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Morphological and Biochemical Factors Associated with Blackgram Resistance to Whitefly, *Bemisia tabaci* and Yellow Mosaic Disease

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ABSTRACT

Background: Yellow Mosaic disease caused by Mungbean yellow mosaic virus (MYMV) is one of the most destructive biotic production constraints in blackgram, responsible for yield losses up to 100 per cent. It is transmitted by whitefly in persistent circulative manner. The host plant resistance has a great importance in MYMV resistance. Morphological and Biochemical characters play a key role in offering resistance to vector and MYMV in the resistant genotypes.

Methods: The Experiment was conducted under glass house conditions using ten blackgram genotypes showed differential reaction to yellow mosaic disease during preliminary field screening. Experiment was laid out completely randomized block deign, replicated thrice.

Result: The results revealed that among the selected genotypes, resistant genotypes (GBG-1 and VBN-7) was recorded the maximum leaf thickness, maximum trichome density with shortest trichome length, maximum total phenolics, total proteins and total amino acids than the remaining genotypes which contributes resistance behavior in the host. Hence it concluded morphological factors are found responsible for vector preference for feeding where as biochemical parameters are crucial for virus infection and multiplication.

Key words: Artificial inoculation, *Bemisia tabaci*, Biochemical, Morphological, MYMV, Urdbean.

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 but in Andhra Pradesh predominantly grown as *rabi* pulse crop. Blackgram is affected by several fungal and viral diseases which cause severe reduction in yield. Among the viral diseases, yellow mosaic disease (YMD) is the major viral disease responsible for yield loss up to 100 percent (Usharani *et al.,* 2004) and it is caused by four distinct viruses (belongs to genus begomovirus) collectively known as yellow mosaic virus (YMV) *i.e.* Mungbean yellow mosaic India virus (MYMIV), Mungbean yellow mosaic virus (MYMV), Horsegram yellow mosaic virus (HgYMV) and Dolichos yellow mosaic virus (DoYMV). Begomoviruses are being transmitted by whitefly (*Bemisia tabaci*) from infected to healthy plants in persistent circulative manner (John *et al.*, 2008). A feasible, effective, economical, environment friendly and sustainable solution to alleviate Yellow Mosaic Disease incidence is to develop and use resistant varieties for both virus and insect vector.

Plant-insect interaction is a dynamic system, subjected to continual variation and change. 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 (War *et al.,* 2012). Besides, plants have also evolved by developing certain chemical defences in the form of induction of defensive proteins and various kinds of secondary metabolites (Kliebenstein *et al.,* 2001).

Understanding the morphological characteristics that lead to vector infestation and the biochemical levels associated with YMD infection is crucial for breeders to develop resistant varieties. The present investigation was conducted to understand the various morphological and biochemical characteristics in blackgram genotypes that contribute to resistance against YMD and the vector *B. tabaci*.

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MATERIALS AND METHODS

The experiment was conducted at department of Entomology, S.V. Agricultural College, ANGRAU, Tirupati andhra Pradesh under glass house conditions during March to May 2022. Genotypes that were showed consistent reaction during field experiments of *rabi* 2020-21 and 2021-22; Total ten genotypes, two genotypes from each category resistant to highly susceptible were selected for studying the morphological and biochemical traits (Table 1).

Seeds of test genotypes were sown in earthen pots and a single plant represents a replicate and each entry was replicated three times. Recommended pot mixture was used in each pot to raise the plants. The experiment was conducted by using completely randomized design (CRD) with three replications. The whitefly population was collected from blackgram fields with the help of an aspirator and released on 20 days old brinjal plants for multiplication in insect rearing cages (72 cm × 88 cm × 77 cm) kept in the glasshouse. Population from experimental area was molecular characterized as described by Singh *et al.,* (2012) Blackgram plants showing conspicuous symptoms of mungbean yellow mosaic virus (MYMV) were collected from naturally infected plants from the farmer fields. Virus culture maintenance and artificial virus transmission studies were done by adopting methodology of Madhumathi *et al.* (2020) (Fig 1).

Morphological characters

Leaf thickness was measured in mid way between the margin and midrib at the widest part of the leaf using digital vernier calipers and expressed in units of mm.From each leaf, three leaf bits each of 5 mm^2 diameter were taken and the trichome density was observed on the lower surface of the leaves using an Olympus stereo microscope (40 X magnification). Data were expressed as the number of trichomes per 5 mm^2 leaf diameter. Same protocol was followed for the trichome length and expressed in µm.

Biochemical parameters

The phenol estimation in healthy and diseased leaf samples was done as per methodology ofSadasivam and Manickam (1996). The protein content was estimated by using the colorimetric method using the Folin reaction and absorbance was read at 520 nm after 30 min (Lowry *et al.,* 1951). Reducing sugars in the leaf samples was estimated as per the procedure of Miller (1972).The extraction and estimation of free amino acids from leaf samples was done according to Moore and Stein (1948) by using Ninhydrin reagent.

RESULTS AND DISCUSSION

The genomic DNA from single whitefly isolated as described by Singh *et al.* (2012) and used for amplification of mitochondrial cytochrome oxidase gene (mtCOI) by gene specific primers. The amplified product of 880 bp was purified and sequenced and deposited in Gene Bank. The mtCOI based molecular analysis revealed that *B. tabaci* population (Accession Number: OP 781729) in the present study was aligning to *B. tabaci* complex spp. Asia- I mitochondrion GenBank ID: JX 993184 (Bapatla) with 96% homology.Studies have shown that the Asia I genetic group of *Bemisia tabaci* transmits the mungbean yellow mosaic virus (MYMV) more efficiently than the Asia II-1 and other biotypes (Archana *et al.,* 2018).

Biophysical factors

The leaf thickness in different genotypes was ranged from 0.30 (LBG-623) to 0.58 mm (VBN-7). The susceptible genotypes (LBG-623 and BG19-15) recorded the lowest leaf thickness 0.30 and 0.33 respectively and on par with each other while, the highest leaf thickness was recorded in resistant genotypes GBG-1 (52.93) and VBN-7 (50.02).

The resistant genotypes possessed thicker leaf lamina than the remaining categories, consequently supporting a smaller whitefly population. The possible

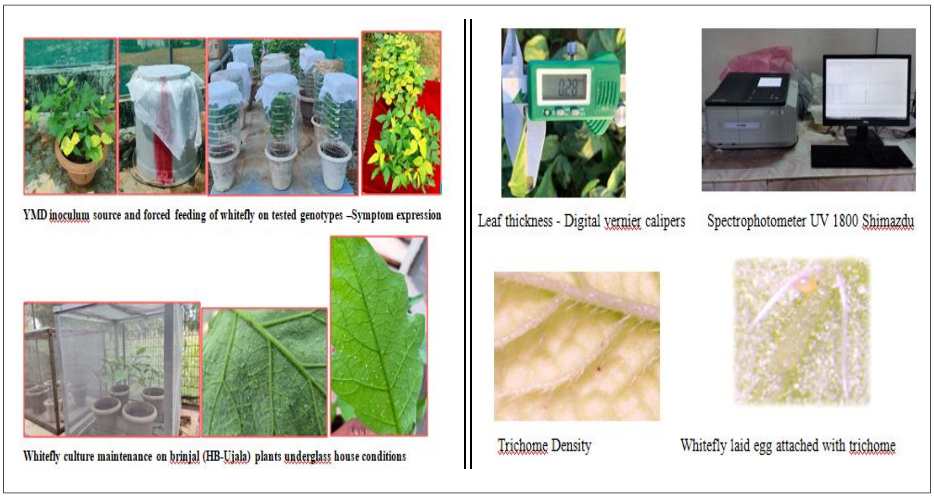

Fig 1: Experimental set up for the studying the morphological and biochemical traits in blackgram genotypes.

reason for this might be that genotypes with thicker leaf lamina were less succulent and thus were less preferred for feeding. These results are in conformity with Sulistyo and Inayati (2016) reported that thickness in leaves of soyabean complicates the stylet of whiteflies to penetrate the epidermis of leaves and interrupt the feeding process.

Trichome density (5 mm dia leaf disc)

The highest trichome density (20.33 and 18.33) per 5 mm diameter was recorded in resistant genotypes VBN-7 and GBG-1 respectively and onpar with each other while, the lowest trichome density (7.67 and 8.67) was recorded in highly susceptible genotypes LBG-623 and BG19-15 (Table 1). Leaf trichome density has defensive character that prevents the infestation of whitefly by deterring or limiting their establishment (Pena *et al.,* 2006) and thus making the movement, feeding and oviposition difficult in resistant genotypes. Similar results were also reported by (Noris and Kogan, 1980).

Trichome length (µm)

Resistant genotypes VBN-7(399.72 ìm) and GBG-1 (438.98 µm) possessed the significantly shortest trichome length followed by moderately resistant genotypes TBG-104 (463.38 µm), PU 1503 (731.85 µm) while, highly susceptible genotypes LBG-623 (960.45 ìm) and BG 19-15 (886.05 ìm) possessed the significantly the longest trichome length among all the genotypes (Table 1).

Biochemical parameters

Total phenols (mg g^{-1})

The total phenols in all the genotypes differed significantly and varied from 0.40 to 0.81mg/g in healthy and 0.44 to 0.90 in diseased leaves of different genotypes. Results revealed that after virus infection amount of total phenols increased in all the genotypes irrespective of their category but there was significant difference in the resistant genotypes, VBN-7 (0.81 to 0.90 mg/g) and GBG-1 (0.76 to 0.84 mg/g) while the lowest rate of increase insusceptible genotypes LBG-623 (0.40 to.044mg/g) and BG 19-15 (0.42 to 0.45 mg/g) (Table 2). Numerous studies have been highlighted the role of plant phenolics in resistance metabolism by impairing insect gut metabolism and prevent the nutrient uptake by covalently binding to dietary proteins, lipids and digestive enzymes. These compounds also inhibit insect growth through oxidative stress by the

Table 1: Biophysical parameters in selected blackgram genotypes under glass house conditions.

Genotype name	Trichome density (5mm^2 leaf disc)	Leaf thickness (mm)	Trichome length (µm)
LBG-623 (HS)	7.67 (2.93)**	0.30	960.45
BG 19-15 (HS)	8.67 (3.10)	0.33	886.05
BGGP 938 (S)	10.00 (3.31)	0.40	836.17
TU-94-02 (S)	10.00 (3.31)	0.36	841.02
BGGP 941 (MS)	10.67 (3.41)	0.43	584.48
BGGP 890 (MS)	11.33 (3.50)	0.47	640.34
PU1503 (MR)	13.00 (3.72)	0.49	731.85
TBG-104 (MR)	15.00 (3.99)	0.48	463.38
VBN-7 (R)	20.33 (4.61)	0.58	399.72
GBG 1 (R)	18.33 (4.39)	0.52	438.98
SE (m) ±	1.12	0.017	79.34
C.D (P=0.05)	3.35	0.051	237.56
CV	15.52	5.83	20.26

*FW- Fresh weight HS: Highly susceptible S: Susceptible MS: Moderately susceptible MR: Moderately resistant R: Resistant.

Table 2: Biochemical parameters inselected blackgram genotypes under glass house condition.

Genotype name	Total phenol (50 DAS) (mg g^{-1} FW)		Total proteins (mg g^{-1} FW)		Reducing sugars (mg g^{-1} FW)		Free amino acids (mg g^{-1} FW)	
	Healthy	Diseased	Healthy	Diseased	Healthy	Diseased	Healthy	Diseased
LBG -623 (HS)	0.40	0.44	0.34	0.36	5.63	5.04	0.29	0.33
BG 19-15 (HS)	0.42	0.45	0.37	0.40	5.29	4.82	0.35	0.39
BGGP 938 (S)	0.50	0.54	0.39	0.46	5.12	4.79	0.40	0.44
TU-94-02 (S)	0.53	0.57	0.40	0.43	4.84	4.35	0.43	0.47
BGGP 941 (MS)	0.57	0.62	0.41	0.45	4.45	4.13	0.46	0.51
BGGP 890 (MS)	0.58	0.63	0.42	0.47	4.21	3.60	0.49	0.54
PU1503 (MR)	0.65	0.72	0.52	0.58	3.86	3.91	0.55	0.62
TBG-104 (MR)	0.77	0.83	0.58	0.66	3.63	3.50	0.62	0.69
VBN-7 (R)	0.81	0.90	0.68	0.76	2.50	2.34	0.68	0.81
GBG-1 (R)	0.76	0.84	0.63	0.71	2.45	2.37	0.75	0.84
SE (m) ±	0.02	0.024	0.031	0.03	0.28	0.35	0.018	0.01
C.D (P=0.05)	0.06	0.07	0.091	0.09	0.86	1.05	0.054	0.05
C.V.	5.86	6.36	11.17	9.92	11.39	14.90	6.19	5.93

*FW- Fresh weight HS: Highly susceptible S: Susceptible MS: Moderately susceptible MR: Moderately resistant R: Resistant.

generation of oxygen and phenoxy radicals (War *et al.,* 2012). In the present study, phenolic content in the resistant genotypes enhances the mechanical activity of the host cell walls by the production of lignin and suberin which are the main components in the formation of physical barriers that block the spread of virus. Present results are in conformity with Mantesh *et al.* (2020) in green gram, Patel *et al.* (2013) in blackgram and Vidyasree *et al.* (2024) in blackgram and greengram.

Total proteins (mg g^{-1})

The higher rate of increase in total proteins from healthy to diseased was recorded in the resistant genotypes VBN-7 (0.68 to 0.76 mg/g) and GBG-1 (0.63 to 0.71 mg/g) were on par with each other followed by moderately resistant genotypes TBG-104 (0.58 to 0.66 mg/g), PU1503 (0.52 to 0.58 mg/g). Highly susceptible genotypes LBG-623 (0.34 to 0.36 mg/g) and BG19-15 (0.37 to 0.40 mg/g) recorded the lower rate of increase and significantly not different.Higher protein content in resistant varieties may be due to the increased synthesis of certain proteins for the activation of enzymes that are essential for various defence activities (Vidyasekaran, 2001). Chauhan *et al.* (2022) reported higher protein content in resistant cotton cultivars than the susceptible cultivars. This may be due to activation of defence mechanism between host and pathogen and concluded that among the primary biochemical compounds, only protein activity proposed as the first line of defence in cotton against cotton leaf curl virus (CLCuV).

Reducing sugars (mg/g)

The data on reducing sugars presented in Table 2 revealed that decreasing trend in total reducing sugars content was observed in diseased leaves compared to healthy leaves in all the genotypes but higher rate of reduction in total reducing sugars in healthy to diseased leaves was recorded in highly susceptible genotypes LBG-623 (5.63 to 5.04 mg/g) and BG 19-15 (5.29 to 4.82 mg/g) followed by susceptible genotypes BGGP 938 (5.12 to 4.79 mg/g), TU94-02 (4.84 to 4.35 mg/g). The resistant genotypes GBG-1 (2.45 to 2.37 mg/g) and VBN-7 (2.50 to 2.34 mg/g) were recorded the lowest rate of reduction in healthy to diseased leaves compared to remaining genotypes.

Sugars are considered as one of the vital nutrients in plants, the difference in the relative amount of sugars between different genotypes with differential susceptibilities to whitefly indicated that these compounds might act as phago-stimulants to whitefly feeding on blackgram (Kadu, 2018) and hence differences in whitefly population and differences in YMD incidence. The present results in accordance with Ramarao *et al.* (2021) and Anil *et al.* (2018) reported that susceptible mungbean genotypes had higher amount of reducing sugars than resistant genotypes.

Total free amino acids (mg/g)

The higher rate of increase in total free amino acids from healthy to diseased was recorded in the resistant genotypes VBN-7 (0.68 to 0.81mg/g) and GBG-1 (0.75 to 0.84 mg/g) followed by moderately resistant genotypes TBG-104 (0.62 to 0.69 mg/g), PU1503 (0.55 to 0.62 mg/g) were on par with each other. Highly susceptible genotypes LBG-623 (0.29 to 0.33 mg/g) and BG19-15 (0.35 to 0.39 mg/g) recorded the lower rate of increase and significantly not different (Table 2).The higher amount of total free amino acids in resistant genotypes due to virus multiplication which entails the synthesis of virus specific abnormal proteins that accumulates an ultimately rises the percentage over susceptible genotypes. Increase in protein content observed in infected leaves may also correlate with respiration. Increased nitrogen uptake by infected plants associated with rapid respiration probably helps in the synthesis of more amino acids (Sinha and srivatsava, 2010) and Mohammed *et al.* (2021).

It can be postulated that biophysical character were responsible for interruption of feeding process (Virus transmission) of viruliferous whitefly adult with host plant thus leading to lower disease incidence in resistant genotypes. Biochemical characters had a significant correlation YMD incidence. During host and virus interaction (incompatible reaction) there is activation of Host Reaction leading to localized cell death, generation of harmful free radicals includes different ROS, such as super oxide radical (O^-_2), H_2O_2 and Hydroxy radicals.

CONCLUSION

The present study results revealed that maximum leaf thickness, maximum trichome density, shortest trichome length and purple petiole colour, maximum total phenolics content, protiens and total amino acids was observed in resistant genotypes (GBG-1 and VBN-7).From the results it can be inferred maximum trichome density, shorter trichome length might be difficult for the whitefly landing, movement, settlement and oviposition, even though whitefly settled on leaf surface, thicker leaf lamina would be less succulent for probing thus interrupt the feeding process or virus transmission. Secondary metabolites like phenols, tannins, flavanoids and total proteins increased upon virus infection leads to biochemical defence mechanism get activated in order to suppress the replication of the virus in the resistant host. Insight of this experiment helps to develop MYMV resistant varieties by using this traits in the future studies.

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Conflict of interest

All authors declared that there is no conflict of interest.

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