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RESISTANCE IN SOYBEAN GENOTYPES TO WHITEFLY *BEMISIA TABACI*

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ABSTRACT

This study demonstrates the defensive responses of eight soybean genotypes based on plant metabolites to the whitefly *Bemisia tabaci.* At two sampling intervals (30 and 50 days after sowing) secondary metabolites viz., total phenols, o-dihydroxy phenols, flavonols and tannins and primary metabolites viz., total soluble sugars, reducing sugars and total soluble proteins have been estimated from uninfested and infested soybean plants (125 whitefly adults/ plant). The genotypes revealed significant variation in secondary metabolites while primary metabolites decreased. The correlation between whitefly and metabolites showed that secondary metabolites were significantly negatively correlated incidence while primary metabolites were significantly positively correlated.

Key words: Soybean, *Bemisia tabaci*, plant resistance, defence metabolites, induced resistance, phenols, flavanols, tanmins, sugars, proteins

Soybean is an essential oil seed crop, which is highly valued for the source of rich protein (Agarwal et al., 2013). The major biotic limiting factor responsible for the decreased soybean production in the Northern Indian region mung bean yellow mosaic disease (MYMD), the transmitted by the whitefly *Bemisia tabaci*. Yield losses up to 80% had been documented under severe incidence (Rani et al., 2016). Whitefly management with insecticides is often challenging because of rapid resistance and resurgence development (Sharma et al., 2012). In IPM host plant resistance is widely compatible with other components. The host plant morphological, biochemical, physiological and molecular characteristics influence the insect interaction (Padilha et al., 2021). Plant response to external stress involves observable changes in the molecular, cellular crosstalk and signalling pathways, and induction of plant secondary metabolites (PSM) (Isah, 2019). Insect defence mechanism observed in plants were mainly related to secondary metabolites and to some extent, primary metabolites (Slansky, 1990; Bi and Felton, 1995; Isah, 2019). To develop insect-resistant genotypes it is essential to understand the variation defensive responses (War et al., 2012). The present study explores the possible roles of certain plant metabolites (PSM and PM) in resistant and susceptible soybean genotypes against *B. Tabaci*. Total phenols, o-dihydroxy phenols, flavonols, tannins, total soluble sugars, reducing sugars and proteins compounds have been assessed.

MATERIALS AND METHODS

Eight soybean genotypes, viz. DS 3105, SL 688, SL 958, SL 1113, PS 1347, PS 1572, SL 1028 and SL 1074 were selected and evaluated in kharif 2018 and 2019 at the Punjab Agricultural University, Ludhiana, Punjab, India (30°54'1.75"N and 75°48'48.06"E.), . The maintenance of *B. tabaci* culture, multiple-choice test and population counts were performed as per the methodology described by Harish et al. (2022). Total phenols (Swain and Hillis, 1959), o-dihydroxy phenols (Nair and Vaidyanathan, 1964), flavonols (Balbaa et al., 1974), total soluble sugars (Dubois et al., 1956), reduce sugars (Nelson, 1944), total soluble protein contents (Lowry et al., 1951) were estimated from the leaf samples using the standard methodologies with minor modifications. The data on the whitefly incidence were analysed in a one-way ANOVA and those biochemical compounds by performing ANOVA with factorial C.R.D. Pearson's correlation analysis along with coefficient of determination was used to determine the relationship. The differences in treatments were compared using the Tukey's HSD test ($p < 0.05$). The graphs were plotted using ggplot 2 package in R program. The statistical analysis was performed using the IBM SPSS 25.0.

RESULTS AND DISCUSSION

The *B. tabaci* incidence viz., the total number of adults settled, eggs and nymphs recorded on the soybean

genotypes showed significant differences. It was significantly lower in moderately resistant genotypes (SL 1074 and SL 1028) compared to the highly susceptible DS 3105, followed by susceptible (SL 688, SL 958, SL 1113) and moderately susceptible (PS 1572, PS 1347) genotypes (Table 1). Many arthropod insect herbivores, including whiteflies, choose the favourable host plants and leave behind the less preferred plants (Firdaus et al., 2011). A positive correlation between adult attractiveness and oviposition preference had been reported (Valle et al., 2012). After selecting the appropriate place, first instar nymphs permanently settle at one place and feeds on the leaf sap contents from the same place till the red-eyed nymph stage (Stansly and Naranjo, 2010). The host-related visual factors that were reported to mainly influence the attractance or repellence in whitefly are various morphological characteristics, plant metabolites and olfactory cues (Firdaus et al., 2011; Cui et al., 2016).

The increase in foliar flavonoids in tomatoes showed to impart antixenosis-based resistance to *B. tabaci* by reducing the performance of oviposition, landing, settling, probing and phloem-feeding (Yao et al., 2019). Several phenolic compounds induction in response to *B. tabaci* feeding damage and their negative effects on the development were reported in tobacco (Zhang et al., 2017). In present study, all the estimated metabolites significantly differed in uninfested and infested genotypes at 30 and 50 DAS. Total phenols increased in infested conditions, with significantly higher total phenols recorded in genotypes SL 1074 and SL 1028 (MR) (Table 2). Total phenols content increased by 11.3-22.6 folds, with resistant genotypes SL 1074 (22.6 and 21.1%) and SL 1028 (21.7 and 22.2%) recorded the highest increase at 30 and 50 DAS, respectively. Significant variation was observed in o-dihydroxy phenols and contents were high in infested genotypes. Among all the genotypes, DS 3105 (HS) revealed significantly lowest o-dihydroxy phenols, while significantly more values were recorded in moderately resistant genotypes SL 1028 and SL 1074. The o-dihydroxy phenols increase was 9.6-29.7 folds, with maximum being in genotypes SL 1074 (29.7 and 21.1%) and SL 1028 (29.1 and 21%) at 30 and 50 DAS, respectively. The flavonols significantly varied and increase in response to whitefly feeding was observed in all genotypes. The flavonols were significantly higher in genotypes SL 1074 and SL 1028 (MR) and were lowest in DS 3105; it was higher in resistant genotypes SL 1074 (23.1 and 21.6%) and SL 1028 (22.3 and 20.9%) at 30 and 50 DAS, respectively (Table 2). The tannin contents increased in genotypes subjected to whitefly stress and varied significantly. Tannins were significantly lowest in genotype DS 3105, followed by SL 688, SL 958, SL 1113. The resistant genotypes SL 1074 (7.3 and 10.2%) and SL 1028 (9.3 and 10.2%) showed the highest increase in tannins at 30 and 50 DAS, respectively (Table 2). Observations on the increase in secondary metabolites in response to *B. tabaci* damage corroborate with studies on various crops (Raghuraman et al., 2004; Taggar et al., 2014; Cui et al., 2012).

Phenolic compounds exhibit direct toxicity against the phytophagous insects and are also involved in the activation of defence signal pathways (Bi and Felton, 1995). Phenols oxidised to quinones, which covalently bind with the gut foliar proteins and affect

Table 1. *B. tabaci* preference and population buildup on soybean genotypes (multiple-choice test under screen-house conditions)

Genotype	*#Adults per trifoliate	*Oviposition trifoliate	*#Nymphs trifoliate	Nymphal survival ratio	*Red eyed nymphs trifoliate	Red eyed nymphal survival ratio
DS 3105	42.42± 0.26^{a}	36.30± 1.10^{a}	22.46± 0.28^{a}	0.62	25.29± 0.11^{a}	0.80
SL 688	32.47± 0.17^{b}	23.63± 0.39^{b}	17.21± 0.20^{bc}	0.73	16.14± 0.57^{bc}	0.68
SL 958	30.90± 0.33^{c}	21.85± 0.44^{bc}	16.33± 0.18^{bc}	0.75	15.32± 0.68^{bc}	0.68
SL 1113	29.46± 0.80^{c}	19.89± 0.51^{c}	15.56± 0.35^{c}	0.78	14.15± 0.04^{c}	0.66
PS 1347	23.32± 0.09^{d}	16.82± 0.19^{d}	12.02± 0.13^{d}	0.71	10.06± 0.43^{d}	0.61
PS 1572	21.80± 0.08^{e}	16.26± 0.37^{d}	11.42± 0.16^{d}	0.70	9.39± 0.04^{d}	0.59
SL 1028	18.64± 0.23^{f}	13.22± 0.21^{e}	8.96± 0.31^{e}	0.68	7.21± 0.06^{e}	0.59
SL 1074	17.88± 0.42^{f}	11.77± 0.88^{e}	8.30± 0.52^{e}	0.71	6.77± 0.11^{e}	0.62

*Mean of three replications recorded over a period of 5 weeks; Mean ± S.E.M (Standard error of mean); #Combined mean of three canopies (N=3) (upper, middle and lower) recorded during July 2018 and 2019; Numbers followed by same letter in same column not significantly different ($p \leq 0.05$; Tukey's HSD test)

Table 2. Total phenols, o-dihydroxy phenols, flavonols and tannin contents in leaves of healthy and *B. tabaci* infested soybean genotypes

Genotypes	Total phenols (mg/g dry wt)				o-dihydroxy phenols (mg g-1 dry weight)			
	30 DAS		50 DAS		30 DAS		50 DAS	
	Healthy plants	Infested plants	Healthy plants	Infested plants	Healthy plants	Infested plants	Healthy plants	Infested plants
DS3105	6.26±0.13c	7.13±0.21e	6.91±0.07c	7.80±0.05d	0.64±0.02f	0.75±0.02f	0.66±0.03d	0.73±0.02g
SL688	6.41±0.08c	7.35±0.01de	7.36±0.01bc	8.84±0.05c	0.73±0.01e	0.87±0.02e	0.75±0.01c	0.83±0.01f
SL958	6.44±0.02c	7.57±0.10de	7.73±0.23b	8.64±0.21c	0.76±0.01de	0.93±0.01d	0.75±0.00c	0.88±0.01e
SL1113	6.50±0.04c	7.74±0.08cd	7.93±0.11b	9.10±0.06c	0.79±0.01cd	1.00±0.00c	0.86±0.01b	1.00±0.00d
PS1347	6.67±0.06bc	8.03±0.05bc	9.72±0.25a	11.54±0.12b	0.81±0.01cd	1.07±0.01b	0.86±0.01b	1.07±0.01c
PS1572	6.95±0.20ab	8.42±0.12a	9.83±0.02a	11.78±0.28b	0.82±0.02bc	1.09±0.01b	0.87±0.01b	1.09±0.01c
SL1028	7.10±0.07a	9.07±0.08a	10.18±0.18a	13.12±0.16a	0.86±0.00ab	1.24±0.02a	0.98±0.01a	1.24±0.02b
SL1074	7.29±0.13a	9.42±0.02a	10.30±0.03a	13.13±0.05a	0.88±0.02a	1.29±0.00a	1.01±0.01a	1.29±0.00a
LSD (5%)	A= 0.10 B= 0.21 AXB= 0.30		A= 0.14 B= 0.29 AXB= 0.42		A= 0.013 B= 0.026 AXB= 0.037		A= 0.013 B= 0.025 AXB= 0.036	
	Flavonols (mg g-1 dry weight)				Tannins (mg g-1 dry weight)			
DS3105	0.51±0.00e	0.56±0.02f	0.65±0.01d	0.74±0.02b	9.91±0.00d	10.26±0.22e	10.70±0.00e	11.10±0.23f
SL688	0.54±0.03e	0.63±0.02ef	0.70±0.03cd	0.80±0.01b	10.77±0.16d	11.30±0.15de	11.57±0.16d	12.26±0.24e
SL958	0.54±0.01e	0.64±0.02ef	0.75±0.04c	0.89±0.02b	10.96±0.38d	11.49±0.48de	11.76±0.38d	12.33±0.26e
SL1113	0.58±0.02de	0.66±0.00ef	0.93±0.01b	1.08±0.02ab	11.11±0.19d	11.71±0.27d	11.91±0.19d	12.57±0.23e
PS1347	0.62±0.03de	0.77±0.03d	0.93±0.00b	1.15±0.02ab	12.55±0.23c	13.30±0.33c	13.35±0.23c	14.18±0.22d
PS1572	0.71±0.02c	0.89±0.02c	0.95±0.01b	1.19±0.01ab	13.97±0.30b	14.77±0.30b	14.77±0.30b	15.57±0.30c
SL1028	1.03±0.01b	1.33±0.03b	1.08±0.00a	1.36±0.02a	14.30±0.18ab	15.76±0.44ab	15.10±0.18b	16.81±0.63b
SL1074	1.11±0.02a	1.44±0.00a	1.10±0.03a	1.40±0.00a	15.34±0.46a	16.55±0.45a	16.14±0.46a	18.00±0.09a
LSD (5%)	A= 0.022 B= 0.043 AXB= 0.061		A= 0.019 B= 0.039 AXB= 0.055		A= 0.32 B= 0.64 AXB= NS		A= 0.30 B= 0.60 AXB= NS	

the digestive process (War et al., 2012). The elevated concentration of phenols induces feeding deterrence. They generate reactive oxygen species which causes oxidative damage to the midgut lipids and proteins resulting in the disruption to the normal growth and development, and extreme toxicity also leads to the death (War et al., 2012). Many hemipteran pests, including whitefly, target the plant vascular system as it consists of higher amounts of sugars and amino acids (Will et al., 2013). The total soluble sugars significantly varied among uninfested genotypes and these decreased genotypes in response to the *B. tabaci* feeding at 30 and 50 DAS; significantly more decrease was recorded in DS 3105 (HS) and SL 688 (S) and decrease was 15.3-31.0 folds (Table 3). The reducing sugars significantly differed among the uninfested genotypes with significantly less reduction being in genotypes SL 1074 (MR) and SL 1028 (MR); decrease was more in genotype DS 3105 (15.4 and 17.5%) at 30 and 50 DAS, respectively. Higher soluble and reducing sugars in the cotton genotypes during their vegetative and reproductive growth stages showed to increase the susceptibility against *B. tabaci* (Raghuraman et al., 2004). The total soluble proteins significantly differed in uninfested genotypes at 30 and 50 DAS and decreased under infested conditions; it was more in genotypes DS 3105 (HS) and SL 688 (S); and (15.1-33.7 folds, with resistant genotypes DS 3105 (17.8 and 15.1%) and SL 688 (20.9 and 16.5%) showing maximum decrease both at 30 and 50 DAS, respectively. Total soluble proteins indirectly serve as a potential source for supplying the dietary nitrogen requirements of *B. tabaci* (Salvucci et al., 1998). A significant decrease in foliar proteins with the damage imposed by *Helicoverpa zea* was reported in soybean (Bi and Felton, 1995).

Insect pests feeding-related changes observed in several crop plants are showed to be strongly correlated with the secondary metabolite contents (Raghuraman et al., 2004; Taggar et al., 2014; Cui et al., 2016). All the foliar phenolic compounds (total phenols, o-di hydroxy phenols, flavonols and tannins) showed a significant negative correlation with the nymphal and adult whitefly population at 30 and 50 DAS. The phenolic compounds at 30 DAS were found to have a significant negative correlation with *B. tabaci* population (nymphs and adults), viz. total phenols (-0.94^{**} and -0.91^{**}), o-dihydroxy phenols (-0.98^{**} and -0.98^{**}), flavonols (-0.83^{**} and -0.83^{**}), tannins (-0.95^{**} and -0.94^{**}), nymphs and adults, respectively (Table 2). A similar trend with was observed at 50 DAS. These data suggest that plants having higher phenolic compounds usually do not support *B. tabaci*. The significant negative correlation between *B. tabaci* and the total phenols, o-dihydroxy phenols, tannins and flavanols with higher activity in moderately resistant genotypes and their possible involvement in imparting resistance in black gram genotypes were documented (Taggar et al., 2014). A significant positive correlation was observed between the primary foliar metabolites (total soluble sugars, reducing sugars and total soluble protein contents) and *B. tabaci* (Table 2). The total soluble sugars (0.94^{**} and 0.92^{**}), reducing sugars (0.96^{**} and 0.95^{**}), and total soluble proteins (0.88^{**} and 0.85^{**}) contents at 30 DAS were significantly positively correlated with nymphs and adults, respectively. The recorded response at 50 DAS also showed similar trend. Therefore, higher amounts of total soluble sugars, reducing sugars and total soluble proteins in soybean genotypes offer more favourable conditions.

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AUTHOR CONTRIBUTION STATEMENT

RS and GKT have designed the experiments. SS has supervised the biochemical analysis of the leaf samples. GNH has conducted the experiment. GNH, RS and GKT wrote the manuscript.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Table 3. Total soluble sugars, reducing sugars and total soluble proteins contents in leaves of healthy and *B. tabaci* infested soybean genotypes

Genotypes	Total sugars (mg/g fresh wt)				Reducing sugars (mg g-1 fresh weight)			
	30 DAS		50 DAS		30 DAS		50 DAS	
	Healthy plants	Infested plants	Healthy plants	Infested plants	Healthy plants	Infested plants	Healthy plants	Infested plants
DS3105	24.32±0.48a	21.09±0.41a	32.62±0.21a	27.05±0.49a	1.07±0.01a	0.92±0.01a	1.54±0.06a	1.31±0.01a
SL688	23.89±0.11a	20.54±0.24a	32.32±0.16ab	26.96±0.73a	1.00±0.02ab	0.87±0.02ab	1.30±0.01b	1.10±0.12b
SL958	21.80±0.56b	18.45±0.32b	30.42±0.42c	24.25±0.22b	0.95±0.04ab	0.77±0.05bcd	1.07±0.00cd	0.89±0.02cd
SL1113	21.87±0.55b	18.55±0.42b	30.92±0.26bc	24.66±0.03b	0.98±0.05ab	0.80±0.01abc	1.12±0.00c	0.93±0.01c
PS1347	18.91±0.33c	15.37±0.41c	27.95±0.20d	21.72±0.61c	0.90±0.01bc	0.71±0.02cde	1.00±0.00cde	0.80±0.02cde
PS1572	19.73±0.73c	16.61±0.45c	28.16±0.32d	22.35±0.31c	0.92±0.03bc	0.71±0.02cde	1.03±0.01cde	0.84±0.01cde
SL1028	18.66±0.10c	15.08±0.32c	27.17±0.08d	21.35±0.28c	0.87±0.05bc	0.66±0.00de	0.96±0.02de	0.77±0.02de
SL1074	15.69±0.34d	12.34±0.17d	25.38±0.35e	19.37±0.57d	0.79±0.04c	0.58±0.03e	0.89±0.02e	0.68±0.02e
LSD (5%)	A= 0.41 B= 0.82 AXB= NS		A= 0.39 B= 0.77 AXB= NS		A= 0.03 B= 0.05 AXB= NS		A= 0.03 B= 0.07 AXB= NS	
	Total soluble proteins (mg g-1 fresh weight)							
DS3105	23.49±0.47a	19.94±0.66a	26.03±0.38a	22.63±0.37a				
SL688	23.31±0.05ab	19.28±0.13ab	25.59±0.13a	21.97±0.33ab				
SL958	22.83±0.68ab	18.30±0.30abc	23.55±0.35b	19.63±0.18c				
SL1113	23.18±0.32ab	18.70±0.10ab	24.98±0.24a	20.96±0.21b				
PS1347	22.04±0.10b	16.81±0.63c	19.57±0.33d	16.01±0.12e				
PS1572	22.38±0.55ab	17.79±0.13bc	21.01±0.23c	17.29±0.18d				
SL1028	19.70±0.32c	15.08±0.04d	18.09±0.04e	14.53±0.65f				
SL1074	17.14±0.48d	12.82±0.77d	16.47±0.14f	13.05±0.25g				
LSD (5%)	A= 0.44 B= 0.89 AXB= NS		A= 0.30 B= 0.60 AXB= NS					

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