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Post Infectional Biochemical Changes Induced by Mealybug *Paracoccus marginatus* (Hemiptera: Pseudococcidae) in Leaves of Superior Seedless Grapes

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Authors' contributions

This work was carried out in collaboration between all authors. Author SM performed the experiment and did statistical analysis. Authors NK and NKA designed the study. Authors SM and NK wrote the first draft of the manuscript. Author NKA managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Mealybug (*Paracoccus marginatus)* belonging to the family Pseudococcideae, is a devastating pest of grapevine in different parts of the world including India. Due to its piercing sucking type feeding behaviour, it injures the vines mechanically and induces several physiological & biochemical alterations in the host. To evaluate reaction of Superior Seedless to this pathogen, this study was undertaken at Punjab Agricultural University, Ludhiana (Punjab) in 2017. The post infectional alterations were compared in healthy and mealybug infested leaves of grapevines under natural conditions in order to decipher the defense response of the plant. A substantial decrease of 3.38, 3.37, 3.36, 36.84, 10.34, 21.23 and 48.02 percent was recorded in chlorophyll a, chlorophyll b, total chlorophyll, carotenoids, total soluble sugars, total soluble proteins and free amino acid content

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respectively in mealybug infested leaves compared to healthy ones, while total phenols, ortho-dihydroxy phenols, flavonoids, peroxidase and polyphenol oxidase activity increased by 31.84, 24.20, 21.60, 17.79 and 10.16 per cent, respectively. Hence, mealybug infestation led to sequence of physiological and biochemical alterations in the host plant.

Keywords: Mealybug; grapevine; pigments; phenols; enzymes.

1. INTRODUCTION

Grape (*Vitis vinifera* L.) is one of the most important horticultural commodities grown worldwide but is affected by various pests and diseases [1]. Among these, the vine mealybug a pest of grapevine growing areas causes huge economic losses even if it exists in low densities [2]. The pest infests wine & table grape varieties and deteriorates the quality by feeding on the grape berries and its excreta honeydew may act as a substrate for sooty mould [3,4] making it a vital tool in virus transmission [5]. The mealybug deteriorates the vigour of vine by feeding on leaves, roots, canes and trunk [6]. The infested leaves turn either brownish red or reddish brown and spread by farm equipments, wind, animals, and field crew. Grape clusters harvested from infested vineyards usually promote the rapid movement of the pest to the new vineyards since some mealybugs can survive the de-stemming process and grower management practices.

Plant protection measures are of limited effectiveness against mealybug because of the waxy covering of its body and its habit of hiding in crevices [7]. Also, frequent use of pesticidal applications may be perilous for human population as well as for the environment. Many natural enemies (parasitic wasps) also play a vital part in the biological control of mealybug [8] but little research on this aspect has been conducted. The pressure to increase agricultural production along with the requisite to reduce the application of insecticides directs us to investigate the survival mechanism of the insect attack. Hence, there is a need to understand the factors governing the relationship between insects and plants as this may lead to unravel the causes of insect pest attack development and its management. The attention now is towards growing cultivars resistant to pests & diseases and finding the physiological and biochemical basis of resistance for future breeding. The present investigation was thus conceived to determine the impact of mealybug infestation on the physiological and biochemical constituents of leaves of superior seedless grapes. Such information will be very useful to devise biologically safe strategy to manage insect attack.

2. MATERIALS AND METHODS

The present studies were conducted in the Fruit Research Farm (FRF) of Department of Fruit Science and the laboratories of Department of Botany, Punjab Agricultural University, Ludhiana during 2017. The vines of Superior Seedless were marked in May 2017 to inspect attack of mealybug. The identification of mealybug (*Paracoccus marginatus*) was made as per Singh and Kaur [9]. The healthy and mealybug infected leaves (Fig. 1) were brought to the laboratories for physiological and biochemical quantifications. The total chlorophyll, carotenoids, total soluble sugars, total soluble proteins, free amino acids, total phenols, total flavonoids, ortho-dihydroxy phenols, and enzymatic antioxidants (peroxidase and polyphenol oxidase) were analysed from the healthy and mealybug infested leaves of the grape variety Superior Seedless. Total chlorophyll content in leaves was evaluated by the method prescribed by Hiscox and Israelstam [10]. The carotenoid content was estimated by the method given by Kirk and Allen [11]. The total soluble sugars, total soluble proteins and free amino acids were estimated by following standard procedures given by Dubois et al. [12] Lowry et al. [13] & Lee and Takashahi [14] respectively. The total phenols, ortho-dihydroxy phenols and flavonoids were estimated according to the method given by Mahadevan and Sridhar [15]. Assay of Peroxidase (PO) (EC 1.11.1.7) and Polyphenol oxidase (PPO) (EC 1.10.3.2) activity was done by the method described by Thomas et al. [16] and Zauberman et al. [17] respectively. Statistical analysis of data given was performed using Microsoft Excel Software (2010).

3. RESULTS AND DISCUSSION

Total Chlorophylls and Carotenoids: The pest attack led to a variation in photosynthetic pigment in grape leaves. A substantial decrease in the content of chlorophyll a, chlorophyll b, total chlorophyll and carotenoids was observed in

mealybug infested leaf tissue of superior seedless grape variety by a deviation of -3.38%, -3.37%, -3.36% and -36.84% respectively in contrast to the healthy leaves (Table 1). The results are in agreement with the findings of Ponmurugan and Baby [18] who reported a decrease in total chlorophyll in tea leaves infected by Phomopsis disease. The infection might have prevented the direct contact of sunlight with the infected leaves leading to increased synthesis of enzyme chlorophyllase and thus reduced chlorophyll. Chlorophylls and carotenoids play a predominant role as protecting pigments against oxidative destruction [19]. Reduction in chlorophyll content may attribute to a reduced photosynthetic efficiency of the host and thereby impaired conducting system.

Total Soluble Sugars, Total Soluble Proteins and Free Amino Acids: The content of total soluble sugars (Table 2) decreased in the mealybug infected leaves of superior seedless grape variety (5.46 ± 0.19 mg g^{-1} of DW) in comparison with the healthy ones (6.09 ± 0.06 mg g^{-1} of DW). This decrease in sugar content of infested leaves could be attributed to enhanced respiratory rate or utilisation of sugar by the pathogen as a respiratory substrate during pathogenesis [20]. Due to pest infestation total soluble proteins and free amino acids could have depleted in the plants. A decrease in total soluble proteins and free amino acid content by -21.23% and -48.02% respectively (Table 2) has been observed. The decreased protein content may be due to blockage of protein synthesis or degradation of proteins in the host plants. Our results corroborate with earlier reports of Kandakoor et al. [21] who found amino acids to be strongly correlated with the incidence of thrips where they reported a decrease in amino acids due to increased incidence of thrips.

Total Phenols, Total Flavonoids and ortho-dihydroxy Phenols: The data for total phenols, total flavonoids and ortho-dihydroxy phenols of non-infested and mealybug infested leaf tissue of Superior seedless grape is presented in table 3. It was observed that as the pathogenesis progressed, there was a gradual increase of phenolic content. The total phenol content was more in the mealybug affected leaves (4.43 ± 0.16 mg g^{-1} of DW) compared to the non-affected ones (3.36 ± 0.17 mg g^{-1} of DW). The insect attack leads to accumulation of phenols in the diseased leaves. This could be due to alteration in phenolic metabolism in response to pest attack [22]. The accumulation of phenolics in the host may lead to inhibition of further advancement of the pathogen. A strong manifestation of resistance induction potential of mealybug in infested plants is exhibited by phenolics deposition which can act as a natural defense mechanism by the host against phytophagous insects [23]. The ortho-dihydric phenols are important in disease reactions and these were higher in infected leaves by a deviation of +24.20% over healthy. They are easily oxidised by the activity of phenol oxidases and the resulting quinines are highly labile and toxic to pathogens and their enzymes [24]. A similar trend was observed in the total flavonoid content. Total flavonoid content was higher in infected samples (3.04 ± 0.10 mg g-1 of DW) when compared to their healthy counterparts (2.50 ± 0.04 mg g-1 DW). Flavonoids help in formation of polymeric compounds, which create a protective physiochemical barrier against pathogens. In similar reports made by Sharma et al. [25] it was observed that total flavonoids were higher in diseased counterparts as compared to the non-affected leaves in pea genotypes in their reaction to powdery mildew caused by *Erysiphe polygoni*.

Peroxidase and Polyphenol oxidase: The data on peroxidase activity (POD) of mealybug infested and healthy leaf tissue of the grape variety Superior Seedless is shown in Table 4. The data presented in Table 4 reveals that the peroxidase (POD) and polyphenol oxidase (PPO) of the mealybug infected leaves of Superior seedless were significantly higher when compared to their healthy counterparts by a deviation of +17.79% and +10.16% respectively over healthy. Enzymes are known to act as defensive agents for the plants under stress conditions and are among the most powerful and ubiquitous proteins in the plants [26]. Any physical injury in host tissue triggers a series of physical and biochemical alterations which are defensive in nature. Previous reports by Korth [27] and Frost [28] suggested that arthropod attack and mechanical wounds might trigger structural defense reactions in plants. Significant higher quantities of defense related proteins i.e. POD and PPO in the tissues infested by insect confer stronger resistance. These antioxidant enzymes are also involved in the biosynthesis of defense related metabolites. The enhanced levels of enzymes could easily validate the enhanced plant resistance against arthropods. The increased defense responses also deter the relish of plant tissue by the feeding insects [29].

Such enzymes may also be relevant as resistance describing mechanism of plants. Our results corroborate earlier reports made by Potter et al. [30] who suggested that the damage caused by insects to the host plants may trigger their metabolism towards the higher synthesis of proteins. Hence, elevated content of defense related enzymes/proteins is the validation of their report. Enhanced PPO and POD activities were reported in plants exposed to various biotic and abiotic inducer activities [31]. The present study reports that the activity of enzymes POD and PPO was higher in mealybug infested leaves than the healthy leaves so it may be concluded that the enzymatic activity is directly linked to the host resistance. Increased POD activity could be correlated with infection in plants as cinnamyl alcohols are polymerised to lignins catabolised by lignification of peroxidase leading to disease resistance [26]. Increased lignin following an increase in peroxidase activity of the plant tissue could be the strengthened physical barrier as a defence mechanism. This boosted toughness of physical barriers of the host could be to make the plant tissues difficult to get digested by herbivores [32].

Fig. 1. Mealybug infested grapevine leaves

Table 1. Chlorophyll a, chlorophyll b, total chlorophyll and carotenoid content of healthy and mealybug infected leaves

Leaves of variety superior seedless	Chlorophyll a (mg g^{-1} FW)	Chlorophyll b (mg g^{-1} FW)	Total chlorophyll (mg g^{-1} FW)	Carotenoids (mg g^{-1} FW)
Healthy	1.48±0.01	2.37±2.29	3.86±0.03	9.5 x 10^{-3} ±0.0002
Infected	1.43±0.02	2.29±0.02	3.37±0.02	6.0 x 10^{-3} ±0.0001
% deviation over healthy	-3.38	-3.37	-3.36	-36.84

Table 2. Total soluble sugars, total soluble protein and free amino acid content of healthy and mealybug infected leaves

Leaves of variety superior seedless	Total soluble sugars (mg g^{-1} DW)	Total soluble proteins (mg g^{-1} DW)	Free amino acids (mg g^{-1} DW)
Healthy	6.09±0.06	1.13±0.07	0.116±0.007
Infected	5.46±0.19	0.89±0.09	0.061±0.004
% deviation over healthy	-10.34	-21.23	-48.02

Table 3. Total phenols, ortho-dihydroxy phenols and total flavonoid content of healthy and mealybug infected leaves

Leaves of variety superior seedless	Total phenols (mg g^{-1} DW)	Ortho-dihydroxy phenols (mg g^{-1} DW)	Flavonoids (mg g^{-1} DW)
Healthy	3.36±0.16	2.52±0.23	2.50±0.04
Infected	4.43±0.17	3.13±0.06	3.04±0.10
% deviation over healthy	+31.84	+24.20	+21.60

Table 4. Peroxidase and polyphenol oxidase activity of healthy and mealybug infected leaves

Leaves of variety superior seedless	Peroxidase (ΔOD/min/g FW)	Polyphenol oxidase (ΔOD/min/g FW)
Healthy	1.63±0.07	0.0118±0.0003
Infected	1.92±0.03	0.0130±0.0007
% deviation over healthy	+17.79	+10.16

**± indicates Standard deviation*

4. CONCLUSION

On the basis of the present investigation, it may be concluded that mealy bug infestation led to an alteration in the physiological and biochemical parameters affecting the grape quality either directly or indirectly, which in turn affected its yield potential adversely. Initiation of a defence mechanism in response to infestation by mealybug was observed in the leaf tissue of Superior Seedless. Breeding grape varieties resistant to mealybug infestation through alleviation of the activity peroxidase and polyphenol oxidase is opined.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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