - 40 Watts S and Kariyat R, Morphological characterization of trichomes shows enormous variation in shape, density and dimensions across the leaves of 14 *Solanum* species. *AoB Plants*, 13 (2021) 1.
  - 41 Galdon AJ, Fullana PM, Mulet PA, Conesa MA, Martin C & Galmes J, The ratio of trichomes to stomata is associated with water use efficiency in tomato. *Plant J*, 96 (2018) 607.
  - 42 Keijzer JD, Van Den Broe LAM, Ketelaar T & Van Lammeren AAM, Histological examination of horse chestnut infection by *Pseudomonas syringae* pv. *aesculi* and non-destructive heat treatment to stop disease progression. *PLoS ONE*, 7 (2012) 39604.
  - 43 Bhuiyan N, Selvaraj G, Wei YD & King J, Gene expression profiling and silencing reveal that monolignol biosynthesis plays a critical role in penetration defence in wheat against powdery mildew invasion. *J Exp Bot*, 60 (2009) 509.
  - 44 Bacete L & Hamann T, The role of mechanoperception in plant cell wall integrity maintenance. *Plants*, 9 (2020) 1.
  - 45 Wan J, He M, Hou Q, Zou L, Yang Y, Wei Y & Chen X, Cell wall associated immunity in plants. *Stress Biol*, 1 (2021) 1.
  - 46 Voxeura A & Hoftea H, Pectin-derived immune elicitors in response to lignin modification in plants. *Proc Natl Acad Sci USA*, 117 (2020) 4442.
  - 47 Nazarov PA, Baleev DN, Ivanova MI, Sokolova LM & Karakozova MV, Infectious Plant Diseases: Etiology, Current Status, Problems and Prospects in Plant Protection. *Acta Naturae*, 12 (2020) 46.