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Biochemical constituents vis-a-vis Leafhopper, *Amrasca biguttula biguttula* (Ishida) tolerance in Sunflower germplasms

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Abstract
Leaf hoppers, *Amrasca biguttula biguttula* Ishida (Homoptera: Cicadellidae) are the important sucking pests of sunflower in India. Both nymphs and adults suck the plant sap and their severe infestation leads to curling of leaves and the characteristic "hopper burn" symptom. Leaf hopper infestation reduces the oil yield. Field screening of 28 sunflower germplsma revealed that six of the germplasms reacted as resistant (GMU-25,339,504,922,570 and GP9-472-4-13), 16 germplasms as moderately resistant (GMU-1, 4, 116, 405, 595, 669, 703, 782, 914, 1029, 243, 327, 556, 696, 776 and AKSFI-46-2,) and six as susceptible (GMU-255, 713, 795, 1093, 112 and 343) to leafhopper pest. Total soluble sugars (r =0.243, 0.933), reducing sugars (r = 0.143, 0.736), non reducing sugars (r =0.289, 0.882) and leaf nitrogen (r =0.194, 0.904) showed positive relationship with number of leafhopper pests and their damage. Phenols (r = -0.187, -0.748) and tannins(r = -0.080, -0.821) showed negative relationship with number and damage of leafhopper pests. Hence, these resistant screened lines may be used for further use in breeding programme and integrated pest managenent programme in sunflower.

Keywords: Sunflower germplasms, leafhopper, *Amrasca biguttula biguttula* (Ishida), phenols, tannins, sugars

Introduction
Sunflower (*Helianthus annuus* L.) is one of the important oilseed crops in the world which ranks third in area after soybean and groundnut. Indian farmers started large scale cultivation of sunflower in 1972 with the introduction of high yielding Russian varieties. Sunflower yield levels of the country are the lowest in the world due to several biotic and abiotic factors and yet the potential of the crop is, far from being exploited. Sunflower insect pests are broadly categorised as seedling pests, sucking pests, soil insects, defoliators and inflorescence pests (Basappa and Prasad, 2005) [1]. Sucking pests like leafhoppers, thrips and whiteflies causes considerable extent of loss to the crop.

Leafhopper, *Amrasca biguttula biguttula* (Ishida) appears in serious form causing crop loss up to 46 per cent (Anonymous, 1997) [2]. The pest is of economic importance in Karnataka. The pest incidence would start from seedling stage and prevails throughout crop period. Damage symptoms of the pest include stunted growth of plant, cupped and crinkling leaves, and burnt appearance of leaf margin(Anonymous, 2000) [3]. Breeding varities which can genetically or physically resist the feeding by leafhoppers is one of the means by which leafhopper damage on sunflower crop can be minimized as antibiosis factors of the host plant have been reported to play an important role in imparting resistance against pests and diseases (Panda and Khush, 1995) [4] and relatively resistant cultivars are known to contain inherently higher amount of secondary metabolites (Dhaliwal and Dilawari, 1993) [5].

Host plant resistance provides an additional component to integrate with other management tactics to minimize yield loss in sunflower. Research in relevance to biochemical characterization of sunflower germplasm lines against leafhopper is lacking though leafhopper is a major sucking insect pest on sunflower. Hence, the present studies were undertaken on biochemical characterization of promising sunflower germplasm lines against leafhopper, *Amrasca biguttula biguttula* (Ishida).

Materials and Methods
Screening of sunflower germplasms: Field screening experiments were conducted during *Rabi* 2016-17 at Main Agricultural research Station, UAS, Raichur, Karnataka, India.

Totally 28 germplasms were screened along with local and standard checks in the field under natural infestation of leafhopper pests to identify the resistant ones. Sunflower germplasms were sown in three replications at 60x30 cm between rows and plants respectively and all the recommended package of practices were followed except plant protection measures. The reaction of sunflower germplasms was assessed by visual grading of damage and insect counts on each test entry and were categorized accordingly.

Collection of samples for biochemical analysis

Leaf samples of resistant, moderately resistant and susceptible group of sunflower germplasms (Totally 15) along with local check were collected and two grams of leaf sample was weighed, cut into small pieces and grounded finely in a mortar and pestle in 80 per cent alcohol and then filtered through Whatman No-41 filter paper and volume was made upto 20 ml. 2 ml of saturated lead acetate and 3 ml of di-sodium hydrogen phosphate was added to the filtrate to clarify the dark colour and filtered through whatman no. 41 and final volume made upto 20 ml using 80 per cent alcohol This constituted the stock solution from which aliquots were drawn for the estimation of total sugars, reducing sugars, phenols and tannins. The absorbance of each chemical constituent in a sample was measured by using spectrophotometer.

Total Sugars: Total sugars were estimated by hydrolyzing non reducing sugars by adding 1 ml of 1 N sulphuric acid to 1 ml of aliquot and heated over boiling water bath for 30 minutes and two drops of phenolphthalein indicator was added after cooling. Acid in the hydrolysate was neutralized by adding 1 N sodium hydroxide drop wise till it developed pink colour. Finally it was made colourless by adding 0.1 N sulphuric acids and volume was made up to 10 ml with distilled water and absorbance was read at 620 nm.

Reducing sugars: To estimate reducing sugars, 1 ml of A+B reagent was added 1 ml of aliquot and then the mixture was heated for 20 minutes. 1 ml of arsenomolybdate solution was added after cooling and finally volume was made up to 10 ml with distilled water. The absorbance was read at 620 nm. Standard curve prepared with glucose was used to calculate total sugars and reducing sugars in the samples.

Non-reducing sugars: The difference between the total soluble sugar and reducing sugar without hydrolysis corresponds to the quantity of non-reducing sugar.

Total phenol content: Folin-Ciocalteau reagent and 2 per cent sodium carbonate were used to estimate total phenols. 1 ml of 1 N FCR and Two milliliters of two per cent sodium carbonate in 0.1 N sodium hydroxide solutions was added to One milliliter of aliquot and then the mixture was stirred and placed on a hot water bath for one minute. Then test tubes were cooled immediately under running water and the volume was made up to 10 ml with distilled water. Colour absorbance was measured at 650 nm. catechol Standard curve was used to determine total phenol in the test samples.

Total tannin content: To estimate tannins, 0.2 ml of aliquot from each sample was taken in a test tube to which 0.5 ml of FDR was added. To each test tube 1 ml of saturated sodium carbonate was added and the volume was made up to 20 ml with distilled water and finally the intensity of colour developed was read at 735 nm. A tannic acid standard curve was used to quantity of tannin in the sample.

Leaf nitrogen: Nitrogen content in the plant sample was determined by using Micro-Kjeldahl technique. 0.5 g of powdered plant sample was digested using 10 ml of concentrated sulphuric acid at 420 ºC for 1.5 hours in the presence of 0.2 g catalyst mixture. The contents were cooled and transferred quantitatively to 50 ml volumetric flask to make up the volume by adding water. Distillation was carried out by adding 10 ml 40 per cent sodium hydroxide (NaOH) in distillation unit and the ammonia evolved in container containing boric acid was mixed with indicator solution. The amount of ammonia trapped in the container is estimated by titrating against 0.2 N sulphuric acid (H_2SO_4).

The per cent Nitrogen is calculated by using the formula

$$\% \text{ Nitrogen} = \frac{\text{Titre value x Normality of } H_2SO_4 \text{ x } 50}{\text{Weight of plant sample X } 10}$$

Statistical Analysis: The data was analysed by following the statistical procedure to work out means and correlation coefficients.

Results and Discussions

Leafhopper pests were found to be active throughout the *rabi* season from November 2016 to January, 2017. Weekly observations revealed that among 28 germplasms screened against leafhopper pests, six were categorized as Resistant, 16 as moderately resistant, six as susceptible (Table1 & 2). Sunflower germplasm screening work against sucking pests was conducted and reported by several workers but the germplasms involved in screening were different. For instance, Suganthy and Uma (2010) [6] reported a maximum of 28 hoppers per plant in Morden. Based on the mean scale index, in first season, four accessions *viz.*, KBSH 1, AHT 14, GK 2002 and GMU 698 had less leaf hopper population (< 1.0 hopper/plant) than other accessions and were grouped as resistant varieties (Table 1). Another six accessions *viz*., AHT 17, IHT 751, GMU 606, GMU 647, K 578 and GMU 621 recorded higher mean population (1.0 to 2.0 hoppers/plant) and based on the mean, these were grouped as moderately resistant varieties. Among the remaining accessions, 95 accessions were rated as susceptible and seven accessions were rated as highly susceptible. Based on this study, the accessions GMU-25, GMU-339, GMU-504, GMU-922, GMU-570 and GP9-472-4-13 recorded the least hopper population and injury grade and hence can be used for further genetic improvement programmes.

Total sugar content of different sunflower germplasms varied from 9.12 mg/g (GMU-25) to 22.85 mg/g (GMU-112) per gram of leaf sample. The highest quantities were noticed in susceptible germplasms. Reducing sugar content of different germplasms varied from 3.10 mg/g (GMU-504) to 10.52 mg (GMU-112) per gram of leaf sample.Non-reducing sugar content of different germplasms varied from 3.68 mg (GMU-25) to 11.60 mg (GMU-112) per gram of leaf sample (Table 3). Similarly, % Leaf Nitrogen content of different germplasms varied from 1.4% (GMU-25) to 3.81% (GMU-112). These contents were positively correlated with

leafhopper pests population and their damage, Total soluble sugars (r =0.243, 0.933), reducing sugars (r = 0.143, 0.736), non reducing sugars (r =0.289,0.882) and leaf nitrogen (r =0.194,0.904) (Table 3 & 4). These results are in line with Nachiappa and Baskaran (1983) [7] whose results revealed that, higher content of reducing sugars and lower phenols were observed in susceptible mango genotypes like padiri, neelum, sindura, peter and mulgoa. Lower content of sugars was witnessed in resistant khader, baneshan, bangalora and chinnarasam genotypes. Similarly, Thimmaiah (1992) [8] also reported that the total sugar content in the leaves, stem tips, squares and boll rind of cotton genotypes reveals that vegetative parts of susceptible genotypes to insect pests invariably have higher levels of sugars, where as the resistant genotypes have comparatively lower levels of sugars. Further, Soundarajan and Baskaran (2001) [9] reported that the sugar content was negatively correlated with resistance to *B. tabaci* in brinjal. The present findings are also accordance with Naga *et al*. (2014) [10] who reported that the total soluble sugars were positively correlated with whitefly population. Nanda *et al* (2000) [11] observed low level of total soluble in the leaf sheath of resistant groundnut genotype Ptb-33 (1.45%) compared with the susceptible genotype TN-1 (2.08%).

The data recorded on phenol content of different germplasms varied from 0.85 mg to 4.43 mg per gram of leaf sample among the susceptible and resistant group, respectively. The highest quantities were noticed in moderately resistant and resistant group. However, lower quantities of phenols were noticed in susceptible sunflower germplasms. These results showed significant differences at 5% level of significance. There was a negative correlation between phenols and Leafhopper pests population and their damage (r = -0.187, -0.748). The phenols and tannin contents showed significant negative correlation with sucking pest population. These results are in confirmation with the findings of Somasekhar *et al*. (2003) [12] where thrips resistant groundnut varities had higher quantities of phenols and tannins compared with the susceptible varities. Rohini *et al*., (2011) [13] reported that the presence of high quantity of biochemical components like tannins, phenols conferred resistance against thrips. Significant negative correlations were obtained between polyphenols and damage indices (r = -0.57), mean adult counts (r = -0.56), and mean larval counts (r = -0.64) of resistant cowpea cultivars, indicating that polyphenols play a significant role in cowpea thrips resistance (Alabi *et al*., 2011) [14].

Tannin content varied from 2.32 to 4.82 mg g^{-1} of leaf sample. Lower tannin content was recorded in susceptible genotypes viz., GMU-112 (2.32 mg g^{-1} of leaf sample) and GMU-795(2.42 mg g^{-1} of leaf sample), while the resistant genotypes had higher quantities of tannins ranging from 3.42 to 4.82 mg g^{-1} of leaf sample. The data showed a significant negative relationship between tannin content and leafhopper population (r= -0.080). A similar trend was observed between tannin contents and per cent leaf damage (r= -0.821) at 5% level of significance (Table 3 & 4). The total phenol and tannin contents in different plant parts (leaves, squares and bolls) of different cotton varities/hybrids showed significant negative relationship with the incidence of thrips (Balakrishnan, 2006) [15]. The results obtained are also in confirmation with earlier findings of Venugopal Rao *et al*. (1990) [16] who reported that the presence of higher concentrations of biochemical components like phenols and tannins conferred resistance to whitefly in cotton. Similarly, findings of Acharya and Singh (2008) [17] revealed that the total content of tannin, phenol and gossypol were negatively correlated with the population density of whiteflies. However, Jindal and Dhaliwal (2009) [18] reported that there was no significant effect of phenols and tannins on population of whitefly.

Table 1: Reaction of Sunflower germplams against leafhopper damage during *rabi* 2016-17

Germplasm	Leafhopper population /6 leaves/Plant	Injury grade
GMU-1	16.60	1
GMU-4	13.30	1
GMU-25	14.30	0
GMU-112	11.20	4
GMU-116	13.50	1
GMU-243	13.70	2
GMU-255	12.10	3
GMU-327	12.60	2
GMU-339	8.90	0
GMU-343	12.80	4
GMU-405	11.90	1
GMU-504	11.00	0
GMU-556	13.20	2
GMU-595	6.50	1
GMU-669	9.10	1
GMU-696	13.10	2
GMU-703	15.40	1
GMU-713	14.20	3
GMU-776	12.80	2
GMU-782	19.90	1
GMU-795	8.70	3
GMU-914	10.10	1
GMU-922	8.00	0
GMU-1029	12.10	1
GMU-1093	11.80	3
GMU-570	11.30	0
GP9-472-4-13	9.20	0
AKSFI-46-2	12.20	1
Morden (SC)	16.70	4
KBSH-44 (LC)	22.10	4

Table 2: Reactions of selected sunflower germplasms to leafhopper damage under field condition during *rabi* 2016-17

Score	Genotype	Reaction
0	GMU-25, GMU-339, GMU-504, GMU-922, GMU-570, GP9-472-4-13	Resistant
1-2	GMU-1, GMU-4, GMU-116, GMU-405, GMU-595, GMU-669, GMU-703, GMU-782, GMU-914, GMU-1029, AKSFI-46-2, GMU-243, GMU-327, GMU-556, GMU-696, GMU-776	Moderately resistant
3-4	GMU-255, GMU-713, GMU-795, GMU-1093, GMU-112, GMU-343, Morden (SC), KBSH-44 (LC)	Susceptible

Table 3: Biochemical constituents of selected sunflower germplasms during *rabi* 2016-17

Germ plasm	Leafhopper population /6 leaves/Plant	Injury grade	Total soluble sugars (mg/g)	Reducing sugars (mg/g)	Non reducing sugars (mg/g)	Phenols (mg/g)	Tannins (mg/g)	Leaf Nitrogen (%)
GMU-339	8.90	0	12.94	7.60	5.17	2.13	3.42	2.58
GMU-504	11.00	0	9.34	3.10	6.03	3.73	4.57	1.52
GMU-25,	14.30	0	9.12	5.35	3.68	4.43	4.82	1.40
GMU-922	8.00	0	10.92	7.10	3.73	4.43	3.97	1.99
GMU-570	11.30	0	10.24	4.47	5.58	2.43	4.22	2.04
GMU-116	13.50	1	14.98	8.88	5.91	1.84	3.08	2.89
GMU-1	16.60	1	14.53	9.13	5.25	1.89	3.18	2.69
GMU-4	13.30	1	15.43	6.75	8.36	1.79	3.13	2.76
GMU-327	12.60	2	16.55	8.25	8.00	1.69	2.98	3.03
GMU-243	13.70	2	15.88	8.38	7.24	1.84	2.93	2.97
GMU-713	14.20	3	16.55	9.15	6.93	1.50	2.67	3.32
GMU-795	8.70	3	17.90	9.65	7.73	1.45	2.42	3.46
GMU-255	12.10	3	17.45	8.40	8.49	1.45	2.77	3.26
GMU-112	11.20	4	22.85	10.52	11.60	0.85	2.32	3.81
GMU-343	12.80	4	20.15	9.27	10.23	1.05	2.47	3.72
KBSH-44 (LC)	22.10	4	19.02	8.37	10.23	1.87	3.12	3.37

Table 4: Correlation of biochemical constituents with leafhopper density during *rabi* 2016-17

Host plant characters	Leafhopper (r values	
	Leafhopper density	Injury to foliage
Total soluble sugars (mg/g)	0.243	0.933
Reducing sugars (mg/g)	0.143	0.736
Non reducing sugars (mg/g)	0.289	0.882
Phenols (mg/g)	-0.187	-0.748
Tannins (mg/g)	-0.080	-0.821
Leaf Nitrogen (%)	0.194	0.904

Conclusion

In the current investigation, 28 sunflower genotypes screened in field and laboratory experiment, only six genotypes may prove promising in breeding programme concerning resistance to leafhopper pests. Phenols and tannins conferred the sunflower genotype resistance to leafhopper pests damage. This suggests that sunflower varities with high concentration of phenols and tannins play a major role against leafhopper pest damage.

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