Peroxidase activity and phenolic content in barley and wheat infested by cecidomyiid insects

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Abstract— Two species of *Mayetiola* (Cecidomyiidae), *Mayetiola destructor* (Say) and *Mayetiola hordei* Keiffer are the most destructive insect pests of wheat and barley, respectively, in Morocco. Infested plants are stunted, will stop growing, and eventually die. The objective of the present study was to understand mechanisms of cereals' responses to induced stress by these pest's attacks and determine the peroxidase activity and phenolic content in infested wheat and barley plants. Two susceptible cultivars were used in this experiment; Nesma, a bread wheat variety, and Kanby, a barely. The peroxidase activity and phenolic content in the infested and check plants were measured. The results showed that peroxidase values of infested barley and wheat plants ranged from 700 to 1850 and from 1380 to 2100 U/g fresh weight, respectively, while the total phenolic content ranged from 200 to 320 and from 300 to 450 µg/g fresh weight, respectively. A linear relationship existed between peroxidase activity and total phenolic content in both barley and wheat infested and not. The present experiment showed that the peroxidase activities and the total phenolic content were significantly increased after infestation of barley and wheat by the two insect species.

Index Terms— Peroxidase activity, phenolic content, Wheat, barley, *Mayetiola destructor*, *Mayetiola hordei*.

1 INTRODUCTION

THE genus *Mayetiola* (Diptera: Cecidomyiidae) causes significant economic losses in cereals. Two sympatric species of *Mayetiola* have been recognized as serious damaging pests in semiarid Morocco. *Mayetiola destructor* (Say) is found on both wheat and barley, but it is predominant on wheat where no gall is formed [1], [2]. *Mayetiola hordei* (Kieffer), the "barley stem gall midge" is found exclusively on barley and produces stem galls. *Mayetiola destructor* infests both bread wheat *(Triticum aestivum L.)* and durum wheat *(Triticum turgidum L. var. durum)* [3], [4]. The damage caused by *Mayetiola destructor* can result in total loss of the wheat crop if high infestation occurs during the early stages of development [5]. The damages caused by *Mayetiola hordei* in barley are not significantly different from those caused by *Mayetiola destructor* [6], [7]. As a group, gall midges produce plant galls on the buds, stems, leaves, flowers, and fruit of dicotyledonous, monocotyledons, gymnosperms, ferns, and mushrooms [8].

The responses of plants and crops to biotic stresses such as bacteria, viruses, parasites and insects are varied and generally involve some metabolic alterations. Every year, those stresses cause considerable losses in crop quality and productivity [9], [10]. The stresses induce some alteration in protein synthesis which include overall changes in protein synthesis or changes in the level of specific proteins [11]. The changes depend generally on the nature, duration and severity of the stress and are characterized by increases or decreases in existing proteins or the novo appearance of proteins [12], [13], [14].

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Plants possess a complex range of enzymatic and several secondary metabolites that can protect cells from damages such peroxidases and phenolic compounds [15], [16], [17]. Evidence is presented showing that peroxidases catalyze the polymerization of phenolic compounds to produce a variety of products which may take part in the defense system of plants against pathogens or parasites [18], [19], [20], [21], [22]. Moreover, the oxidation of phenolic compounds generally leads to the production of quinines [23], [24], which are highly toxic compounds responsible for the generation of reactive oxygen species [25]. Phenolic compounds are intermediates in the phenylpropanoid production and lignin biosynthesis [26], [27]. Phenolic alcohols are cross-linked into the cell wall matrix by the activity of peroxidase enzymes [28]. Peroxidases are free radical scavengers that utilize hydrogen peroxide as a substrate [27], [29], [30]. Peroxidase activity can be induced under biotic and abiotic stresses to accommodate lignin biosynthesis and other stress response pathways [31], [32], [33], [34]. Some indicators of stress response can be measured using biochemical assays for total phenolic compounds and peroxidase enzyme activity [35].

The aim of this study was to understand the changes in selected metabolic and biochemical parameters in wheat and barley plants under infestation by Hessian fly and the barley stem gall midge, respectively. The specific objective was to understand mechanisms of cereals' responses to induced stress by these pest's attacks. Biochemical indicators concerned by this research are peroxidase activity, phenolic compounds and total protein.

2 MATERIALS AND METHOD

2.1 Plant material and growth conditions

2.1.1 Insects

The insects used in this study were *Mayetiola destructor* and *Mayetiola hordei*. The insects were taken from a culture maintained at the Entomology Laboratory of the National Institut

of Agronomic Research-regional center of Settat. These cultures originated from puparia collected from bread wheat and barley fields in Sidi El Aidi Agricultural experiment station (INRA-Settat). Infested plants are maintained in wooden flats at 20±2°C until adult emergence.

2.1.2 Plant material

Two susceptible cultivars were used in this experiment; a bread wheat *(Triticum aestivum L.)*, cv. Nesma and a barley *(Hordeum vulgar L.)*, cv. Kanby. They were seeded in separate standard wooden flats (54 x 28 x 8 cm), containing soil and vermiculite. The flats were kept in a greenhouse under a temperature of 20±2°C, and were watered two to three times a week.

2.1.3 Infestation protocol

The infestation was carried out when the plants reached the second-leaf stage. Each flat of wheat or barley was caged separately with a cheesecloth tent. Approximately, 50 newly mated females of *Mayetiola* spp. were released under each tent. The infestations were made in the morning between eight and ten o'clock corresponding to the mating period of the insect. Three days later, the cheesecloth tent was removed. Three kinds of infestation were realized. For barley, ten flats were infested by *Mayetiola hordei* and ten others by *Mayetiola destructor*. For wheat, ten flats were infested by *Mayetiola destructor*. No infestation was done with *Mayetiola hordei* on wheat as it is not a host. For each cultivar of barley and wheat, ten flats were kept as checks with no infestation, and were grown under the same environmental conditions.

2.1.4 Plant sampling

The different plant samplings for the laboratory analysis were carried out at different age of the cultivars, corresponding to the life cycle of larval development. Thus, three stages: 15th, 25th and 35th days of plant age were chosen corresponding respectively to the first, second and third instars of *Mayetiola* ssp. [6], [7]. The second (25th) stage was named the Feeding-Stage. After the 15th day of plant growth, larvae reached the base of the stem and began feeding. The third stage (35th) was named the Nonfeeding stage [6], [7].

Several plant samplings were withdrawn from the wooden flats at the 15th, 25th and 35th day of plant growth in both infested and check flats. The fresh plant material was kept in ice and the laboratory analysis was carried out immediately after.

2.2 Total Phenolic concentration

2.2.1 Extraction

The total phenolic assay was performed by a version of the method of Singleton et al., [36]. Fresh material (1g) was homogenized and ground in a cold mortar containing 3 ml of a mixture of methanol-water (80/20, v/v). The homogenate was then centrifuged at 10 000 g for 10 min at 4°C and the supernatant was used for the phenolic concentration measure.

2.2.2 Assay for total phenolics

About l ml of supernatant was taken in a test tube and the following reagents were added: 1 ml methanol, 5 ml deionized water and 0.5 ml of 50% (v/v) Folin-Ciocalteau reagent. The same procedure was followed for all samples, with 1 ml of methanol for the blank. Tubes were covered and placed in the dark for more than 30 min, then swirled. Absorbance was measured at 725 nm, using a blank as a baseline. Standard curves were prepared using cafeic acid.

2.3 Total enzymatic activity of peroxidase

2.3.1 Extraction

Peroxidase was extracted from the plant tissue under buffered, cold conditions according to Baaziz and Saaidi method [37]. Fresh material (1g) was homogenized and ground in a mortar in 3 ml of 0.005 M phosphate buffer (pH 7) containing 0.1 M 2-mercaptoethanol. The homogenate was then centrifuged at 20000 g for 20 min at 4°C. The supernatant was used for the enzyme activity, for the protein assay and for peroxidase electrophoretic analysis.

2.3.2 Assay for guaiacol peroxidase and total protein

Peroxidase activity was assayed at 30°C as described by Souza and MacAdam [38], using guaiacol as the substrate. The assay mixture for spectrophotometer determination of peroxidase activity consisted of 1 ml 0.1 M acetate buffer (pH 5.4), 2 ml 30 mM guaiacol (freshly prepared) and 0.1 ml enzyme buffer extract diluted; the reaction was initiated by the addition of 0.05 ml of 3% hydrogen peroxide continuously for 6 mn (backman spectro). The activity was measured at 470 nm and expressed on a fresh weight basis (unit per gram fresh weight) (U/g FW). The unit was defined as the amount of enzyme that gave a change in absorbance of 0.1 in 1 min.

2.4 Total proteins

The total protein was determined with Folin-phenol regent according to Lowry et al., method [39], using bovine serum albumin as a standard.

2.5 Statistical analysis

Statistical differences between infested and control plants were determined by an LSD test at the 5 or 1 % level. The limit of significant level was accepted at $p<0.05$. The significance of correlations between peroxidase activity and total phenolic content in plants were studied using the Spearman method.

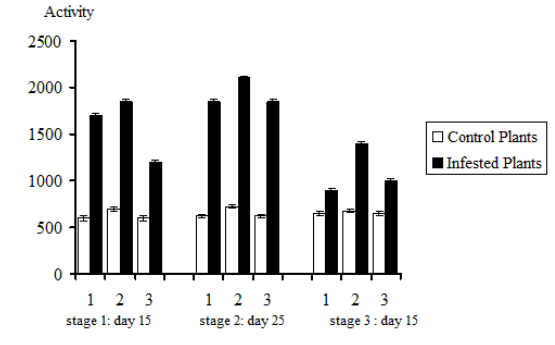

Fig. 1. Change in peroxidase activity in barley and wheat infested by *Mayetiola* spp. 1: Barley with *Mayetiola hordei*, 2: Wheat with *Mayetiola destructor*, 3: Barley with *Mayetiola destructor*.

3 RESULTS

3.1 Effect of larval feeding on peroxidase activity

The effects of cecidomyiid larval feeding on peroxidase activity in barley and wheat are shown in figure 1. The activity of this enzyme was similar in check plants of both barley and wheat, and did not exceed 700 U/g FW for the three growth stages tested. However, the analysis of infested plants showed that the peroxidase activity increased significantly ($p<0.005$) as compared to the control in both barley and wheat plants. The increase occurred at the first period (to day 15) of infestation and continued to increase during the second period (to day 25) and the values recorded increased more than three fold. In the last period (to day 35), a light decrease of peroxidase activity was noted but was significantly higher than that recorded in control plants. In general, the peroxidase activity in both wheat and barley infested by *Mayetiola* spp. presented similar evolution during all the growth stages tested. However, the peroxidase activity was significantly higher in wheat than in barley ($p<0.05$); the maximum values observed were 2100 U/g FW in wheat and only 1850 U/g FW in barley. Those values were recorded at day 25 after infestation. The result showed also a significant difference in peroxidase activity between the barley infested by *Mayetiola hordei* and barley infested by *Mayetiola destructor*. This difference was observed during the first stage (day15) (1700 vs 1200 U/g FW, respectively) and during the third stage (day 35) (900 vs 1000 U/g FW, respectively).

3.2 Phenolic compounds concentration

The mean changes in phenolic compounds concentration in barley and wheat infested by *Mayetiola* spp. are given in figure 2. The mean total phenolic compounds values in both barley and wheat check plants and during the three stages (15, 25 and 35th days) were similar; no significant difference was observed. However, after infestation, a significant difference in phenolic compounds concentration was noted, between the control and infested plants, in both wheat and barley ($p<0.005$). The increase in total phenolic compounds concentration was observed from the first stage and reached a maximum particularly during the second stage (day 25) (450 and 320 µg/g FW in wheat and in barley, respectively). During the last stage of the experiment, the total phenolic compounds concentration decreased but stayed higher than the control. However, the comparison among the three infestations showed a significant difference in total phenolic compounds in wheat and in barley. The phenolic compounds concentration was significantly higher in wheat than in barley ($p<0.05$). This significant difference was observed especially during the second stage of the experiment (450 vs 320 µg/g FW). The result also showed that the infestation of barley by *Mayetiola hordei* or by *Mayetiola destructor* induced a higher increase in total phenolic compounds but no significant difference between the two species infestations was observed.

3.3 Assay for total proteins

The effect of infestation by *Mayetiola* spp. on protein concentration in wheat and barley is presented in figure 3. The mean protein concentration in all control plants was similar in barley and wheat and estimated between 8 and 9 mg/g FW during the three stages of the experiment. When the infestation was carried out on wheat and barley, the amount of the protein concentration significantly increased ($p<0.005$). The increase in the protein concentration was observed from the first stage to the last stage of the experiment and the concentration peak was noted during the second stage (day 25). In this period, the recorded concentrations in protein were up to 2.5 and 2 fold in wheat and in barley infested plants, respectively. As shown in figure 3, the protein concentration in barley infested by *Mayetiola hordei* or *Mayetiola destructor* showed similar patterns and no significant difference was observed.

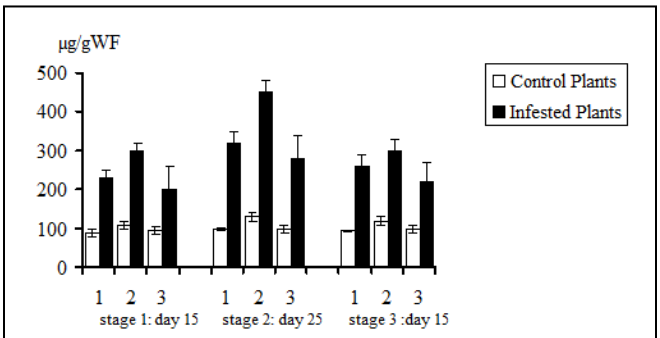

Fig. 2. Change in phenolic compounds in barley and wheat infested by *Mayetiola* spp. 1: Barley with *Mayetiola hordei*, 2: Wheat with *Mayetiola destructor*, 3: Barley with *Mayetiola destructor*.

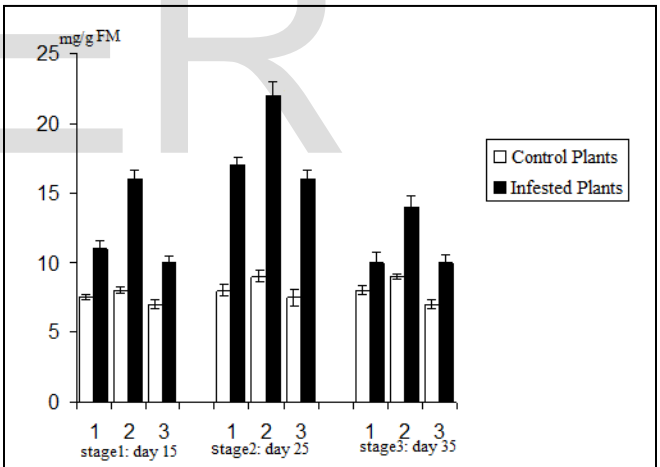

Fig. 3. Change in protein in barley and wheat infested by *Mayetiola* spp. 1: Barley with *Mayetiola hordei*, 2: Wheat with *Mayetiola destructor*, 3: Barley with *Mayetiola destructor*.

3.4 Relationship between peroxidase activity and phenolic compounds

We determined the correlation coefficient between peroxidase activity and phenolic compounds in both barley and wheat infested and not infested (figure 4). We considered all samples (28 x 3). The linear correlation coefficient obtained was 0.92 ($p<0.001$).

3 DISCUSSION

Plants are continually exposed to a vast range of potential parasites and pathogens [40], [41]. As a result, they have

evolved intricate mechanisms to recognize those threats and protect themselves by setting up defense responses to restrain the invading agents [42], [43], [44]. In this study, the biotic stress was realized by the infestation of barley and wheat plants by *Mayetiola* spp. During the course of cecidomyiid larvae penetration in wheat and barley cells, besides the chemical secretion, the larvae may causes mechanical signal by the physical pressure on the plant cell. Response of the plant to *Mayetiola spp* attack was little documented. To our knowledge, this study was the first report on the change in some biochemical parameters in cereals infested by *Mayetiola* spp. in Morocco.

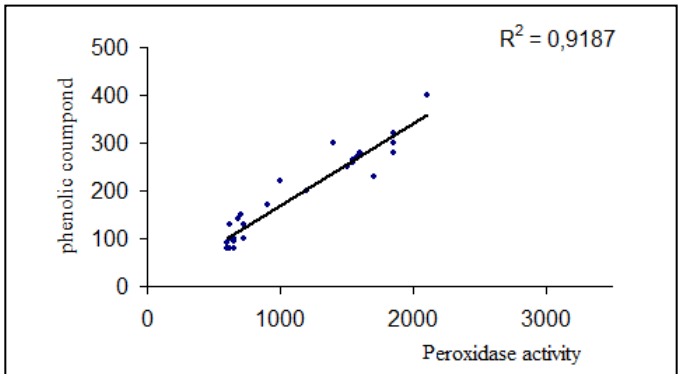

Fig. 4. Correlation between peroxidase activity and phenolic compounds in both barley and wheat infested and not infested by *Mayetiola spp*.

It is known that peroxidases activities, phenolics and protein concentrations are the principal components to undergo modifications in plants subject to biotic and abiotic stresses [45], [46], [47], [48]. Phenolic compounds are among the most widely distributed secondary products in plants [49], [50], since they are known to accumulate in response to infections in several species. It has also been suggested that they play a potential role in disease resistance [51]. Our result indicates that the concentration of phenolic components obviously increased after induced cecidomyiid stress in barley and wheat. The results suggest that phenolic concentrations are sensitive to the stress stimulus. Our result corroborates those of the studies on *Brassica napus L.* infested by *Pieris brassica* larvae [52], and tomato plants infested by by *Fusarium oxysporum f. sp. lycopersici* which also induce a significant increase in phenolic compounds [53]. It has also been shown that host plants infested by *Agrobacterium tumefaciens* developed gall formation and an increase in total phenolic compounds [54], [55], [56].

High concentrations of antioxidant enzymes have been found in responses to stress [57]. The present experiment showed that the peroxidases activities were significantly increased after infestation of barley and wheat by *Mayetiola spp.* Changes in peroxidases activities seem to be related to the interaction between barley or wheat and *Mayetiola* spp. This result was consistent with the evidence by Vanacker et al., [58] which showed in barely that the number of antioxidative compounds and enzymes changes in the apoplast of barley leaves inoculated with powdery mildew. This observation would suggest that in cereals, the peroxidases may be involved in the defense response against insect parasitism. Peroxidase activity in date palm showed an increase, correlating with the level of resistance to the Bayoud disease [37]. Also, it has been observed that peroxidase levels increase following chinch bug and aphid feeding in tolerant buffalo grass, sorghum, and barley [59], [60], [61], [62]. Recently, it was demonstrated that peroxidase activity increased with increasing number of sawfly, *Pontania vesicator*, galls per leaf of *Salix fragilis*. Similarly, peroxidase activity in leaves of *Acer saccharinum* increased along with increasing level of infestation with *Vasates quadripes* [63]. The enzymatic activity of peroxidases was elevated at the attack site of rice seedlings [64].

In previous studies, it has been suggested that the protein accumulation was observed in plants infested by insect pathogens and play a role in limitation of pathogen propagation [65]. In the present study, barley and wheat infestations with cecidomyiid species induce a higher protein accumulation. This accumulation could be explained by the mechanisms of plant defense responses resulting from plant-insect interaction.

The total phenolic compounds, the total proteins and activity of peroxydases in wheat and barley infested plants presented similar evolutions during a three-periods experiment. Increases of concentrations and activity of these compounds were noted at 15th day after infestation, and it reached a peak on the 25th day after infestation. After this stage, the concentrations and activity of these compounds showed a decrease trend. This study showed that during the first days of the infestation, there were no visible morphological changes in the infested plants despite the increase in phenolics and proteins concentrations and peroxidases activities. After this period and during the second stage (day 25), also named the feeding stage, a discoloration and gall formation were seen on the stem of the barley and wheat infested plants. These symptoms corresponded to the attained peak of the three components in barley and wheat infested plants as compared to the check plants. This could be in relation with gall formation. The wheat-Hessian fly *(Mayetiola destructor)* interaction has much to offer to both plant pathologists and entomologists as a model for investigations of insect-plant interactions and insect-induced plant gall formation [66].

The increase in the studied compounds was higher in wheat than in barley. A formed gall was more visible in barely than in wheat and seems in accordance with the difference of concentration of these three components in the two cereal species. We suggest that in barley a synthesis of those components occurred during the time of the gall formation. Effectively, it is well known that peroxidases play a role in lignin and associated wall formation. The results confirm that peroxidases could be used in gall lignifications. Similar observations were recorded in tobacco [67], *Arabidopsis thaliana* [68] and switch grass *(Panicum virgatum L.)* [69], suggesting that some peroxidases appear to have a specialized role in lignification.

Otherwise, in another study on barley and wheat infested by *Mayetiola spp* and in the same conditions [70], the change of some biochemical parameters showed also a significant change. The amount of total soluble and reduced carbohydrates, total free proline and phenyl ammonia lyase activity increased in infested plants and suggest that the accumulation of those metabolites is a response to the stress induced by

cecidomyie in barley and wheat [70].

On the other hand, the larvae density in wheat was higher than in barely and it is possible that the number of larvae would be responsible for this difference. Wheat is the preferred host for the Hessian fly [71], but larvae can also live on barley *(Hordeum vulgare)* and other wheat-related species, although larval growth is slow and mortality is high [72].

During the third stage, the concentration of the phenolic compounds, protein concentration and peroxidase activity decreased in wheat and in barley. This could be explained by the fact that during this period, the larvae stopped the feeding. The recent study revealed that metabolites and enzymes are depleted from leaf tissue of *Pongamiapinnata (L.)* during gall formation as a consequence of the invasion of the parasite [73].

A correlation between peroxidases activity and phenolic compounds levels has been proposed for various crops [74], [75]. Peroxidases are capable of oxidizing different phenols [76], [77], [78], [79] and it would seem plausible that these enzymes may be involved in the insolubilization of phenylpropanoids [80], [81]. The correlation coefficient between peroxidase activity and phenolic compounds observed in barley and wheat seems to be high: 0.92. These results were consistent with the findings of many research groups who reported such positive correlation between total phenolic content and antioxidant activity [82], [83], [84]. The observation that peroxidase activity increases in barley and wheat at 25 day after infestation at the same time that phenol concentration reaches maximum levels would suggest that this enzyme may be involved in the defense response. These results confirm that in cereals, the peroxidase activity is well-correlated to phenolic compounds concentration. However, the role played by peroxidase enhancement in the resistance to plant pathogens has not been established unequivocally and it is still not clear whether it is a cause or a consequence of this phenomenon [85].

4 Conclusion

In conclusion, the results of the present work showed that the peroxidases activities and total phenolic content increased after induced stress caused by cecidomyiid attacks in barley and wheat. Changes in peroxidases activities and phenolic content seem to be related to the interaction between barley or wheat and the cecidomyiid species.

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