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Peroxidase and Polyphenol Oxidase Activity in Moderate Resistant and Susceptible *Vicia faba* Induced by *Aphis craccivora* (Hemiptera: Aphididae) Infestation

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ABSTRACT. Two faba bean, *Vicia faba* L., cultivars, Gazira2 and Misr1, representing cultivars moderately resistant and susceptible to aphids, were analyzed for peroxidase (POD) and polyphenol oxidase (PPO) activities induced by cowpea aphid, *Aphis craccivora* Koch. infestation. Two tissue types (whole plant [WP] and detached leaf [DL]), two infestation status (infested and uninfested), and three aphid infestation durations (1, 3, and 5 d) were considered in POD and PPO data analysis. Factorial analysis showed that only cultivar factor has a significant effect on both POD and PPO activity, especially on the first day after aphid infestation (P : 0.0003 and 0.001, respectively). Tissue type has no significant effect, indicating either DL or WP can be used for measuring POD and PPO activities. While the insignificant different of infestation status reflecting a constitutive resistant character in Gazira2. Mann–Whitney U -test showed that POD and PPO activities in Gazira2 were higher significantly when compared with Misr1 with P value 0.0006 and 0.0015 for POD and PPO, respectively. Repeated measures analysis indicates that the POD and PPO activities on Gazira2 were significantly higher when compared with Misr1. Additionally, POD activity changed significantly over the time in 1, 3, and 5 d after aphid infestation. We concluded that higher activity of POD and PPO in cultivar Gazira2 is strongly associated with their resistant characters.

Key Words: ROS, biochemical marker, Gazira2, constitutive

Plants have an ability to respond to insects feeding by altering the state of their enzymes, by either constitutive or inducible (Hildebrand et al. 1986, Nabity et al. 2006, Chen et al. 2009, Giordanengo et al. 2010, He et al. 2010). These enzymes finally affect insect feeding success (Lukasik et al. 2012). Overproduction of harmful reactive oxygen species (ROS) is the first mechanism in plants when they are injured by insect feeding, resulting in oxidative stress, which may cause cell and tissue damage (Michalak 2006, Gill and Tuteja 2010, Živković et al. 2010). Plants overcome this situation by producing an efficient enzymatic antioxidant defense system, which prevent and protect plant cells from oxidative damage by scavenging the ROS (Mittler et al. 2004, Gill and Tuteja 2010). Peroxidase (POD) and polyphenol oxidase (PPO) are among the enzymes, which play an important role in plant stress caused by insect feeding (Lattanzio et al. 2006, Jaiti et al. 2009, Ramirez et al. 2009, He et al. 2010). Both enzymes are widely distributed among plant Kingdom (Lattanzio et al. 2006, Dogan et al. 2007, Zhang et al. 2008).

POD is heme-containing monomeric glycoproteins that utilize either H_2O_2 or O_2 to oxidize a wide variety of molecules (Yoshida et al. 2003). Their activity is localized in the cytoplasm and cell wall (Heng-Moss et al. 2004, Chen et al. 2009). POD has a specific role in lignifications and strengthening the plant cell wall that is highly resistant to biodegradation (Schoemaker and Piontek 1996, Lee et al. 2007, Jaiti et al. 2009). PPO also contributes to lignifications (Ralph et al. 2008) and together with POD it consumes oxygen and produces quinones, which may reduce plant digestibility for the insect (Duffey and Stout 1996, Lattanzio et al. 2006, Jaiti et al. 2009, Ranger et al. 2009).

Artificially, PPO and POD activities can be elevated by increasing salinity (Wang et al. 1997, Nabity et al. 2006), adding jasmonic acid (Jaiti et al. 2009), soluble silicon (Gomes et al. 2005, Ranger et al. 2009), arsenate (Lin et al. 2008), or by introducing a gene to plants. Transgenic tobacco anionic POD is an example, which can express approximately up to 400 times higher POD activity than corresponding tissues of wild-type plants (Behle et al. 2002, Dowd and Lagrimini

2006). Tomato *TPX2* gene or sweet potato *swpa1* gene are other examples in which overexpression of the gene could confer increased salt tolerance or oxidative-stress tolerance (Botella et al. 1993, Yoshida et al. 2003).

The increased activities of these enzymes in a plant are considered as a resistant state of the plant to the insect pest (Wei et al. 2007, Ramirez et al. 2009, Gulsen et al. 2010). However, some other studies reported that there were no differences in the PPO activities between infested and uninfested buffalo grasses challenged to Chinch bug, *Blissus occiduus* (Heng-Moss et al. 2004). Similarly, no differences were reported in POD activities between some noninfested and infested cereal genotype to Russian wheat aphid *Diuraphis noxia* (Kurdjumov; Ni et al. 2001). Therefore, the biochemical response during plant–insect interaction might be specific, either increased and or decreased, depending on the plant or insect species (Chen et al. 2009).

Once the specific POD and PPO responses have been revealed, they can be used as biomarker (Heng-Moss et al. 2004) and to elucidate the mechanism relies on resistant plant (Gulsen et al. 2010). This biochemical response has been used for selecting Chinch bug resistant turf grasses and black pecan aphid, *Melanocallis caryaefoliae* (Davis) resistance in pecan germplasm (Chen et al. 2009) also in aphid resistance of alfalfa (Wei et al. 2007).

The cowpea aphid, *Aphis craccivora* Koch. is one of the most important pests of Faba bean, *Vicia faba* L. (Laamari et al. 2008, Larocca et al. 2011). Pesticides are the most common way to control the aphids (Sadeghi et al. 2009). However, aphids have an ability to develop pesticide resistance due to the small size, high reproductive capacity, and strong adversity adaptability. Nontarget effects of the use of pesticides are an important issue (Wei et al. 2007). The promising alternative is using plant resistance as a sustainable, safe, and economical alternative (Heng-Moss et al. 2004).

Recently, some resistant faba bean cultivars to aphids have been found, such as *V. faba* Minor, which is tolerant to *Aphis fabae* (Shannag and Ja'far 2007) and *V. faba* landrace V51, which is resistant

to *A. craccivora* (Laamari et al. 2008), but caution should be addressed for resistant-breaking biotypes, which have occurred in several plants–aphid systems (Dogimont et al. 2010). Unfortunately, the availability of insect-resistant crops is still rare (Klingler et al. 2001). One of the problems is the undesirable large scale insect bioassays, which have to be incorporated in breeding programs. Therefore, developing new means such as a biochemical marker for evaluating insect resistance efficiently is a challenge (Schoonhoven et al. 1998).

This study was conducted to establish a baseline biochemical information regarding the response of two enzymes (POD and PPO) in two faba bean cultivars (Gazira2 and Misr1) infested by *A. craccivora*. Both cultivars have been reported as moderately resistant and susceptible cultivars, respectively. Colony development study for some faba bean cultivars showed that Gazira2 was less preferred for *A. craccivora*, indicated by significantly fewer numbers of aphids after 14-d infestation. Feeding behavior studies using Electrical Penetration Graph (EPG) suggested that the longer duration of waveform F, which means more stylet penetration difficulties into the cell, was the possible resistant mechanism in Gazira2 (Soffan and Aldawood 2014a,b). The relations of the above results with POD and PPO activity were expected to get an understanding of resistant mechanism in Gazira2.

Materials and Methods

POD and PPO analysis were conducted in the Microbiology laboratory of Plant Protection Department, College of Food and Agriculture Sciences, King Saud University, Riyadh, Kingdom of Saudi Arabia. Faba bean cultivars were maintained in growth chambers with environment setting $26 \pm 0.03^\circ\text{C}$, $44 \pm 0.12\%$ relative humidity (RH, means \pm SE), and a photoperiod of 16:8 (L:D) h (recorded by HOBO data loggers, ONSET Co., Bourne, MA).

Plant Material. Faba bean cv. Gazira2 and cv. Misr1 were used for experiments, representing the moderately resistant and susceptible cultivars, respectively. The seeds were obtained from the Legume Research Unit Plant Production Department, College of Food and Agriculture Science, King Saud University. Seeds were germinated in a mixture of sand and peat moss (1:1) growth medium after soaking in water for 48 h. After 1 wk, seedlings were transplanted to plastic pots (diameter: 11 cm, height: 14 cm). Four granules per pot of complete fertilizer (N: 12%, P: 12%, K: 17%; BASF-Asoco Agro, Limburgerhof, Germany) was applied in the growth medium once at seedling stage (19-d-old plant). Watering was by drenching the pot for 150 ml once at every 2 d.

Both cultivars were prepared for POD and PPO analysis using 23–28-d-old plants having five true leaves. Only three true leaves, consisted of two leaf blade, laid between the most base and upper part of the plant were used.

Cowpea Aphids. Cowpea aphids were used to induce the activity of POD and PPO in the plant. They were obtained from a colony which was collected from alfalfa plants grown in Al Amaria, Riyadh, Kingdom of Saudi Arabia ($46^\circ 31'5.5518''$ N; $24^\circ 48'40.179''$ E). Single mother of an apterous adult aphid was used for the initiation of cowpea aphid culture on the faba bean cv. Misr. Before the beginning of the experiment, the cowpea aphid culture had been running for 8 mo. New faba bean seedlings were provided continuously to replace the old plant for the maintenance and continuous growth of aphid cultures.

Apterous nymphs were collected from the pure culture above. One hundred of the apterous nymphs were introduced on each true leaf, distributed equally in each leaf blade. A 60-ml transparent plastic cups (d: 7 cm, h: 2.5 cm) were used as a leaf cage only in whole plant (WP) to prevent aphid escaping from the chosen leaf. Aphid infestation on detached leaf (DL) following the same procedure as in WP. The same 60-ml transparent plastic cup was used to accommodate the DL. DL was kept its freshness by wrapping the leaf base with cotton. Water was filled into a plastic cup to keep the cotton wet. Uninfested plants were prepared with the same procedure as mentioned above without aphid infestation.

Plant Sampling. Three true leaves from each plant were sampled on 1, 3, and 5 d, respectively, to indicate the possible difference in PPO and POD activities across the infestation duration period. One leaf blade in each true leaf was considered as a single sample for both WP and DL. The sampled leaves were collected on a certain day according to infestation duration, and it was used directly for enzyme analysis. Each sample had six replications.

Enzyme Extraction. Fresh leaf blade having about 0.1–0.2 g was used for enzyme extraction. Each fresh leaf was grounded into powder using a mortar and pestle by immersing it first with liquid nitrogen. Phosphate buffer (100 mM, pH 6.0) 1.2 ml was mixed with the sample powder in a reaction tube, followed by incubation for overnight at 4°C . Centrifugation was conducted after incubation at 12,000 rpm for 15 min at 4°C . Supernatant obtained after centrifugation was used directly for enzyme analysis (Kavitha and Umesh 2008, Kumar et al. 2011).

POD Activity Measurement. POD activity was assayed according to Ramanathan et al. (2001) with modification. Enzyme extracts for each sample were taken (0.4 ml) then reacted with 3.2 ml 50 mM pyrogallol in 50 mM phosphate buffer (pH 6) and 0.4 ml of 3% hydrogen peroxide as an initiator. Directly, after adding the initiator, enzyme activity was measured as change of absorbance at wave length 430 nm for 1 min using a spectrophotometer (JENWAY 6051, Staffordshire, UK) at room temperature (Groppa et al. 1999). POD activity was expressed as change of absorbance/min/g fresh leaves (Kavitha and Umesh 2008).

PPO Activity Measurement. Ninety-six-well micro plates were used as a reaction container. Each well contained the reaction mixture of 150 μl 50 mM catechol in 50 mM phosphate buffer (pH 6.5) and 50 μl of enzyme extract. Catechol was used as a substrate for the enzyme. First reading was done before incubation in a micro plate reader (BIOTEK ELx808, Winooski, VT, US) at 490 nm and at room temperature to get an initial absorbance. Second reading was done after 1-h incubation at 38°C . Each reading was repeated twice for the means value. PPO activity units per leaf gram were expressed as change of absorbance at 490 nm (A490/g of leaf) from the first and second reading (Ni et al. 2001, Heng-Moss et al. 2004).

Experimental Design and Statistical Analysis. The experimental design was an entirely randomized factorial model (2 by 2 by 2). The two faba bean cultivars (Gazira2 and Misr1) were combined with two infestation status (infested and uninfested) and two tissue type (DL and WP). All the data were collected on three levels of aphid infestation duration (1, 3, and 5 d). Six sample data were collected as the replication number.

Data analysis was conducted using SAS ver. 9.2 (SAS Institute Inc. 2008). Each parameter in the experiment was tested for normality distribution using PROC UNIVARIATE with Shapiro–Wilk method. PROC GLM was used to evaluate the factorial analysis of variance (ANOVA) 2 by 2 by 2 (cultivar factor, infestation factor, and tissue factor) followed by Mann–Whitney *U*-test for means separation using PROC NPAR1WAY. Repeated measures analysis was conducted with PROC MIX to evaluate effects of cultivars, tissue type, and infestation status across the aphid infestation duration days (Madden et al. 1982). Square root (\sqrt{x}) transformation was applied prior analysis (Wilkinson and Douglas 1998, Osborne 2010).

Results

POD and PPO activities of two cultivars were analyzed firstly using factorial analysis 2 by 2 by 2, considering three main effects, which were cultivars (Gazira2 and Misr1), infestation status (infested and uninfested), and tissue type (DL and WP; Table 1). This test was conducted to confirm the possibility of interaction among the factors.

In overall factorial ANOVA result (Table 1), the model showed that only on the first day after aphid infestation, both POD and PPO have a significant *P* value (0.014 and 0.005, respectively), it means that the means weights of the eight groups were significantly different. While in 3rd day and 5th day after aphid infestation, it did not show the same result as in the day 1.

Table 1. Overall ANOVA factorial analysis (2 by 2 by 2) for cultivars (Misr1 and Gazira2), tissue type (WP and DL), and infestation status (infested and uninfested) related to POD and PPO activity at 3 d of aphid infestation durations

Source	Enzyme	Days after aphid infestation											
		1				3				5			
		DF	MS	F	P	DF	MS	F	P	DF	MS	F	P
Model	POD	7	1.30	2.88	0.014	7	0.96	0.98	0.457	7	1.25	1.38	0.239
	PPO	7	3.46	3.36	0.005	7	0.96	1.3	0.272	7	0.76	1.11	0.373
Error	POD	48	0.45			44	0.98			43	0.91		
	PPO	48	1.03			46	0.74			43	0.68		
Corrected total	POD	55				51				50			
	PPO	55				53				50			

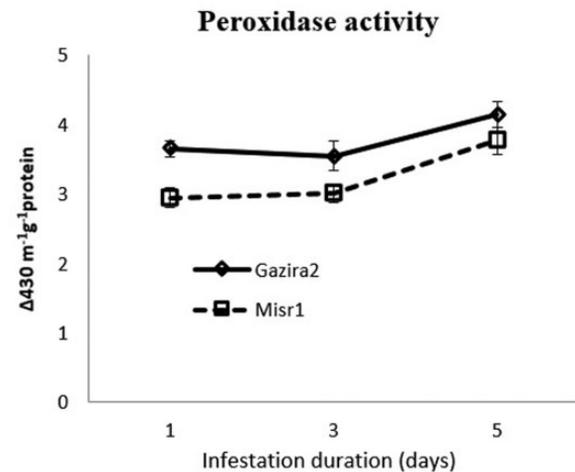
Table 2. Factorial analysis (2 by 2 by 2) for cultivars (Misr1 and Gazira2), tissue type (WP and DL), and infestation status (infested and uninfested) related to POD and PPO activity at 3 d of infestation durations

Source	Enzyme	DF	Days after aphid infestation								
			1			3			5		
			MS	F	P	MS	F	P	MS	F	P
Cultivar (Cv)	POD	1	6.84	15.07	0.0003	4.35	4.45	0.04	1.92	2.12	0.15
	PPO	1	12.36	11.98	0.001	1.31	1.76	0.19	3.33	4.87	0.03
Tissue (Ts)	POD	1	0.64	1.41	0.24	1.18	1.21	0.28	2.04	2.25	0.14
	PPO	1	3.62	3.50	0.07	0.88	1.19	0.28	0.22	0.32	0.57
Infestation (If)	POD	1	0.96	2.11	0.15	0.11	0.12	0.74	1.84	2.03	0.16
	PPO	1	0.08	0.07	0.79	0.43	0.58	0.45	0.04	0.06	0.81
Cv*Ts	POD	1	0.25	0.56	0.46	0.03	0.03	0.87	1.06	1.17	0.29
	PPO	1	2.07	2.01	0.16	0.39	0.53	0.47	0.11	0.16	0.69
Cv*If	POD	1	0.22	0.49	0.49	1.29	1.32	0.26	1.47	1.63	0.21
	PPO	1	2.80	2.71	0.11	1.16	1.56	0.22	0.06	0.09	0.77
Ts*If	POD	1	0.10	0.22	0.64	0.01	0.01	0.92	0.12	0.14	0.71
	PPO	1	0.59	0.57	0.45	0.003	0.0	0.95	0.28	0.40	0.53
Cv*Ts*If	POD	1	0.08	0.19	0.67	0.62	0.63	0.43	0.44	0.49	0.49
	PPO	1	1.31	1.27	0.27	3.04	4.09	0.05	1.35	1.97	0.17

More detail interaction effect is presented in Table 2. This analysis measured whether or not three factors (cultivar, infestation status, and tissue type) react differently. It showed that most of the main factor and its interaction have P value > 0.05 on both POD and PPO. P value < 0.05 only shown in the day 1 for POD and PPO (P : 0.0003 and P : 0.001, respectively) and in the day 5 for PPO (P : 0.03). Figure 1 showed a confirmation on this result, in which the two lines representing different cultivars were far away. Therefore, it can be concluded that only cultivar effect was an important factor, which should be considered, and there was no assumption of interaction. Further analysis then only concern about the main effect of cultivars factors. Mean comparison was conducted using nonparametric Mann-Whitney U -test to reveal which cultivars have a more POD and PPO activities (Table 3).

Mann-Whitney U analysis as showed in Table 3 confirmed the factorial analysis result, in which only cultivars factor gave a significant different regarding POD and PPO activities, especially in 1 d and 5 d after aphid infestation. On 1 d after aphid infestation, the P value was 0.0006 and 0.002 for POD and PPO, respectively, in which Gazira2 has higher activity as also shown in Figs. 1 and 2, whereas on 5 d after aphid infestation, the P value was 0.16 and 0.009 for POD and PPO, respectively. Using alpha 0.1, we may conclude that Gazira2 had significantly higher POD and PPO activities on 5 d after aphid infestation. It was noticed also in the Table 3, that, numerically, all POD and PPO values for Gazira2 were higher compared with Misr1.

Different tissue type and infestation status did not give any effect on the POD and PPO activities, most of P value in days 1, 3, and 5 have no significant different (Table 3). However, it is important to note that in tissue type, DL numerically has higher POD and PPO activities when compared with WP tissue, whereas infestation status has a slight effect

**Fig. 1.** POD activity between Gazira2 and Misr1, over three duration of aphid infestation (1, 3, and 5 d).

on the POD and PPO activities. Numerically, aphid infestation induced the elevation of POD and PPO activities especially as shown in the day 5 after aphid infestation (Table 3).

To get the overall view of the POD and PPO analysis over the time of infestation duration, the repeated measurement analysis was conducted (Table 4; Figs. 1 and 2). For POD, there was two important fact. First, the P value in all within subject (time) test was < 0.005 , indicating that the enzyme activity for POD do change over time. Second, among

Table 3. Mann–Whitney *U* analysis for POD and PPO activity at three duration of aphid infestation and three main effect (cultivar, tissue type, and infestation status; means \pm SE (*n*))^a

Source	Days after aphid infestation					
	1		3		5	
	POD	PPO	POD	PPO	POD	PPO
Gazira2	3.7 \pm 0.1 (32)	2.8 \pm 0.2 (32)	3.6 \pm 0.2 (29)	2.3 \pm 0.2 (30)	4.2 \pm 0.2 (27)	2.7 \pm 0.2 (27)
Misir1	2.9 \pm 0.1 (24)	1.8 \pm 0.1 (24)	3.0 \pm 0.1 (23)	2.1 \pm 0.1 (24)	3.8 \pm 0.2 (24)	2.2 \pm 0.1 (24)
<i>P</i> value ^b	0.0006*	0.0015*	0.12	0.39	0.16	0.09
DL	3.5 \pm 0.1 (28)	2.6 \pm 0.3 (28)	3.5 \pm 0.2 (24)	2.3 \pm 0.2 (26)	4.2 \pm 0.2 (23)	2.5 \pm 0.1 (24)
WP	3.3 \pm 0.2 (28)	2.1 \pm 0.1 (28)	3.2 \pm 0.2 (28)	2.1 \pm 0.1 (28)	3.8 \pm 0.2 (28)	2.4 \pm 0.2 (27)
<i>P</i> value	0.35	0.19	0.25	0.85	0.12	0.52
Uninfested	3.5 \pm 0.1 (28)	2.4 \pm 0.3 (28)	3.4 \pm 0.2 (24)	2.1 \pm 0.2 (26)	3.8 \pm 0.2 (24)	2.4 \pm 0.2 (24)
Infested	3.2 \pm 0.1 (28)	2.3 \pm 0.1 (28)	3.3 \pm 0.2 (28)	2.3 \pm 0.1 (28)	4.2 \pm 0.2 (27)	2.5 \pm 0.2 (27)
<i>P</i> value	0.29	0.27	0.81	0.17	0.13	0.72

^aNumber in bracket representing number of replication (*n*).

^b*P* value indicating significant difference between source of variation.

*Significant different at α : 0.05.

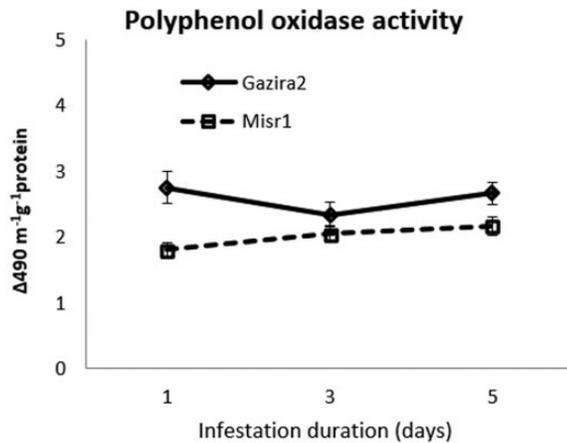


Fig. 2. PPO activity between Gazira2 and Misir1, over three duration of aphid infestation (1, 3, and 5 d).

the between groups test, only cultivars factor which has *P* value < 0.05. For cultivar factor, the *P* value of between groups (cultivars) and within subject (time) were 0.01 and <0.0001, respectively, and the interaction between them was not significantly different. Both significant value of between-group and within-subject test indicating that the cultivar factor has significant different over the time; consequently, in the Fig. 1., the lines for the two cultivars (Gazira2 and Misir1) were far apart and do change over time in a parallel way as a result of no significant interaction.

While for PPO activity, the between groups test which has *P* value < 0.05 were only cultivars factor. The within-subject test (time) and the interaction between cultivar and time were not significantly different. This result indicating that PPO activity was different in both cultivars, but it did not reflect a strong dynamic over the time (Fig. 2), as indicated by the *P* value for time, which is 0.05 (Table 4).

Discussion

Efficient, cheap, and reliable aphid plant-resistant markers are essential to support breeding programs. Biochemical markers were proposed and developed with an understanding that plants have the ability to respond insect infestation by changing biochemical states (Chen et al. 2009). POD and PPO are among the enzyme, which are released as a response to cell damage (Esteban-Carrasco et al. 2001, Michalak 2006, Ashry and Mohamed 2011). The presence of those enzymes had been used as a marker for resistance cultivars, e.g., in rice cultivar

Table 4. Repeated measurement analysis for POD and PPO activity over three duration of aphid infestation (1, 3, and 5 d), at three source of variation (cultivars, tissue type, and infestation status)^a

Source	POD				PPO			
	DF	MS	F	P	DF	MS	F	P
Cultivar (CV)	1	0.61	6.59	0.01	1	0.95	7.35	0.01
Time (T)	2	0.60	19.62	<0.0001	2	0.11	3.03	0.05
T*CV	2	0.02	0.71	0.49	2	0.06	1.78	0.17
Error (CV)	48	0.09			49	0.13		
Error(T)	96	0.03			98	0.04		
Infestation (IF)	1	0.00	0.03	0.86	1	0.16	1.12	0.29
Time (T)	2	0.55	18.75	<0.0001	2	0.11	2.99	0.06
T*IF	2	0.07	2.38	0.10	2	0.01	0.28	0.76
Error (IF)	48	0.10			49	0.14		
Error(T)	96	0.03			98	0.04		
Tissue (Ts)	1	0.14	1.36	0.25	1	0.04	0.3	0.58
Time (T)	2	0.60	19.79	<0.0001	2	0.11	2.88	0.06
T*Ts	2	0.02	0.75	0.48	2	0.00	0.03	0.98
Error (Ts)	48	0.10			49	0.15		
Error(T)	96	0.03			98	0.04		

^aData were transformed using square root prior to analysis.

against rice strip virus (Hao et al. 2011), resistant turf grass against chinch bug (Gulsen et al. 2010), and in alfalfa against *Aphis medicaginis* Koch. (Wei et al. 2007).

In this study, a factorial analysis showed that the difference of POD activity was related to cultivars factor, particularly in 1 d after aphid infestation. Mann–Whitney *U*-test confirmed that Gazira2 considerably has higher POD activity when compared with Misir1, especially on 1 d after aphid infestation, whereas on 3 and 5 d after aphid infestation, they did not differ significantly. However, numerically Gazira2 has the same trend as on day 1. Across the different time after aphid infestation, repeated measurement analysis showed that the higher activity in Gazira2 over Misir1 was occurred over the time (1, 3, and 5 d after infestation), especially on POD.

Upper regulation of POD activity for resistant cultivar was in agreement with the study of *A. medicaginis* Koch on resistant alfalfa (Wei et al. 2007) and Chinch bug, *Blissus occiduus* Barber on buffalo grasses *Buchloe dactyloides* (Gulsen et al. 2010). Higher POD activity might explain the possible mechanism of resistant Gazira2 against aphid. As previously reported in feeding behavior and biological studies, aphid population number was less with cv. Gazira2 after 14 d feeding compared with Misir1. Feeding behavior study showed that this may be due to longer duration of waveform F, which was present in Gazira2, indicating more difficulties of Aphid when penetrating their stylet into

cells (Soffan and Aldawood 2014a,b). Therefore, it was suggested that longer waveform F duration had strong relationship with increased activity of POD, which act to strengthen the cell walls. The resistant character of cv. Gazira2 seems a constitutive not inducible, supported by statistically insignificant value of the infestation status factors (Table 2). Noninducible POD activities by infestation status were in contrary with Zhang et al. (2008), who reported that *Bemisia tabaci* infestation could induce PPO and POD of cucumber seedling. It also reported the increase of POD by *Spilosoma virginica* on the halophyte, *Atriplex subspicata* (Nabity et al. 2006). In our result, it was suggested that the level of aphid infestation or the feeding behavior of *A. craccivora* did not reach levels where it could induce the overproduction of ROS, which finally may result in oxidative stress or cell damage, indicated by no symptom appeared in the faba bean after *A. craccivora* infestation. This fact, along with the finding that for symptomless aphid feeding (*Rhopalosiphum padi*) did not elicit any changes of POD activity in cereals compared with the control. While Russian Wheat aphid, *Diuraphis noxia*, which caused chlorosis during feeding, could elicit a 9-fold increase of POD on susceptible “morex” barley and a 3-fold on resistant “Halt” in comparison with control leaves. It was suggested that *D. noxia* feeding probably result in oxidative stress to the wheat plant (Ni et al. 2001). Regarding the tissue type, there were no significant differences between WP and DL assays as presented in Table 1. Which means both types of tissue can be used to measure the POD activity.

PPO had similar activity as POD regarding the response to aphid feeding. Factorial analysis and Mann–Whitney *U*-test showed that cultivar factor was the only factor affecting the PPO activity in which Gazira2 has higher PPO activity when compared with Misr1 (Tables 1 and 2). This result was in agreement with the increasing response of PPO activity in resistant hybrid poplar against the aphid *Chaitophorus leucomelas* Koch. (Ramírez et al. 2009) also in *Macrosiphoniella sanbourni* Gillette aphids on resistant chrysanthemum plant (He et al. 2010).

Through this study, it might be concluded that POD and PPO enzymes have a potential to be used as a biochemical marker to indicate the resistant cultivars among faba bean cultivars.

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Conflict of Interest

The author(s) declare that they have no competing interests.

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