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BIOCHEMICAL RESPONSES IN RESISTANT AND SUSCEPTIBLE SUNFLOWER ACCESSIONS ELICITED BY LEAFHOPPER, *AMRASCA BIGUTTULA BIGUTTULA* ISHIDA FEEDING

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ABSTRACT : The study aimed to understand the defense mechanism of sunflower germplasm accessions against leafhopper, *Amrasca biguttula biguttula* Ishida. Changes in biochemical parameters, *viz*., total sugars, total free amino acids, total phenols, chlorophyll and moisture content were studied in relation to the expression of reaction in seven resistant, four susceptible germplasm accessions and the susceptible check, Morden due to leafhopper feeding. The results indicated that resistant accessions had higher total phenols (153.04 77 µg g^{-1} of GAE) and chlorophyll (36.71) than the susceptible accessions whereas, total free amino acid (316.94 mg g^{-1} fresh weight), total sugars (93.27 mg g^{-1} dry weight) and moisture content (83.61%) were higher in susceptible accessions. The leafhopper nymphal population showed a highly significant and negative correlation with total phenols (r= -0.919) and chlorophyll (r=-0.870), while total free amino acids (r=0.698) and total sugars (r=0.928) showed a highly significant and positive correlation with the leafhopper population.

Key words : Chlorophyll, leafhopper, phenols, sunflower, total sugars.

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INTRODUCTION

Sunflower (*Helianthus annuus* L.) is an important oilseed crop. It has shown distinct superiority over other oilseed crops owing to its wider adaptability to different agro-climatic conditions, highest oil production per unit area, short duration, high yield potential, ability to withstand drought, photoperiod insensitivity, lower seed rate, high seed multiplication ratio and high-quality edible oil (Sindagi and Virupakshappa, 1986). In sunflower, leafhopper *Amrasca biguttula biguttula* Ishida (Homoptera : Cicadellidae) is one of the economically important pests as it is causing complete crop failure in case of severe infestation (Rana and Sheoran, 2004).

Amino acids are essential for insect growth and development while sugars act as feeding stimulants in insects (Thorteinson, 1960). Among phytochemicals, phenolic compounds (simple phenols, phenolic acids, hydroxycinnamic acid derivatives and flavonoids) are generated in plants as secondary metabolites through the shikimic acid pathway. The biosynthesis of phenolic compounds from the aromatic amino acid phenylalanine was found to be responsive to biotic and abiotic stresses (Cartea *et al*, 2011). Phenolic resins are general protein poisoners (Rhoades and Cates, 1976). The presence of free amino acids, sugars and phenols availability in the host plant may affect the insect population among the cultivars. Various studies have been conducted to identify the biochemical factors contributing to resistance in host plants against sucking pests. Taylo and Bernardo (1996) reported that total free sugars correlated positively to the leafhopper population in okra. Ramandeep (2016) observed a highly significant and negative correlation between the jassid nymphal population and total phenols in okra. Murugesan and Kavitha (2010) reported that protein content correlated positively with the leafhopper population in cotton. Breeding varieties that can genetically or physically resist the feeding by leafhoppers is one of the means by which leafhopper damage on the crop can be minimized as antibiosis factors of the host plant has been reported to play an important role in imparting resistance against pests and diseases (Panda and Khush, 1995). Relatively, resistant cultivars are known to contain an inherently higher amount of secondary metabolites (Dhaliwal and Dilawari, 1993). Research with relevance to the biochemical characterization of sunflower germplasm accessions to leafhopper is scanty. In the present study, certain biochemical responses due to leafhopper feeding and its impact on chlorophyll and moisture content in resistant and susceptible sunflower plants were taken up.

MATERIALS AND METHODS

Eleven germplasm accessions of sunflower *i.e.*, seven resistant accessions (GMU-339, GMU-696, TSG-197, TSG-401, GMU-504, TSG-HA-430-B and AKSFI-46-2); four susceptible accessions (PSECO-86, PSMO-53-B-1, PSCRM-127 and PSCIM-186) and a susceptible check, Morden were included in the experiment. Each of the accessions was sown in a single row of 4 m length with a spacing of 60 × 30 cm during late *rabi*, 2020-21 at ICAR IIOR farm, Rajendranagar, Hyderabad, India. The experiment was carried out in a completely randomized block design with three replications.

Observations on leafhopper population and injury were recorded on three randomly selected plants per replication on 65 days old crop when leafhopper population was at the highest. The nymphs of leafhopper were counted on top, middle and lower leaves from each sampled plant. Leafhopper injury was scored and grade was awarded on a 0-5 scale (0: Free from leafhopper injury; 1: Slight yellowish on the edges up to 30%; 2: Yellowing and curling up to 40% leaves; 3: Yellowing and curling up to 60% leaves; 4: Yellowing and curling up to 80% leaves and 5: Maximum, yellowing, cupping and curling up to 100%).

On the basis of injury grade, Mean Scale Index (MSI) was determined as under:

$$MSI = \frac{(G_0 \, x \, P) + (G1 \, x \, P) + (G2 \, x \, P) + (G3 \, x \, P) + (G4 \, x \, P) + (G_5 x P)}{TP}$$

Where,

G - Leafhopper Injury Grade (0 to 5)

P - The number of plants under the grade for each category

TP- Total number of plants taken for observation

Based on MSI, the accessions were grouped into five categories (0: Highly resistant; 0.1-1.0: Resistant; 1.1-2.5: Moderately resistant; 2.6-3.5: Susceptible and 3.6-5.0: Highly susceptible) (Ingale *et al*, 2019).

The plant samples were collected at 55 and 65 days after sowing (DAS) from each replication for the estimation of biochemical compounds. Plant samples were brought to the laboratory and analyzed for the total sugars, total free amino acids and total phenols. The quantity of total phenols was determined by the method of Ainsworth and Gillespie (2007). The amount of total free amino acids was estimated using the procedure developed by Moore and Stein (1948). Total sugars were estimated following the anthrone method (Yoshida *et al*, 1976). SPAD ("Scientific Plant Analysis Division" (APOGEE) MC-100 Plus Model) chlorophyll meter readings were recorded in the field from lower, middle and upper leaves of three randomly selected plants from each accession.

For the determination of moisture percentage, leaves from the top, middle and bottom parts of three plants were collected from all accessions. Leaves were cleaned with a muslin cloth, weighed and kept in a drying oven run at 65°C for 72 hours. After drying, the leaves were weighed again and put back into the oven at the same temperature, for another six hours. The leaves were taken out from the oven and kept in a desiccator for 10 minutes and weighed. After the weight of the dry material became constant, the moisture percentage was calculated according to the following formula:

$$\text{Moisture percent} = \frac{\text{Fresh weight of leaf} - \text{Dry weight of leaf}}{\text{Fresh weight of leaf}} \times 100$$

The means of the biochemical parameters were calculated and analysis of variance (ANOVA) was carried out using OP STAT software. T-test was done using data analysis in MS-Excel software. A simple correlation was made between the population of leafhoppers during the peak activity *i.e.*, 65 DAS and biochemical factors using data analysis Software Windowstat Version 9.1.

RESULTS AND DISCUSSION

The data regarding the nymphal population and biochemical parameters at 55 and 65 DAS is presented in Tables 1 and 2 and discussed hereunder.

Nymphal population

At 55 DAS average nymphal population ranged between 3.0 (TSG-401) and 12.5 (PSMO-53-B-1) and the susceptible check, Morden recorded 10.8 with MSI ranging from 1.0 to 3.2. The average nymphal population at 65 DAS ranged between 6.5 (TSG-197) and 17.6 (PSMO-53-B-1), whereas the susceptible check, Morden recorded 18.3 with MSI ranging from 1.0 to 3.5.

Total phenols

Significant differences were observed in the mean

Table 1 : Biochemical constituents in resistant and susceptible sunflower germplasm accessions of leafhopper at 55 days after sowing (*Rabi*, 2020-21).

S. no.	Germplasm	Average no. of leafhopper nymphs/3 leaves/ plant*	MSI	Category	Total phenols (µg g^{-1} of GAE)	Total free amino acids (mg g^{-1} fresh weight)	Total sugars (mg g^{-1} dry weight)	SPAD chlorophyll meter reading	Moisture content (%)
1	GMU-339	4.4	1.0	Resistant	133.16^{a}	183.57^{bc}	42.93^{a}	38.56^{c}	82.68^{c}
2	GMU-696	3.4	1.0	Resistant	113.73^{c}	188.71^{c}	49.32^{c}	41.33^{bc}	83.60^{cd}
3	TSG-197	3.6	1.0	Resistant	129.67^{b}	186.41^{bc}	45.05^{b}	42.55^{b}	80.61^{a}
4	TSG-401	3.0	1.0	Resistant	121.99^{b}	184.60^{bc}	40.69^{a}	45.74^{a}	82.00^{bc}
5	GMU-504	4.9	1.0	Resistant	105.59^{d}	168.72^{a}	54.13^{d}	40.58^{bc}	81.92^{bc}
6	TSG-HA-430B	3.8	1.0	Resistant	108.25^{c}	164.80^{a}	53.71^{d}	39.83^{c}	83.95^{d}
7	AKSFI-46-2	3.8	1.0	Resistant	111.57^{c}	165.17^{a}	51.60^{c}	38.16^{c}	82.66^{c}
8	PSMO-53-B-1	12.5	3.0	Susceptible	85.31^{e}	178.06^{b}	80.38^{f}	40.53^{bc}	81.60^{b}
9	PSECO-86	9.6	3.2	Susceptible	89.44^{e}	249.35^{e}	86.17^{g}	33.95^{d}	83.93^{d}
10	PSCRM-127	8.1	2.8	Susceptible	88.82^{e}	186.19^{bc}	65.42^{e}	41.03^{bc}	85.13^{e}
11	PSCIM-186	9.4	2.8	Susceptible	86.26^{e}	182.18^{bc}	66.87^{e}	42.03^{bc}	84.11^{de}
12	Morden (SC)	10.8	3.0	Susceptible	88.05^{e}	237.84^{d}	90.45^{h}	38.63^{c}	84.92^{e}
	S.Em.±	-	-	-	2.15	2.97	0.99	0.81	0.32
	LSD (0.05%)	-	-	-	6.35	8.77	2.95	2.39	0.96
	Mean (Resistant)	3.8	-	-	117.71	177.43	48.20	40.96	82.49
	Mean (Susceptible)	10.0	-	-	87.58	206.72	77.86	39.24	83.94
	t-cal	-	-	-	6.87	0.0018	2.48	0.05	0.001
	t- tab (p=0.05)	-	-	-	1.69	1.69	1.69	1.69	1.69

*Mean of three plants; SC = Susceptible check; GAE = Gallic acid equivalent; MSI = Mean scale index
In vertical columns, means followed by same letter do not differ significantly by DMRT (P=0.05).

total phenols of resistant and susceptible accessions of sunflower at both the 55 and 65 DAS.

At 55 DAS, total phenols among the resistant accessions ranged between 105.59 and 133.16 µg g^{-1} of gallic acid equivalent (GAE). Significantly highest total phenols were found in GMU-339 (133.16 µg g^{-1} of GAE) followed by TSG-197 (129.67 µg g^{-1} of GAE), TSG-401 (121.99 µg g^{-1} of GAE) and GMU-696 (113.73 µg g^{-1} of GAE). The total phenols were significantly lower in susceptible accessions ranging between 85.31 and 89.44 µg g^{-1} of GAE while the susceptible check, Morden recorded 88.05 µg g^{-1} of GAE. Resistant accessions had significantly higher mean total phenols (117.71µg g^{-1} of GAE) as compared to susceptible accessions (87.58 µg g^{-1} of GAE) (t-cal, 6.87, p=0.05).

At 65 DAS, total phenols ranged from 147.36 to 157.84µg g^{-1} of GAE in resistant accessions. Among the resistant accessions, GMU-339, TSG-197, GMU-696 and TSG-401 recorded significantly higher total phenols (154.55 to 157.84 µg g^{-1} of GAE), which were on par with each other. Lower total phenols (93.49 to 96.77 µg g^{-1} of GAE) were observed in susceptible accessions whereas, susceptible check, Morden had total phenols of 89.62 µg g^{-1} of GAE. Resistant accessions had significantly higher mean total phenols (153.04 µg g^{-1} of GAE) as compared to susceptible accessions (94.86 µg g^{-1} of GAE) (t-cal, 5.63, p=0.05).

Total Free amino acids (FAA)

At 55 DAS non-significant differences between the mean total FAA of resistant and susceptible accessions were observed. Total FAA ranged between 164.80 to 249.35 mg g^{-1} fresh weight whereas, the susceptible check, Morden recorded a total FAA of 237.84 mg g^{-1} fresh weight.

Total FAA among the resistant accessions at 65 DAS ranged from 241.37 to 300.09 mg g^{-1} fresh weight. The lowest total FAA among the resistant accessions

Table 2 : Biochemical constituents in resistant and susceptible sunflower germplasm accessions during the peak activity of leafhopper at 65 days after sowing (*Rabi*, 2020-21).

S. no.	Germplasm	Average no. of leafhopper nymphs/3 leaves/ plant*	MSI	Category	Total phenols (µg g^{-1} of GAE)	Total free amino acids (mg g^{-1} fresh weight)	Total sugars (mg g^{-1} dry weight)	SPAD chlorophyll meter reading	Moisture content (%)
1	GMU-339	7.7	1.0	Resistant	157.84^{a}	244.77^{a}	51.24^{b}	36.73^{a}	82.77^{bc}
2	GMU-696	8.3	1.0	Resistant	155.43^{a}	241.37^{a}	56.54^{c}	37.99^{a}	83.86^{c}
3	TSG-197	6.5	1.0	Resistant	157.69^{a}	250.79^{ab}	51.22^{b}	36.84^{a}	80.65^{a}
4	TSG-401	8.8	1.0	Resistant	154.55^{a}	243.36^{a}	48.66^{a}	37.61^{a}	82.35^{b}
5	GMU-504	9.7	1.0	Resistant	148.23^{b}	296.19^{c}	63.35^{e}	33.67^{b}	80.55^{a}
6	TSG-HA-430B	10.4	1.0	Resistant	147.36^{b}	300.09^{cd}	59.69^{d}	35.70^{ab}	83.67^{c}
7	AKSFI-46-2	9.1	1.0	Resistant	150.15^{b}	269.42^{b}	60.38^{d}	38.44^{a}	82.54^{b}
8	PSMO-53-B-1	17.6	3.2	Susceptible	95.71^{cd}	340.59^{d}	85.56^{g}	28.57^{c}	81.28^{ab}
9	PSECO-86	12.3	3.4	Susceptible	93.49^{cd}	323.31^{d}	100.67^{i}	30.77^{bc}	83.67^{c}
10	PSCRM-127	12.5	2.8	Susceptible	96.77^{c}	285.06^{bc}	89.15^{h}	30.74^{bc}	84.97^{d}
11	PSCIM-186	13.9	3.0	Susceptible	95.38^{cd}	291.85^{bc}	83.66^{f}	32.84^{bc}	83.20^{bc}
12	Morden (SC)	18.3	3.5	Susceptible	89.62^{d}	343.92^{d}	107.33^{j}	30.24^{c}	84.96^{d}
	S.Em.±	-	-	-	2.09	7.89	0.448	1.01	0.36
	LSD (0.05%)	-	-	-	6.18	23.29	1.32	2.99	1.07
	Mean (Resistant)	8.4	-	-	153.04	263.71	55.87	36.71	82.34
	Mean (Susceptible)	14.9	-	-	94.86	316.94	93.27	30.63	83.61
	t-cal	-	-	-	5.63	3.60	7.21	2.23	0.007
	t- tab (p=0.05)	-	-	-	1.69	1.69	1.69	1.69	1.69

*Mean of three plants; SC= Susceptible check; GAE=Gallic acid equivalent; MSI=Mean scale index.
In vertical columns, means followed by same letter do not differ significantly by DMRT (P=0.05).

were found in GMU-696 (241.37 mg g^{-1} fresh weight) followed by TSG-401 (243.36 mg g^{-1} fresh weight), GMU-339 (244.77 mg g^{-1} fresh weight) and TSG-197 (250.79 mg g^{-1} fresh weight) which remained on par with each other. The FAA ranged between 285.06 and 340.59 mg g^{-1} fresh weight in the susceptible accessions whereas, susceptible check, Morden had a total FAA of 343.92 mg g^{-1} fresh weight. Resistant accessions had significantly lower mean FAA (263.71 mg g^{-1} fresh weight) as compared to susceptible accessions (316.94 mg g^{-1} fresh weight) (t-cal, 3.60, p=0.05).

Total sugars

Significant differences were observed in mean total sugars in resistant and susceptible accessions of sunflower at both the 55 and 65 DAS.

At 55 DAS, total sugars among the resistant accessions ranged between 40.69 and 54.13 mg g^{-1} dry weight. Significantly lowest total sugars were found in TSG-401 (40.69 mg g^{-1} dry weight) which was on par with GMU-339 (42.93 mg g^{-1} dry weight) followed by TSG-197 (45.05 mg g^{-1} dry weight) and GMU-696 (49.32 mg g^{-1} dry weight). Total sugars ranged between 65.42 and 86.17 mg g^{-1} dry weight in the susceptible accessions while the susceptible check, Morden recorded 90.45 mg g^{-1} dry weight. Resistant accessions had significantly lowest total sugars (48.20 mg g^{-1} dry weight) as compared to susceptible accessions (77.86 mg g^{-1} dry weight) (t-cal, 2.48, p=0.05).

Total sugars among the resistant accessions ranged from 48.66 to 63.35 mg g^{-1} dry weight at 65 DAS. TSG-401 recorded the lowest total sugars of 48.66 mg g^{-1} dry weight followed by TSG-197 (51.22 mg g^{-1} dry weight), GMU-339 (51.24 mg g^{-1} dry weight) and GMU-696 (56.54 mg g^{-1} dry weight). Significantly higher total sugars (83.66 to 100.67 mg g^{-1} dry weight) were observed in susceptible accessions whereas, susceptible check, Morden had total sugars of 107.33 mg g^{-1} dry weight. Resistant accessions had significantly lower mean total sugars (55.87) as compared to susceptible accessions (93.27) (t-cal, 7.21, p=0.05).

SPAD chlorophyll meter reading

The rate of depletion of chlorophyll was lower in resistant accessions with mean SPAD chlorophyll meter readings of 40.96 and 36.71 as compared with susceptible accessions, 39.24 and 30.63 at 55 and 65 DAS, respectively. At 55 DAS non-significant difference between the mean SPAD readings of resistant and susceptible accessions was observed. The SPAD reading among the resistant accessions ranged between 38.16 and 45.74, in susceptible accessions, it ranged from 33.95 to 40.53 whereas, the susceptible check, Morden recorded SPAD reading of 38.63.

Significant difference (t-cal= 2.23, p=0.05) was observed in mean SPAD reading of resistant (36.71) and susceptible accessions (30.63). SPAD reading among the resistant accessions ranged from 33.67 to 38.44 where, highest SPAD reading was found in AKSFI-46-2 (38.44) followed by GMU-696 (37.99), TSG-401(37.61), TSG-197 (36.84), GMU-339 (36.73) and TSG-HA-430-B (35.70) and GMU-504 (33.67). Chlorophyll was depleted more in susceptible accessions where SPAD reading ranged from 28.57 to 32.84 whereas, susceptible check, Morden had SPAD reading of 30.24, respectively at 65 DAS.

Moisture content

The non-significant difference was recorded in the mean percent moisture content of resistant and susceptible accessions although among the accessions significant differences were recorded at 55 and 65 DAS. The percent moisture content ranged from 80.61 to 85.13% and 80.55 and 84.97% whereas, the susceptible check, Morden recorded moisture content of 84.92 and 84.96%, respectively at 55 and 65 DAS.

Correlation between leafhopper nymphal population and biochemical parameters

The correlation between leafhopper nymphal population and biochemical parameters (n=36) during the peak activity of leafhopper at 65 DAS was worked out (Table 3). The leafhopper nymphal population showed a highly significant and negative correlation with total phenols (r= -0.919) and chlorophyll (r=-0.870). However, total free amino acids (r=0.698) and total sugars (r=0.928) showed a highly significant and positive correlation, while a non-significant and positive correlation with moisture content (r=0.349).

The results indicated that there was a significant difference between resistant and susceptible accessions with respect to biochemical constituents. The total phenols and chlorophyll were significantly higher in resistant accessions compared to susceptible accessions. The present results are in agreement with those of Krishnaananda (1973), who reported total phenolic compounds have been found to play a decisive role in the defense mechanisms of plants against infectious agents. Anusha *et al* (2016) reported that the maximum biochemical response was of phenols (104 % increased) to leafhopper infestation indicating their major role in defense against mango leafhopper infestation. Similarly, Gadad *et al* (2014) reported that biochemical analysis of leaves of all the test varieties indicated that phenol was negatively and significantly correlated with thrips incidence in groundnut. The present results also get support from the findings of Murugesan and Kavitha (2010), who also reported that chlorophyll was negatively associated with leafhopper oviposition and damage. Total free amino acids and sugars were significantly lower in resistant accessions compared to susceptible accessions. Diets with increased specific free amino acids increase the fecundity and ovipositional capacity and efficiency of insects (Rahul, 2020). Kandakoor *et al* (2014) reported that amino acids (r= 0.830, 0.723) showed a positive relationship with the number of thrips and their percent damage in groundnut. Our results regarding the positive correlation of total sugars with the leafhopper population are in line with Taylo and Bernardo (1995), who also reported a positive correlation between total sugars and leafhopper susceptibility. However, Singh and Agarwal (1988), Hooda *et al* (1997) and Iqbal *et al* (2011) reported that total sugars had a negative correlation with the leafhopper population and that the resistant varieties had higher sugars. The mean moisture content showed no significant difference in resistant and susceptible accessions although among the accessions significant differences were recorded and in susceptible accessions, the moisture content was more. The present findings are in conformity with those of Iqbal *et al* (2011a), who reported that moisture content of the middle leaves showed a positive but significant correlation with the jassid

Table 3 : Correlation coefficients of leafhopper, *Amrasca biguttula biguttula* incidence with biochemical parameters in sunflower germplasm accessions (*Rabi*, 2020-21).

Biochemical parameters	Correlation coefficient (r)
Total phenols	-0.919***
Total free amino acids	0.698*
Total sugars	0.928***
SPAD chlorophyll meter reading	-0.870***
Moisture content	0.349^{NS}

***Significant at <0.01, *Significant at 0.01; NS: non-significant.

population in okra.

CONCLUSION

Higher total phenols, lower total free amino acids, total sugars and moisture content conferred resistance to the sunflower germplasm accessions against leafhopper. Higher total phenol and lower total sugars and total free amino acids in sunflower accessions *viz*., TSG-197, GMU-339, TSG-401 and GMU-696 seem to be associated with resistance. This revealed that a sole factor is not responsible for the resistance but the amalgamation of various parameters together plays an important role in offering resistance in these accessions.

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