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Biochemical Basis of Resistance to Sesame Phyllody Transmitted by Leafhopper in Sesame

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ABSTRACT

Sesame is an important oilseed crop and India happens to be the largest producer. However, its productivity is quite low due to the infestation of insect pests and diseases and one of the important diseases is sesame phyllody transmitted by the leafhopper, *Orosius albicinctus* Distant. The biochemical basis of resistance to leafhoppers and sesame phyllody in selected genotypes revealed that the total phenols in leaves ($r = -0.771^{**}$), shoot ($r = -0.764^{**}$) and pods ($r = -0.762^{**}$) showed a significant and negative relationship with phyllody incidence. While, total sugars, reducing sugar, amino acids and crude proteins showed a positive relationship with phyllody incidence.

Introduction

Sesame (*Sesamum indicum* L.) commonly known as 'Til' is an important oil seed crop grown in India and the oldest oilseed crop in the world. The crop is grown in a wide range of environments, extending from semi arid tropics and sub tropics to temperate regions.

The primary centre of origin could be placed in fertile crescent or Indian sub continent or in Iran Afghanistan area. Since, most of the wild species in the genus are located in Africa, it has been

suggested that Ethiopia is the centre of origin (Anon., 2006).

Materials and Methods

The estimation of biochemical constituents viz., total phenols, total sugars, reducing sugars, crude proteins and total free amino acids in leaves collected from 60 day old plants of different resistant and susceptible genotypes was done to establish the relationship between various biochemical constituents and to compare it with resistance and susceptibility.

Extraction of plant tissues in alcohol

The healthy vegetative shoot, leaves and pods from 60 days old plants of test entries were collected and thoroughly washed with distilled water and dried under shade (Plate 8). One gram of plant sample pieces of all the genotypes were taken in separate conical flask and 15 ml of 80 per cent ethanol was added. It was refluxed for 30 minutes on hot water bath. After boiling, the extract was cooled and the pieces of tissues was ground thoroughly in a mortar with pestle in slight ethanol. The supernatant was decanted in to another flask and residue again re-extracted with small quantity of hot ethanol and decanted. This extract was filtered through Whatman No. 1 filter paper and made up to a known volume with 80 per cent ethanol. The ethanol part of (alcoholic) extract was stored in refrigerator at 4 °C, and used for the estimation of total sugars, reducing sugars and phenols by using standard procedures and protocols.

Total phenol

The estimation of total phenols was carried out by following the method suggested by Bray and Thorpe (1954). Five gram of fresh tissue was taken in 50 ml of 80 % methanol and heated in a boiling water bath for 10 minutes. The tissue was ground in an electric mixer and filtered 0.1 to 0.5 ml of the supernatant was taken in tube and volume made up to 1 ml by adding distilled water. 0.5 ml of folin – ciocalteu reagent was added followed by 1 ml of Na₂CO₃ solution. The tube was shaken and heated in a boiling water bath for exactly 1 minute. After cooling under a running tap water, the blue solution was diluted to 25 ml with distilled water and its absorbance was measured at 650 nm in a spectrophotometer. Total phenol was calculated with the help of reference curve using catechol (100 mg / 100 ml) as standard and expressed as mg/g.

Crude proteins

The estimation of crude proteins was done by following Micro-Kjeldahl method (Sadasivam and

Manickam, 1996). This method gives an estimate of total nitrogen content present in the samples. Later, this was converted to crude protein by using conversion factor. The method adopted was detailed here under.

Preparation of reagents

Sodium hydroxide solution

About 400 g of sodium hydroxide was dissolved in one liter of distilled water.

Indicator solution

Twenty grams of methyl red and 100 mg of bromocresol green were dissolved in 60- ml of ethanol.

Digestion mixture

Ninety nine grams K₂SO₄, 4.1 gm of HgO and 0.8 gm of CuSO₄ were ground in a mortar to prepare the digestion mixture.

Boric acid solution

Twenty grams of boric acid was dissolved in 500 ml of warm distilled water.

One gram of finely ground dry plant sample was placed in a boiling tube to which a pinch of digestion mixture and 15 ml of concentrated H₂SO₄ was added. These tubes were kept for digestion in Kjehl-Plus (KPS-012L) provided with vacuum pump for about one hour. This was later allowed to cool and transferred to distillation apparatus. Conical flask of capacity 100 ml containing 25 ml of 4 per cent boric acid with a few drops of mixed indicator was placed under the condenser, the tip of which was dipped in boric acid solution. Required amount of NaOH solution was added to the sample. Later the distillate was titrated against 0.02 N H₂SO₄ till the original pink-red color is restored. The amount of nitrogen present in a given sample was calculated by the following formula:

$$\begin{aligned} & \text{Nitrogen (\%)} \\ & \text{Titre value} \times \text{Normality of acid} \times \\ & \quad 0.014 \times \text{volume of digested sample} \\ & = \frac{\text{-----}}{\text{Weight of the sample (gm)} \times \text{Aliquot taken}} \times 100 \end{aligned}$$

Crude protein was calculated by the formula:

Crude protein (%) = % N x 5.30 (conversion factor for sesame).

Then, the crude proteins in the sample expressed as per cent.

Total free amino acids

The amount of total free amino acids present in the samples was estimated by following Ninhydrin method developed by Moore and Stein (1948).

Total and reducing sugars

Reducing and total sugars in the extracts were estimated by following the procedure of Somogyi (1952). For estimating the total sugars, hydrolysis of non-reducing sugars to reducing sugars was done by adding one ml of 1.0 N hydrochloric acid to one ml of plant extract and were heated on a boiling water bath at 50 °C for 20 minutes. Later, it was cooled and a drop of phenolphthalein indicator solution was added. Then 1.0 N sodium hydroxide was added drop wise till the solution turned pink due to excess alkali. The excess alkali was reneutralised with 0.1 N hydrochloric acid, which was added drop wise till the solution turned colorless and was made up to known volume. One ml of hydrolysate for total sugars and one ml of plant extract for reducing sugars was taken separately in boiling tubes to which one ml of freshly prepared alkaline copper reagent was added and boiled in a water bath for exactly 20 minutes. After cooling under running tap water, one ml of arseno molybdate reagent was added with immediate mixing. The volume was made up to 15 ml with distilled water and the blue color developed was read at 520 nm. Suitable blanks prepared which were used to adjust the light

transmission to 100 per cent. A standard curve was prepared with glucose, which was used to calculate the unknown. The quantities were expressed as milligrams per gram of the plant sample.

Results and Discussion

Total phenols

In general the total phenols were higher in resistant genotypes compared to susceptible. The total phenol contents in the leaf tissues of resistant genotypes varied between 17.70 to 35.70 mg/g, while in susceptible genotype it varied between 3.70 to 8.00 mg/g (Table 21). Among the resistant genotypes, OSC-207 recorded maximum amount of total phenols (35.07 mg/g) in leaf tissue. The correlation studies showed a significant negative ($r = -0.77^{**}$) relation with total phenols and phyllody incidence (Table 2). Similarly, in pod tissues a significant difference among the genotypes for total phenols was noticed. The amount of total phenols in pod tissues ranged from 8.90 to 28.60 mg/g in resistant genotype and 1.00 to 2.68 mg/g in susceptible genotypes (Table 2). The amount of total phenols was higher (28.60 mg/g) in the pods of resistant genotype OSC-207 and was low (1.00 mg/g) in the pods of susceptible genotype AT-249. The correlation study revealed that the phenol content in the pods of sesame was found to be negatively ($r = -0.76$) associated with sesame phyllody (Table 2).

The phenol content in the shoots of sesame genotypes also varied to some extent in different genotypes. In the shoots of resistant genotype the total phenols varied from 11.60 to 31.30 mg/g, while in susceptible genotype it varied between 1.66 to 4.10 mg/g. Among the resistant genotypes, the shoots of OSC-207 recorded maximum amount of total phenols (31.30 mg/g). The correlation studies showed a significant negative ($r = -0.76^*$) relation with total phenols and phyllody incidence (Table 2).

The result of the present investigations are in close agreement with Thangjam (2015) who reported total phenol content of diseased samples was found to be

30 and 25 mg/g however, the healthy samples consistently recorded low phenol content (18 and 22 mg/g). Likewise, the results of present investigation are also in line with findings of Vijaykumar *et al.*, (2009 and 2012) who reported higher amount of total phenols in gall midge resistant rice genotypes compared to susceptible.

In majority of plants, phenols acts as prime biochemical factor for resistance due to their anti-feedant as well as antibiosis property on growth and reproduction.

Total sugars

The results revealed a significant difference among the genotypes for total sugars in leaves. The amount of total sugars ranged from 6.00 to 12.66 mg/g in resistant genotype and 14.83 to 21.66 mg /g in susceptible genotypes (Table 21). The total sugar was higher (21.66 mg/g) in the leaves of susceptible genotype GT-1 and was lowest (6.00 mg/g) in the leaves of resistant genotype OSC-207. Similarly, in shoots of sesame significant difference among the genotypes for total sugars was noticed. The amount of total sugars in shoots ranged from 4.50 to 11.16 mg/g in resistant genotype and in susceptible genotype it varied between 12.66 to 20.16 mg/g. The total sugar content were higher (20.16 mg/g) in the shoot of susceptible genotype, GT-1 and were lowest (4.50 mg/g) in the shoots of resistant genotype OSC-207 (Table 1).

Pods of sesame genotypes also followed same trend that total sugars in pods ranged from 2.50 to 9.16 mg/g in resistant genotype and in susceptible genotype it varied between 10.66 to 18.16 mg/g.

The total sugars were higher (18.16 mg/g) in the pods of susceptible genotype, GT-1 and were lowest (2.50 mg/g) in the pods of resistant genotype OSC-207 (Table 21). An increasing trend of total sugar content in leaves, shoots and pods of different genotypes showed a significant positive impact on leaves ($r = 0.83^{**}$), shoots ($r = 0.83^{**}$) and pods ($r = 0.84^{**}$) and phyllody incidence (Table 2).

The present results are in close agreement with Zafari *et al.*, (2012) who reported that Total soluble sugar and reducing sugar contents decreased in lime leaves under phytoplasma infection when compared with controls and accumulation of carbohydrates has been reported in coconut palms affected by lethal yellowing (Maust *et al.*, 2003) and in corn plants affected by maize bushy stunt (Junqueira *et al.*, 2004), but some variations depending on the virulence of the phytoplasma isolate and on the host/phytoplasma association, were also described (Lepka *et al.*, 1999).

Patil *et al.*, (2011) reported that resistant genotypes of wheat exhibited more amount of mean total sugar as compared to susceptible genotypes during the growth. The present findings are in accordance with Kandakoor *et al.*, (2013) who reported a significant positive correlative with thrips incidence and total sugars in groundnut. Further Kumar *et al.*, (2015) also indicated that total sugars had a significant positive correlation with damage by pod fly in pigeon pea. Bhavani *et al.*, (2012) reported that sugarcane genotypes susceptible to *Chilo infuscatellus* with higher per cent of total sugars than the resistant ones. This is considered as a secondary effect of infection and can be explained through an inhibition of phloem transport. As a consequence, photosynthetic product accumulation in chloroplasts, inhibits the photosynthesis and reduces the supply of sugars from source leaves to roots. The phytoplasmas are confined to the phloem and are found in sink tissues that actively import sugars from sources. Phytoplasmas could alter the carbohydrate metabolism of the plant and thus affect sugar transport (Maust *et al.*, 2003). These prokaryotes, in fact, contain a minimal genome and lack genes coding for ATP syntheses, sugar uptake and use, making them dependent on their host (Christensen *et al.*, 2005).

Reducing sugars

The amount of reducing sugars in leaves of resistant genotypes varied from 3.00 to 6.33 mg/g. In susceptible genotypes it varied between 8.08 to

12.00 mg/g. Among the resistant genotypes, OSC-207 recorded lower amount of reducing sugars (3.00 mg/g) and was on par with VS-07-023 (3.66 mg/g), while the susceptible genotype, GT-1 recorded higher amount of reducing sugars (12.00 mg/g) in the leaves (Table 21). Similarly, the amount of reducing sugars in shoots of resistant genotypes varied from 1.25 to 4.58 mg/g. In susceptible genotypes it varied between 5.33 to 9.08 mg/g. Among the resistant genotypes OSC-207 recorded lower amount of reducing sugars (1.25 mg/g) and was on par with VS-07-023 (1.91mg/g) and RT-363 (3.08 mg/g), while the susceptible genotype GT-1 had higher amount of reducing sugars (9.08 mg/g) (Table 1).

In sesame pods the amount of reducing sugars of resistant genotypes varied from 2.25 to 5.58mg/g. In susceptible genotypes it varied between 6.83 to 10.08 mg/g. Among the resistant genotypes, OSC-207 recorded lower amount of reducing sugars (2.25 mg/g) and was on par with VS-07-023 (2.91mg/g) and RT-363 (4.08mg/g), while the susceptible genotype GT-1 had higher amount of reducing sugars (10.08 mg/g) (Table 1).

An increasing trend of reducing sugar in leaves, shoots and pods of different genotypes showed a significant positive impact on per cent phyllody damage ($r = 0.817^{**}$) and $r = 0.83^{**}$), ($r = 0.838$), respectively (Table 2).

The result of the present investigation are in close agreement with Thangjam (2015) who reported higher reducing sugar recorded in diseased sesame samples compared to healthy samples. Similarly, Junqueira *et al.*, (2004) also reported the increased level of reducing sugars in leafhopper inoculated maize plants towards changes in host metabolism due to the sesame phytoplasma infection.

Since, reducing sugars are considered to be an essential component in insect nutrition, and it plays a vital role in host selection by phytophagous insects. Hence their concentration in plant is positively associated with feeding behaviour of

insects and also phytoplasmas are confined to the phloem and are found in sink tissues that actively import sugars from sources. Phytoplasmas could alter the carbohydrate metabolism of the plant and thus affect sugar transport (Maust *et al.*, 2003). These prokaryotes, in fact, contain a minimal genome and lack genes coding for ATP syntheses, sugar uptake and use, making them dependent on their host (Christensen *et al.*, 2005).

Total free amino acids (TFA)

The total free amino acids in the leaves of resistant genotypes varied from 2.39 to 1.90 mg/g and in susceptible genotypes it varied between 3.09 to 7.16 mg/g. The susceptible genotype GT-1 recorded significantly higher amount of aminoacid (7.16 mg/g) and significantly lower amount (1.90 mg/g) was found in the resistant genotype OSC-207 (Table 21).

The difference in TFA among genotypes were significant and showed an increasing trend with susceptibility and exhibited significant positive relationship with sesame phyllody (Table 21).

Similarly, the total free amino acids in shoots of resistant genotypes varied from 1.03 to 2.41 mg/g and in susceptible genotypes it varied between 2.63 to 6.58 mg/g. In the pods of resistant genotypes it varied between 1.70 to 2.23mg/g. The susceptible genotype GT-1 recorded significantly higher amount of TFA (6.97 mg/g) and significantly lower amount (1.70 mg/g) was found in the resistant genotype OSC-207 (Table 21). An increasing trend of TFA in leaves, shoot and pods of tested genotypes showed a significant positive impact on per cent phyllody ($r = 0.70^{**}$, 0.70^{**} , 0.71^{**}) (Table 2).

The present study is in confirmation with reports of Trandafirescu *et al.*, (2011) where the most susceptible varieties recorded higher contents of TFA. The results are in confirmity with Anantharaju and Muthiah (2008) who reported a significant positively association with spotted pod borer and total amino acids.

Table.1 Biochemical constituents in leaves, shoot and pods of sesame genotypes against sesame phyllody

| Sl. No. | Genotypes | Category | Leaf hopper Population | Phyllody (%) | Total phenols (mg/g) | | | Total sugars (mg/g) | | | Reducing sugars (mg/g) | | | Crude proteins (%) | | | Total free aminoacids (mg/g) | | |
|---------|------------|----------|------------------------|--------------|--------------------------------|-------------------------------|--------------------|--------------------------------|-----------------------|----------------------------------|------------------------|----------------------|----------------------|----------------------------------|-----------------------|----------------------------------|------------------------------|--------------------------------|--------------------|
| | | | | | Leaves | Shoot | Pods | Leaves | Shoot | Pods | Leaves | Shoot | Pods | Leaves | Shoot | Pods | Leaves | Shoot | Pods |
| 1 | OSC-207 | HR | 0.5 | 0.00 | 35.7 _{0^d} | 31.3 _{0^a} | 28.60 ^a | 6.00 ^d | 4.50 ^f | 2.50 ^g | 3.00 ^f | 1.25 ^g | 2.25 ^g | 13.66 ^g | 11.65 _g | 12.40 ^g | 1.9 ^f | 1.03 ^f | 1.70 ^f |
| 2 | VS-07-023 | HR | 0.05 | 0.00 | 32.7 _{0^{cd}} | 28.3 _{0^a} | 25.60 ^a | 7.33 _{3^{cd}} | 5.83 ^f | 3.83 ^g | 3.66 ^f | 1.91 ^{fg} | 2.91 ^g | 15.00 _g | 12.99 _g | 13.74 _g | 2.12 _f | 2.13 _f | 1.93 ^{ef} |
| 3 | RT-363 | R | 1.8 | 2.90 | 25.2 _{0^{bc}} | 20.8 _{0^b} | 18.10 ^b | 9.66 _c | 8.16 ^{ef} | 6.16 ^{fg} | 5.00 ^{ef} | 3.08 ^{efg} | 4.08 ^{fg} | 17.16 _{fg} | 15.15 _{efg} | 15.90 _{fg} | 2.09 _f | 2.4 ^{ef} | 1.90 ^{ef} |
| 4 | JLS-9707-2 | R | 1.4 | 1.60 | 22.7 _{0^{ab}} | 18.3 _{0^b} | 15.60 ^b | 8.50 _b | 7.00 ^{ef} | 5.00 ^{efg} | 4.25 ^{ef} | 2.50 ^{ef} | 3.50 _g | 16.66 _{efg} | 14.65 _{defg} | 15.40 _{d^{efg}} | 2.43 _f | 1.91 _{5^{ef}} | 2.23 ^{ef} |
| 5 | G-TIL-2 | MR | 2.65 | 51.00 | 16.0 _{0^{ab}} | 11.6 _{0^c} | 8.90 ^c | 12.6 _{6^{ab}} | 11.16 _{de} | 9.16 ^{def} | 6.33 _e | 4.58 _e | 5.58 _{ef} | 20 ^{cdef} | 17.99 _{cdef} | 18.74 _{c^{def}} | 2.37 _f | 2.40 _{5^{ef}} | 2.18 ^{ef} |
| 6 | RT-367 | MR | 2.50 | 16.00 | 17.7 _{0^{ab}} | 13.3 _{0^c} | 10.60 ^c | 11.8 _{3^{ab}} | 10.33 _{cde} | 8.33 ^{cde} | 6.00 _e | 4.16 _{de} | 5.16 _{def} | 19 ^{bcd} | 16.99 _{bcd} | 17.74 _{b^{cde}} | 2.39 _f | 2.08 _f | 2.19 ^{ef} |
| 7 | RT-366 | S | 3.35 | 22.80 | 8.00 ^a | 4.10 _{cf} | 2.68 ^d | 16.1 _{6^a} | 13.66 _{bcd} | 11.50 _{b^{cde}} | 8.08 _d | 5.75 _{cd} | 6.83 _{cde} | 26.6 _e | 24.59 _{bcd} | 25.34 _{b^{cde}} | 3.09 _e | 2.63 _e | 2.9 ^{de} |
| 8 | RT-368 | S | 4.10 | 26.80 | 5.30 ^e | 1.88 _d | 1.10 ^d | 15.8 _{3^e} | 14.33 _{bcd} | 12.33 _{cd} | 7.91 _{cd} | 6.16 _{cd} | 7.166 _{bcd} | 24.58 ^a | 22.57 _a | 23.32 ^a | 4.03 _e | 3.08 _e | 3.84 ^{de} |
| 9 | AT-231 | S | 4.55 | 19.50 | 6.40 ^f | 2.50 _f | 2.04 ^d | 14.8 _{3^f} | 12.66 _{abcd} | 10.66 _{bcd} | 7.41 _{cd} | 5.33 _{abcd} | 6.33 _{bcd} | 20.83 _b | 18.82 _{ab} | 19.57 _b | 3.23 _d | 2.61 _{5^{cd}} | 3.03 ^{cd} |
| 10 | GT-1 | HS | 5.10 | 45.60 | 3.30 ^f | 1.66 _f | 1.62 ^d | 21.6 _{6^f} | 20.16 _{abc} | 18.16 _{6^{abc}} | 12.00 _{abc} | 9.08 _{abc} | 10.08 _{abc} | 22.16 _{6^{bcd}} | 20.15 _{bcd} | 20.90 _{cd} | 7.16 _c | 6.33 _c | 6.97 ^{bc} |
| 11 | DS-5 | HS | 5.55 | 39.50 | 4.70 ^f | 2.90 _{de} | 2.16 ^d | 20.3 _{3^f} | 17.66 _{ad} | 15.66 _b | 10.75 _{ab} | 7.83 _{ab} | 8.83 _{ab} | 23.5 ^{abc} | 21.49 _{abc} | 22.24 _{bc} | 6.33 _b | 6.58 _b | 6.14 ^{ab} |
| 12 | AT-249 | HS | 5.00 | 37.50 | 3.70 ^f | 1.90 _f | 1.00 ^d | 18.1 _{6^f} | 16.66 _a | 14.66 ^a | 9.08 ^a | 7.33 ^a | 8.33 ^a | 21.16 _{6^{abc}} | 19.15 _{abc} | 19. ^{abc} | 5.12 ^a | 4.58 ^a | 4.92 ^a |
| SEM± | | | | | 0.85 | 0.33 | 0.55 | 0.85 | 0.83 | 0.85 | 0.47 | 0.43 | 0.55 | 0.51 | 0.54 | 0.53 | 0.37 | 0.39 | 0.38 |
| CD @ 5% | | | | | 1.47 | 3.14 | 2.88 | 1.47 | 1.41 | 1.47 | 1.47 | 0.80 | 0.70 | 0.71 | 1.13 | 1.13 | 0.51 | 0.51 | 0.51 |

Means in the column followed by same letters are not significantly different at p=0.05(F-test)

Table.2 Correlation coefficient and regression equation of biochemical components of sesame leaves, pods and shoot against sesame phyllody

| Biochemical parameters | Correlation coefficient (r) | | | R ² | Regression equation |
|--------------------------------------|-----------------------------|------------------------|-------------------------|----------------|--|
| | Leaves (X ₁) | Pods (X ₂) | Shoot (X ₃) | | |
| Total sugars (mg/g) | 0.827** | 0.830** | 0.838** | 0.71 | Y=16.58- 4.06X ₁ - 5.81X ₂ +13.22X ₃ |
| Reducing sugars (mg/g) | 0.817** | 0.838** | 0.830** | 0.72 | Y=40.60- 8.13X ₁ + 53.5X ₂ -38.06X ₃ |
| Total phenols (mg/g) | -0.771** | -0.762** | -0.764** | 0.70 | Y=53.52- 8.97X ₁ + 18.25X ₂ -10.85X ₃ |
| Total free amino acids (mg/g) | 0.700** | 0.71** | 0.700** | 0.43 | Y=42.3- 83.48X ₁ + 78.01X ₂ +5.69X ₃ |
| Crude proteins (%) | 0.662** | 0.662** | 0.662** | 0.42 | Y=32.12- 0.01X ₁ + 18.25X ₂ -10.85X ₃ |

N=15; *Significant at P ≤ 0.05; ** Significant at P ≤ 0.01

This fact might be explained both by the reduction in the protein synthesis and the increase in the protein decomposition due to the disturbance induced by the pathogenic agent.

Crude proteins (%)

The crude proteins in the leaves of resistant and susceptible genotypes varied between 13.66 to 20.00 mg/g and 20.83 to 26.60 mg/g, respectively. The susceptible genotype RT-368 recorded significantly higher amount of crude proteins (24.58 mg/g) and significantly lower amount of crude proteins (13.66 mg/g) was found in the resistant genotype OSC-207 (Table 21). An increasing trend of crude proteins in leaves of selected genotypes was observed with increase in susceptibility of phyllody incidence. A similar trend was observed in the tissues of pod samples, wherein, susceptible genotypes recorded significantly higher crude proteins compared to resistant genotypes. The crude proteins in the pod tissue of susceptible and resistant genotypes varied between 25.34 to 19.00 mg/g and 12.40 to 18.74 mg/g, respectively. The susceptible genotype RT-366 recorded significantly higher amount of crude proteins (25.34 mg/g) and significantly lower amount of crude proteins was recorded in resistant genotypes (12.40 mg/g) (Table 21). The susceptible genotype RT-366 recorded significantly higher amount of crude proteins (24.59 mg/g) in comparison with resistant genotypes (11.65 mg/g) (Table 21). The correlation studies revealed an increasing trend of crude proteins in leaves ($r = 0.66^{**}$), pods ($r = 0.66^{**}$) and shoots ($r = 0.66^{**}$) of different genotypes had a significant positive impact on per cent phyllody (Table 2).

The present study is in confirmation with reports of Junqueira *et al.*, (2004) who have reported that the increased in protein contents in leafhopper inoculated maize plants point out changes in host metabolism due to the phytoplasma. Senthil *et al.*, (2010) also reported that the quantity of phenols in infected leaves was found to be higher compared to healthy leaves in *Cucumis sativus* (Linn.) affected by leaf spot disease caused by *Penicillium notatum*.

Similar results were also obtained by Patil and Patil (1989) where, reduction in crude proteins (17.44 per cent) content has been observed in phyllody infection. Similar results were also reported in other phytoplasma diseases (Carling and Milliken, 1977).

The result of the present investigation were in close agreement with Thangjam (2015) who reported diseased sample from 1st and 2nd date of sowing recorded highest content of total proteins (60 and 58 mg/g) while healthy samples recorded 46 and 39 mg/g of total proteins, respectively. Irrespective of the dates of sowing, the diseased samples recorded total proteins content than the healthy samples.

Elevated level of different biochemical parameters in diseased samples indicate that the reaction of plants to phytoplasma infection which is induced rather than constitutive.

Conflict of interest

The authors declare no conflict of interest.

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