

Characterization of Antixenosis and Antibiosis to Whitefly, *Bemisia tabaci* (Gennadius) Asia-I in Blackgram Genotypes

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ABSTRACT

Present investigation was aimed to evaluate the potential resistance mechanisms to whitefly, *Bemisia tabaci* Asia-I in ten selected blackgram genotypes under green house and laboratory conditions. Total ten genotypes showed differential response, resistant to highly susceptible were selected from the preliminary field screening during *rabi* 2020-21 and 2021-22. We evaluated the adult attractiveness, ovipositional preference, incubation period, duration of nymphal stages, total duration of juvenile phase and nymphal survival (%). The results showed that among the ten genotypes, GBG-1 and VBN-6 showed the least preference to adult attractiveness and oviposition in free choice test, indicating antixenosis mechanism of resistance. The genotype GBG-1 showed prolonged the developmental period from egg to adult (~10 days) and lowest percent of nymphal survival (35.0%) followed by VBN-6 (~8 days and 37.50%), suggesting high levels of Antibiosis. These genotypes may be helpful in breeding programs aimed at developing commercial cultivars resistant to whitefly.

Keywords: Non-preference, Resistance mechanisms, Silverleaf whitefly, Host plant resistance, Vinga Mungo.

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INTRODUCTION

Blackgram (*Vigna mungo* (L). Hepper) or urdbean is an important short duration; self-pollinated *Kharif* pulse crop cultivated in almost all parts of India. India currently represents the largest producer of blackgram accounting for more than 70 per cent global production followed by Myanmar, Pakistan and Thailand (Sasidhar, Singh, & Sanodiya, 2022). The production of blackgram is hampered by an array of abiotic and biotic stresses and among these issues, the problems associated to pests and diseases are very critical. Out of an array of insect pests reported on blackgram, polyphagous sweet potato whitefly, *Bemisia tabaci* (Gennadius) (Aleyrodidae: Hemiptera), the most devastating, transmits Mungbean yellow mosaic virus (MYMV) in pulses responsible for 5 to 100 % yield losses by Usharani, Surendranath, & Haq (2014).

B. tabaci is considered a complex of cryptic species that are morphologically indistinguishable, with a total of 43 identified species (Ellango et al., 2015). Chemical control is still the key strategy in the management of *B. tabaci* (Mansaray & Sundufu, 2009). However, due to the rapid selection of insects resistant to insecticides, this practice has proven to be inefficient. In addition, the excessive use of insecticides has caused serious environmental imbalances, stimulating the search for less aggressive methods to control this whitefly (Desneux, Decourtye, & Delpuech, 2007).

Among alternative methods to chemical control, the use of resistant genotypes is a method proven to be efficient and which allows the pest population below the level of economic damage being highly compatible with other management tactics (Panda & Khush, 1995). Plant resistance is based on one three mechanisms: antibiosis, antixenosis and tolerance. Antixenosis negatively affect insect feeding and oviposition behavior, causing inhibition of insect activity during the colonization process on the plant (Smith, 2005). In antibiosis, the plant may adversely affect the biology of the insect that attempts to use it as a host (Panda & Khush, 1995).

However, studies involving the characterization of blackgram genotypes on whitefly still have not evaluated a wide range of germplasm, which motivated the conduction of the present study in order to detect the mechanisms of resistance against whitefly, *B. tabaci* in blackgram genotypes under greenhouse and laboratory conditions.

MATERIAL AND METHODS

Studies on mechanisms of resistance *i.e.* Non-preference for oviposition and antibiosis were carried out at greenhouse of the Department of Entomology, S.V. Agricultural College, Tirupati, Andhra Pradesh during February to April 2022. Genotypes that showed consistent resistance reaction in field screening of *rabi* 2020-21 and 2021-22 were selected and used in the present study.

Antixenosis

Whitefly culture maintenance

Whitefly population was maintained on brinjal plants var. HB- UJALA, inside the cages (72 cm length x 88 cm width x 77 cm height) covered by a muslin cloth on the top

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portion for proper aeration and light and remaining all sides of the cage was wrapped by the tissue paper to prevent the escape of whiteflies. Adult whiteflies were collected from farmer fields on brinjal plants near Cherlopalli, Tirupati, Chittoor district, Andhra Pradesh. Every fortnight insect free fresh plants aged 30-40 days was regularly introduced inside the culture cages for maintenance and continuity of insect population. From this nucleus population, freshly hatched whiteflies that were free from YMD viruses were collected by using a plastic tube of size 10cm × 4 cm and used for further studies. Population from culture was molecular characterized as described by Singh et al. (2012) using mtCOI primers, forward primer C1-J-2 (5-TTGATTTTTTGGTCATCCAGAAGT-3) reverse primer L2- N-3014 (5-TCCAATGCACTAATCTGCCATATTA-3).

Test genotypes were sown in 3 L pots, containing soil and vermin compost in the proportion of 3:1. At the fully opened trifoliate stage, three pots (three replicates) for each genotype were selected (uniform growth and insect free condition) and were arranged symmetrically in completely randomized block design inside the screened cage (2.0 m base x 1.8 m height) in which 50 pairs of the whiteflies were released in the center of the cage, methodology recommended by Jesus, Junior, Pitta, Campos, & Tagilar (2011). The sexing of adults of *B. tabaci* was done based on the morphological characters viz., females are bigger with a blunt abdominal tip, while males are smaller with a pointed abdominal tip (Kedar, Saini, & Kumarang, 2014). After 72 hours of the release, three trifoliate leaves (Top, middle and bottom) from each genotype were removed and taken to the laboratory and eggs count were taken through Olympus stereo zoom binocular microscope, Dewinter Calipers Pro version 4.6 software. For no choice conditions each genotype was individually covered in a plastic chimney, ten pairs of newly emerged adults were released into each cage. After 72 hours of release, the adults were removed and eggs deposited were counted.

For antibiosis studies, ten test genotypes were grown in earthen pots up to the fully opened trifoliate stage in completely randomized design (CRD) with three replications in green house to avoid any outside whitefly infestation. Leaf cages were prepared by following Kaler (1999) to study the antibiotic effects of these genotypes on *B. tabaci*. Three leaf cages were attached to each genotype, in each leaf cage; five pairs of freshly emerged adults were released from the nucleus population. After 48 hours, adults were removed along with the leaf cages and the leaf portion under each leaf cage in detached leaf was marked and observed under a stereomicroscope (40 x) to count the number of eggs laid. Only 10 eggs were selected and the rest were removed with a needle and a fine brush. The marked area on each leaf was separated and again covered with a leaf cage to prevent additional oviposition by whitefly from the outside and crawlers moving outside the marked area. The marked area was observed daily under a stereomicroscope (40x) to determine the duration of the incubation period, nymphal period (after every two days fresh leaf sample was taken from leaf cage-up to confirmation in each instar), viability of nymphs and developmental period (from egg to adult).

Incubation period

To determine the hatching percentage and incubation period, the marked eggs on the abaxial surface of leaves were observed till hatching. Observations on the incubation period and the number of nymphs hatched out were recorded.

Nymphal period

To determine the number and duration of different nymphal instars, nymphs were marked individually and observed under the microscope from the hatching of eggs till pupation. The observation of nymphal instars, their duration, total period, and mortality were recorded.

Total developmental period

The total developmental period from egg to adult was computed by counting the data obtained from observations and the data regarding the duration of various stages.

Data from antixenosis and antibiosis experiments were analyzed by using a one-way analysis of variance (ANOVA). The mean values were separated using Duncan's Multiple Range Test (DMRT) ($P=0.05$)

RESULTS

Genomic DNA from a single whitefly was extracted following the method described by Singh *et al.* (2012) and used to amplify the mitochondrial cytochrome oxidase I (mtCOI) gene with gene-specific primers. The resulting 880 bp amplified product was purified, sequenced, and submitted to GenBank. Molecular analysis of the mtCOI gene revealed that the *B. tabaci* population in this study (Accession Number: OP781929) shared 96% homology with the Asia-I mitochondrion of the *B. tabaci* complex (GenBank ID: JX 993184, Bapatla). Previous studies have shown that the Asia-I genetic group of *B. tabaci* is a more efficient transmitter of mungbean yellow mosaic virus (MYMV) compared to Asia II-1 and other biotypes (Archana, Mandal, & Subramanian, 2018).

Antixenosis mechanism

Free choice test- Adult attractiveness

The tested genotypes differed significantly in their attractiveness to *B. tabaci* adults. After 24 hours of release, maximum number of adults were settled on PU 1503(MR) (16.00/plant) followed by VBN-6(R) (12.00/plant) and BG 19-15(HS) (10.00/plant) whereas, minimum number was settled on GBG-1(R) (5.00/plant). After 48 hours of release, maximum number of adults was recorded on LBG-623(HS) (12.25/plant) differed significantly from other varieties. This was followed by BG 19-15(HS) (10.25/plant) which was on par with BGGP 938(S) (9.50/plant). The adult population was minimum on GBG-1(R) (3.50/Plant) followed by BGGP 941(MS) (5.00/plant) and BGGP 890(MS) (5.75/plant) were on par with each other.

Oviposition preference

The highest mean no of eggs were deposited on the leaves of highly susceptible genotypes LBG -623 (49.00/trifoliolate leaf) and BG 19-15 (42.00/ trifoliolate leaf) after 72 hours of infestation while, the lowest mean of eggs (4.67 and 7.67/ trifoliolate) was observed on resistant genotypes VBN 6 and GBG-1 respectively. These were followed by moderately resistant genotypes TBG-104 (9.40/trifoliolate leaf) PU 1503 (10.92/

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trifoliolate leaf) and moderately susceptible genotypes BGGP 941(15.92/ trifoliolate) and BGGP 890 (18.08/ trifoliolate leaf) (Table.1).

Table 1. Adult attractiveness & oviposition preference test- Mean (\pm SE) number of adults of *B. tabaci* on selected blackgram varieties 24 and 48 hours after release and mean number (\pm SE) of eggs 72 hours after release (in free-choice test).

Genotype Name	Mean of adults (no.)		Mean no. of eggs per trifoliolate leaf
	(24 hours)	(48 hours)	(72 hours)
LBG-623 (HS)	8.25 \pm 1.43 *(3.00 \pm 0.26)bcd	12.25 \pm 0.85 (3.63 \pm 0.11)a	49.00 \pm 4.79 (7.04 \pm 0.33)a
BG 19-15(HS)	10.0 \pm 1.08 (3.30 \pm 0.15)bc	10.25 \pm 0.85 (3.34 \pm 0.13)ab	42.00 \pm 4.24 (6.53 \pm 0.31)ab
BGGP 938 (S)	6.75 \pm 1.37 (2.75 \pm 0.24)cde	9.50 \pm 1.25 (3.22 \pm 0.20)ab	31.50 \pm 4.19 (5.66 \pm 0.38)b
TU-94-02 (S)	7.25 \pm 1.60 (2.83 \pm 0.26)cde	8.00 \pm 1.15 (2.98 \pm 0.19)bc	27.25 \pm 3.03 (5.29 \pm 0.29)bc
BGGP 941(MS)	6.50 \pm 1.70 (2.67 \pm 0.34)cde	5.00 \pm 0.70 (2.43 \pm 0.14)cd	15.92 \pm 4.66 (4.01 \pm 0.62)bcd
BGGP 890(MS)	5.20 \pm 0.70 (2.22 \pm 0.15)e	5.75 \pm 0.62 (2.58 \pm 0.12)cd	18.08 \pm 4.95 (4.27 \pm 0.62)bcd
PU1503(MR)	16.0 \pm 1.22 (4.11 \pm 0.14)a	7.75 \pm 0.85 (2.94 \pm 0.14)bc	10.92 \pm 2.37 (3.41 \pm 0.36)cd
TBG-104(MR)	8.4 \pm 1.20 (3.02 \pm 0.20)bcd	6.25 \pm 1.03 (2.67 \pm 0.19)cd	9.40 \pm 1.70 (3.20 \pm 0.27)d
VBN-6(R)	12.0 \pm 0.70 (3.60 \pm 0.10)b	8.00 \pm 1.15 (2.98 \pm 0.19)bc	4.67 \pm 0.88 (2.36 \pm 0.18)d
GBG1(R)	5.00 \pm 0.40 (2.44 \pm 0.08)de	3.50 \pm 1.32 (2.02 \pm 0.36)d	7.67 \pm 1.23 (2.92 \pm 0.20)d
F	7.03*	5.87**	7.10**
CV	14.05	13.44	18.63

Means followed by the same lower case letter in each column do not differ by DMRT (P = 0.05) *Values in parenthesis are square root transformed. HS: Highly susceptible S: Susceptible MS: Moderately susceptible MR: Moderately resistant R: Resistant.

No- choice test

The data regarding the ovipositional preference of whitefly on different blackgram genotypes under no choice conditions after 72 hours of insects release presented in Table 2 and revealed that there was a significant variation in among the genotype es in the oviposition. The highest mean of eggs was observed on highly susceptible genotypes LBG-623 (39.00eggs/trifoliolate leaf), BG 19-15(33.50 eggs/trifoliolate leaf) followed by susceptible genotypes BGGP-938 (26.75 eggs/trifoliolate leaf), TU94-02 (21.50 eggs/trifoliolate leaf) and moderately susceptible genotypes BGGP 941(20.00 eggs/ trifoliolate leaf) and BGGP 890 (17.25 eggs/trifoliolate leaf). The resistant genotypes VBN-6 and GBG-1 recorded the lowest mean number of eggs 8.50 eggs/ trifoliolate leaf and 11.75 eggs/trifoliolate leaf respectively.

Table 2. Mean (\pm SE) number of eggs of *B. tabaci* on selected blackgram varieties 72 hours after release (in no-choice test).

S. No.	Genotype Name	Mean number of eggs per trifoliolate leaf (72 hours)
1	LBG -623 (HS)	39.00 \pm 3.31 (6.30 \pm 0.26)a
2	BG 19-15 (HS)	33.50 \pm 3.77 (5.76 \pm 0.32)b
3	BGGP 938 (S)	26.75 \pm 2.68 (5.24 \pm 0.26)bc
4	TU-94-02 (S)	21.50 \pm 1.50 (4.73 \pm 0.15)cd
5	BGGP 941(MS)	20.00 \pm 0.81 (4.58 \pm 0.08)d
6	BGGP 890(MS)	17.25 \pm 2.28 (4.24 \pm 0.27)de
7	PU1503(MR)	15.50 \pm 2.21 (4.03 \pm 0.28)de
8	TBG-104(MR)	12.25 \pm 2.78 (3.58 \pm 0.37)ef
9	VBN-6 (R)	8.50 \pm 0.95 (3.07 \pm 0.16)ef
19	GBG 1 (R)	11.75 \pm 1.03 (3.56 \pm 0.14)g
	F	2.82**
	CV	13.40

HS: Highly susceptible S: Susceptible MS: Moderately susceptible MR: Moderately resistant. R: Resistant Values followed by same letter in each column are not significantly different by DMRT. Values in paranthesis are square root transformed CV: Coefficient of variation.

Antibiosis mechanism

Antibiosis mechanism of resistance generally expressed in terms of duration of nymphal period, total developmental period (egg to adult) and nymphal survival (%) of whitefly on different blackgram genotypes. The incubation period of whitefly eggs and mean duration of first instar on the leaves of selected blackgram genotypes ranged between 5.53 to 6.11 and 2.41 to 3.47 days respectively. There was no statistically non-significant between the selected genotypes. In case of 2nd, 3rd and 4th instars minimum duration was observed in LBG-623 (3.53, 3.56 and 3.90 days) followed by BG 19-15 (3.74, 3.82 and 3.67 days respectively) while maximum duration was observed on GBG-1 (6.50, 6.27 and 5.77 days) followed by VBN-6 (6.14, 5.63 and 5.42 days) (Table 3).

Table 3. Mean (\pm SE) duration of the incubation period, nymphal stages and nymphal period of *B. tabaci* on selected blackgram genotypes under glass house conditions (30 ± 2 C; 70 ± 10 % RH; photoperiod of 12:12 L: D).

Genotype name	Incubation period (days)	1 st Instar (days)	2 nd Instar (days)	3 rd Instar (days)	4 th instar (days)	Nymphal period (days)	Total development period (egg-adult) (days)	Nymphal survival (%)
LBG -623(HS)	5.33 \pm 0.33a	2.41 \pm 0.08b	3.53 \pm 0.24c	3.56 \pm 0.48c	3.90 \pm 0.66bc	13.40 \pm 1.20e	18.73 \pm 1.17g	85.00 \pm 2.88a
BG 19-15(HS)	5.43 \pm 0.40a	2.64 \pm 0.22ab	3.74 \pm 0.36c	3.82 \pm 0.30c	3.67 \pm 0.72c	13.90 \pm 0.79de	19.33 \pm 0.96g	82.50 \pm 2.50a
BGGP 938(S)	5.30 \pm 0.38a	2.48 \pm 0.11b	4.13 \pm 0.41c	4.01 \pm 0.43bc	4.13 \pm 0.41bc	14.75 \pm 0.86cde	20.06 \pm 0.89efg	80.00 \pm 4.08a
TU-94-02(S)	5.65 \pm 0.32a	2.86 \pm 0.26ab	4.27 \pm 0.36c	4.32 \pm 0.23bc	4.37 \pm 0.31abc	15.83 \pm 0.91cde	21.49 \pm 1.11fg	80.00 \pm 4.08a
BGGP 941(MS)	5.45 \pm 0.32a	3.24 \pm 0.38ab	4.85 \pm 0.34bc	4.39 \pm 0.39bc	4.83 \pm 0.50abc	17.32 \pm 1.09bc	22.14 \pm 0.63cdef	77.50 \pm 7.50ab
BGGP 890(MS)	5.60 \pm 0.37a	3.12 \pm 0.32ab	4.76 \pm 0.30bc	4.17 \pm 0.32bc	4.46 \pm 0.24abc	16.53 \pm 0.70cd	22.17 \pm 0.87cde	75.00 \pm 2.88ab
PU1503(MR)	6.21 \pm 0.46a	2.95 \pm 0.28ab	4.24 \pm 0.49c	4.83 \pm 0.85abc	5.33 \pm 0.55ab	17.36 \pm 0.86bc	23.58 \pm 0.79bcd	62.50 \pm 8.53b
TBG-104(MR)	5.45 \pm 0.45a	3.41 \pm 0.27a	5.75 \pm 0.30ab	5.15 \pm 0.75abc	5.02 \pm 0.23abc	19.35 \pm 0.91ab	24.80 \pm 1.20bc	37.50 \pm 4.78c
VBN-6 (R)	6.00 \pm 0.51a	2.75 \pm 0.20ab	6.14 \pm 0.60a	5.63 \pm 0.36ab	5.42 \pm 0.36ab	19.95 \pm 0.80ab	25.96 \pm 1.13ab	37.50 \pm 4.78c
GBG 1(R)	6.11 \pm 0.43a	3.47 \pm 0.31a	6.50 \pm 0.57a	6.27 \pm 0.53a	5.77 \pm 0.20a	22.02 \pm 0.70a	28.13 \pm 0.69a	35.00 \pm 6.45c
SE (d)	0.57	0.37	0.59	0.71	0.64	2.60	1.37	7.38
C.D. (P=0.05)	NS	NS	1.21	1.46	1.32	10.54	2.81	15.16

HS: Highly susceptible S: Susceptible MS: Moderately susceptible MR: Moderately resistant.

R: Resistant Values followed by same letter in each column are not significantly different by DMRT.

Values in paranthesis are square root transformed CV: Coefficient of variation.

Maximum duration of total nymphal period was recorded on GBG-1(R) (22.02 days) followed by VBN-6(R) (19.95 days) which was statically on par with TBG-104(MR) (19.35 days) while, minimum duration of total nymphal period was recorded in LBG-623(HS) (13.40 days) followed by BG 19-15(HS) (13.90 days) and BGGP 938 (S) (14.75days). The total developmental period from egg to adult was significantly different among the blackgram genotypes. The total development period was minimum of 18.73 days on LBG-623(HS) which was statistically on par with the BG19-15(HS) (19.33 days). However, the maximum developmental period of 28.13 days was observed on GBG-1(R) followed by VBN-6(R) (25.96 days) and TBG-104 (MR) (24.80 days). Duration of total developmental period on remaining genotypes TU 94-02(S), BGGP 938(S), BGGP 890(MS), BGGP 941(MS) and PU1503(MR) it was 21.49, 20.06, 22.14, 22.17 and 23.58 days respectively (Table 3.)

The survival percentage of various immature stages on the blackgram genotypes varied from 35 to 85.00 per cent. Maximum per cent of nymphal survival was recorded on LBG-623(HS) (85.00%) which was statistically on par with BG19-15 (HS) (82.50 %). Minimum per cent of nymphal survival was observed on GBG-1(R) (35.00%) which was statistically on par with VBN-6(R) (37.50%). The genotypes TU 94-02(S) (80.00%), BGGP 938(S) (80.00%) and BG 19-15(HS) (82.50 %) were on par with each other and also genotype BGGP 941 (MS) (75.00%) on par with BGGP 890(MS) (77.50%) The

present results are in agreement with Rodrigues, Junior, Farias, & Jesus (2012) reported that nymphal survival variability in different cowpea genotypes ranged between 52.5 and 90.0 %. Similar results were also reported by Cruz, Baldin, & Castro (2014) observed the significant differences in nymphal survival on cowpea genotypes (45.5 to 89.1%).

CONCLUSIONS AND DISCUSSIONS

In the present study, resistant genotypes (VBN-6 and GBG-1) were recorded the lowest number of eggs in the both free- choice and no- choice conditions 4.67 and 7.67/ trifoliolate and 8.50 and 11.75 eggs/trifoliolate leaves respectively, it indicates the presence of antixenosis mechanism of resistance. The present results are in line with Toscano, Boica, & Maruyama, (2002) evaluated four wild tomato genotypes, LA 716, PI 127826, PI 127827 and PI 134417 along with two commercial genotypes santaclara and Bruna VFN hybrid for ovipositional preference of whitefly in free and no choice tests and observed that wild genotypes, LA 716 and PI 134417 were least preferred in both free choice and no choice tests with lowest number of eggs. The present results were concurred with Baldin & Beneduzzi (2010); Junior, Campos, Lourencao, & Campos (2007); Silva, Baldin, Souza, & Lourencao (2012) ; Fekri, Samih, Imani, & Zarabi (2013) in pumpkin, cotton, soyabean and tomato genotypes respectively.

In the present study, in the free choice condition even though the higher number of whiteflies settled on resistant genotypes (VBN-6 and PU 1503) after 24 hours of release, recorded the lowest number of eggs 10.92 and 4.67 eggs per trifoliolate leaf after 72 hours of release, indicating the occurrence of non- preference for oviposition in those genotypes. Present results are in accordance with Jindal & Dhaliwal (2011) reported that higher number of adults was settled on cotton genotypes LD 694 and PA 183 after 6 hours of release, but those genotypes recorded the lowest number of eggs after one week. Similar results also reported by Tamilselvan, Mahalingam, Mohankumar, & Senguttuvan (2020) in cotton.

The settling behavior of the whitefly is much important for the insect to establish progenies by utilizing the host plants for feeding, oviposition and shelter. The cues emanating from the host plant mediate the preferences by the insects. The leaf architecture and color, leaf pubescence, cuticle thickness and volatile compounds released from plant play a role as repellent or attractant for the whiteflies. In the present study, resistant genotypes (VBN-6 and GBG-1) possessed lower trichome density, greater values of leaf thickness and dark green leaves compared to susceptible genotypes (Table 4). Higher trichome density and longer length of trichomes might be responsible for the higher oviposition rate in susceptible genotypes (Fig. 1). Trichome density acts as a defensive trait that deters whitefly infestation by limiting their ability to establish, making movement and feeding difficult (Pena et al., 2006). Potential effects of trichomes on whiteflies may vary depending on trichome angle to the leaf surface, length and type, all factors potentially affecting adult oviposition, and immature attachment and feeding. Leaf thickness hinders whitefly stylets from penetrating the epidermis, disrupting the feeding process (Sulistyo & Inayati, 2016). These characters might be responsible for low attractiveness to adult whiteflies and also for lower oviposition.

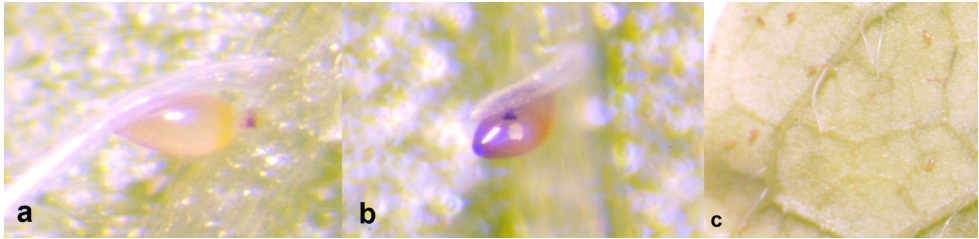


Figure 1. Influence of trichome density and length on egg laying pattern of whitefly. a) Proper anchoring of eggs at the base of longer trichomes. b) Egg associated with shorter trichomes. c) Higher Oviposition on highly susceptible genotype-LBG 623.

In the present free choice and no choice experiments it has been observed that in the free-choice conditions, highly susceptible genotypes recorded significantly higher oviposition than no-choice conditions. However resistant genotypes had somewhat higher oviposition in no choice conditions compared to free choice conditions. These results clearly indicated the capability of whitefly for choosing the host for feeding and oviposition and also for fitness of offspring (parental care) under adverse condition. In case of free choice condition as the adult whiteflies have a choice to choose the host for oviposition fewer eggs were deposited compared with more number of eggs in no choice condition where the adult whiteflies do not have any other choice except to lay the eggs on the host that is available. In case of no-choice conditions there was no chance for explore a new host; it may be reason for higher oviposition on genotypes in no choice condition. According to Blua, Yoshida, & Toscano (1995) behavioral changes of the whitefly adults for oviposition preference is attributed to several factors that modify insect preference as the confinement conditions are different for free-choice and no-choice.

During period of study, it was also observed that maximum numbers of eggs were laid in upper trifoliate leaf than middle trifoliate and lower trifoliate leaf. Similar reports were given by Campos, Junior, Lourencao, & Campos (2005) who reported that highest number of eggs laid by the whitefly on the apex leaf on cotton and cowpea cultivars (Rodrigues et al., 2012). According to Van-Lenteren and Noldus (1990) preference for the youngest parts of the bean plant may be related to the highest concentration of nutrients (amino acids), reducing sugars, thinner and softer cuticle, as well as a higher amount of water. These characteristics may facilitate the whitefly oviposition and eggs hydration, providing a higher survivorship of the nymphs.

In this study, mean duration of incubation period was statistically non-significant between the selected genotypes. It indicated that these varieties do not affect the insect's embryonic stage. The present findings confirm with the findings of Baldin, Vendramim, & Lourencao, (2005); Lima & Lara (2004); Rodrigues et al. (2012) reported that there was no significant differences in the mean duration of eggs incubation on tomato genotypes (6 days), on soybean plants (6.4 to 6.6 days) and cowpea cultivars (5.53 to 6.72 days) respectively. Baldin, Silva, & Pannuti (2015) reported non-significant difference in the mean duration of first nymphal instar on melon genotypes (2.8 to 4.4 days).

Characterization of antixenosis and antibiosis to Whitefly

According to Munthali, (1992) among several biological characteristics, the duration of development of an insect is most useful to categorize accessions as resistant and susceptible. In present study, the longest period of development (egg-to-adult), the longest period of nymphal development and the lowest per cent of nymphal survival (%) of whitefly was observed on resistant genotypes VBN-6 and GBG-1 indicating presence of antibiosis mechanism in these genotypes. According to Panda (1979) the period of development in resistant varieties is delayed due to anomalies caused by the ingestion of compounds (mainly enzymes and proteins), which are inadequate to the insect's biology. Whitefly nymphs on the susceptible varieties LBG-623 and BG 19-15 (Fig. 2) were looked like healthy while, those confined to VBN-6 and GBG-1 showed deformities or died before adulthood, thus confirming the occurrence of antibiosis on those varieties (Panda, 1979).

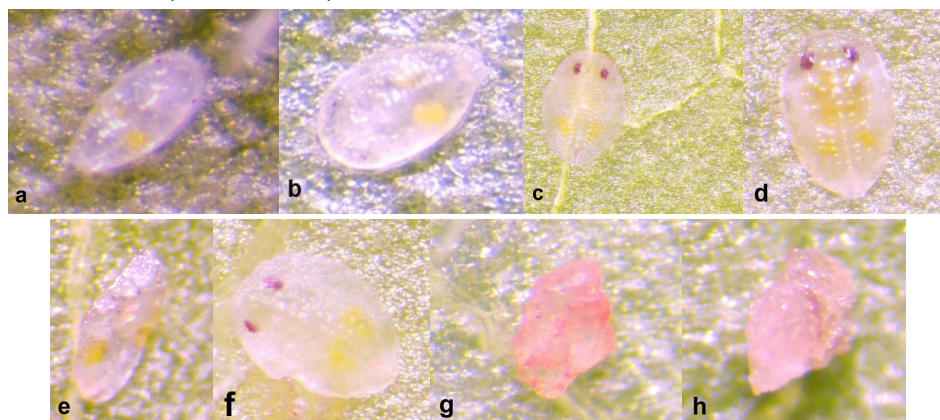


Figure 2. Occurrence of Antibiosis-Antibiosis factors (e to h) in resistant genotypes VBN-6 and GBG-1. a) Healty 1st Instar, b) 2nd instar, c) 3 rd instar, d) 4th instar or pupal stage in highly susceptible genotype-LBG 623, e)Size reduction in 2nd instar, f) size reduction in 3 rd instar, g) Dead and deformed , h) mortality in nymphs before reaching adulthood.

The longest prolongation from egg to adult was observed on the resistant genotype GBG-1 (28.13 days), which required approximately 10 days more to complete the life cycle compared with the susceptible genotype LBG-623 (18.73 days). Present results are in accordance with Tamilselvan, Mahalingam, Kumar, & Senguttavan (2019); Niveditha, Sree, Das, & Neelesh (2020); Rodrigues et al.(2012); Cruz et al. (2014); reported that mean of total developmental period was 16.24 to 20.42 days on cotton genotypes, 16.59 to 23.99 days on soyabean, 17.3 to 23.6 days on cowpea cultivars and 16.4 to 19.3 days on cowpea genotypes respectively. Jindal, Dhaliwal, & Dhawan (2007); Santos et al.(2019) reported the antibiosis mechanism in cotton and bean entries by recording prolonged total developmental time (~ more than 10 days) and lowest per cent of nymphal viability (~30%) compared to susceptible entries respectively.

The inadequate ingestion of nutrients from the plants might be responsible for low survival of nymphs on resistant genotypes VBN-6 and GBG-1. Whitefly mortality on resistant plants could be caused by starvation resulting from phenols, flavonoids

and tannins produced by cowpea plants responsible for the antibiotic effect on the black aphid (Jackai & Daoust, 1986). The present results are in agreement with Fekri, et al. (2013) observed that antibiosis mechanism of resistance in tomato genotype CAL-JN3 with highest per cent of nymphal mortality 33.97. Rodrigues et al. (2012); Cruz et al. (2014) and Baldin & Benduzzi (2010) reported lowest nymphal survival (%) on resistant genotypes in cowpea and squash genotypes respectively.

In the present study, resistant genotypes (VBN-6 and GBG-1) had higher amounts of phenolics, total proteins and total free amino acids, might be responsible for the antibiotic effects in resistant genotypes (Table 4.). Taggar, Gill, Gupta, & Singh (2014) who found that *B. tabaci* feeding in blackgram cultivars, increased peroxidase activity and tannin content in the genotypes. Production of phenoxy and other oxidative radicals by the PODs, acting together with tannins directly deter the feeding by insects and/or produce toxins that reduce the plant digestibility, which leads to nutrient deficiency in insects with drastic effects on their growth and development (War et al., 2012).

Table 4. Morphological and Biochemical parameters in selected blackgram genotypes.

Genotype name	Trichome density (5mm ² leafdisc)	Leaf thickness (mm)	Total phenols (mg g ⁻¹ FW)	Total proteins (mg g ⁻¹ FW)	Reducing sugars (mg g ⁻¹ FW)	Free Amino acids (mg g ⁻¹ FW)
LBG -623 (HS)	19.00 (4.47)**	0.29	0.35	0.182	4.39	0.24
BG 19-15 (HS)	18.83 (4.45)	0.33	0.42	0.238	4.20	0.35
BGGP938 (S)	13.50 (3.80)	0.44	0.57	0.303	4.13	0.48
TU-94-02 (S)	12.34 (3.65)	0.42	0.67	0.368	3.92	0.56
BGGP 941 (MS)	10.17 (3.33)	0.47	0.78	0.410	3.27	0.65
BGGP890 (MS)	10.00 (3.31)	0.49	0.91	0.507	3.10	0.71
PU1503 (MR)	9.00 (3.15)	0.51	1.04	0.567	2.93	0.84
TBG-104 (MR)	9.17 (3.18)	0.70	1.36	0.605	2.71	0.91
VBN-6 (R)	7.67 (2.94)	0.74	1.47	0.708	2.48	0.96
GBG 1 (R)	7.00 (2.82)	0.68	1.48	0.772	2.28	0.98
SE (d)	0.77	0.31	0.098	0.023	0.085	0.059
C.D.						
(P=0.05)	1.78	0.07	0.029	0.099	0.253	0.175
C.V.	6.65	6.10	8.81	12.19	4.38	10.12

HS: Highly susceptible S: Susceptible MS: Moderately susceptible MR: Moderately resistant.

According to the present study, the genotypes, GBG-1 and VBN-6 were least preferred for oviposition when *B. tabaci* adults were given free choice and no-choice tests, indicating antixenosis mechanism of resistance. These genotypes also exhibited antibiosis resistance factors, increased duration of nymphal developmental period, a greater prolongation of the egg to adult period and increased casualties in the nymphal stage. However, the resistance factors associated with these materials must be better investigated by characterizing the detailed biochemical composition and enzymatic activity of these genotypes.

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