Madras Agric. J., 99 (4-6): 349-351, June 2012
https://doi.org/10.29321/MAJ.10.100083

**Short Note**

# Biochemical Basis of Resistance in Brinjal to Papaya Mealybug, *Paracoccus marginatus* (Williams and Granara de Willink)

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**Changes in biochemical parameters like total carbohydrates, total sugars, total chlorophyll, moisture, ash, total phenolic content, peroxidase (PO), polyphenol oxidase (PPO), phenylalanine ammonia-lyase (PAL), catalase (CAT) were compared with susceptible (CO 2 variety) and less susceptible (*Solanum viarum*) to papaya mealybug. Total carbohydrates, total sugars and total chlorophyll were higher in susceptible variety (CO2). Low moisture content and high ash content was observed in less susceptible cultivar *S. viarum.* The resistance in brinjal varieties was characterized by the presence of defense enzymes like PO, PPO, PAL, CAT and total phenols in different levels. There was a clear correlation between the levels of biochemical constituents and mealybug incidence.**

**Key words:** Brinjal, *Paracoccus marginatus*, resistance, peroxidase, polyphenol oxidase, phenylalanine ammonia lyase, catalase.

Brinjal (*Solanum melongena* L.) holds a coveted position among vegetables. India is the second largest producer accounting for 26% of world output with about 84.5 lakh tonnes in production of brinjal (FAO, 2008). Due to its low calorific value (24 kcal 100 $g^{-1}$) and high potassium content (200 mg 100 $g^{-1}$), it is suitable for diabetes, hypertensive and obese patients. Brinjal crop is prone to damage by various insects and mites. The major pests include eggplant fruit and shoot borer, leafhopper, whitefly, thrips, aphid, spotted beetles, leaf roller, stem borer, blister beetle, red spider mite, and disease like little leaf. Recently, papaya mealybug *Paracoccus marginatus* Williams and Granara de Willink (Pseudococcidae; Hemiptera) has been observed to cause damage to brinjal from the early stage of the crop growth to harvest. Chemical control of mealybug may reduce the pest attack to a greater extent, but it causes adverse effects on the environment and human health. Thus utilization of resistant varieties for the control of the pest is inevitable.

Identification of resistance is possible through quantifying the biochemical components present in the genotype. The biochemical constituents like glycoalkaloid (solasodine), phenols, phenolic oxidase enzymes namely poly phenol oxidase (PPO) and peroxidase (PO) are available in brinjal and these biochemical constituents possess insect resistance properties (Kalloo, 1988). With this background, an experiment, the first of its kind was carried out to study the biochemical basis of resistance to papaya mealybug in brinjal.

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## Materials and Methods

### *Plant material*

Based on the study conducted for screening brinjal genotypes resistant against papaya mealybug, susceptible (CO 2) and less susceptible (*S. viarum*) were selected for the experiment. Seeds of brinjal cultivars, *S. viarum* and CO 2 were obtained from Horticultural College and Research Institute, TNAU, Coimbatore. Seeds were sown in nursery and thirty day old seedlings were transplanted in the field. Two weeks later, natural incidence of *P. marginatus* started on young leaves of CO 2. Since *S. viarum* was not affected by *P. marginatus,* ten plants were artificially infested by releasing the mealybug adults. Infested and healthy plants of the same size and age were chosen and the leaves were collected. The biochemical components determining the resistance of the plant to insect attack were analysed and estimated in the two genotypes.

### *Metabolites and enzymes estimation in brinjal cultivars*

Green leaves were plucked from 45 days old plants and cut into small uniform pieces. Representative samples of 500mg of each cultivar were utilized for the estimation of total carbohydrates and total sugars (Hedge and Hofreiter, 1962), total phenol (Bray and Thorpe, 1954) and chlorophyll content (Arnon, 1949). The ash and moisture content of the leaf bits were determined as per the procedure of Ranganna (1979) and ISTA (1999).

Peroxidase (PO) activity was assayed spectrophotometrically as per Hammerschmidt *et al.* (1982). Polyphenol oxidase (PPO) and Phenylalanine ammonia-lyase (PAL) activity were determined as per Mayer *et al.* (1965) and Dickerson *et al*. (1984) respectively. Catalase activity was estimated by following the method adopted by Barber (1980). All the estimations were done in triplicate and the results were statistically analyzed and reported.

## Results and Discussion

The results of the biochemical analysis revealed that *S. viarum* showed lower quantity of total sugars (11.6 mg/g) as compared to CO 2 (20 mg/g).Total chlorophyll content and total carbohydrate content were higher in CO 2 (1.343 and 73.8 mg/g respectively) compared to *S. viarum* (1.048 mg/g and 51.5 mg/g respectively) (Table 1). Kalode and Pant (1967) reported that insect susceptible plant parts had higher concentration of sugars which acted as feeding stimulant in the susceptible varieties. Besides, CO 2 had high moisture content (83.42%) and low ash content (2.79%) compared to *S. viarum* (78.75% and 2.95% respectively) (Table1). Similar correlation was reported by Elanchezhyan *et.al.* (2008).

**Table 1. Biochemical contents of *S. viarum* and CO 2**

| Biochemical contents | Mean ±SD | |
|---|---|---|
| | *S. viarum* | *S. melongena* (CO2) |
| Total carbohydrates (mg/g)* | 51.5±1.65 | 73.8±0.92 |
| Total sugars (mg/g)* | 11.6±1.65 | 20.0±0.36 |
| Total chlorophyll (mg/g)* | 1.048±0.16 | 1.343±0.08 |
| Moisture (%)* | 78.75±1.25 | 83.42±0.07 |
| Ash (%)* | 2.95±0.07 | 2.79±0.03 |

*Mean of three replications;

As shown in Table 2, total phenolic content of healthy leaves of *S. viarum* was significantly higher (165 mg $g^{-1}$) than that of $CO_2$ (150 mg $g^{-1}$). This result provides evidence that total phenolic content in leaves plays an important role in imparting resistance against mealybug because of direct toxicity. Goodman (1986) reported that the cassava variety with a high phenolic acid level in its extracellular fluids was less preferred by *Phenacoccus manihoti*. Increased total phenols were observed in mealybug infested leaves of CO 2 and *S. viarum* (293.75 and 178 mg/g respectively) in comparison to healthy counterparts (150 and 165 mg/g respectively). Hence, the increased quantity of phenolics in the affected leaves may contribute to the resistance against mealybug.

The herbivory of brinjal leaves by mealybugs increased activities of polyphenol oxidase, peroxidase and phenylalanine ammonia lyase in both *S. viarum* and CO 2. Peroxidase (PO) and polyphenol oxidase (PPO) activity were increased in completely infested leaves of *S. viarum* (21.40 and 13.65% over healthy leaves respectively) and CO 2 (160.38 and 105.91% over healthy leaves respectively), respectively (Table 2). These results agree with many previous reports that PO specific activity levels increased after herbivore infestation (Khattab, 2007). The association of PPO activity with host plant resistance to insects has been reported in tomato (Thipyapong *et al*. 2006). In the present case phenylalanine ammonia lyase (PAL) was maximum in infested leaves of *S. viarum* (48.57% over healthy leaves) as well as in CO 2 (4.88% over healthy leaves), respectively (Table 2). Activation of PAL and subsequent increase in phenol contents were general responses associated with resistance mechanism in plants (Ghosal *et al*., 2004).

**Table 2. Changes in phenols and antioxidant enzymes in healthy and infested leaves of brinjal genotypes**

| Genotype | Type of material | Total phenol (mg $g^{-1}$) | Peroxidase (PO) | Polyphenol-oxidase (PPO) | Phenylalanine ammonia lyase (PAL) (nmol trans-cinnamic acid $min^{-1}$ $g^{-1}$) | Catalase (CAT) (µg of $H_2O_2$ $g^{-1}$ $min^{-1}$) |
|---|---|---|---|---|---|---|
| | | | (∇OD $min^{-1}$ $mg^{-1}$ protein) | | | |
| *S. viarum* | Healthy | 165 | 25.7 | 31.5 | 0.35 | 153.43 |
| | Infested | 178 (+5.33)* | 31.2 (+21.40) | 35.8 (+13.65) | 0.52 (+48.57) | 145.20 (-5.36) |
| *S. melongena* (CO2) | Healthy | 150 | 21.2 | 22 | 0.41 | 231.20 |
| | Infested | 293.75 (+95.83) | 55.2 (+160.38) | 45.3 (+105.91) | 0.43 (+4.88) | 130.05 (-43.75) |

*Figures in the parenthesis are % increase in values in infested leaves over healthy

In the present study, reduced CAT activity (153.43 µg of $H_2O_2$ $g^{-1}$ $min^{-1}$) was observed in *S. viarum* compared to CO 2 (Table 2). This is in line with the findings of Park *et al*. (2006) who reported that reduced CAT activity would help the plant to maintain high hydrogen peroxide levels thus causing damage to the insect midgut.

The salient findings of the present study are in line with Kalloo (1988) who reported that the biochemical constituents like phenols, phenolic oxidase enzymes namely polyphenol oxidase and peroxidase available in brinjal possess insect resistant properties.

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Received: July 11, 2011; Accepted: April 16, 2012