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ARTIFICIAL SCREENING OF SELECTED BLACKGRAM GENOTYPES AGAINST YELLOW MOSAIC DISEASES (YMD) UNDER GLASSHOUSE CONDITIONS

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ABSTRACT

Yellow Mosaic Disease (YMD), caused by the *Mungbean yellow mosaic virus* (MYMV), is a major biotic constraint affecting blackgram production. Field screening for YMD resistance is often unreliable, as plants may escape infection even under high inoculation pressure, making it challenging to accurately identify resistant lines. Four resistant and eight moderately resistant genotypes identified through field screening during the *rabi* seasons of 2020-21 and 2021-22 were re-evaluated using whitefly-mediated virus transmission studies to validate their resistance sources. The experiment was conducted in a completely randomized design (CRD) with three replications under glasshouse conditions at S.V. Agricultural College, ANGRAU, Tirupati, Andhra Pradesh. The results revealed that per cent disease index (PDI%) ranged from 4 to 88.56 with disease rating scale 1 to 9. Only three genotypes, GBG-1, VBN-6 and VBG 12-062 found to be resistant with 1 disease rating scale and low per cent disease index values (4%, 5% and 8.5%) respectively, remaining genotypes were found to be moderately resistant to highly susceptible. The identified resistant lines can be used in breeding programme to develop MYMV resistant cultivars.

***Key words*:** Artificial screening, YMD, Asia- I, Glass house conditions, resistant source, blackgram genotypes, MYMV

Introduction

Blackgram (*Vigna mungo* (L.) Hepper) commonly known as urdbean, mash or black mapte is a short duration and highly remunerative pulse crop grown in most parts of India traditionally as *kharif* crop. India currently represents the largest producer of blackgram accounting for more than 70 per cent global production (Sasidhar *et al*., 2022). Despite of its importance, the substantial constraints in mungbean productivity are primarily due to biotic stresses. Among them, viral diseases are widely devastating and cause heavy yield loss (Paul *et al*., 2013) and particularly the most important damage amongst the virus is found to be Mungbean Yellow Mosaic Virus (MYMV). MYMV belongs to begomovirus, the largest genus of the family Geminiviridae (Dhakar *et al*., 2010), which is characterized by its monopartite or bipartite (DNA A and DNA-B) genome and is transmitted by whitefly, *Bemisia tabaci* in a circulative and persistent manner (Sidhu *et al*., 2009). The disease resulted in yield losses ranging from 5 to 100%, depending on crop age, cultivar susceptibility, and whitefly population (Mahalakshmi *et al*., 2015).

Field screening under diverse environmental conditions is the first step in identifying resistant lines. However, this approach is time-consuming, requires evaluation in 'hot spot' areas, and is often inefficient due to plants escaping infection even under heavy inoculation pressure (Selvi *et al*., 2006). MYMV symptoms may not always appear in the field due to factors such as environmental changes, whitefly genotypes, and host factors, leading to failed infections and making it difficult to identify truly resistant lines. Therefore, it is essential to screen genotypes using forced feeding methods, which ensure a 100% infection rate and standardized inoculum

pressure. In the present study, thirteen blackgram genotypes selected from preliminary field screening were evaluated under glasshouse conditions for resistance to yellow mosaic disease through whitefly-mediated artificial inoculation to identify resistant sources for use in breeding programs.

Materials and Methods

Whitefly-mediated transmission studies were conducted in a glasshouse at the Department of Entomology, S.V. Agricultural College, Tirupati, Andhra Pradesh, from March to May 2022. Genotypes classified as resistant, moderately resistant, moderately susceptible, susceptible, and highly susceptible were selected based on preliminary field screening from the *rabi* seasons of 2020-21 and 2021-22. Whiteflies were collected from brinjal fields in Tirupati using an aspirator and released onto 20-day-old brinjal plants for multiplication in insect-rearing cages (72 cm × 88 cm × 77 cm) kept in the glasshouse. Old brinjal plants were regularly replaced with healthy ones to maintain a vigorous culture. After one cycle, freshly hatched, virus-free whiteflies were used for transmission studies. The whitefly population from the experimental area was molecularly characterized following Singh *et al.*, (2012) using *mtCOI* primers: forward primer C1-J-2 (5'-TTGATTTTTTGGTCATCC AGAAGT-3') and reverse primer L2-N-3014 (5'-TCCAATGCACTAATCTGCCATATTA-3'). *mtCOI*based molecular analysis showed that the *B. tabaci* population (Accession Number: OP781729) aligned with the species Asia-I (GenBank ID: JX993184, Bapatla) with 96% homology.

Source of virus inoculum and maintenance of yellow mosaic virus (YMV) culture

Blackgram plants showing distinct symptoms of mungbean yellow mosaic virus (MYMV) were collected from naturally infected plants at the Dryland Farm, S.V. Agricultural College, Tirupati. *B. tabaci* whiteflies were allowed a 24-hour virus acquisition period before being transferred to 15-day-old healthy blackgram plants of the susceptible variety LBG-623 in a glasshouse. After a 24-hour inoculation access period, the inoculated plants were placed in insect-proof cages to allow MYMV symptoms to develop, serving as the stock culture (Naveesh *et al.*, 2020).

Raising of healthy blackgram seedlings

Seeds of the test genotypes were sown in earthen pots, with each plant serving as a single replicate, and every entry replicated three times. The plants were grown in the recommended potting mixture to ensure optimal conditions. The experiment followed a Completely Randomized Design (CRD) with three replications. No pesticides were applied to the seedlings, maintaining the integrity of the screening process. At 10-15 days post-germination, these seedlings were selected as test plants. Each genotype was securely enclosed in a plastic chimney (4.7 cm diameter at the top, 7.2 cm at the bottom, with a bulging middle and a height of 21 cm), inverted with the mouth pressed into the soil and the base covered with 100-micron muslin cloth to prevent the escape of whitefly adults. The chimneys were carefully anchored into the moist soil to ensure stability, setting a controlled environment for rigorous screening.

Whitefly transmission

Ten to fifteen *B. tabaci* adults were collected in a vial-like plastic tube from the maintained culture using an aspirator and starved for 3 hours. After starvation, the whiteflies in the plastic tubes were released onto the YMV-diseased blackgram variety LBG-623, which was used as the stock culture, and allowed to feed for an acquisition period of 24 hours. Following the 24-hour acquisition access period (AAP), the *B. tabaci* adults were removed from the stock culture and transferred into separate insect-free cages containing healthy blackgram plants of the tested varieties for an inoculation access period (IAP) of 24 hours. After the 24-hour IAP, the *B. tabaci* adults were removed, and the plants were sprayed with the insecticide imidacloprid (17.8 SL @ 0.4 ml/L) (Madhumati *et al.*, 2020). The spread of MYMV was recorded at weekly intervals until maximum infection was achieved. The number of infected genotypes per week was calculated, and the genotypes were scored based on the degree of MYMV incidence using a 1-9 rating scale to classify them into different infection categories.

Rating scale used for scoring against Mungbean Yellow Mosaic Virus (MYMV) (Singh *et al.*, 1992)

Rating	Percentage foliage affected	Infection category
1	No visible symptoms or minute yellow specks 0.1%-5% leaf area	Resistant
3	Mottling of leaves covering 5.1-15% leaf area	Moderately resistant
5	Yellow mottling and discoloration of 15.1-30% leaf area	Moderately susceptible
7	Pronounced yellow mottling and discoloration of leaves and pods, reducing in leaf size, stunting of plants, 30.1%-75% foliage affected	Susceptible
9	Severe yellow mottling and discoloration of leaves, stunting of plants, failure of flowering and fruit setting 75.1-100% foliar affected	Highly susceptible

Table 1: List of genotypes that showed consistent reaction to YMD across the two seasons during late *rabi* 2020-21 and 2021-22 under field conditions.

S. No.	Category	No. of. Genotypes	Late *rabi* 2020-21	Late *rabi* 2021-22
1	Resistant	5	GBG-1, VBN-6 ,VBN-7, PU15-27, VBG12-062	GBG-1, VBN-6, VBN-7, PU15-27, VBG 12-062
2	Moderately resistant	8	TBG-104, PU 15-03, LBG 961, TBG104, MBG 1051, BG GP806, BGGP 904,BGGP 822	PU15-03, LBG 961, TBG-104, TBG -104, MBG1051, BGGP 806, BGGP 904,BGGP 822
3	Moderately susceptible	9	BGGP 927, LBG 20-1, BGGP 815, LBG965, BG GP 808, BGGP 912, BGGP 941, BGGP 890, PU-6	BGGP 927, LBG 20-1, BGGP 815, LBG965, BG GP 808, BGGP 912, BGGP 941, BGGP 890, PU-6
4	Susceptible	32	BG 19-13, BGGP 868,BGGP 648, BGGP 889, TU-40, BGGP 850, BGGP 803, Shekar 2, BGGP 892, BG 19-06, LBG 752,BGGP 938, GBG81, LBG 800,IPU 17-2, IPU 11-6, LBG 971, BGGP 968, GBG81,BGGP 803, BGGP 809,GBG 99, ACM14-001,GBG-45, TU94-02, OBG 38, BGGP 807, VBG 17-012 BG 19-02, BG 19-14, GBG 92, LBG-752	BGGP 960, PU 1504, MBG 1037, BGGP 968, ACM 14-001, ABG -04, LBG 971, IPU 11-6, GBG 99, LBG 800, BGGP 938, GBG 79, BG 19-06,BG 19-14, BG GP 805, BGGP 803, Shekar2, BGGP 892 , BG 19-02, BGGP 648, BGGP 889, BGGP 807, BGGP 868, OBG 38, TU94-02, BG 19-13, GBG-81, GBG-45, LBG -752, IPU 17-2, LBG 752, GBG 92, BGGP 850, TU-40, VBG 17-012, BGGP 809
5	Highly Susceptible	5	TU-67,BGGP 645, BGGP 685, LBG-623, BG 19-15	TU-67, BGGP 645, BGGP 685, BG 19-15, LBG-623

Percent disease index was calculated by using the formula given by Wheeler (1969).

$$\text{Percent disease Index} = \frac{\text{Sum of all the numerical ratings}}{\text{Number of observations} \times \text{Maximum disease rating}} \times 100$$

Results and Discussions

Field screening experiment was conducted with 70 blackgram genotypes during the *rabi* seasons of 2020-21 and 2021-22. Genotypes were categorized based on the disease rating scale of Alice and Nadarajan (2007). The results revealed that out of 70 genotypes, five were resistant and eight consistently showed a moderately resistant reaction across both seasons (Table 1 and Fig. 1). These resistant and moderately resistant genotypes were further screened against YMD under glasshouse conditions to determine the stability of their resistance (Table 2).

Percent disease index (PDI) in the tested genotypes ranged from 4.0 to 88.56%. Among the thirteen genotypes,

Fig. 1: Genotypes which showed consistent resistant reaction to YMD during late *rabi* 2020-21 and 2021-22 under field conditions

Table 2: Screening of selected blackgram genotypes against YMD under glass house conditions.

S. No.	Genotype Name	No. of days for symptom develop-ment	PDI (%)	Rating scale	Disease reaction
1	GBG-1	19	4.0	1	Resistant
2	VBN-6	15	6.0	1	Resistant
3	VBG 12-062	16	8.5	1	Resistant
4	PU1527	12	18.50	5	Moderately resistant
5	VBN-7	14	20	3	Moderately resistant
6	TBG-104	16	24	3	Moderately resistant
7	PU1503	12	52.46	7	Susceptible
8	LBG-961	10	64.52	7	Susceptible
9	MBG 1051	13	71.46	7	Susceptible
10	BGGP806	13	65.75	7	Susceptible
11	BGGP941	15	58.80	7	Susceptible
12	BGGP822	12	88.56	9	Highly susceptible
13	BGGP904	14	85.00	9	Highly susceptible

three genotypes GBG-1, VBN-6 and VBG 12-062 were found to be resistant with 1 disease rating scale and PDI (4%, 6% and 8.5%). Three genotypes VBN-7, PU 15-27 and TBG-104 exhibited moderately resistant reaction (20.0, 18.5, 24.0% and disease rating scale 3). Five genotypes *viz.*, PU1503, LBG-961, MBG 1051, BGGP 806and BGGP 941 showed susceptible reaction with 7 disease rating scale. Two genotypes *viz.*, BGGP 822 and BGGP 904 found to be highly susceptible with disease rating scale 9 and 88.56 and 85.0% PDI respectively (Table 2 and Fig. 2).

In the present study, VBN-7 and PU15-27 were found to be moderately resistant, though they appeared resistant under field screening. Except for TBG-104, all moderately resistant genotypes were susceptible or highly susceptible (BGGP 822 and BGGP 941) when compared to field screening results. Only three genotypes GBG-1, VBN-6, and VBG 12-062 consistently exhibited resistance under both conditions, showing small yellow flecks (Disease Rating Scale 1), despite strong virus inoculum pressure and the presence of the efficient virus-transmitting cryptic whitefly species, ASIA-I. This variation may be attributed to factors such as geographical location, weather conditions, genotype differences, virulent virus strains, existing whitefly cryptic species, and their feeding preferences for specific germplasm.

Kalyankumar *et al.*, (2021) reported that the *B. tabaci* cryptic species Asia II-8 was responsible for the higher incidence of yellow mosaic disease (YMD) in Tamil Nadu. In contrast, Archana *et al.*, (2018) found that Asia I was a more efficient transmitter of mungbean yellow mosaic virus (MYMV) than Asia II-1 in blackgram. According to Nair *et al.*, (2017), Asia II-1 is dominant in Northern India, while Asia II-8 is predominant in Southern India. Habib *et al.*, (2007) observed that mungbean is more susceptible to MYMV at the early growth stage than at maturity. These findings highlight that the initial 3-4 weeks are critical for YMD development due to the early arrival of viruliferous whiteflies. Furthermore, disease development can be inconsistent because whitefly populations vary based on planting location and season (Laosatit *et al.*, 2020).

Under field conditions, higher temperatures lead to increased whitefly populations, whereas high rainfall and humidity negatively affect whitefly build-up (Rahman *et al.*, 2006; Islam *et al.*, 2008). Due to these environmental constraints, natural field screening may not accurately differentiate resistance levels. In contrast, under screen house conditions, resistant genotypes may show moderate resistance, and moderately resistant genotypes may

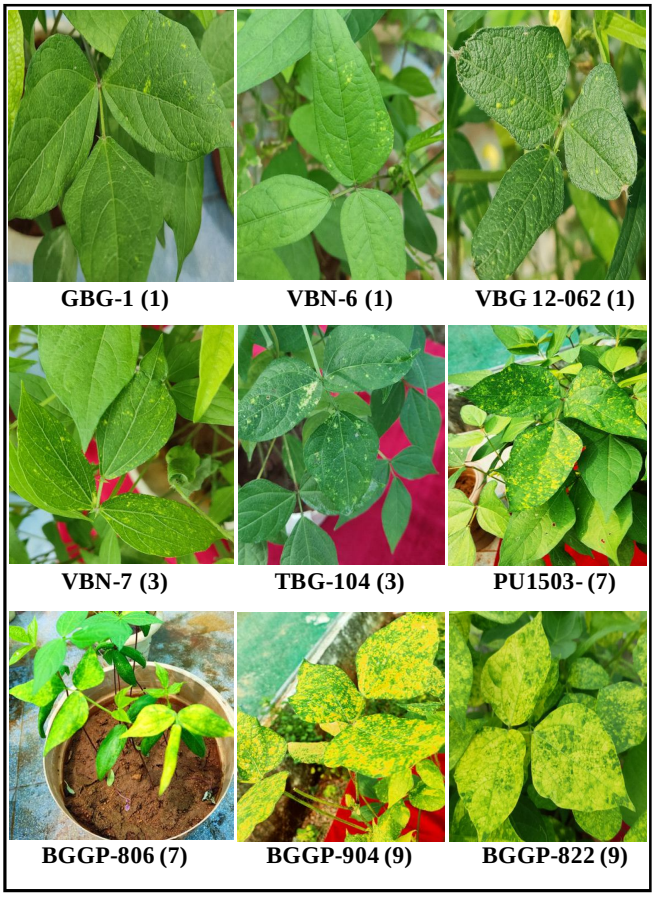

Fig. 2: YMD Symptom expression in genotypes screened under glass house conditions.

exhibit susceptibility or high susceptibility. This variation is attributed to factors such as the presence of highly infective cryptic whitefly species, strong disease inoculum pressure, and the forced feeding of viruliferous whiteflies on specific genotypes. Screen house conditions eliminate the chance of avoiding whitefly feeding, allowing for a true expression of resistance or susceptibility to YMD.

The forced feeding method is an effective tool for validating resistance sources or confirming resistance in field-screened genotypes. This method has been widely used to screen pulses for MYMV resistance (Kundragami *et al*., 2009). The present findings align with those of Ambarish *et al*., (2023), who reported that, out of 19 field-resistant genotypes screened through artificial inoculation, none were completely free from MYMV. However, three green gram genotypes RM-16-20, JNG-18 and TK-6-1 exhibited a resistant reaction to MYMV. Similarly, Suman *et al*., (2018) observed that while the Pusa-9531, HUM-12, and Meha cultivars were moderately resistant under field conditions, they were moderately susceptible under screen house conditions. Bachkar *et al*. (2019) screened nine field-resistant genotypes under glasshouse conditions and found that only three PS-1589, PS-1587, and SL1104 showed resistance to soybean yellow mosaic virus (SYMV). Das *et al*., (2018) noted that, out of 60 horse gram germplasm lines, only two genotypes, Arka Arjun and Jade-5058, displayed resistance to HgYMV under both natural and artificial epiphytotic conditions. Naveesh *et al*., (2020) screened 43 soybean genotypes for SYMV resistance in glasshouse conditions using whitefly-mediated transmission. None of the genotypes were resistant, though 11 showed moderate resistance. Similar evaluations of soybean genotypes against SYMV have been documented by Kumar *et al*., (2008), Talukdar *et al*., (2013) and Baruah *et al*., (2014).

MYMV is a significant constraint to legume cultivation and production in Asia, including India. Managing this disease remains a considerable challenge. Recent outbreaks of whitefly, coupled with resistance to commonly used insecticides (Ahmed *et al*., 2010), have led to an increase in MYMV incidence in various crops, including legumes (Karthikeyan *et al*., 2014; Nene, 1972). Developing and using resistant cultivars offers the best solution for mitigating yellow mosaic disease, with field screening serving as the foundation for further research. In this study, only three genotypes GBG-1, VBN-6, and VBG 12-062 exhibited resistance, despite exposure to strong inoculums of MYMV, pure cultures, and the dominant insecticide-resistant cryptic whitefly species, ASIA-1. The continuous exploitation of MYMV-resistant sources in blackgram is essential; thus, these three resistant lines can be utilized in breeding programs and studies focusing on the morphological and biochemical factors associated with resistance to both the disease and its vector.

Conclusion

Three genotypes (GBG-1, VBN-6 and VBG 12-062) were found to be resistant under field conditions and screen house conditions, these genotypes can be used in resistance breeding programme against YMD. High Yield, YMD resistance and other agronomic characters can be considered to develop varieties suitable for different agro ecological zones.

Declaration

The authors confirm that they do not have any conflicts of interest.

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