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Biochemical basis of host-plant resistance to shoot and fruit borer, *Diaphania caesalis* Wlk. in jackfruit (*Artocarpus heterophyllus* Lam.)

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ABSTRACT: Shoot and fruit borer, *Diaphania caesalis* Wlk. is a major pest of jackfruit, which feeds on tender shoots, flowers, leaf buds and fruits that leads to reduce yield and quality. Studies were conducted to understand the relationship between host-plant resistance and biochemical attributes. Total phenol, antioxidant activity and epicuticular wax were evaluated in contrasting jackfruit accessions to understand the biochemical basis of resistance and susceptibility against *D. caesalis*. The study revealed that total phenol content and radical scavenging activity (DPPH) were higher in resistant accessions. However, on the contrary, higher wax content was observed in susceptible accessions compared to the resistant ones. Correlation studies between percent bud damage caused by *D. caesalis* with these biochemical parameters unveiled that total phenols and DPPH activity had a significant negative correlation, while epicuticular wax had a significant positive relationship with percent bud damage. These results suggest that a combination of these biochemical attributes like phenol and DPPH activitymay contribute to plant resistance, which might be used as markers in selection of resistant jack fruit sources against the target pest.

Keywords: Antioxidant activity, *Diaphania caesalis*, epicuticular wax, jackfruit, phenols

INTRODUCTION

Jackfruit (*Artocarpus heterophyllus* Lam.) is a tropical evergreen tree prominently known as poor man's fruit. It is native to Western Ghats of India (Ochas *et al.,* 1981). India and Bangladesh are considered to be the world's largest producers of jackfruit. In India it is cultivated in an area of 1,53,000 hectares with an annual production of 1742000 MT (Anon., 2017). The major jackfruit growing states in India include Tamil Nadu, Kerala, West Bengal, Bihar, Uttar Pradesh, Orissa and Assam.Jackfruit is a multi-purpose species providing food, timber, fuel, fodder, and medicinal and industrial products. Nutritionally jackfruit is rich in carbohydrates, proteins, potassium, calcium, iron and vitamin A, B and C. The presence of isoflavones, antioxidants, and phytonutrients in the fruits indicate that jack fruit also has cancer-fighting properties (Anonymous., 2012).

In spite of such a vast potential and usefulness, the marketable yield of jackfruit is low which is attributed to various biotic and abiotic factors. Insect pests are major constraint. As many as 39 species of insects are known to attack jackfruit in India (Butani, 1979). However, the shoot and fruit borer, *Diaphania caesalis* (Wlk.), is considered as an economically important pest of jackfruit (Karim, 1995). Since it feeds on all parts of the jackfruit including tender shoots, fruits, flowers and buds, it causes reduction in quality and yield. Farmers primarily rely on chemicals to keep the pest population below economic injury level.With rising environmental awareness and disadvantages of insecticides, host-plant resistance serves as a viable component of integrated pest management strategy. There are reports on the existence of significant variability in the preference of borer to different genotypes (Madhukar *et al*., 2015; Sajjad *et al*., 2011). Hence the present study was undertaken to investigate the biochemical basis of resistance and susceptibility in jackfruit against *D. caesalis*, which could help breeders in taking forward jackfruit improvement.

MATERIALS AND METHODS

Present investigation was carried out during 2016-17 at ICAR- Indian Institute of Horticultural Research, Bengaluru. Based on screening of jackfruit germplasm by Soumya *et al.* (2015), four resistant jackfruit accessions with zero field infestation (G-61, G-72, G-73 and G-74) and four susceptible accession having >20% infestation (G-12, G-11, G-31 and G-7) were selected for this study. All the trees were in one contiguous block so that no differential environmental factors affected them.To understand the relationship between the biochemical attributesand the genotype resistance or susceptibility, four important biochemical parameters *viz.,* epicuticular wax, total phenols and antioxidant activity by FRAP (Ferric reducing antioxidant potential) and DPPH (Diphenyl-1-picryl hydrazyl radical scavenging ability) were estimated in immature leaves of selected accessions. For this study, the immature leaves were sampled during peak infestation period *i.e.,* duringSeptember monthfrom three canopy levels (upper, middle and lower) from all

Table 1. Biochemical constituents inleavesof resistant and susceptible jackfruit genotypes for *D. caesalis*

Category	Genotype	Total phenols (mg GAE/100g)	FRAP AEAC/100g)	(g DPPH AEAC/100g)	(g Epicuticularwax (µg/ cm^2)
Resistant	G-61	307.53^{a}	5.61^{a}	6.73^{a}	19.53^{cd}
	G-72	304.83^{a}	1.52^{a}	3.61^{b}	10.29^{d}
	G-73	255.90^{a}	1.68^{a}	4.10^{b}	8.36^{d}
	G-74	247.25^{a}	2.60^{a}	6.01^{a}	9.98^{d}
Susceptible	G-12	133.84^{b}	1.25^{a}	3.15^{b}	35.52^{bc}
	G-11	106.40^{b}	1.23^{a}	3.31^{b}	45.77^{b}
	G-31	153.45^{b}	1.31^{a}	2.64^{b}	53.04^{ab}
	G-7	135.51^{b}	1.28^{a}	2.59^{b}	67.85^{a}
SE m±		23.75	1.07	0.54	6.70
CD @ p= 0.05		71.20	1.51	0.76	9.48

Values in the column followed by common letters are non-significant at p = 0.05 as per DMRT

Table 2. Estimated mean values of total phenol, FRAP, DPPH and epicuticular wax based on tolerance level of jackfruit genotypes

Genotype	Total phenols (mg GAE/100 g)	FRAP(g AEAC/100 g)	DPPH (g AEAC/100 g)	Epicuticular wax (µg/cm^2)
$(Resistant)_4$	278.9	2.85	5.1148	12.037
$(Susceptible)_4$	132.3	1.2660	2.9213	50.545
LSD (0.05)	45.52*	2.32 ns	1.88*	17.74*
GLM Procedure (Pr > F)	0.0002	0.1455	0.0291	0.0018

*Significance of $p \leq 0.05$.

Table 3. Relationship between biochemical constituents and percent bud damage by *D. caesalis* in jackfruit

Biochemical parameters	Correlation coefficient
Total Phenols (mg GAE/100g)	-0.961*
FRAP (g/100g)	-0.560
DPPH (g/100g)	-0.735*
Epicuticularwax (µg/cm^2)	0.866*

*Significant @ p=0.05

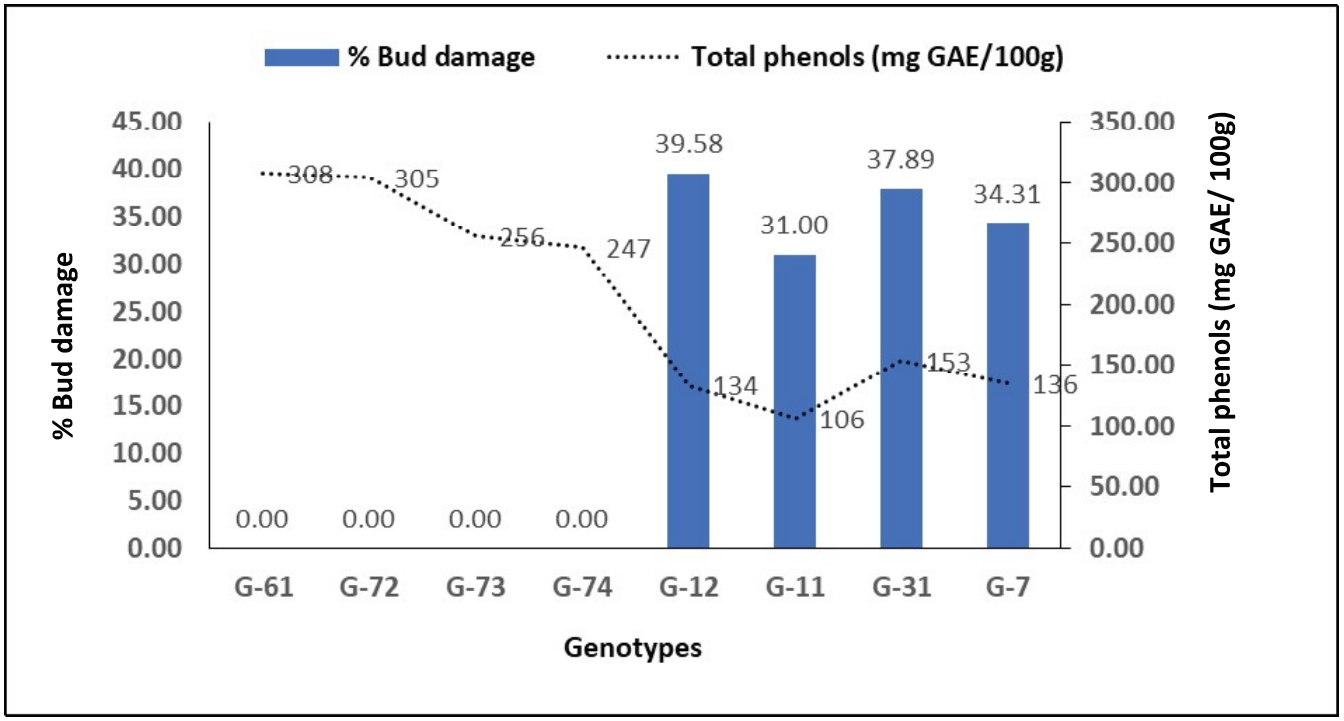

Fig 1. Relationship between percent bud damage caused by *D. casealis* and total phenols

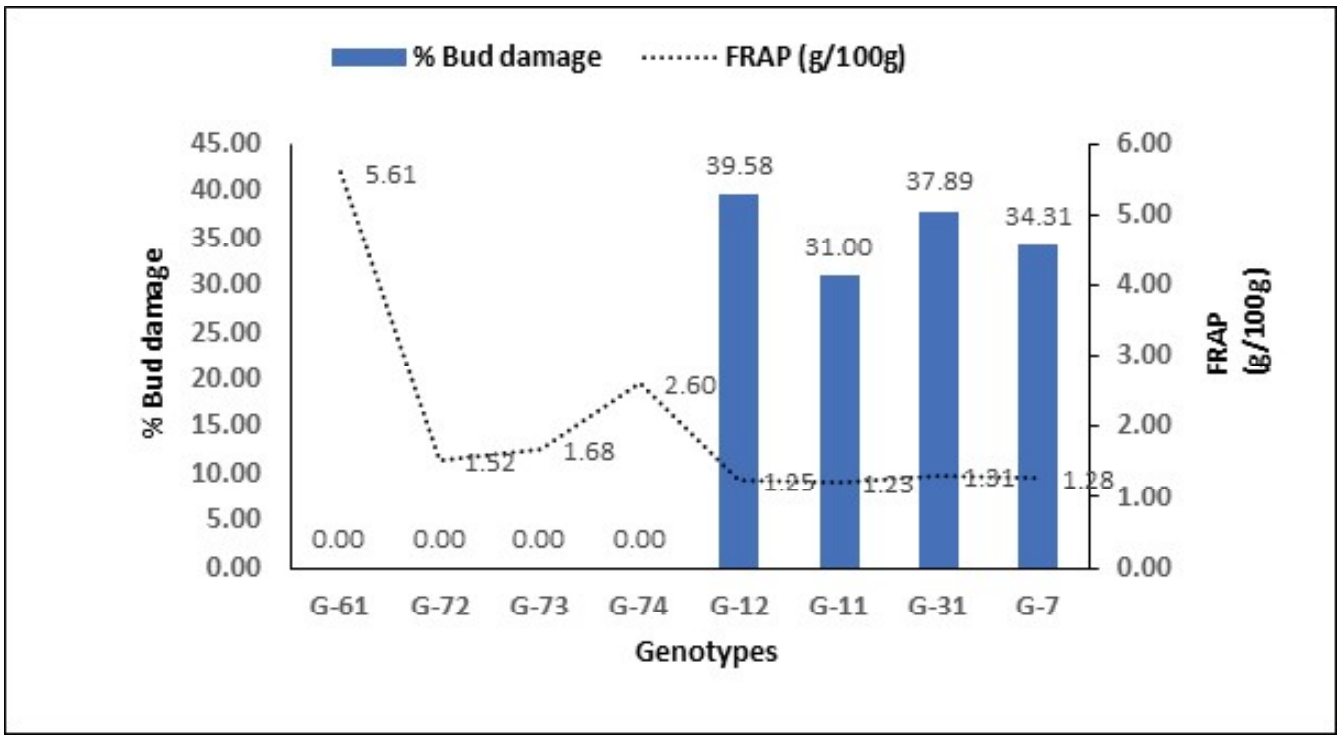

Fig 2. Relationship between percent bud damage caused by *D. casealis* and FRAP`

the selected accessions.

Extraction of plant tissue

Fresh immature leavessamples of test entries were collected and the samples were grinded thoroughly in liquid nitrogen.Leaf samples (5 g) from all the accessions were taken in separate conical flasks and 15 ml of 80% methanol was added. Next day after through homogenization, the extracts were filtered and volume made up to 50 mL with 80% methanol. The methanolic extract was stored at 4ºC and further used for the estimation of total phenol and antioxidant activity.

Total phenol

Total phenol were estimated by modified Folin-Ciocalteau method suggested by Singleton and Rossi (1965).To 0.5 ml of extract, 3.3 ml of deionized water, 1 ml of Folin-Ciocalteu reagent and 1.0 ml of 20% Na_2CO_3 solution were added. After 30-45 min the blue color developed was measured by spectrophotometer (T80+ UV/VIS Spectrometer) at 700 nm. Calibration curve was prepared using standard gallic acid and concentrations of phenol present in different samples were expressed as gallic acid equivalent (mg GAE/100 g fw).

Antioxidant activity by FRAP assay

Antioxidant activity by FRAPassay was performed according to the method described by Benzie and Strain (1996). The FRAP reagent included 300 mM acetate buffer (pH 3.6), 10 mM TPTZ in 40 mMHCl and 20 mM $FeCl_3$ in the ratio 10:1:1 (v:v:v). 1.8mL of FRAP reagent was mixed with 0.2 ml of plant extract, incubated at 37 ºC for 30 min in a water bath and blue color was measured in a spectrophotometer at 593 nm. The standard curve was prepared using ascorbic acid and antioxidant activity was expressed as ascorbic acid equivalent antioxidant capacity (AEAC).

Free radical scavenging activity using DPPH assay

Free radical scavenging activity using DPPH assay was performed as described by Kang and Saltveit (2002). A 2.5 ml aliquot of a 0.2mM DPPH solution in methanol (95%) was added to 0.2 ml of methanol sample extract and 0.3ml of acetate buffer, and shaken vigorously. Change in the absorbance was measured at 515 nm for 30 min. The percentage inhibition of DPPH of the test sample was calculated by the following formula: %Inhibition = $100 \times (A_0 - A) / A_0$ where A_0 was the initial absorbance, and A was the final absorbance of the sample extract at 515 nm. Methanol (95%) was used as a blank. Results were expressed as ascorbic acid equivalent antioxidant capacity (AEAC).

Estimation of Epicuticular wax

Leaf epicuticular wax content was estimated following colorimetric method given by Ebercon *et al.* (1977).The leaf discs (1 cm^2) from expanded leaves were taken and 16 number of discs were immersed in 15 mL of chloroform for 15 sec. After evaporation of extract, acidic $K_2Cr_2O_7$ was added, contents boiled and optical density was measured at 590 nm. The concentration of leaf wax was estimatedusing the standard curve of polyethylene glycol (PEG 6000) and expressed as µg/cm^2.

Statistical Analysis

The data obtained was subjected to Analysis of Variance, ANOVA and means were separated by Duncan's multiple range test (DMRT). Further correlation studies were made to establish the relationship between biochemical constituents and percent damage caused by *D. caesalis* and for this study per cent bud damage data of Soumya *et al.* (2015) was used. All these analysis were done by SPSS 16.0. Differences between means of resistant and susceptible genotypes were resolved for significance at $P < 0.05$ using the PROC GLM procedure by Statistical Analysis System (SAS) software (9.3 version).

RESULTS AND DISCUSSION

Total Phenols

Significant differences among the jackfruit accessions for total phenol was noticed.Total phenols were higher in resistant accessions (247.25 to 307.53 mg GAE/100 g) compared to susceptible ones (106.40 to 153.45 mg GAE/100 g) (Table 1). Among the resistant accessions, G-61 recorded higher (307.53mg GAE/100g), while susceptible accession G-11had lower total phenol (106.40 mg GAE/100g) content (Table 1). Data analysis based on the tolerance level of genotypes (resistant and susceptible) established that the tolerant genotypes recorded higher phenol content (Table2). Significant differences ($P \leqslant 0.05$) were recorded for phenol content based on genotype tolerance level.The correlation studies revealed a significantnegative relationship between total phenoland percent bud damage ($r = -0.961$) (Table 3, Fig. 1). The present findings are in conformity with Prasad *et al.* (2014) who reported that the total phenol in brinjal genotypes had a significant negative impact on percent shoot infestation by *L. orbonalis.* Similar reports are available in chickpea for pod borer (Girija *et al*., 2008) and in rice for gall midge (Anantharajuand Muthiah, 2008). Phenols act as prime biochemical factor for protection because of their combative role against insect damage and additional antibiosis property on development and reproduction (Durbey and Sarup, 1984), so higher levels of phenol provide better resistance to plants.

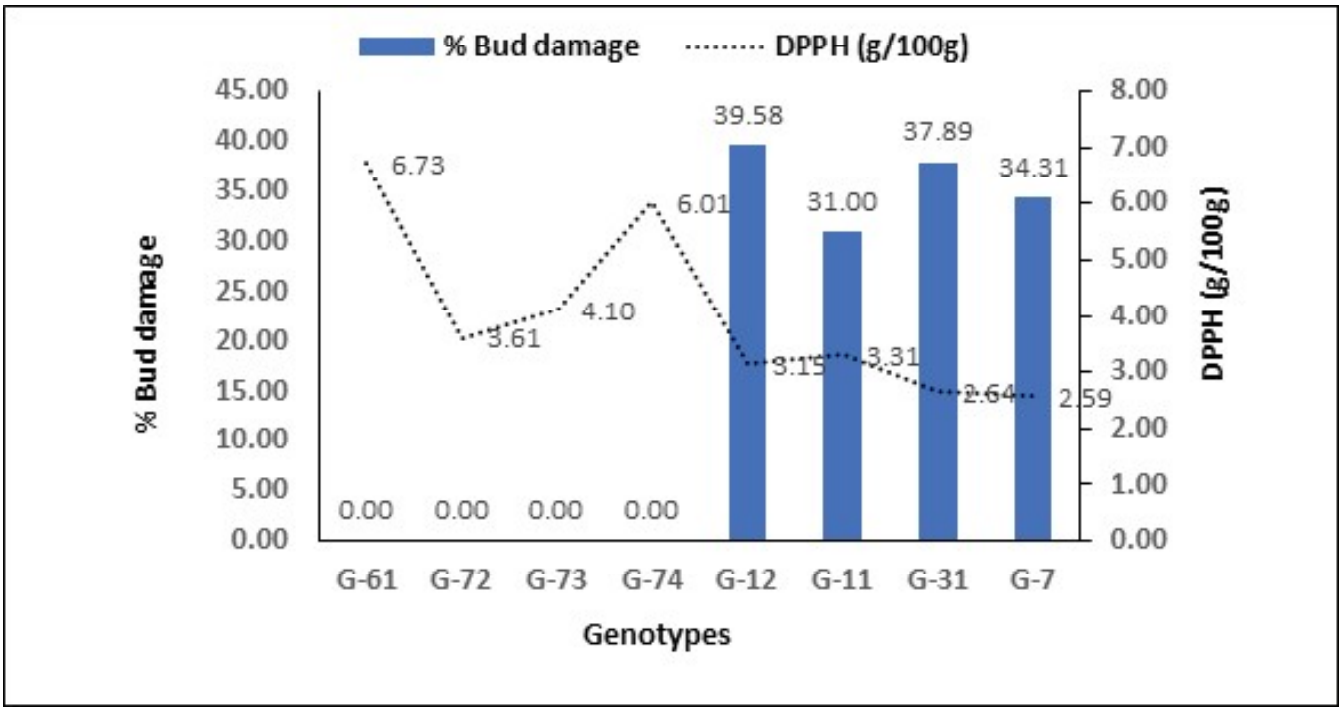

Fig 3. Relationship between percent bud damage caused by D. casealis and DPPH

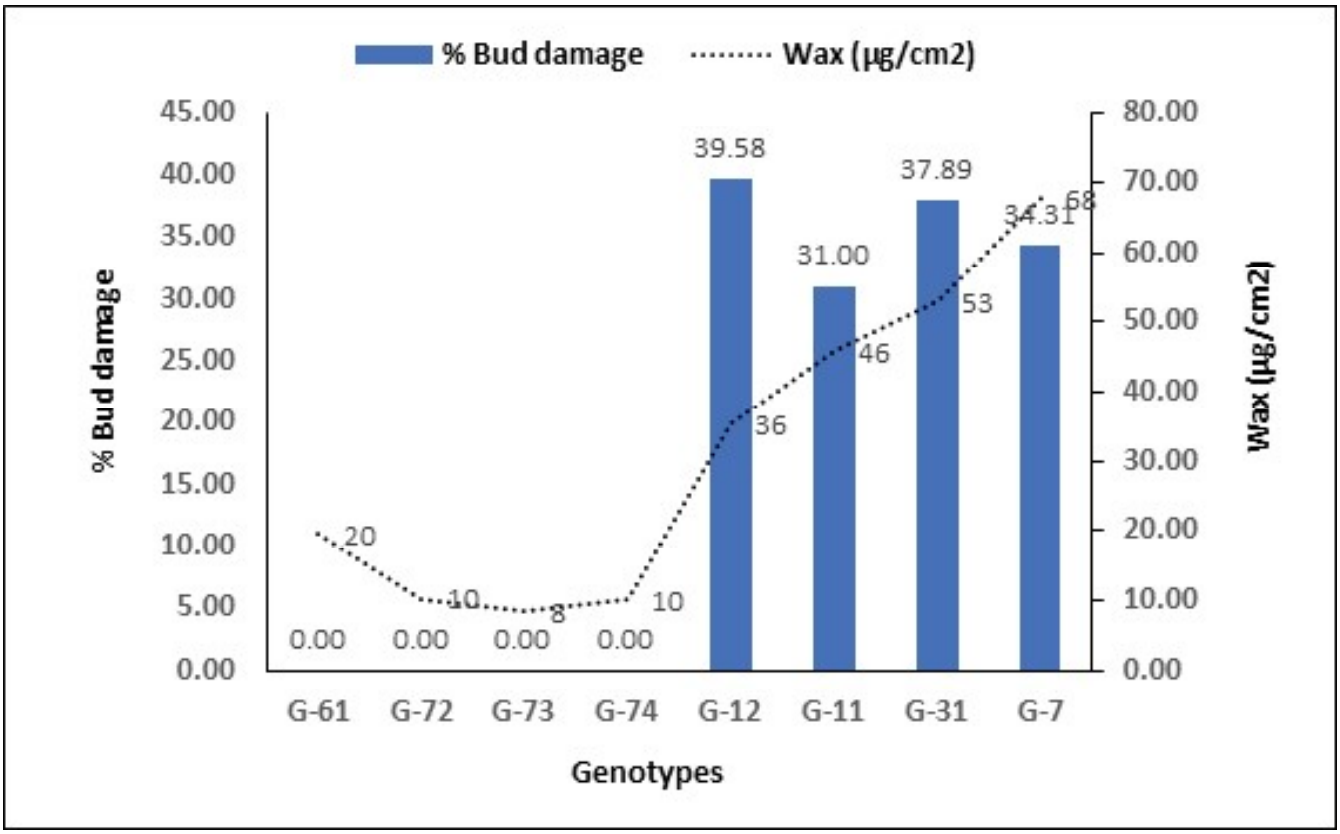

Fig 4. Relationship between percent bud damage caused by D. casealis and wax

Total antioxidant capacity

Antioxidant activity assay in jackfruit extracts were evaluated using two complementary*in-vitro* assays namely, FRAP and DPPH. There was a non-significant variations among the accessionconcerning the antioxidant activity (FRAP) in leaves of jackfruit. Among the accessions, resistant genotype G-61had maximum antioxidant activity (5.61 g AEAC/100g) which was followed by G-74 (2.60 g AEAC/100g) whereas, the lowest antioxidant activity (1.23 g AEAC/100g) was noticed in G-11, a susceptible genotype (Table 1). The correlation studies were carried out in order to establish the possible association between antioxidant activity and shoot and fruit borer incidence. The results revealed a non-significant negative association (r = -0.560) with the antioxidant activity (FRAP) and incidence of *D. casealis* (Table 3, Fig. 2).

Free radical scavenging activitywas evaluated using DPPH assays which measure the abilityof antioxidants in a sample to scavenge pre-formed radicals. The correlation studies revealed a significant negative association between the percent bud damage and antioxidant scavenging activity in jackfruit leaf buds (r = -0.735) (Table 3, Fig.3). The antioxidant scavenging activity showed a marked variation among the accessions tested. Higher activity was recorded in resistant accessions (3.61 to 6.73g AEAC/100 g) and lower values in susceptible accessions (Table 1).The effect of tolerance level of genotypes (resistant and susceptible) on antioxidant activity was investigated. The genotypes differed significantly ($P \leqslant 0.05$) for DPPH; whereas no significant differences ($P \geqslant 0.05$) were recorded for FRAP assay (Table 2.) Surekha *et al.* (2013) reported that among various plant defenses, antioxidant system plays crucial role in mitigatingthe pathogen mediated oxidative stress,however not much work was carried on the role of antioxidant activity in relation to pest damage to negate or validate our findings.

Epicuticular waxes

The epicuticular waxes showed significant variation among the genotypes and it was on higher side in susceptible accessions (35.52 to 67.85 µg/cm^2) compared to resistantones (8.36 to 19.53 µg/cm^2) (Table 1). Differentiation of genotypes were also revealed by PROC GLM and results showed that significant differences ($P \leqslant 0.05$) were recorded for wax content based on genotype tolerance level (Table 2.) The correlation studies revealed a significant positive association with the percent bud damage by *D. caesalis* and wax content on leaves (r = 0.866) (Table 3, Fig. 4). The maximum load of wax was recorded in susceptible genotype G-7 (67.85µg/cm^2), while minimum was recorded in resistant genotype G-73 (8.36 µg/cm^2). These results are in accordance with Damon *et al.* (2014) who reported significant positive relationship between thrips damage and wax content in onion.The results are in close conformity with Eigenbrode and Shelton (1990) who made studies on behaviour and survival of diamond back moth larvae on *Brassica* and revealing that the percent larval survival was more on normal leaf type having greater wax load compared to glossy leaf type with lesser wax load. The presence of epicuticular wax mediate interaction between plants and insect herbivores (Eigenbrode *et al.*, 1991) and it also acts a phagostimulant (Adati and Matsuda,1993). These epicuticular waxes may contain some aliphatic components, sugars and amino acids as well as auxiliary metabolites which might act as phagostimulant. From this it can be concluded that epicuticular waxes play important role in insect host specificity since it influences the insect survival and behaviour. Present study establishes the relationship between biochemical parameters and host-plant resistance in jackfruit and shows potential of phenols and DPPH activity as a viable biochemical markers for selection of resistant lines at initial stages of plant development to support breeding programme of fruit resistance in jack fruit.

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