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# The Defense Mechanism of Cabbage Plant Against Phloem-Sucking Aphid (*Brevicoryne brassicae* L.)

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**Abstract:** The current work aims to study the defense mechanism of cabbage plant ( *Brassica olereacea* var. *capitata*) against phloem sucking aphid *( Brevicoryne brassicae L.*). A significant reduction in the percentage of epicuticular wax, dry weight, sugar, amino acids levels were found with aphid feeding. Furthermore, aphid herbivory elicit an effect on the uptake of some nutrient elements such as P, K, Ca, Mg and Fe. Insect feeding significantly increased the uptake of $Ca^{2+}$ and $K^{+}$ while, the other measured elements were markedly decreased. In addition lipid peroxidation measured as malondialdehyde was markedly increased after insect feeding. The levels of antioxidant compounds (glutathione, ascorbic acid, carotenoids and phenols) were changed in response to aphid feeding. The level of ascorbic acid was significantly increased by infestation. The oxidative enzyme activities (superoxide dismutase, ascorbate peroxidase, ascorbate oxidase) were significantly reduced while polyphenol peroxidase and oxidase activities were enhanced by insect infestion. Moreover, total soluble protein content of the infested senscing cabbage leaves was markedly declined. No qualitative changes were found in the polypeptide pattern of both healthy and infested plants. However, quantitative differences in polypeptides were obtained in control and infested plants. The findings suggest that aphid feeding probably results in oxidative stress in cabbage. Ascorbic acid, proline, phenol peroxidases, oxidases as well as $Ca^{2+}$and $K^{+}$ may play a role in the defense mechanism of aphid infested cabbage leaves, thereby delay their death.

**Key words:** Aphid - cabbage- pigments- carbohydrates - amino acids - elements - antioxidants

## INTRODUCTION

Insects and diseases represent potential biotic stresses to their host plants. Plants challenged by insects respond through changes in the composition and physical properties of the cell wall as well as the biosynthesis of secondary metabolites (Hpkins and Hüner, 2004). Plants protect themselves from herbivory through the formation of wax barriers (Taiz and Zeiger, 1998). Infestation by *Rhopalosiphum padi* L. caused a significant reduction in dry weight, leaf area and number of leaves of barley plants (Matttson and Addy, 1975). Chlorosis is the most obvious plant injury symptom on aphid *(Blissus occiduus*) infested wheat plants, which is indicative of chlorophyll loss (Heng-Moss *et al*., 2004).

It was reported that, herbivory of plants generally, stimulated an accumulation of proline whereas total carbohydrate content was decreased and there was no significant effect on the phenolics (Miles, 1989). Recent studies have also shown that *Nilaparvata lugens* infestation reduces the nutrient uptake of rice plants especially phosphorous and potassium uptake (Wu *et al*., 200*).* Modification in plant protein profiles and alteration in plant oxidative enzyme levels have been reported to be among a plant's first response to insect herbivory (Felton *et al*., 1994; Rafi *et al*., 1996; Chaman *et al.,* 2001 and Ni *et al*., 2001). Phloem-feeding alfalfa hoppers (*Spissistilus festinus)* increase the activities of several oxidative enzymes and lipid peroxidation of soybean plants (Felton et al., 1994 ). Reports indicate that plants have different mechanisms of aphid resistance as several enzymes respond differently to aphid infestation (Chaman *et al*., 2001 and Ni *et al*., 2001). The previous authers as well as Azzouz *et al.* (2005) suggest that the mechanisms of aphid resistance may be interpreted by increased synthesis or expression of specific plant proteins, which enhance plant resistance to insects.

The aim of the present work was, therefore, to study the defense mechanism of cabbage plant against phloem-feeding aphids particularly, the oxidative responses since they have an important role as antiherbivores. The investigation aimed also, to study the effect of the cabbage herbivore on the gene level as reflected in protein profiles.

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## MATERIALS AND METHODS

Seedlings of 16 days old cabbage (*Brassica oleracea* var. *capitata*) were obtained from the Egyptian Ministry of Agriculture, Giza, Egypt. The seedling were transplanted in the field at Giza Governate, Egypt. Two months later few populations of phloem sucking aphids *(Brevicoryne brassicae l.*) started to attack the outer most leaves of some sensitive cabbage plants. However some other plants are not infested. Some infested and healthy plants of the same size and age were chosen and covered with transparent plastic network with narrow openings. The chosen cabbage plants were kept in the field under the natural environmental conditions (around 25/13 ºC day/night, 13h photoperiod, 70% humidity) for 13 days. After 13 days, the visual symptoms of chlorosis were appeared on the infested leaves. The infested senscing outermost cabbage leaves as well as the healthy green ones (control) were collected for physiological and biochemical measurements, particularly the antioxidants which serve as antiherbivory agents.

***Fresh and Dry Weights:***

The whole fresh healthy and infested leaves were washed then dried in oven at 70C for 2 days. The fresh weights and dry weights of the leaves were recorded.

***Wax Content:***

For quantitative estimation of epicuticular waxes, the outermost fresh leaves of similar age were taken and washed in chloroform for 15 seconds (Schuck, 1976). The chloroform extracts were filtered and dried in order to calculate the percentage of wax.

***Mineral Analysis:***

The mineral ions of the ground dry leaves extracted by wet ashing as described by Chapman and Pratt (1978). Phosphorus, potassium, calcium, magnesium and iron are determined simultaneously by Atomic Absorption (Perkin Elemer 3100, USA).

***Pigment Determination:***

Chlorophyll a, chlorophyll b and total carotenoids were extracted from one gram of longitudinal sections of fresh leaves in 85% acetone and measured spectrophotometrically according to Metzner *et al.* (1965) and their levels were calculated according to the formula of Lichtenthaler (1987).

***Carbohydrate Determination:***

Total soluble carbohydrates and polysaccharides were extracted according to the methods described by Naguib (1963) and determined using anthrone reagent (Fairbairn, 1953).

***Free Amino Acid Determination:***

Free amino acids were extracted according to the method of Vartanain *et al.* (1992) and estimated using ninhydrin (Yemm and Cocking, 1955). Proline was determined by the method described by Bates *et al.* (1973).

***Lipid Peroxidation:***

The degree of membrane damage as indicated by the degree of Lipid peroxidation. Malondialdehyde was determined as a product of lipid peroxidation in the frozen leaves (Minotti and Aust, 1987).

***Total Phenol Determination:***

Total phenols of fresh leaves were extracted and estimated according to Malik and Singh (1980).

***Enzymatic and non Enzymatic Antioxidants:***

Antioxidant substances e.g., ascorbic acid and glutathione were determined by the methods of Kampfenkel *et al.* (1995) and Griffith (1985) respectively.

Antioxidant enzymes were extracted from frozen healthy and infested cabbage leaves by using a known volume of phosphate buffer (PH 7)( 1:4 w/v). The crude extracts were used for enzyme assays. Cu-Zn superoxide dismutase (Cu-Zn SOD) was measured according to Giannopolitis and Ries (19775). Polyphenol peroxidase (PX) activity was determined according to the method described by Bergmeyer (1974). Polyphenol oxidase (PPO) activity was measured by the method of Gonzalez *et al.* (1991). Catalase activity assayed following the method of Chen *et al.* (2000). Ascorbate peroxidase (APX) and oxidase (AO) activities were measured by the methods of Cao *et al.* (2004) and Maxwell and Bateman (1967) respectively.

***Total Prolein Electrophoresis:***

Total soluble protein concentration was measured by using BIO-RAD protein assay dye reagent by the method of Bradford (1676). The protein profiles were characterized and identified by using one-dimensional SDS-PAGE according the method of Laemmli (1970) and each band was analyzed by gel documentation system (GDS 8000, California, USA).

***Statistics:***

The significance in variation of healthy and infested means was assayed using paired student's t-test at $P \leq 0.05$ (Motulsky and Schouest, 1989).

## RESULTS AND DISCUSSIONS

Plants protect themselves from herb ivory by the formation of a layer of lipid material called thick cuticle (a waxy outer layer) which reduce water loss and work as a defense against some insects (Taiz and Zeiger, 1998). The infested cabbage leaves exhibited a decline in the percentage of epicuticular wax as compared to that of healthy one (Table. 1). Such effect may be attributed to the enzymatic effects of aphid lipases which digest the wax to facilitate phloem-sap sucking process occurred by aphid (Hamouda, under publication).

Infestation of cabbage leaves by phloem-sucking aphid caused a significant reduction in their dry weights (Table. 1) this might be due to the drain of phloem sap assimilates towards the insect a way from the other plant parts which may contribute to metabolic reduction (Miles, 1989).

Chlorosis is the most obvious plant injury symptom on cabbage leaves after aphid feeding and is indicative of chlorophyll loss. Such effect may be cleared by the significant reduction in chlorophyll a and b contents as well as carotenoids determined in the infested cabbage leaves (Table. 1). The decrease in the photosynthetic pigments may be due to the inhibition of pigment biosynthesis which may results from the alteration in mineral nutrition or lack of assimilates which drain towards the insect or to the effect of reactive oxygen species on these pigments (Stacey and Keen, 1996). The chlorotic symptoms were also, observed on wheat plants infested by aphid (Heng-Moss *et al.,*2004; Wang *et al*., 2004), which was interpreted to be due to unbalanced chlorophyll biosynthesis and degradation (Wang *et al.,* 2004).

There was a significant decrease in the levels of magnesium, phosphorous and iron of the infested leaves as compared with healthy ones. However, calcium and potassium levels of infested leaves were significantly increased relative to the controls (Table 2). The increase in $Ca^{2+}$ and $K^{+}$ might be due to an increase in their uptake under infestation. It could suggested that they may play an important role in the defense mechanism of cabbage plants against aphid. Moreover, $Ca^{2+}$ is immobile element (Ziegler, 1975) so it may not be ingested by phloem-feeding aphid. It is reported that $Ca^{2+}$ protects membranes from damage results from stress, thereby maintaining membrane integrity and minimize efflux of $K^{+}$ (Salisbry and Ross, 1992). The reduced levels of magnesium, phosphorus and iron of infested cabbage leaves may be due to the reduction of their uptake and/or drain of phloem sap by aphid. The decrease in $Mg^{2+}$ ion levels was concomitant with the low pigment contents of infested cabbage leaves. Other study has shown that *Nilaparvata lugens* infestation reduces the nutrient uptake of rice plants particularly phosphorous and potassium (Wu *et al*., 2004).

The decrease in p is closely related to many physiological and biochemical process in plants such as protein biosynthesis, growth, and photosynthesis (Wang, 2000).

The phloem-feeding aphid continually controls and/or modifies the metabolic substances levels of the surrounding tissues. This is supported in the current work where the levels of total soluble carbohydrates, polysaccharides, free amino acids and total soluble proteins of infested cabbage leaves were lower than those of the healthy ones (Table 2). Such effect might be due to the drain of the assimilates towards the aphids and/or decrease in their biosynthetic pathways induced by aphids. It was reported that strong and persistent flow of host assimilates created by the continual removal of metabolites and breakdown of insoluble reserves by insects (Miles, 1989 and Khattab, 2005). On the other hand, free proline content of infested cabbage leaves was greater than that of control ones (Table 2). This might be an indicator for biotic stress experienced by cabbage plants. Similar results have been reported in xylem-feeding infested eucalypt leaves where proline content was determined (Khattab, 2005). Proline is a universal osmolyte accumulated in response to several stresses (Öncel *et al*., 1996) and may have a role in plant defense reactions (Kuznetsov and Shevyakova, 1997).

Herbivory of cabbage leaves significantly increased lipid peroxidation (malondialdehyde content) compared with that of healthy one (Table 3). Biotic stress stimulate the production of active oxygen which causes lipid peroxidation and subsequently membrane damage of the host membranes. (Baker and Orlandi, 19963). The increase in lipid peroxidation may be due to the uncapability of antioxidants to scavenge all the active oxygen species resulted from this biotic stress. In the current work lipid peroxidation was releated to carotenoid and chlorophyll loss (Table 1) which agrees with the results of Hildebrand *et al.* (1986).

**Table 1:** Effects of phloem sucking aphid *(Brevicoryne brassicae)* on the epicuticular wax, water content, dry weight and pigment levels of healthy and infested cabbage leaves. Each value is the mean of three different variables ± SE.

| Parameter | Healthy leaf | Infested leaf |
|---|---|---|
| Wax (g/100g DW) | 2.6 ± 0.03 | 1.9 ± 0.11 |
| Dry weight (g) | 7.61 ± 0.12 | 5.24 ± 0.83 |
| **Pigments (mg/gFW)** | | |
| Chlorophyll a | 4.73 ± 0.13 | 1.32 ± 0.92 |
| Chlorophyll b | 4.13 ± 1.56 | 1.35 ± 0.03 |
| Carotenoids | 0.65 ± 0.20 | 0.16 ± 0.07 |

**Table 2:** Effects of phloem sucking aphid *(Brevicoryne brassicae)* on the mineral content, total soluble and insoluble carbohydrates, total free amino acids, proline and total soluble protein of healthy and infested cabbage leaves. Each value is the mean of three different variables + SE.

| Parameter | Healthy leaf | Infested leaf |
|---|---|---|
| **Mineral content (mg/100g DW)** | | |
| Potassium (K) | 2100 ± 11.15 | 2480 ± 5.80 |
| Calcium (Ca) | 1200 ± 10.70 | 2300 ± 2.30 |
| Magnesium (Mg) | 1680 ± 11.80 | 580 ± 2.15 |
| Phosphorous (P) | 410 ± 5.80 | 250 ± 1.20 |
| Iron (Fe) | 5.41 ± 0.23 | 2.71 ± 0.11 |
| Total soluble carbohydrates (mg/gFW) | 124.74 ± 9.70 | 64.21 ± 2.19 |
| Polysaccharides (mg/gFW) | 14.70 + 3.98 | 11.7 ± 0.41 |
| Total free amino acids (mg/gFW) | 5.01 ± 2.12 | 3.5 ± 0.29 |
| Proline (m mol/g FW) | 1.41 ± 0.31 | 1.65 ± 0.85 |
| Total soluble proteins (mg/gFW) | 4.86 ± 0.21 | 3.21 ± 0.12 |

**Table 3:** Effects of phloem sucking aphid *(Brevicoryne brassicae)* on the lipid peroxidation, antioxidant compounds and antioxidant enzymes of control healthy and infested cabbage leaves. Each value is the mean of the three different replicates variables +.

| Parameter | Healthy leaf | Infested leaf |
|---|---|---|
| Malondialdehyde (nmol/g FW) | 1.03 ± 0.041 | 3.81 ± 1.12 |
| **Antioxidant compounds** | | |
| Ascorbic acid (mg/g FW) | 2.81 ± 0.17 | 4.40 ± 0.12 |
| Glutathione (mg/gFW) | 1.40 ± 0.19 | 1.09 ± 0.13 |
| Total phenols ( mg/gFW) | 8.12 ± 1.31 | 7.10 ± 2.10 |
| **Antioxidant enzymes (U/gFW)** | | |
| Superoxide dismutase (SOD) | 13.4 ± 0.18 | 11.2 ± 0.32 |
| Catalase (CAT) | 38.0 ± 1.15 | 20 ± 0.21 |
| Ascorbate peroxidase (APX) | 6.42 ± 0.55 | 2.91 ± 0.12 |
| Ascorbate oxidase (APO) | 5.50 ± 1.4 | 4.17 ± 2.90 |
| Polyphenol peroxidase (POX) | 5.71 ± 0.12 | 8.51 ± 0.11 |
| Polyphenol oxidase (PO) | 5.41 + 0.23 | 11.2 + 3.71 |

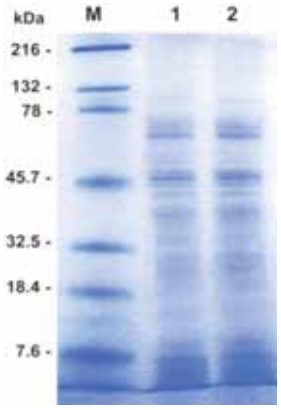


**Fig 1:** Protein-banding pattern of healthy and severely damage (densely infested by aphids) cabbage leaves. Lane M: protein markers. Lane 1, healthy leaves. Lane 2, severly damaged leaves.

The level of ascorbic acid was significantly increased in the infested cabbage leaves (Table 3). Such increase was about 57% greater than that measured in healthy one. In contrast to ascorbic acid, the levels of glutathione and total phenols were significantly decreased in the infested leaves compared to healthy uninfested ones (Table 3). The decrease in the total phenol content of the infested leaf was concomitant with the high activities of both phenol oxidases and peroxidases (Table 3). Total phenols act as antioxidant enzyme substrates. Ascorbate may, therefore, play a role in antioxidant defense and thus as antiherbivory agent (Ayers *et al*., 1997 and Smirnoff *et al*., 2001).

The herbivory of cabbage leaves by the phloem-feeding aphids increased the activities of polyphenol oxidase and peroxidase enzymes while reduces the activities of superoxide dismutase, catalase, ascorbate peroxidase and oxidase enzymes (Table 3). The enhanced activities of polyphenol oxidase and polyphenol peroxidase enzymes may increase the scavenging capacity for free oxygen species. Similar results have been recorded by Stout *et al.* (1999); Ni *et al*. (2001) and Chaman *et al.* (2001) in different plant species. A number of reports have suggested that peroxidases play an important role in herbivore resistance in crop plants (Chittoor *et al.*, 1999 and Constabel, 1999).

The infested cabbage leaves exhibit low level of soluble protein compared with control one (table 2) which might be attributed to the decline in its biosynthesis that resulted from drain of assimilates such as amino acids towards the phloem sucking aphid. Moreover, the reduction in total soluble protein in the infested leaves was concomitant with P level which affected protein synthesis. Similar results were reported by Singla and Grover (1994) who found that the rate of protein synthesis declines during stress condition. The infested and the control healthy cabbage leaves exhibited similar protein profiles (Figure 1 and Table 4). However, polypeptide analysis revealed the presence of 21 polypeptide chains in both healthy (control) and infested cabbage leaves (Figure 1). Quantitative differences in some polypeptide chains were observed. The electrophoretic patterns of infested cabbage leaves showed an increase in polypeptide chains with MW 8.2, 22.4, 25.6, 37 and 38 KDa and a decrease in the amount of polypeptide chains with MW 4.3, 4.9, 6.1 and 39 KDa compared with their respective control (Table 4). Heng-Moss *et al*. (2004) reported that no differences in protein profiles were observed between the control and insect infested buffalograsses. However, Rafi *et al*., (1996) and Jerez (1998) demonstrated changes in protein profiles in resistant plants after insect feeding. It was suggested that synthesis or increased expression of specific plant proteins may serve to enhance the plant resistance to stresses (Ni *et al*., 2001).

In conclusion, phloem feeding aphid induce an oxidative stress which diminished by increasing the concentration of ascorbate as well as the activities of phenol oxidase and peroxidase enzymes. The high levels of calcium, potassium as well as proline also, may probably played significant roles in cabbage defense mechanism against aphid. Moreover, the increasing in the synthesis of certain polypeptides may serve to enhance the resistance of cabbage plant thereby delay their death.

**Table 4:** Comparative analysis of relative concentrations, MW and RF of the different types of polypeptide chains of healthy (control) and aphid-infested cabbage leaves by SDS-PAGE technique and detected by GDS analyesr.

| Lane Band number | Healthy leaf | Infested leaf | MW (kDa) | RF |
|---|---|---|---|---|
| 1 | 0.70 | 0.72 | 94.74 | 0.19 |
| 2 | 1.11 | 0.60 | 71.20 | 0.25 |
| 3 | 2.14 | 2.15 | 67.15 | 0.27 |
| 4 | 4.40 | 4.50 | 62.51 | 0.30 |
| 5 | 0.90 | 0.70 | 54.18 | 0.35 |
| 6 | 0.90 | 0.80 | 52.37 | 0.36 |
| 7 | 1.90 | 1.90 | 46.96 | 0.40 |
| 8 | 4.60 | 3.20 | 45.49 | 0.41 |
| 9 | 1.68 | 0.95 | 43.81 | 0.43 |
| 10 | 1.47 | 0.92 | 42.09 | 0.45 |
| 11 | 1.10 | 0.80 | 39.00 | 0.50 |
| 12 | 2.20 | 3.40 | 38.00 | 0.51 |
| 13 | 0.90 | 3.40 | 37.31 | 0.52 |
| 14 | 1.90 | 0.94 | 33.12 | 0.58 |
| 15 | 1.90 | 3.80 | 25.47 | 0.64 |
| 16 | 2.3 | 5.80 | 022.42 | 0.67 |
| 17 | 5.3 | 8.50 | 8.16 | 0.87 |
| 18 | 9.6 | 7.60 | 6.11 | 0.93 |
| 19 | 15.0 | 15.60 | 5.70 | 0.94 |
| 20 | 19.0 | 14.50 | 4.88 | 0.97 |
| 21 | 21.0 | 18.50 | 4.33 | 1.0 |
| Sum | 100 | 100 | | |

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