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Induced changes in the antioxidative compounds of *Vigna mungo* genotypes due to infestation by *Bemisia tabaci* (Gennadius)

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Abstract

Antioxidative compounds were quantified from the leaves of nine black gram (*Vigna mungo* (L.) Hepper) genotypes over a period of two years, for potential whitefly, *Bemisia tabaci* (Gennadius) (Hemiptera: Aleryrodidae) resistance. Oviposition preference, nymphal and adult development were evaluated under screen-house conditions. Biochemical analysis revealed that higher per cent increase in the total phenol and *o*-dihydroxy phenol contents both at 30 and 50 days after sowing was evident in moderately resistant genotypes NDU 5-7 (49.6 and 50.8%, respectively) and KU 99-20 (47.8 and 50.8%, respectively) under whitefly stress conditions as compared to non-stressed plants. Tannin and flavonol contents in leaves increased to varying degrees (up to 11.1 and 7.1%, respectively) in resistant plants after whitefly infestation, indicating that the changes in tannin and flavonol contents were closely associated with the resistance to whitefly. Correlation studies relating leaf content of black gram antioxidative compounds from different genotypes with whitefly population were also worked out. Total phenols (r= -0.71 & -0.88), *o*-dihydroxy phenols (r= -0.56 & -0.76), flavonols (r= -0.80 & -0.81) and tannins (r= -0.16 & -0.26) showed significant negative correlation with whitefly population (nymphs and adults) suggesting that enhanced level of these biochemicals may contribute to bioprotection of black gram plants against *B. tabaci* infestation. Comparatively higher level of resistance in genotype NDU 5-7 and KU 99-20 can serve as base for genetic improvement of black gram, focusing on the development of resistant varieties to *B. tabaci*.

Key words

Bemisia tabaci, Black gram, Correlation, *O*-dihydroxy phenols, Phenols, Resistance, Whitefly

Introduction

Host plant resistance, as a component of integrated pest management, has advantage over other tactics in view of the effects of use of insect resistant cultivars that are cumulative over time and sustainable. Various biochemical compounds present in the plant parts play an important role in offering host plant resistance against insect pests. Plant-insect interaction is a dynamic system, subjected to continual variation and change. Plants challenged by insects respond through changes in the composition and physical properties of the cell wall as well as biosynthesis of secondary metabolites (Hopkins and Hüner, 2004). In order to reduce insect attack, plants have developed different defence mechanisms including physical barriers such as leaf surface wax, thorns or trichomes, and cell wall thickness and lignification which form the first physical barrier to feeding by herbivores (Hanley *et al.,* 2007; War *et al.,* 2012). Besides, plants have also evolved by developing certain chemical defences in the form of induction of defensive proteins (Haruta *et al.,* 2001) and various kinds of secondary metabolites (Baldwin, 2001; Kliebenstein *et al.,* 2001). Plant defense against insect pests could either be constitutive, which is always present irrespective of the external stimuli or induced, which occurs once the plant is under stress and/or stimulated by external factor. Although these defensive strategies adopted by plants prevent them against most of the herbivores, some insect pests have successfully adapted to the plant defensive systems (Mello and Silva-Filho, 2002).

Black gram (*Vigna mungo* (L.) Hepper) is one of the important pulse crops grown all over the world. It is a major component of daily Indian diet, serving as a protein source and is mainly grown during summer season in India. The principal limiting factor affecting production of this crop in northern India is whitefly, *Bemisia tabaci* (Gennadius) (Hemiptera) which is a member of the Aleyrodidae, a family with about 126 genera and over 1,000 species. As a phloem feeder, whitefly causes direct injury by extracting sap from the plant tissue and reducing the productivity by directly consuming the carbohydrates and other nutrients through the plant's vascular system. However, the most serious damage takes place when the insect acts as a vector of several geminivirus types and is considered the world's most important viral pathogen transmitter (Brown *et al.,* 1996; Drost *et al.,* 1998).

Plant antioxidative compounds, such as phenolics, are believed to play an important role in chemical defence against herbivores (Appel, 1993). One mechanism by which their toxicity is thought to arise is via their propensity to produce reactive by-products when oxidized (Appel, 1993; Barbehenn and Martin, 1994). It has been well documented that as a result of insect attack, the metabolism of total phenols and *o*-dihydroxy phenols changes considerably in the plant (Marmit *et al.,* 2008). The recognized importance of such secondary metabolites mediating insect plant interactions led to the present investigation aiming to estimate the induction of various antioxidative compounds in black gram genotypes infested by *B. tabaci*. The present study aimed at comparing total phenol, *o*-dihydroxy phenol, flavonol and tannin profiles of resistant and susceptible genotypes of black gram in response to feeding by whitefly, *B. tabaci*. The findings of the work would help in the early screening and selection of desirable genotypes in whitefly resistance breeding programmes.

Materials and Methods

Black gram genotypes : Nine black gram genotypes were used in the multiple-choice test conducted inside the screen-house. These test genotypes had shown different levels of resistance to *B. tabaci*, based on the whitefly resistance index (WRI) (Taggar and Gill, 2012; Taggar *et al.,* 2013). Genotypes KU 99-20 and NDU 5-7 were moderately resistant, whereas genotypes KU 7-504 and KU 7-505 were highly susceptible to *B. tabaci* under screen-house conditions. Other black gram genotypes evaluated were susceptible to *B. tabaci*.

Whitefly colonies : Field collected *B. tabaci* adults were released on disease-free black gram variety Mash 1-1, grown in earthen pots under unsprayed, natural conditions simulated under screen-house. Non-viruliferous whiteflies were transferred to the insect-transfer cabin for sexing. For raising the insect culture, paired individuals were aspirated from the glass pan and released on brinjal plants (*Solanum melongela* L.), grown in pots and covered with split-cages. Whiteflies were collected from these colonies and re-introduced on black gram variety Mash 1-1 for conditioning. The whiteflies were allowed to develop and multiply on these plants till newly emerged adults appeared on the plants. Old plants were replaced by new ones, whenever needed. The whiteflies from this culture were further used to conduct the experiments.

Multiple-Choice Test : Test genotypes, each replicated thrice, were sown in plastic pots (10 cm in diameter) with single plant per pot and arranged in completely randomized design (CRD) inside the screen-house, during July 2009 and July 2010. Five pots (one plant/pot) of each genotype represented one replication. Whitefly adults were aspirated and released @ 100 adults per plant on the test genotypes at 3rd trifoliate leaf stage, randomly at different places inside the screen-house for their uniform distribution. For studying the biochemical basis of resistance, leaf samples representing fully-formed trifoliate leaf from the upper canopy of 30 and 50 day old plants were used in each replication (Taggar *et al.,* 2012). Test genotypes, kept free from whitefly infestation (sown in a separate screen house), served as control.

Pest population counts : Whitefly population counts were made from three randomly tagged plants, from each genotype, per replication. The total number of eggs laid per trifoliate leaf was recorded from an area of 1 cm^2 of each leaflet, from the lower surface of the top fully-formed trifoliate leaf at weekly interval, using binocular stereomicroscope (40x) for a period of five weeks. The fourth instar red-eyed nymphs (RENs) were recorded from an area of 2 cm^2 of each leaflet, from the lower surface of the trifoliate leaf in the middle canopy, using magnifying lens at weekly interval for a period of five weeks. Adult whiteflies were counted from the lower surface of three fully-formed trifoliate leaves, one each from the upper, middle and lower canopy, using an ordinary mirror at weekly interval for a period of five weeks. Mean pest population of two seasons was pooled and the combined mean of the two seasons is depicted in Table 1 and 2.

Biochemical characteristics : The leaf biochemical investigations were carried out from leaves of nine test genotypes of black gram from both whitefly-stressed (initial stress of 100 whitefly adults per plant) and non-stressed (no whitefly infestation) plants.

Extraction and estimation of antioxidative compounds: Leaves were dried in a hot air oven, first at 100° C for 15 min to denature the enzymes, and then at 60° C to constant weight. Leaves were then crushed manually in a pestle and mortar. Powdered leaf tissue (400 mg) was refluxed with 80 % aqueous methanol for 1 hr. The refluxed material was filtered and the volume was made to 10 ml by washing with hot 80 % methanol. The extraction was repeated two times and from the combined filtrate, total phenols (Swain and Hillis, 1959), *o*-dihydroxy phenols (Nair and Vaidyanathan, 1964) and flavonols (Balabaa *et*

al., 1974) were estimated. For tannins, 50 mg of powdered leaf tissue was taken in test tube and 7.5 ml of water was added to it. The test tubes were kept in boiling water bath for 30 min. The contents were centrifuged at 10,000g for 20 min and the supernatant was collected. The volume was made to 10 ml with water. Tannins were estimated using the method of Sadasivam and Manickam (1992).

Statistical analysis : The replicated data were pooled together and the results were expressed as means ± SD of three replicates. Differences between treatments were analyzed by ANOVA using CPCS1 software package. Tukey's post-hoc test was applied (SPSS 16.0) to determine difference between the genotypes. Correlation analysis along with coefficient of determination was used to identify a possible relationship between whitefly nymph or adult density and biochemical leaf characteristics.

Results and Discussion

The perusal of data in Table 1 indicated a significant difference for the number of whitefly eggs among genotypes in both the years. Seasonal means of whitefly egg numbers were lowest on KU 99-20 and NDU 5-7, and highest on KU 7-504 and KU 7-505. The moderately resistant genotypes KU 99-20 and NDU 5-7 supported lower nymphal population as compared to other genotypes, suggesting that these two genotypes were less colonized by *B. tabaci*. On the other hand, the highly susceptible genotypes KU 7-504 and KU 7-505 recorded higher nymphal population during the study period. The remaining genotypes viz., IPU 02-043, KU 7-618, KU 7-605, KU 7-602 and Mash 1-1 recorded intermediate number of eggs and nymphal population. As far as the colonization by whitefly adults was concerned, KU 99-20 and NDU 5-7 harbored significantly less number of whitefly adults per trifoliate leaf than all other genotypes and were the least attractive to adults (Table 2). On the other hand, genotypes KU 7-504 and KU 7-505 recorded more number of whitefly adults than all other genotypes.

In multiple-choice test, genotypes NDU 5-7 and KU 99-7 were least attractive to *B. tabaci* as far as oviposition and feeding due to nymphs and adults was concerned (Tables 1 and 2), indicating the occurrence of feeding non-preference in these

Table 1 : Whitefly eggs and red-eyed nymphs across different *V. mungo* genotypes in multiple-choice test under screen-house conditions

Genotype	*Number of eggs per trifoliate	*Number of red-eyed nymphs per trifoliate
KU 7-504	20.43 ± 1.79^{c}	52.67 ± 9.37^{d}
KU 7-505	20.00 ± 2.08^{c}	52.18 ± 9.83^{d}
KU 7-602	11.27 ± 0.61^{b}	16.31 ± 1.88^{c}
KU 7-605	10.89 ± 0.88^{b}	15.13 ± 2.36^{c}
KU 7-618	11.04 ± 0.81^{b}	15.88 ± 2.26^{c}
KU 99-20	3.87 ± 0.49^{a}	10.70 ± 2.08^{a}
IPU 02-043	11.24 ± 0.60^{b}	16.40 ± 1.78^{c}
NDU 5-7	4.19 ± 0.49^{a}	11.31 ± 1.79^{a}
Mash 1-1	13.27 ± 1.57^{b}	39.69 ± 7.67^{b}

*Mean of three replications at weekly intervals recorded over a period of five weeks (Combined mean of two seasons); Treatments in columns with same letter(s) are not significantly different at $P \leq 0.05$; Mean ± S.E.M = Standard error of mean (Taggar *et al*., 2012)

Table 2 : Whitefly adult population across different *V. mungo* genotypes in multiple-choice test under screen-house conditions

Genotype	*Adult whitefly population per trifoliate			Mean ± S.E.M
	Upper canopy	Middle canopy	Lower canopy	
KU 7-504	68.26 ± 2.77^{d}	69.53 ± 2.69^{d}	68.99 ± 2.91^{d}	68.93 ± 0.29^{d}
KU 7-505	68.63 ± 2.66^{d}	69.09 ± 2.81^{d}	68.63 ± 2.79^{d}	68.78 ± 0.12^{d}
KU 7-602	37.66 ± 2.86^{b}	37.76 ± 2.96^{b}	37.93 ± 9.26^{b}	37.78 ± 0.06^{b}
KU 7-605	37.99 ± 2.99^{b}	37.86 ± 2.89^{b}	37.73 ± 2.97^{b}	37.86 ± 0.06^{b}
KU 7-618	37.69 ± 2.85^{b}	38.52 ± 3.18^{b}	38.53 ± 2.89^{b}	38.25 ± 0.22^{b}
KU 99-20	13.83 ± 0.78^{a}	13.62 ± 0.64^{a}	13.39 ± 0.71^{a}	13.61 ± 0.10^{a}
IPU 02-043	40.33 ± 2.81^{b}	37.99 ± 2.79	38.43 ± 3.00	38.91 ± 0.58
NDU 5-7	13.23 ± 0.68^{a}	13.26 ± 0.75^{a}	13.92 ± 0.65^{a}	13.47 ± 0.18^{a}
Mash 1-1	51.96 ± 2.28^{c}	51.53 ± 2.38^{c}	52.02 ± 2.46^{c}	52.11 ± 0.14^{c}

*Mean of five replications (each replication represents population at weekly interval) (Combined mean of two seasons); Treatments in columns with same letter(s) are not significantly different at $P \leq 0.05$; Mean ± S.E.M = Standard error of mean (Taggar *et al*., 2012)

genotypes. Low infestation suggested the presence of biochemical compounds on the leaves, which could repel insects or affect host selection, indicating the existence of a possible chemical resistance factor in the variety. On the other hand, physical factors such as leaf area, pubescence and lamina thickness must also be taken into account regarding host selection and might play a role in imparting resistance in black gram plants to *B. tabaci* (Taggar and Gill, 2012).

There was a non-significant variation in total phenol content among all the non-stressed black gram genotypes at 30 and 50 DAS during the study period (Fig. 1 and 2). However, total phenols increased in all the genotypes under whitefly stress conditions. Resistant genotypes NDU 5-7 and KU 99-7 recorded higher total phenols under whitefly stress conditions during both the years. The highly susceptible genotypes, KU 7-504 and KU 7-505 recorded least increase in total phenol contents upon whitefly feeding. The remaining genotypes viz., IPU 02-043, KU 7-618, KU 7-605, KU 7-602 and Mash 1-1 recorded intermediate total phenol contents after whitefly infestation. As far as per cent increase in the total phenol content of the genotypes is concerned, KU 99-20 (47.8 and 50.8%) and NDU 5-7 (49.6 and 50.8%) recorded highest per cent increase both at 30 and 50 DAS, respectively.

Among all the non-stressed black gram genotypes at 30 and 50 DAS, there was a non-significant variation in the *o*-dihydroxy phenol content (Fig. 3 and 4). However, the level of

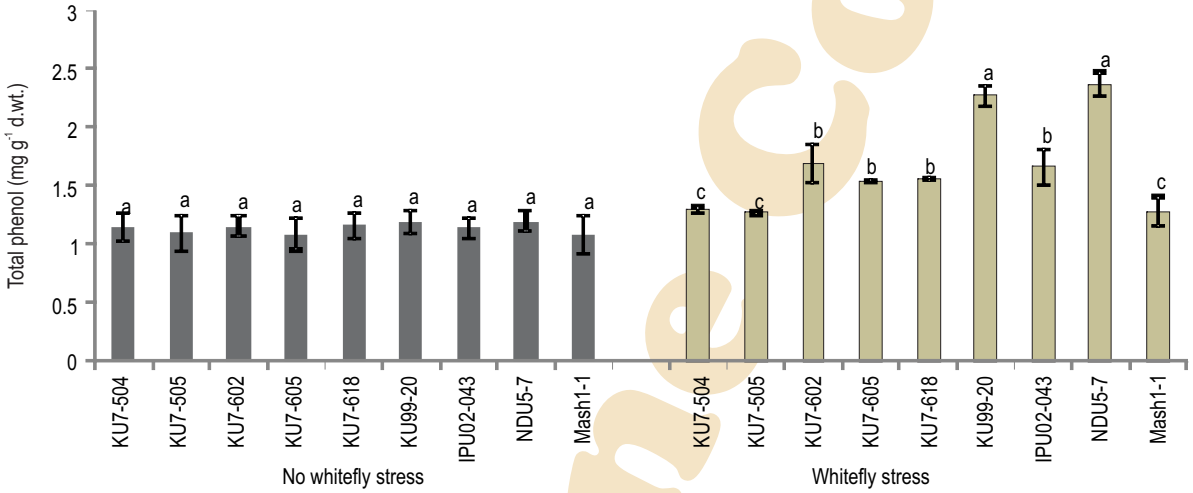

Fig. 1 : Total phenol content (30 DAS) in *V. mungo* leaves as influenced by *B. tabaci* feeding. Bars with same letter(s) are not significantly different at P ≤ 0.05, analyzed by ANOVA

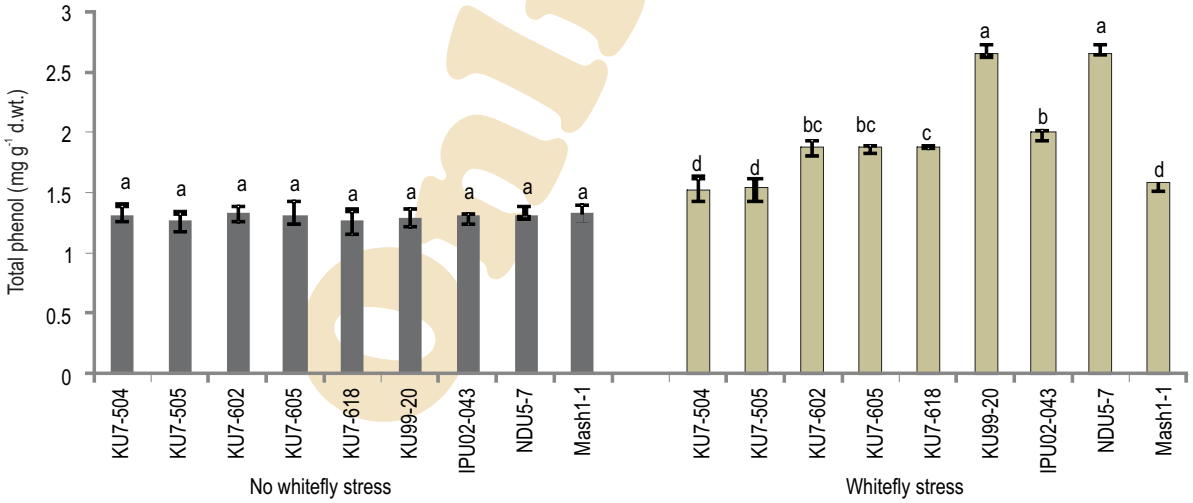

Fig. 2 : Total phenol content (50 DAS) in *V. mungo* leaves as influenced by *B. tabaci* feeding. Bars with same letter(s) are not significantly different at P ≤ 0.05, analyzed by ANOVA

these biochemical compounds increased in all the genotypes under whitefly stress conditions, with resistant genotypes NDU 5-7 and KU 99-7 recording significantly high *o*-dihydroxy phenol contents during both the years. The lowest induction in *o*-dihydroxy phenol contents was observed in the highly susceptible genotypes KU 7-504 and KU 7-505 upon whitefly feeding. The remaining genotypes viz., IPU 02-043, KU 7-618, KU 7-605, KU 7-602 and Mash 1-1 recorded intermediate induction in *o*-dihydroxy phenol contents. The resistant genotypes KU 99-20 (15.7 and 16.3%) and NDU 5-7 (18.4 and 15.4%) recorded highest per cent increase in *o*-dihydroxy phenol contents both at 30 and 50 DAS, respectively.

There were significantly large differences owing to whitefly infestation both at 30 and 50 DAS among black gram genotypes with respect to induction of total phenols and *o*-dihydroxy phenols. Higher quantities of total phenols have been reported to impart resistance to *B. tabaci* in many crops such as cotton (Raghuraman *et al.,* 2004; Balakrishnan, 2006; Acharya and Singh 2008; Jindal and Dhaliwal, 2009) and brinjal (Soundararajan and Baskaran, 2001). In addition to this, induction of resistance by feeding of whitefly nymphs, resulting in accumulation of phenolic compounds in tomato seedlings has also been reported (Murugan and Dhandapani, 2007). Total phenols also act as substrates of antioxidants like peroxidases. Total phenol contents, increased significantly upon infestation of whitefly, particularly in the resistant black gram genotypes as compared to susceptible ones. One of the possible reasons for this may be the fact that phenolics, in fairly large concentration, could ward-off insect pests because of their direct toxicity (Rao and Panwar 2001).

The resistant genotypes NDU 5-7 and KU 99-20 accumulated comparatively higher amount of *o*-dihydroxy phenols after whitefly infestation. *o*-dihydroxy phenol contents were generally found to be higher in whitefly-stressed leaf samples as compared to non-stressed leaves. With growth of plants (at 50 DAS), the resistant genotypes had significantly

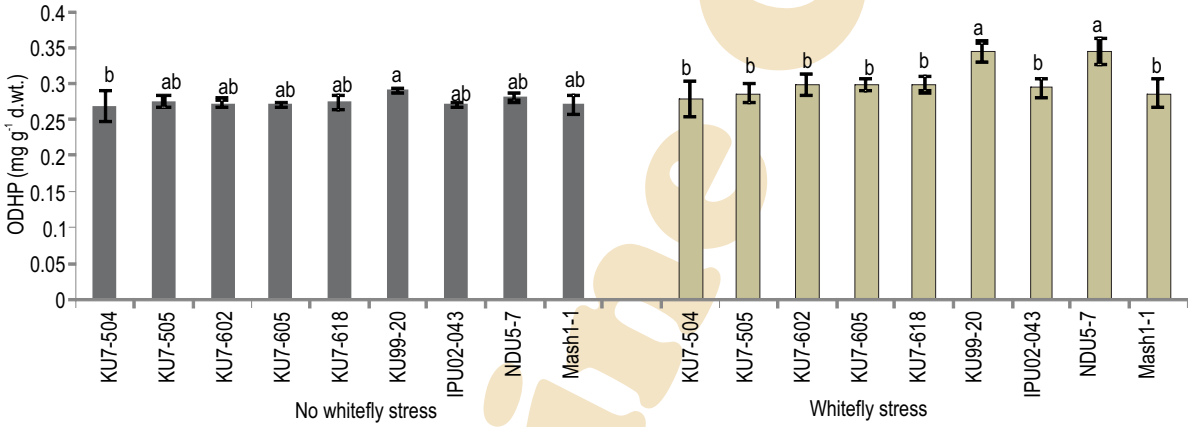

Fig. 3 : *o*-dihydroxy phenol content (30 DAS) in *V. mungo* leaves as influenced by *B. tabaci* feeding Bars with same letter(s) are not significantly different at P≤0.05, analyzed by ANOVA

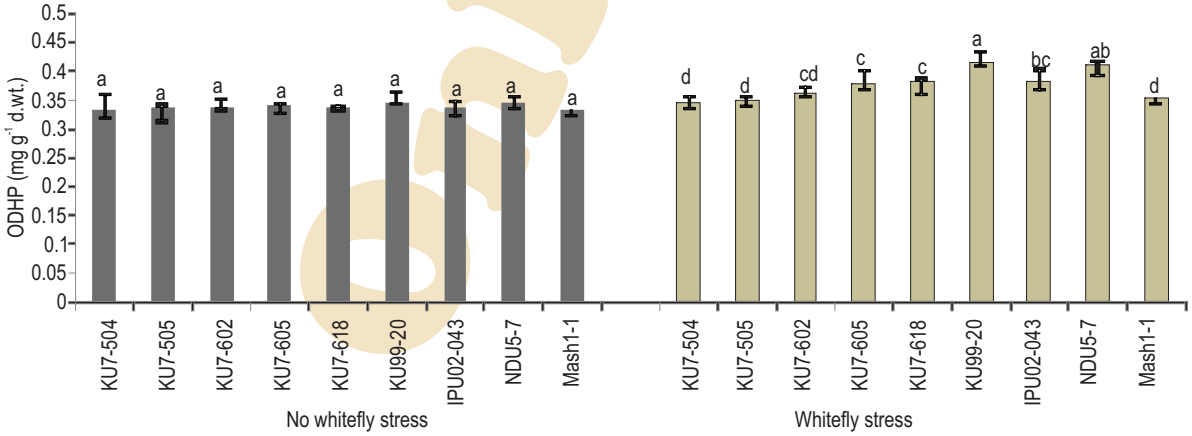

Fig. 4 : *o*-dihydroxy phenol content (50 DAS) in *V. mungo* leaves as influenced by *B. tabaci* feeding Bars with same letter(s) are not significantly different at P≤0.05, analyzed by ANOVA

higher *o*-dihydroxy phenols and total phenols, both under no-whitefly as well as whitefly stressed conditions. However, the level of these biochemicals was much higher in resistant genotypes than the susceptible ones, and they maintained their high levels even after infestation as compared with the susceptible genotypes. It seems that in resistant genotypes, the *o*-dihydroxy phenols and total phenols were continuously produced and maintained their level to provide protection from invading whiteflies. Comparatively higher level of resistance in genotype NDU 5-7 and KU 99-20 can serve as base for genetic improvement of black gram, focusing on the development of resistant varieties to *B. tabaci*. Higher levels of *o*-dihydroxy phenol contents have also been documented to impart resistance to *B. tabaci* in crops such as cotton (Raghuraman *et al.*, 2004) and brinjal (Balaji and Veeravel, 1994). Higher levels of *o*-dihydroxy phenols were observed after infestation of whitefly in resistant genotypes because these compounds are released and oxidized by polyphenol oxidase and peroxidase to the corresponding quinones. These oxidative products help in combating pest infestation in the resistant genotypes. Raghuraman *et al.* (2004) also reported that *o*-dihydroxy phenols had significant negative correlation with *B. tabaci* nymphs and adults in cotton.

Non-significant variations were observed in flavonol content among all the non-stressed black gram genotypes at 30 and 50 DAS during the study period (Fig. 5 and 6). However, flavonol content increased in all the genotypes under whitefly stress conditions. Resistant genotypes NDU 5-7 and KU 99-7 recorded higher flavonol content under whitefly stress conditions during both the years. The highly susceptible genotypes KU 7-504 and KU 7-505 recorded least increase in flavonol content upon whitefly feeding. The remaining genotypes viz., IPU 02-043, KU 7-618, KU 7-605, KU 7-602 and Mash 1-1 recorded intermediate flavonols contents after whitefly infestation. As far as per cent increase in the flavonol content of the genotypes is concerned, KU 99-20 (7.1 and 6.3%) and NDU 5-7 (7.0 and 6.3%) recorded the highest per cent increase both at 30 and 50 DAS, respectively.

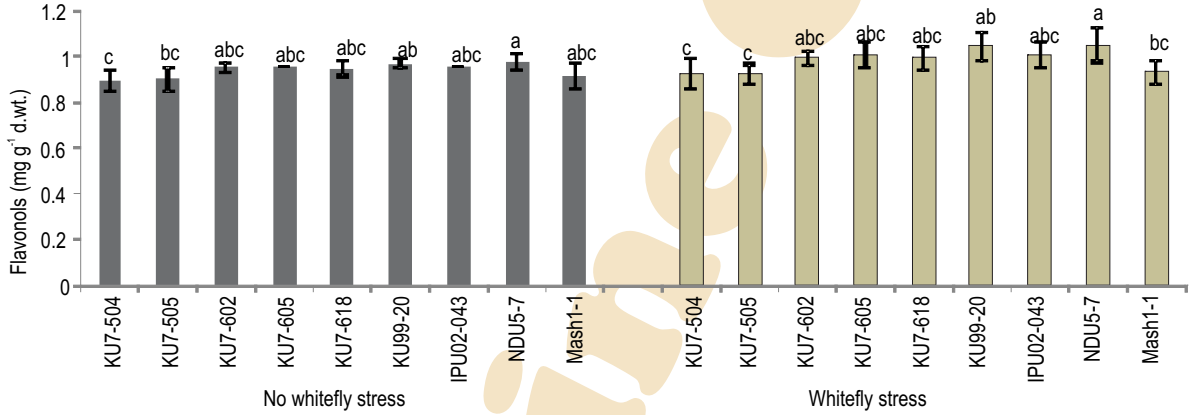

Fig. 5 : Flavonol content (30 DAS) in *V. mungo* leaves as influenced by *B. tabaci* feeding. Bars with same letter(s) are not significantly different at P ≤0.05, analyzed by ANOVA

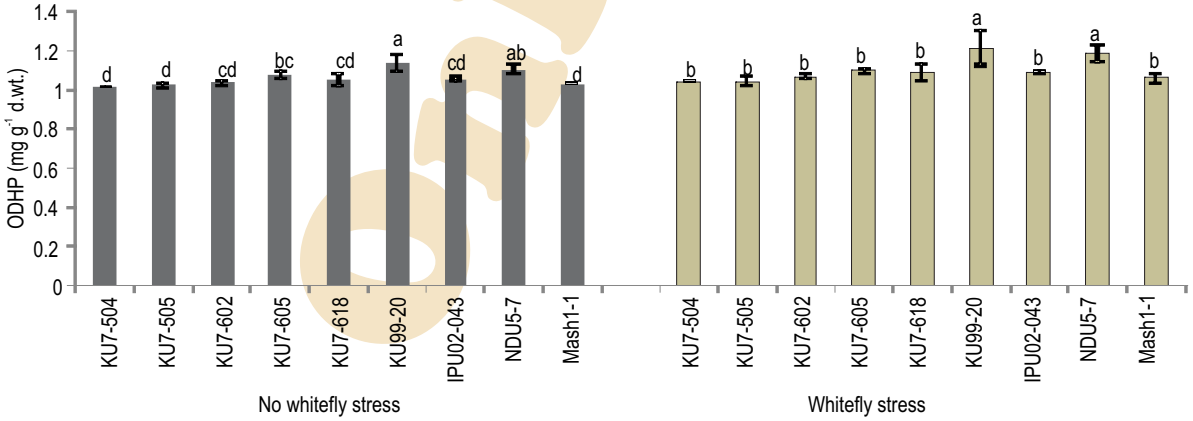

Fig. 6 : Flavonol contents (50 DAS) in *V. mungo* leaves as influenced by *B. tabaci* feeding. Bars with same letter(s) are not significantly different at P ≤ 0.05, analyzed by ANOVA

Among all the non-stressed black gram genotypes at 30 and 50 days after sowing, there was a non-significant variation in the tannin contents (Fig. 7 and 8). However, the level of these biochemical compounds increased in all the genotypes under whitefly stress conditions, with resistant genotypes NDU 5-7 and KU 99-7 recording significantly high tannin contents during both the years. The lowest induction in tannin contents was observed in the highly susceptible genotypes KU 7-504 and KU 7-505 upon whitefly feeding. The remaining genotypes viz., IPU 02-043, KU 7-618, KU 7-605, KU 7-602 and Mash 1-1 recorded intermediate induction in tannin contents. The resistant genotypes KU 99-20 (5.6 and 10.6%) and NDU 5-7 (7.4 and 11.1%) recorded the highest per cent increase in tannin contents both at 30 and 50 DAS, respectively.

There were significant differences among black gram genotypes, as far as flavonol and tannin contents were concerned, owing to whitefly infestation, both at 30 and 50 DAS. Flavonol content increased drastically in the resistant genotypes of black gram upon whitefly infestation as against the susceptible ones, however, no such earlier report is available in black gram. Under whitefly stress conditions, almost similar trends were observed in tannin content as well. In the present studies, both tannins and flavonols were negatively correlated with whitefly population. The increased tannin content in resistant genotypes may be due to the in-built defence mechanism of the plant which increased the tannin content after attack of whitefly, as happened in the case of tomato crop (Bialczyk *et al.,* 1999). Studies have also focused on the relationship of biochemical constituents of a host plant with whitefly populations. High concentration of tannins have also been reported to impart resistance to *B. tabaci* in cotton (Raghuraman *et al* ., 2004; Balakrishnan 2006; Acharya and Singh 2008; Jindal and Dhaliwal, 2009). However, Raghuraman *et al.* (2004) reported that the susceptible variety of cotton recorded lowest tannin content as compared to the resistant counterpart; and a significant negative correlation existed between the tannin content and *B. tabaci* nymphs and adults.

As indicated in Table 3, all the biochemical leaf characteristics showed significant negative correlation with

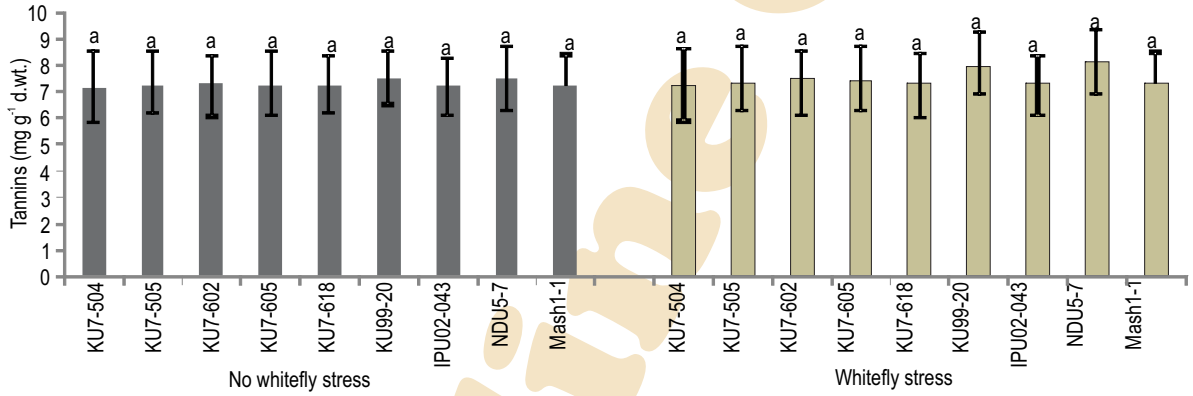

Fig. 7 : Tannin content (30 DAS) in *V. mungo* leaves as influenced by *B. tabaci* feeding. Bars with same letter(s) are not significantly different at P ≤ 0.05, analyzed by ANOVA

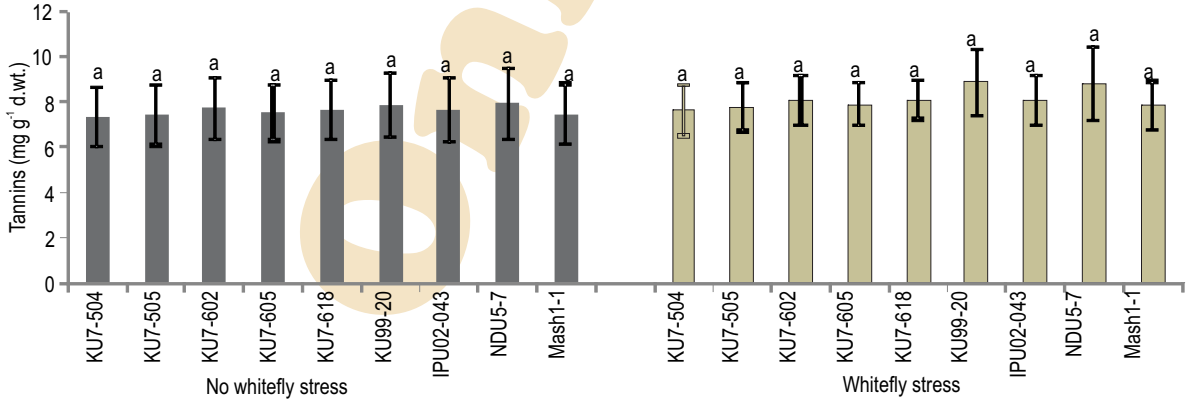

Fig. 8 : Tannin content (50 DAS) in *V. mungo* leaves as influenced by *B. tabaci* feeding. Bars with same letter(s) are not significantly different at P ≤ 0.05, analyzed by ANOVA

Table 3 : Correlations of biochemical leaf characteristics with whitefly population

Biochemical		Red-eyed nymphs		Adults	
		r	R^2	r	R^2
Total phenols	30 DAS	-0.71**	0.51	-0.88**	0.78
	50 DAS	-0.77**	0.59	-0.94**	0.89
o-dihydroxy phenols	30 DAS	-0.56*	0.32	-0.76**	0.59
	50 DAS	-0.71**	0.51	-0.83**	0.70
Flavonols	30 DAS	-0.80**	0.65	-0.81**	0.67
	50 DAS	-0.67**	0.45	-0.85**	0.72
Tannins	30 DAS	-0.16	0.02	-0.26	0.07
	50 DAS	-0.28	0.07	-0.37	0.13

*, **Significant at 5% and 1%, respectively; r = Correlation coefficient; R^2 = Coefficient of determination; DAS = Days after sowing

whitefly population (nymphs and adults). Total phenols (at 30 DAS) were found to have highly significant negative correlation with whitefly nymphs (r = -0.71**) and adults (r = -0.88**). Similar trend was observed at 50 DAS as well. Thus, greater the total phenol content, lesser would be the number of whitefly nymphs and adults and higher the resistance level. There was significantly negative correlation between *o*-dihydroxy phenols (both at 30 and 50 DAS) and whitefly population (nymphs and adults). The correlation values of *o*-dihydroxy phenols (at 30 DAS) with whitefly nymphs and adults were r=-0.56* and -0.76**, respectively. Similar trend was observed at 50 DAS as well. These results indicated that higher the contents of *o*-dihydroxy phenols, lower would be the number of nymphs and adults and therefore higher the level of resistance. Flavonols (at 30 DAS) were found to have highly significant negative correlation with whitefly nymphs (r = -0.80**) and adults (r = -0.81**). Similar trend was observed at 50 DAS as well. Thus, greater the flavonol content, lesser would be the number of whitefly nymphs and adults and higher the resistance level. Negative, but non-significant correlation was observed between tannins (both at 30 and 50 DAS) and whitefly population (nymphs and adults), thus indicating that tannins did not influence the whitefly resistance in black gram.

Antioxidative compounds, particularly phenols have been associated extensively with the chemical defense of plants against microbes, insects and other herbivores (Metraux and Raskin, 1993). These compounds have the ability to form insoluble complexes with proteins, act as enzyme inhibitors or are oxidized to toxic quinones. Thus, rapid accumulation of phenols in resistant genotypes, following whitefly infestation, highlights inducible biochemical pathway of expression of host resistance probably involving synthesis of phenolic precursors and their further oxidation into toxic quinones.

In conclusion, phloem feeding whitefly induces oxidative stress in black gram plants and induction of high levels of antioxidative compounds may probably play a significant role in black gram defence thus, contributing to bioprotection of black gram plants against *B. tabaci* infestation. Taking into account, these results would enable breeding programs to incorporate resistance to whiteflies, one of the most important pests of black gram in north India, within elite lines in the near future. The information generated in this study would contribute to the establishment of breeding programs, that include introgression of traits found in the resistant genotypes.

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References

Acharya, V.S. and A.P. Singh: Biochemical basis of resistance in cotton to the whitefly, *Bemisia tabaci* Genn. *J. Cott. Res. Dev.,* **22**, 195-199 (2008).

Appel, H.: Phenolics in ecological interactions: The importance of oxidation. *J. Chem. Ecol.,* **19**, 1521–1552 (1993).

Balabaa, S.I., A.Y. Zake and A.M. Elshamy: Total flavonoids and rutin content of the different organs of *Japonica* L. *J. Assoc. Analyt. Chem.,* **57**, 752-755 (1974).

Balaji, K. and R. Veeravel: Leaf morphological and biochemical studies on the four varieties of brinjal in relation to whitefly (*Bemisia tabaci* Genn.) incidence. *Prog. Hort.,* **26**, 45-52 (1994).

Balakrishnan, N.: Influence of allelochemical contents in plants on the incidence of major pests of cotton. *Indian J. Pl. Prot*., **34**, 202-205 (2006).

Baldwin, I.T.: An ecologically motivated analysis of plant-herbivore interactions in native tobacco. *Plant Physiol.,* **127**, 1449-1458 (2001).

Barbehenn, R.V. and M.M. Martin: Tannin sensitivity in larvae of *Malacosoma disstria* (Lepidoptera): Roles of the peritrophic envelope and midgut oxidation. *J. Chem. Ecol.,* **20**, 1985–2001 (1994).

Bialczyk, J., Z. Lechowski and A. Libik: The protective action of tannins against glasshouse whitefly in tomato seedlings. *J. agric. Sci.,* **133**, 197-201 (1999).

Brown, J.K., D.R. Frohlich and R.C. Rosell: The sweet potato or silverleaf whiteflies: Biotypes of *Bemisia tabaci* or a species complex? *Ann. Rev. Entomol.*, **40**, 511–534 (1996).

Drost, Y.C., J.C. van Lenteren and H.J.W. van Roermund: Life history parameters of different biotypes of *Bemisia tabaci* (Hemiptera: Aleyrodidae) in relation to temperature and host plant: A selection review. *Bull. Entomol. Res.,* **88**, 219–229 (1998).

Hanley, M.E., B.B. Lamont, M.M. Fairbanks, and C.M. Rafferty: Plant structural traits and their role in antiherbivore defense. *Perspec. Plant Ecol. Evol. Syst.,* **8**, 157-178 (2007)

Haruta, M., I.T. Major, M.E. Christopher, J.J. Patton and C.P. Constabel: A Kunitz trypsin inhibitor gene family from trembling aspen (*Populus tremuloides* Michx.): cloning, functional expression, and induction by wounding and herbivory. *Plant Mol. Biol.*, **46**, 347-359 (2001).

Hopkins, W.G. and N.P.A. Hüner: Introduction to Plant Physiology. John Wiley & Sons. Inc. USA. p: 479-481 (2004).

Jindal, V. and G.S. Dhaliwal: Bases of resistance in cotton to whitefly, *Bemisia tabaci* (Gennadius): Biochemical factors. *Indian J. Ecol.,* **36**, 166-169 (2009).

Kliebenstein, D.J., J. Kroymann, P. Brown, A. Figuth, D. Pedersen, J. Gershenzon and T. Mitchell-Olds: Genetic control of natural variation in *Arabidopsis* glucosinolate accumulation. *Plant Physiol.*, **126**, 811-825 (2001).

Marmit, K.S., V.P. Meena and S.L. Sharma: Quantitative estimation of phenolics and related enzymes in insect induced leaf galls of *Mangifera indica. Ann. Pl. Protec. Sci.,* **16**, 306-308 (2008).

Mello, M.O. and M.C. Silva-Filho: Plant-insect interactions: An evolutionary arms race between two distinct defense mechanisms. *Braz. J. Plant Physiol.*, **14**, 71-81 (2002).

Metraux, J.P. and I. Raskin: Role of phenolics in plant disease resistance. In: In Plant Disease Control (Eds.: C. Ihan). Wiley-Liss, Inc., London. p. 191–209 (1993).

Murugan, M. and N. Dhandapani: Induced systemic resistance activates defense responses to interspecific insect infestations on tomato. *J. Veg. Sci.,* **12**, 43-62 (2007).

Nair, P.M. and C.S. Vaidyanathan: A calorimetric method for the determination of pyrocatechol and related compounds. *Ann. Biochem.,* **7**, 315-321 (1964).

Raghuraman, M., G.P. Gupta and R.P. Singh: Biochemical constituents imparting resistance in upland cotton (*Gossypium hirsutum*) to whitefly (*Bemisia tabaci*). *Indian J. Agric. Sci.,* **74**, 505-507 (2004).

Rao, C.N. and V.P.S. Panwar: Biochemical plant factors affecting resistance to *Atherigona* spp. in maize. *Ann. Pl. Prot. Sci.,* **9**, 37-42 (2001).

Sadasivam, S., and A. Manickam: Phenolics. In: Biochemical Methods for Agricultural Sciences. Wiley Eastern Ltd., New Delhi, India, pp. 187-188 (1992).

Soundararajan, R.P. and P. Baskaran: Mechanisms of resistance in brinjal (*Solanum melongena* L.) to whitefly *Bemisia tabaci* (Gennadius). *Madras Agric. J.,* **88**, 657-659 (2001).

Swain, T. and W.E. Hillis: The phenolic constituents of *Prunus domestica*: The quantitative analysis of phenolic constituents. *J. Sci. Fd. Agric.,* **10**, 63-68 (1959).

Taggar, G.K. and R.S. Gill: Preference of whitefly, *Bemisia tabaci*, towards black gram genotypes: Role of morphological leaf characteristics. *Phytoparasitica,* **40**, 461-474 (2012).

Taggar, G.K., R.S. Gill and J.S. Sandhu: Evaluation of black gram (*Vigna mungo* (L.) Hepper) genotypes against whitefly, *Bemisia tabaci* (Gennadius) under screen-house conditions. *Acta Phytopath. Entomol. Hung.*, **48**, 53-62 (2013).

Taggar, G.K., R.S. Gill, A.K. Gupta and J.S. Sandhu: Fluctuations in peroxidase and catalase activities of resistant and susceptible black gram (*Vigna mungo* (L.) Hepper) genotypes elicited by *Bemisia tabaci* (Gennadius) feeding. *Plant Signal. Behav.,* **7**, 1-9 (2012).

War, A.R., M.G. Paulraj, T. Ahmad, A.A. Buhroo, B. Hussain, S. Ignacimuthu and H.C. Sharma: Mechanisms of plant defense against insect herbivores. *Plant Signal. Behav.,* **7**, 1306-1320 (2012).