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Research Article

Biochemical Changes and Leaf Photosynthesis of *Erwinia mallotivora* Infected Papaya (*Carica papaya*) Seedlings

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Abstract

Background and Objective: Papaya (*Carica papaya*) is a well-known tropical fruit consumed worldwide but its expansion is seriously impaired by many diseases including dieback caused by *Erwinia mallotivora*. In this study, development of dieback and changes in total phenol, total sugar, total protein, peroxidase activity, polyphenol oxidase activity and leaf photosynthesis following the inoculation of *E. mallotivora* on two papaya cultivars, Eksotika dan Eksotika II were reported. **Methodology:** *Erwinia mallotivora* infection (1×10^8 CFU mL⁻¹, 50 μ L injection) to the 8 week old seedlings at the 5th node from the apex caused dieback disease to occur as early as 3 days after inoculation. The inoculated plants were completely destroyed on day 11 after inoculation. **Results:** Both papaya cultivars had similar trend in their physiological and biochemical changes toward the infection. Total sugar in leaves of infected plants was higher than those in the non-infected plants, but the stem and the roots of infected plants contained markedly less sugar than those of healthy plants. *Erwinia mallotivora* infected tissues contained higher concentration of total phenol and total protein and these changes were coupled with higher activity of peroxidase and polyphenol oxidase. **Conclusion:** Plant experiencing dieback disease also had a lower leaf photosynthetic rate with reduced stomatal conductance.

Key words: *Carica papaya*, papaya dieback, *Erwinia mallotivora*, biochemical changes, photosynthesis

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Papaya (*Carica papaya*) is a well-known tropical fruit consumed in most part of the world. In Malaysia, development of papaya production is being checked by many diseases and one of those diseases is dieback, caused by *Erwinia mallotivora*¹. This disease was first detected in Malaysia in 2003. Once the papaya plant is infected by the disease, the control is almost impossible as advanced stage of infection lead to dieback and death of trees².

Using *C. papaya* cv. Sekaki in their pathogenicity testing Amin *et al.*¹ showed that the injected isolates formed brown spots on the leaves, water soaked lesions and greasy spots on the stem after 4-5 days of inoculation around the sites of inoculation and death of the plant occurred about 15 days after inoculation. Working on phytoplasma related dieback of papaya in Australia Gonzalez *et al.*³ reported that plant apical death can be observed within 2 weeks after the first visible external symptom expression and 3 weeks of first detection of phytoplasma in host tissue. Leaf photosynthetic rate of mature leaves of the infected plants was reduced approximately by 50% relative to healthy plants by the time of first visible symptom expression. Carbohydrate levels increased in leaf tissue of diseased plants but decreased in stem and root tissues.

Upon sensing stimuli following invasion of foreign bodies into the system, either from abiotic or biotic agents, among responses shown in plant cells is generation and accumulation of metabolites. Okem *et al.*⁴ reported that *Hypoxis hemerocallidea* exposed to moderate concentration of Cd (5 mg L⁻¹) had accumulated higher concentration of phenolics and flavonoids. This phenomenon was coupled with a significant increase in diphenylpicrylhydrazyl (DPPH) antioxidant scavenging activity in most of the metal-treated plants compared to the positive control ascorbic acid. The extracts obtained from Cd treated plants (2 mg L⁻¹) was found to be effective in reducing activity *Staphylococcus aureus*.

Apart from playing roles in protecting plant against abiotic stresses, plant metabolites are also known to be important in enhancing defense of plant against pests and diseases. In addition to pre-formed chemical defenses, plants employ antimicrobial compounds that are induced only upon pathogen or pest attack⁵ and such compounds are known as phytoalexins. The generation of these compounds is mediated by a pathogen triggered activation of enzymes⁶, which possess unspecific inhibitory effects on a wide range of different pathogens. The compounds that involve in

increasing defense ability against pathogens in plants are formed via various metabolic pathways and can be broadly categorized in three major groups: Alkaloids, isoprenoids and shikimates (e.g., flavonoids)⁷.

Despite of increase in defense mechanism of disease infected plants, apparently, photosynthesis and other related processes such as transpiration and stomatal conductance tend to be reduced. Working with sweet orange plants (*Citrus sinensis*), Ribeiro *et al.*⁸ reported that plant infected by *Xylella fastidiosa* had lower rate of CO₂ assimilation transpiration and stomatal conductance. There were no differences between healthy and infected intact leaves regarding chlorophyll a fluorescence parameters were observed, except for the potential quantum yield of photosystem II which was higher in infected plants. Values of photosynthetic oxygen evolution, effective quantum yield of photosystem II, apparent electron transport rate and photochemical fluorescence quenching were higher in healthy leaves than those in the infected ones. The results suggested that low photosynthetic rates of sweet orange leaves infected with *Xylella fastidiosa* were linked with low stomatal conductance, biochemical alterations of photosynthetic machinery.

In this study, dieback disease development and changes in total phenol, total sugar, total protein, peroxidase activity, polyphenol oxidase activity and leaf photosynthesis following the inoculation of *E. mallotivora* on two papaya cultivars, Eksotika dan Eksotika II were monitored and documented.

MATERIALS AND METHODS

Plant material: Papaya (*Carica papaya*) cvs. Eksotika and Eksotika II seeds obtained from Seeds Production Unit of the Malaysian Agricultural Research and Development Institute (MARDI) were used in the study. Seeds were surfaced sterilized with NaOCl solution (1% chlorine concentration) for 30 sec, rinsed in sterile water were germinated in plug trays (5×5 cm cell) on peat moss. Three week old seedlings were transplanted to plastic pots (15 cm pots) filled with peat moss as substrate. A compound fertilizer (N, P₂O₅, K₂O: 8, 8, 8; 8 g plant⁻¹) was inserted into the pot at every 3 weeks. Plants were grown under greenhouse conditions with daily temperature means of 24°C (min) and 33°C (max).

Bacterial inoculum and plant inoculation: Pure culture of the *E. mallotivora* isolates obtained from Biotechnology Research Centre, MARDI was used a source of inoculum. The culture of the *E. mallotivora* was then sub-cultured to get more single colonies. The isolate was streaked onto Luria

Bertani agar media (Lennox, Becton, Dickinson and Company, Sparks, USA) and grown for 48 h at 30°C and colonies obtained was then transferred onto slant media and kept as stock culture.

Inoculation of bacteria on papaya seedlings was conducted as described by Schaad⁹. The inoculation was carried out on 8 week old papaya plants by using sterile needle attached to a syringe containing 50 µL calibrated *E. mallotivora* suspension at concentration of 1×10^8 CFU mL⁻¹ to the seedlings at the 5th node from the apex of young seedlings. Control plants were similarly inoculated with sterile water. Infected plants were then grown under greenhouse conditions, as described in earlier.

Measurement of disease development: Development of disease was monitored in two different techniques, by measuring the length of lesion and by recording visual symptoms. Length of lesions developed caused by the disease was measured on the stem and results were taken on day 3, 7, 9 and 11 after bacterial inoculation were carried out. Lesion length was measured as the distance from the points of inoculation to the farthest macroscopically visible extent of the lesion.

Disease symptom was assessed daily starting from the 2nd day of inoculation at the point of injection to the area surrounding the point of injection. The symptoms developed were recorded. The symptom was described for 14 days after inoculation with bacteria. Only physical observation via naked eyes was carried out to determine the symptom of this disease.

Total sugar: Total sugar content of papaya leaves was determined by using the Anthrone reagent method¹⁰. Samples from the whole third leaf from the apex, stem around the infection site and whole roots were taken. A 0.1 g of fresh samples were transferred to boiling tube and then hydrolysed in boiling water bath for 3 h with 5 mL of 2.5 N HCl. The volume was made up to 100 mL and centrifuged. To 1 mL of this supernatant, 4 mL of Anthrone reagent was added and then heated in boiling water bath for 8 min. The boiling mixtures were cooled rapidly by putting all the tubes on ice and the absorbance was read at 630 nm on spectrophotometer (Shimadzu UV-3101PC Spectrophotometer). The total sugar was calculated from a standard curve developed using glucose.

Total phenol: Total phenol in samples of whole third leaf from the apex, stem around the infection site and whole roots were estimated using Folin-Ciocalteu assay¹¹.

A 0.5 g fresh sample was digested with 30 mL methanol and then evaporated to dryness. The residue was dissolved in 0.5 mL methanol and volume was made up to 25 mL with distilled water. One milliliter of the extract was diluted to 6 mL with distilled water and 0.5 mL Folin-Ciocalteu reagent (1:1 diluted) was added. After 3 min, 1 mL of 35% Na₂CO₃ was added to the mixture and final volume was made up to 10 mL. The tubes were kept in darkness for 30 min and absorbance was recorded at 600 nm on a spectrophotometer. The total phenol content was calculated from a standard curve using gallic acid. Total phenol was expressed as milligram gallic acid equivalent.

Total protein: Total protein content in samples was determined by using the Bradford method¹², using Bovine Serum Albumin (BSA) as standard. Bradford reagent was prepared by dissolving 100 mg of Coomassie Blue G in 50 mL of ethanol and 100 mL of phosphoric acid (85%). The mixture was stirred for approximately 10 min. The solution was then diluted to 200 mL with distilled water and filtered. Then, the working solution of 10 mL aliquot of the Bradford concentrate solution was diluted (1:5) with water. A 100 mL of 1.2, 1.0, 0.8, 0.6, 0.3 and 0.1 mg mL⁻¹ BSA solutions was added to the respective tubes. A blank tube was included which was added with 100 mL of distilled water. A 5 mL of diluted Bradford reagent was added to each of the mixtures. The mixture was stirred and incubated for 20 min. Absorbance was read at 595 nm on spectrophotometer. The amount of protein in unknown tubes was determined by its absorbance and comparison to the standard protein curve.

Protein extraction for enzyme assays: Protein extraction was carried out by grinding 2 g plant tissues in a chilled mortar (chilled overnight in the refrigerator) with 1 mL cold 0.05 M phosphate buffer (pH 7). The homogenate was then centrifuged at 15000 g for 1 h at 4°C. Supernatant was stored at -20°C prior to enzyme assays.

Peroxidase activity: Peroxidase activity was estimated by following the changes in the appearance of the brown coloration resulting from guaiacol oxidation in the presence of hydrogen peroxide¹³. The reaction mixture containing 4 mL of readily prepared 0.2 M phosphate buffer (pH 5.8), 0.1 mL 0.02 M guaiacol, 1 mL 0.3 M H₂O₂ and 200 µL supernatant. Optical density was read at 470 nm for 1 min using spectrophotometer.

The unit of peroxidase activity was calculated by the empirical formula of Kokkinakis and Brooks¹⁴. Peroxidase activity was calculated using the formula below and expressed in unit/mg protein:

$$\text{Total activity} = \frac{\Delta\text{OD}/\text{min} \times \text{dilution factor}}{\text{Volume of enzyme used in the assay}} \times 1000$$

Polyphenol oxidase activity: Polyphenol oxidase activity was determined by following the intensity of dark-coloured polymeric compounds formed from catechol¹⁵. The reaction mixture containing 0.5 mL protein supernatant and 2.3 mL of 0.1 M phosphate buffer at pH 6.2. In the reaction mixture, 0.2 mL of 0.2 M catechol was added after 5 min pre-incubation and afterwards optical density was recorded at 420 nm.

The number of units of PPO activity in extracts was calculated using the following relationships:

$$\text{Enzyme activity} = V_{\text{max}} \times \text{Volume of enzyme assay}$$

The specific activity of PPO was calculated by dividing the enzyme activity by the total number of gram of protein present in an assay:

$$\text{Specific activity} = \text{Enzyme activity}/\text{gram protein}$$

and the activity was considered as one unit which is equal to 0.001 $\Delta\text{OD min}^{-1} \text{g}^{-1}$ fresh weight.

Gas exchange: Net photosynthesis, stomatal conductance and transpiration rate were measured on the most recent mature leaves (the third leaves from the top) on day 7 after inoculation using a portable closed system photosynthesis measurement system (Infra-Red Gas Analyzer, Li 6400, LiCor, Lincoln, Nebraska, USA). The measurement was taken around 12:00 mid-day using with 5 measurements for each treatment.

Experimental design and statistical analysis: The study was conducted in a Randomized Completely Block Design (RCBD) with four replications. Each plot consisted of 6 plants. Data obtained were subjected to analysis of variance (ANOVA) using Statistical Analysis System ver. 9.2 (SAS Institute Inc., Cary, NC., USA). Treatment means were compared using Least Significant Different (LSD) at $p < 0.05$.

RESULTS

Lesion length: Lesion length indicates the degree of symptom severity after bacterial inoculation. Results show that lesion length on the stem of papaya seedlings increased linearly with duration after inoculation (Fig. 1) for both cultivars. There was no significant difference observed among means of lesion

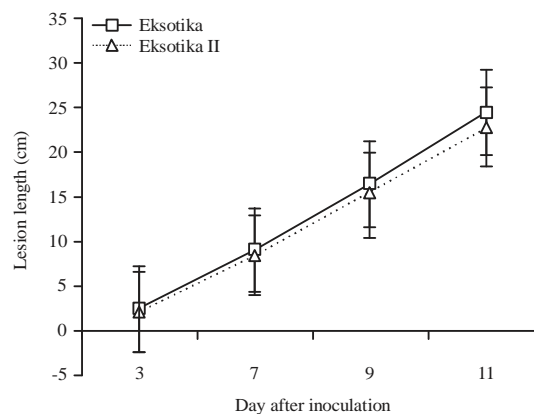


Fig. 1: Lesion length of papaya seedlings for papaya cvs. Eksotika and Eksotika II on day 3, 7, 9 and 11 after inoculation with *Erwinia mallotivora* (1×10^8 CFU mL^{-1})

length for the two cultivars and the trend continued until day 11 after inoculation. At day 3 after inoculation, the average stem lesion length was 2.28 cm and it has increased to 23.68 cm at day 11.

Visual symptoms: The visual symptoms of the dieback disease for both cultivars were the same. Papaya seedlings infected with 50 μL of 1×10^8 CFU mL^{-1} *E. mallotivora* suspensions showed 100% infection whereas, those inoculated with sterilised distilled water (control) did not show any infection. Symptoms can be seen starting from 3rd day after inoculation with greasy and small water soaked lesion at the point of inoculation. At 7 days after inoculation, the symptom became more obvious and lesion size was bigger. Dieback of the infected shoot started to occur at 9 day after inoculation with the wilting of upper part of the plant. By day 11 after inoculation, the plant was fully wilted and dead. After 11 days after inoculation, dieback had infected the whole plant and caused overall destruction to the plants. Table 1 shows the overall summary of disease symptoms recorded in this study. As the visual symptom was the similar for the two cultivars, only symptoms for one cultivars (cv. Eksotika) are shown in Fig. 2.

Total sugar: There was significant difference ($p < 0.05$) in total sugar in leaves for both cvs. Eksotika and Eksotika II (Table 2). Papaya cv. Eksotika II had higher leaf total sugar compared to cv. Eksotika. Inoculated plants contained higher total sugar (46.6 mg g^{-1}) compared to control plants (37.60 mg g^{-1}) in leaf of cv. Eksotika. For cv. Eksotika II, leaf total sugar of inoculated plant was 55 mg g^{-1} and the total sugar in control plant was

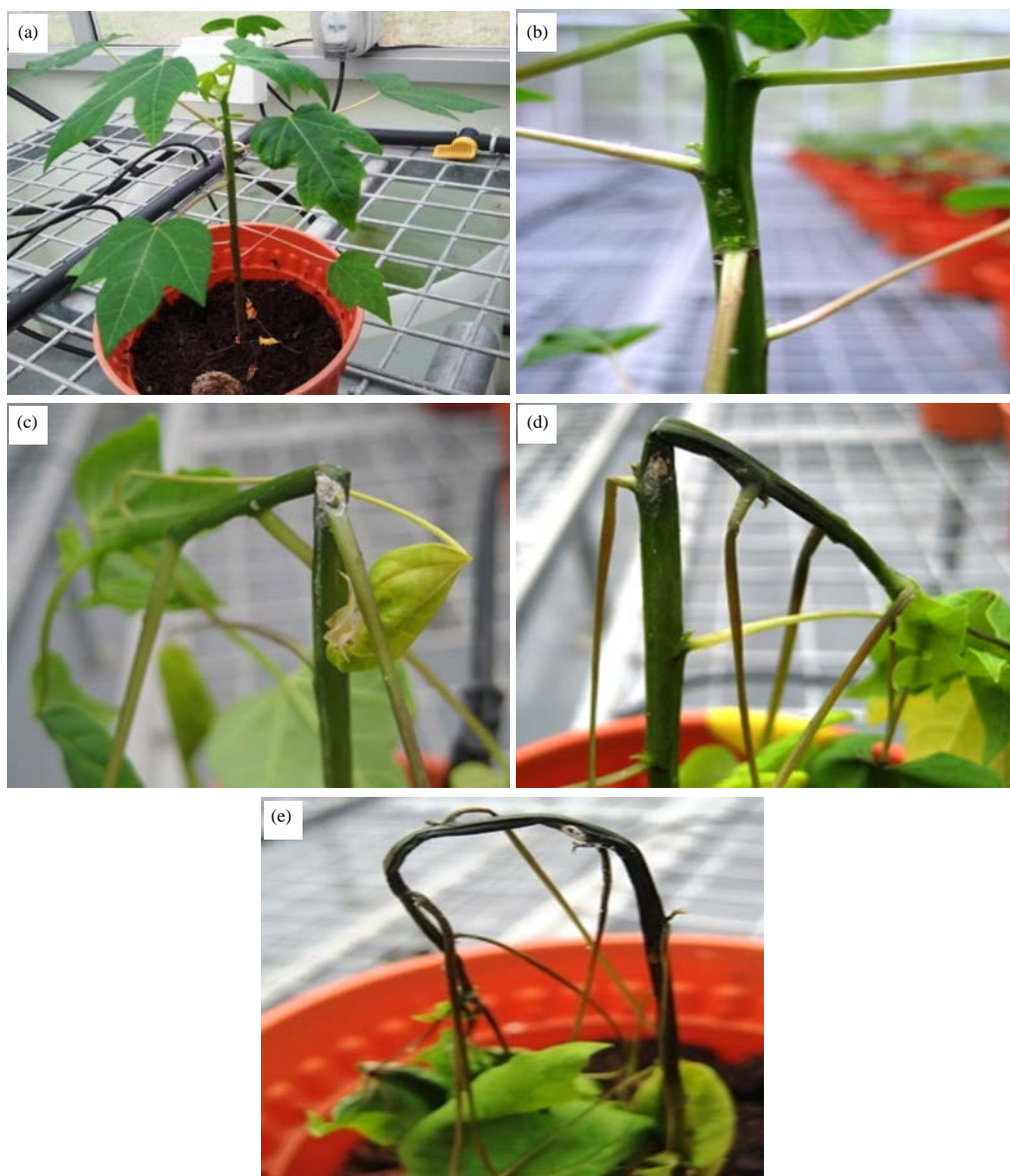


Fig. 2(a-e): Symptoms of disease on papaya cv. Eksotika when inoculated with 1×10^8 CFU mL⁻¹ *Erwinia mallotivora* (a) Control (sterile distilled water), (b) 3 days after inoculation (DAI), (c) 7 DAI, (d) 9 DAI and (e) 11 DAI. All plants were dead on day 11 of inoculation

Table 1: Summary of disease symptoms for papaya seedlings inoculated with *Erwinia mallotivora* (1×10^8 CFU mL⁻¹)

Days	Symptoms
1	No symptom
3	Small water soaked lesion at point of inoculation, about 3 cm in length
7	Increased in lesion size and greasy appearance, about 8 cm in length
9	Lesion size about 17cm, dieback of shoot and upper plant part wilt
11	Plant fully wilted and dead

43.5 mg g⁻¹. Total sugar in stem of inoculated plant was significantly lower with an average of 26.8 mg g⁻¹ compared to control plant (33.45 mg g⁻¹) for both cultivars (Table 3).

Results also showed there was no significant difference in root total sugar among cultivars (Table 4). Control plants had significantly higher root total sugar (29.55 mg g⁻¹) compared

Table 2: Total sugar, total phenol, total protein, peroxidase activity, polyphenol oxidase activity in leaves of papaya cvs. Eksotika and Eksotika II infected by *Erwinia mallotivora*

Cultivars	Treatment	Total sugar (mg g ⁻¹)	Total phenol (mg g ⁻¹)	Total protein (µg g ⁻¹)	Peroxidase activity (ΔOD min ⁻¹ g ⁻¹)	Polyphenol oxidase activity (ΔOD min ⁻¹ g ⁻¹)
Eksotika	Inoculated	46.60	85.14	19.54	0.25	0.48
	Control	37.60	51.14	18.95	0.23	0.35
Eksotika II	Inoculated	55.00	68.43	20.21	0.26	0.43
	Control	43.50	53.00	19.51	0.24	0.30
F-test						
Cultivar		*	ns	**	*	**
Treatment		**	ns	**	**	**
Cultivar×Treatment		ns	ns	ns	**	**

ns, *, ** indicate non-significant and significant at p<0.05 and p<0.01, respectively

Table 3: Total sugar, total phenol, total protein, peroxidase activity, polyphenol oxidase activity in stem of papaya cvs. Eksotika and Eksotika II infected by *Erwinia mallotivora*

Cultivars	Treatment	Total sugar (mg g ⁻¹)	Total phenol (mg g ⁻¹)	Total protein (µg g ⁻¹)	Peroxidase activity (ΔOD min ⁻¹ g ⁻¹)	Polyphenol oxidase activity (ΔOD min ⁻¹ g ⁻¹)
Eksotika	Inoculated	26.40	31.43	18.73	0.37	0.27
	Control	32.80	19.71	18.43	0.28	0.20
Eksotika II	Inoculated	27.20	33.14	19.29	0.34	0.26
	Control	34.10	23.86	18.68	0.29	0.21
F-test						
Cultivar		ns	*	**	ns	ns
Treatment		*	**	**	**	**
Variety×Treatment		ns	ns	**	**	**

ns, *, ** indicate non-significant and significant at p<0.05 and p<0.01, respectively

Table 4: Total sugar, total phenol, total protein, peroxidase activity, polyphenol oxidase activity in root of papaya cvs. Eksotika and Eksotika II infested by *Erwinia mallotivora*

Cultivars	Treatment	Total sugar (mg g ⁻¹)	Total phenol (mg g ⁻¹)	Total protein (µg g ⁻¹)	Peroxidase activity (ΔOD min ⁻¹ g ⁻¹)	Polyphenol oxidase activity (ΔOD min ⁻¹ g ⁻¹)
Eksotika	Inoculated	22.60	19.86	18.42	0.13	0.16
	Control	29.20	17.83	17.88	0.12	0.15
Eksotika II	Inoculated	23.30	22.43	18.59	0.16	0.18
	Control	29.90	19.86	17.99	0.14	0.16
F-test						
Cultivar		ns	ns	*	**	**
Treatment		**	ns	**	**	**
Cultivar×Treatment		ns	ns	ns	**	**

ns, *, ** indicate non-significant and significant at p<0.05 and p<0.01, respectively

to the inoculated plants (22.95 mg g⁻¹) for both cultivars. Overall, total sugar was highest in leaves followed by stem and roots. There was no marked interaction detected between cultivars and inoculation treatment for total sugar content in leaves, stem and roots.

Total phenol: There was no significant difference in total phenol in leaf and root of both papaya cultivars (Table 2, 4). The results also revealed that there was no significant difference in total phenol between inoculated plants and control plants in leaves and roots.

However, total phenol in stem of cv. Eksotika II was markedly higher (p<0.05) compared to those in cv. Eksotika (Table 3). There was significant difference (p<0.01) between inoculation treatments whereby inoculated plants (31.43 mg g⁻¹) contained higher total phenol concentration

compared to control plants (19.71 mg g⁻¹) in stem of cv. Eksotika. For papaya cv. Eksotika II, stem total phenol of inoculated plants was 33.14 mg g⁻¹ and the content in control plants was 23.86 mg g⁻¹. Overall, the highest total phenol was found in leaves, followed by stem and roots.

Total protein: Papaya cv. Eksotika II had significantly higher leaf total protein compared to papaya cv. Eksotika (p<0.05) (Table 2) with the respective average of 19.86 and 19.24 µg g⁻¹. Similarly, total protein in stem of papaya cv. Eksotika II was higher with average of 18.99 µg g⁻¹ compared to 18.58 µg g⁻¹ total protein in stem of cv. Eksotika (Table 3). Among the inoculation treatment, results showed that stem of diseased plants contained higher total protein (p<0.01). Similar trend of results were also observed for roots (Table 4).

Peroxidase activity: Overall, leaves of papaya cv. Eksotika II possessed higher PO activity compared to the PO activity in leaves of cv. Eksotika and its activity in the infected plants was higher compared to those in healthy plants (Table 2). Similarly, PO activity in the root of cv. Eksotika II ($0.148 \Delta\text{OD min}^{-1} \text{g}^{-1}$) was higher than PO activity in root of cv. Eksotika ($0.124 \Delta\text{OD min}^{-1} \text{g}^{-1}$). Differences in PO activity in stem among the cultivars however was not differed. Similar to leaves and roots, the stem of inoculated plants had a higher PO activity. For example, PO activity for cv. Eksotika was $0.37 \Delta\text{OD min}^{-1} \text{g}^{-1}$ for inoculated plants compared to $0.28 \Delta\text{OD min}^{-1} \text{g}^{-1}$ for non-inoculated plants (Table 3). Based on results shown in Table 3 and 4, there were interaction effects of cultivars and inoculation on PO activity ($p < 0.01$) but the effects were marginal as seen in Fig. 3.

Polyphenol oxidase activity: The PPO activity was highest in leaves compared to stem and root for both inoculated plant and control plant. The PPO activity was significantly increased

in inoculated plant compared to control in all plant parts for both papaya cultivars (Table 2-4, Fig. 4). Leaves of papaya cv. Eksotika had higher PPO activity ($0.413 \Delta\text{OD min}^{-1} \text{g}^{-1}$) than those in leaves of papaya cv. Eksotika II ($0.363 \Delta\text{OD min}^{-1} \text{g}^{-1}$). The PPO activity in the root of papaya Eksotika II was higher than PPO activity in root of papaya cv. Eksotika with their respective values of 0.169 and $0.153 \Delta\text{OD min}^{-1} \text{g}^{-1}$. There was no significant different in PPO activity in stem for both cv. Eksotika and cv. Eksotika II.

Leaf net photosynthesis, stomatal conductance and transpiration:

Results on the effects of cultivars and bacterial inoculation on gas exchanges are shown in Fig. 5. Leaf net photosynthesis of cv. Eksotika II was higher ($9.33 \mu\text{mol CO}_2 \text{m}^{-2} \text{sec}^{-1}$) compared to papaya cv. Eksotika ($8.48 \mu\text{mol CO}_2 \text{m}^{-2} \text{sec}^{-1}$). Inoculation of leaves with *E. mallotivora* markedly reduced leaf photosynthetic rate whereby the average value of leaf photosynthesis for the inoculated plant was $6.86 \mu\text{mol CO}_2 \text{m}^{-2} \text{sec}^{-1}$ whilst the

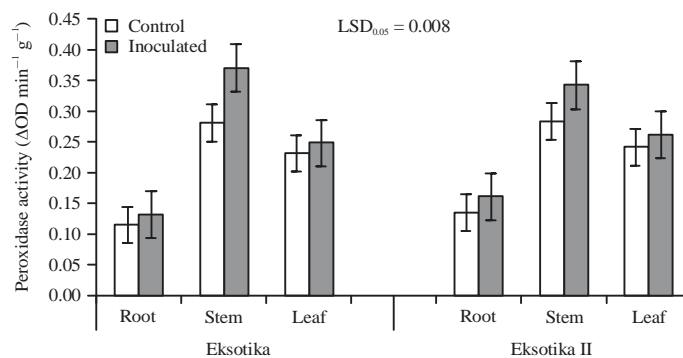


Fig. 3: Peroxidase activity in different part of papaya seedlings (roots, stem and leaves) of papaya cvs. Eksotika and Eksotika II inoculated with *Erwinia mallotivora* ($1 \times 10^8 \text{ CFU mL}^{-1}$)

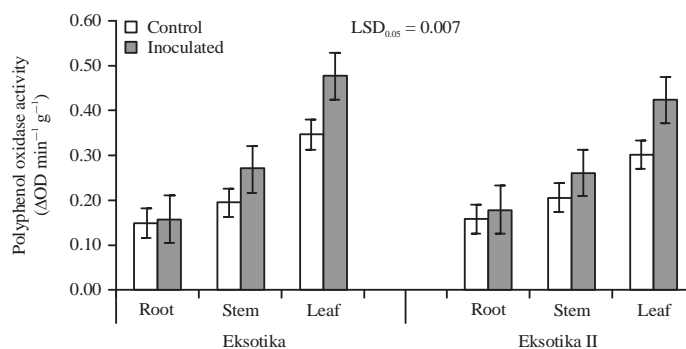


Fig. 4: Polyphenol oxidase activity in different part of papaya seedlings (root, stem and leaves) of papaya cvs. Eksotika and Eksotika II inoculated with *Erwinia mallotivora* ($1 \times 10^8 \text{ CFU mL}^{-1}$)

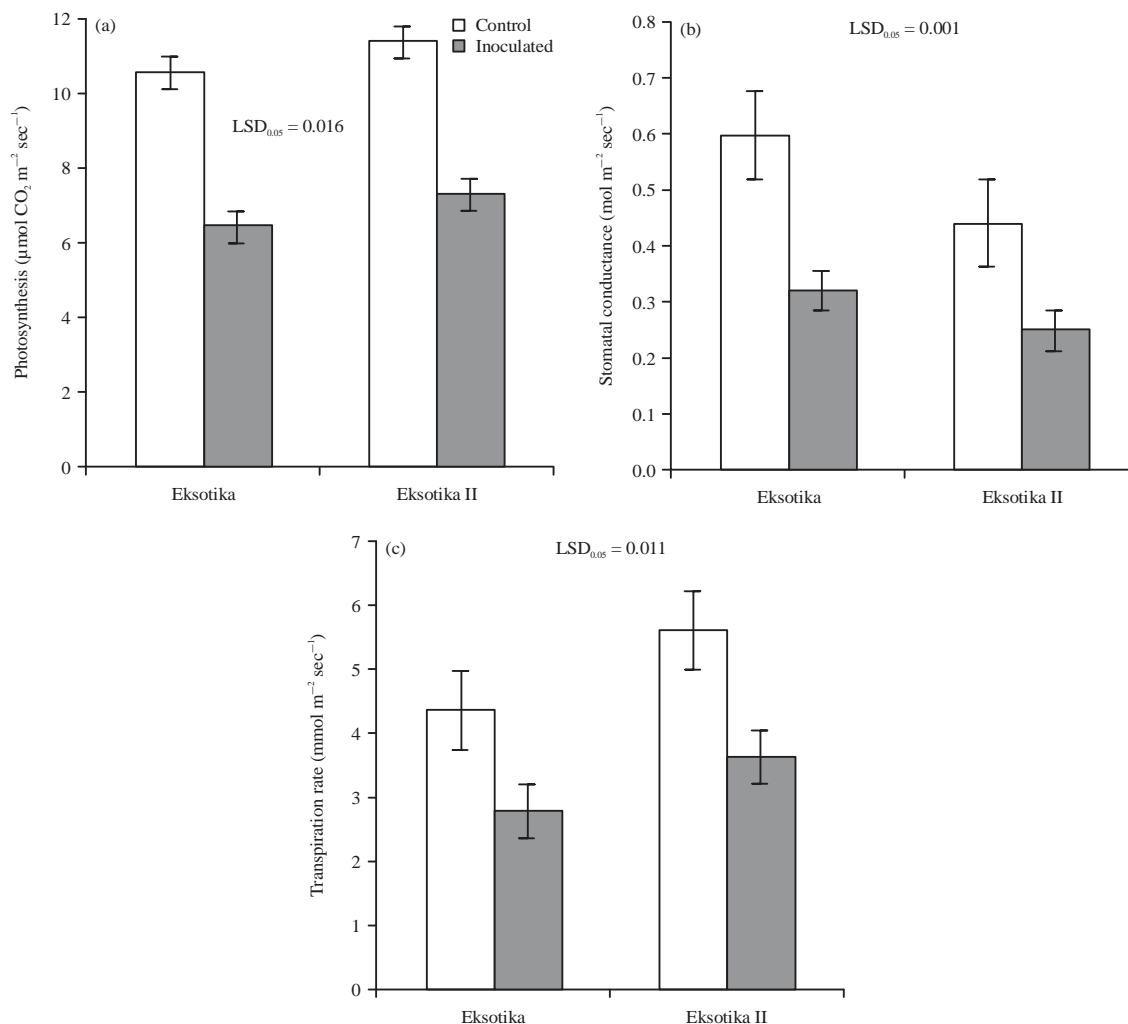


Fig. 5(a-c): (a) Photosynthesis, (b) Stomatal conductance and (c) Transpiration rate of papaya cvs. Eksotika and Eksotika II inoculated with *Erwinia mallotivora* (1×10^8 CFU mL⁻¹)

respective value for the non-infected was $10.96 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ sec}^{-1}$, a reduction of 37.4%. Similar to photosynthesis, stomatal conductance of inoculated plants was lowered by 44.2% ($0.29 \text{ mol m}^{-2} \text{ sec}^{-1}$) compared to the control plants ($0.52 \text{ mol m}^{-2} \text{ sec}^{-1}$). Similar trends of results were also true for leaf transpiration rate.

DISCUSSION

Papaya inoculated with bacteria *E. mallotivora* showed dieback symptom starting from 3 days after inoculation, with small water soaked lesion. At 7 days after inoculation, plant had a severe symptom with increased in lesion size and the greasy appearance starts to form. Lesion size started to develop even more and wilting of the upper plant parts occurred at 9 days after inoculation. The plants dies on day 11

after inoculation. The result recorded here was in line with that recorded by Amin *et al.*¹. Symptoms typical of dieback include bending of the growing tip as observed by Olabiyi¹⁶ indicating that the breakdown of cell wall and rotting of plant tissues mediated by pectolytic enzyme generated by the bacteria.

Leaves of the papaya plant used in the study contained the highest total sugar and this was followed by those in stem and root for both inoculated and control plant. Among the treated plants, total sugar in the leaves of inoculated plant was higher compared to control. The results recorded were paralleled with the earlier reports involving papaya plants, whereby carbohydrate content in diseased leaf tissue was higher than the carbohydrate in diseased stem and roots¹⁷. The phenomenon suggested that sugar could have been accumulated in leaves but cannot

be transported efficiently to the lower plant parts due the blockage or destruction of phloem. Similar observation were reported by Lepka *et al.*¹⁸ whereby the carbohydrate and starch in leaves were increased but decreased in root of tobacco and *Catharanthus roseus*.

There was an increase in total phenol in the inoculated plant compared to control plant in stem part. A significant increase in phenolic compounds was also seen earlier on the phytoplasma infected plum and apple that may contribute to plant defence system¹⁹. Accumulation of phenolic compound in disease infected plants was also observed in many other plant species including *Zea mays*²⁰, *Mimosa eleng*²¹ and *Solanum tuberosum*²². Pradeep and Jambhale²³ postulated that phenolic compounds and related oxidative enzymes are mostly considered as one of the important biochemical parameters for disease resistance and their accumulation at the infection site is proven to have a positive role in restricting disease development. Beside being toxic to pathogens, phenolic compounds may impede pathogen infection by increasing the mechanical strength of the host cell wall²⁴.

Higher total protein in inoculated plants shown here is consistent with the results obtained by other researchers²⁰. Generally, increase in protein content of disease infected plant is due to the activation of the defence mechanisms from the pathogen²⁵, called Pathogenesis Related-protein (PR-proteins) which include enzymes such as peroxidases, chitinases and β -1,3-glucanase²⁶. Proteins play an important role in plant defence in the form of various defence enzymes and other protein based non-enzymatic compounds²⁷.

Peroxidase could acts as plant expression of non-specific defence against stress and presence of injuries at tissue subcellular/molecular level²⁸. Peroxidase also help in lignification, wound healing as well as defence against pathogens and other biotic and abiotic factors²⁹. Results revealed that, the activity of the phenol oxidizing peroxidase and polyphenol oxidase was higher in disease infected plant tissue than in uninfected ones^{21,30,31}. In rice Paranidharan *et al.*³² detected higher peroxidase activity in leaf sheaths infected with *Rhizoctonia solani* and the results suggest that the increase in disease resistant of the plant against the pathogen can be associated with polymerization of cinnamyl alcohols to lignin, which is mediated by peroxidase. Similar trend of results was also observe for PPO activity in response to *E. mallotivora* infection which indicated that PPO is also involved in plant defence mechanism. There is no clear mechanism on how PPO negatively affect the growth of pathogens, but it may involve one or more of

these mechanisms: Generation of toxic compounds of PPO-generated quinones to pathogens and plant cells thus accelerating cell death, alkylation and reduced bioavailability of cellular proteins to the pathogen; cross-linking of quinones with protein or other phenolics; forming a physical barrier to pathogens in the cell wall and/or via quinone redox cycling leading to generation of H₂O₂ and other reactive oxygen species³³.

As expected, despite of many evidence shown in this study on the enhancement of defence mechanism of papaya following infection of the pathogen, but it is insufficient to counter the pathogenic effect of the bacteria. Beside the physical degradation of plants that could be seen visually, disruption of certain physiological process by the pathogen could have occurred much earlier before the physical damage. This has been shown here in the reduction of leaf photosynthesis, stomatal conductance and transpiration of the inoculated plants (Fig. 5). Reduction in photosynthesis might be attributable to stomatal closure which reduced the concentration of CO₂ within leaves. The presence of toxins, ethylene and/or hormones produced by pathogen may induce stomatal closure³⁴. The reduction in transpiration can be due to adhesion of bacterial colonies to the vessels, which may partially block xylem vessel, thus limiting water supply to mesophyll, thus affecting stomatal opening and photosynthetic biochemical reaction³⁵. Apparently, reduction of photosynthesis in inoculated plants do not totally shut-down the production of photosynthates that become the building block for synthesis of many beneficial compounds (sugars, phenols, protein and enzymes) discussed above. If the rate of disease development can be reduced, for example by boosting plant resistance, then magnitude of photosynthetic losses can be minimized, thus enhancing the resistance capability of the plant against the pathogen.

CONCLUSION

Erwinia mallotivora infection caused dieback disease symptom which occurred as early as 3 days after inoculation with the bacteria and plant started to die at 11 days after inoculation. Both papaya cvs. Eksotika and Eksotika II had the same physiological changes toward the infection. There were significant changes for both cultivars in biochemical and physiological parameters such as the total sugar, total protein, PO and PPO activity. *Erwinia mallotivora* infection also caused a reduction in photosynthesis, stomatal conductance and transpiration rate compared to those in the non-infected plants.

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