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Influence of Morphological and Biochemical Parameters for Infection of *Mungbean Yellow Mosaic Virus* and *Bemisia tabaci* in Mungbean and Urdbean

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ABSTRACT

Background: Mungbean and Urdbean are the important pulses in India which are regarded as prospective protein source for human daily diet and rich source of protein and carbohydrate. *Mungbean Yellow Mosaic Virus* is one of the destructive diseases to pulses and transmitted by whitefly. Among the pulses, mungbean and urdbean are severely affected by MYMV in India. Present investigation was aimed to determine the congenial and mitigating factors of mungbean and urdbean crops with reference to infection of MYMV and its vector.

Methods: The morphological and biochemical analysis was carried out by factorial RCBD consisting of two factors *viz*., factor 1: Crops (V) (Mung bean and Urd bean) and factor 2: observation at 15 days interval up to 90 DAS (S) for recording different morphological and biochemical parameters.

Result: Morphological parameters of urdbean are able to mitigate the vector incidence and indirectly reduced the MYMV infection in urdbean than mungbean. Further, the biochemical parameters levels were more in urdbean than mungbean and their levels increased with the increased MYMV incidence. Hence, it is concluded that the morphological characters are found responsible for vector infestation where as biochemical levels are important for the infection of MYMV in both the crops.

Key words: Biochemical, Morphological, MYMV, Vector.

INTRODUCTION

Mungbean and urdbean are important pulse crops in the world as well in India. The productivity is hampered by a variety of causes, one among them is diseases (Alice and Nadarajan, 2007). Mungbean and urdbean are 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 in these crops responsible for yield loss up to 100 per cent (Usharani *et al*., 2004).

The development of resistant varieties against MYMV in mungbean and urdbean is most prominent way to alleviate the disease and its vector. Every host has inherent capacity to resist and overcome the biotic and abiotic stresses. Similarly, urdbean and mungbean also have different morphological and biochemical characters which impact differently on MYMV infection and vector population and offer the resistance.

Understanding the morphological characters responsible for vector infestation and biochemical levels for the infection of MYMV in mungbean and urdbean are very important to develop resistant varieties by the breeders. Present investigation was carried out to understand different morphological and biochemical characters in mungbean and urdbean which contribute for resistance against MYMV and its vector (*B. tabaci)*.

MATERIALS AND METHODS

Location and season

The experiment was conducted at College of Agriculture, V.C. Farm, Mandya during *Kharif,* 2020-21. MYMV susceptible varieties of mungbean (BGS-9) and urdbean (Rashmi) were selected and experiment was conducted in glass house condition.

Maintenance of MYMV culture

The non viruliferous whiteflies maintained on cotton plants were collected in a plastic bottle and released on the MYMV infected mungbean and urdbean crops to feed and acquire MYMV for 12 hours (Acquisition access period) in the glass house. After the acquisition access period (AAP), the viruliferous whiteflies were collected and used for further artificial infection on mungbean and urdbean (Fig 1).

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Inoculation of seedlings

The healthy mungbean and urdbean seedlings with trifoliate leaves in the field were inoculated with 15-20 viruliferous whiteflies which were collected from the plastic bottles with help of an aspirator. After 24 hr inoculation access period, the seedlings were kept in an insect-proof cage for symptom development (Fig 2). The presence of MYMV in infected mungbean and urdbean plants were confirmed by molecular detection through CP-primers by using CTAB method (Lodhi *et al*., 1994).

Evaluation of morphological and biochemical characters

The mungbean and urdbean infected and healthy leaves were collected from the artificial inoculated plants in the controlled field condition. The experiment was carried out in factorial randomized complete block design (Factorial - RCBD) consisting of two factors *viz*., Factor 1: Crops (V) (Mungbean and Urdbean) and Factor 2: Observation at 15 days interval up to 90 DAS (S) (15 DAS, 30 DAS, 45 DAS, 60 DAS, 75 DAS and 90 DAS).

Evaluation of morphological characters

The leaf thickness was measured with a digital vernier caliper (Fig 3) in 1 cm^2 hand-cut sections of the leaves and trichome density was estimated on both upper and lower surface of mungbean and urdbean by cut into leaf discs of 1 cm diameter and counted the number of hairs under stereo microscope and the average value of the trichome density was calculated as cm^2 leaf area which outlined by Taggar and Gill (2012).

Leaf epicuticular wax content was determined as per the procedure given by Ebercon *et al.* (1977).

Evaluation of biochemical levels

Phenol

The phenol estimation was conducted according to the Folin Ciocalteau method which is based on the reaction of oxidizing agent phosphomolybdate which forms blue complex, based on the colorimetric results phenol content was measured under spectrophotometer at 650 nm absorbance Sadasivam and Manickam (1996).

Protein

The protein content of infected and healthy plants 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).

Total sugar

The total sugars in the samples was estimated by using arsenomolybdate reagent as per the procedure of Hedge and Hofreiter (1962) where in the blue color developed was read at 510 nm.

Tannin

Tannin content of infected and healthy plant sample was estimated by Vanillin Hydrochloric acid method by using Vanilin-HCL reagent and intensity of the colour developed was read in the spectrophotometer at 500 nm (Price *et al*., 1978).



Fig 1: A) Non-viruliferous whiteflies on cotton plants. B) Non-viruliferous whiteflies acquiring MYMV from infected plants.



Fig 2: Healthy and MYMV infected mungbean and urdbean plants.

Peroxidase activity

Enzyme was extracted from the 1 g of infected and healthy leaf tissues in 3 ml of 0.1 M phosphate buffer at pH 7 by grinding with pre-cooled mortar and pestle. Centrifuge the homogenate at 18000 g at 5°C for 15 min. To this supernatant 3ml of phosphate buffer, 0.05 ml guaiacol solution, 0.03 ml hydrogen peroxide solution was added and mixed thoroughly and absorbance was taken at 436 nm under spectrophotometer and absorbance taken at every 30 sec (Hartree, 1955).

Polyphenol oxidase activity

Enzyme was extracted from the infected and healthy 25 g frozen tissue in two portion of 100 ml cold acetone Centrifuge or filter under vaccum. The homogenate was air dried to remove acetone. The resulting dry powder was weighed and mixed with 6.5 ml 0.2 M citrate phosphate buffer, 1% Triton, 6.5 ml water and 500 mg polyamide and shake for an hour and filtered. The supernatant was pippeted into cuvette 1.4 ml citrate phosphate buffer, 0.5 ml TNB and 1 ml substrate solution was added and immediately absorbance was observed at 412 nm (Mayer *et al*., 1965).

RESULTS AND DISCUSSION

Molecular detection of MYMV in mungbean and urdbean

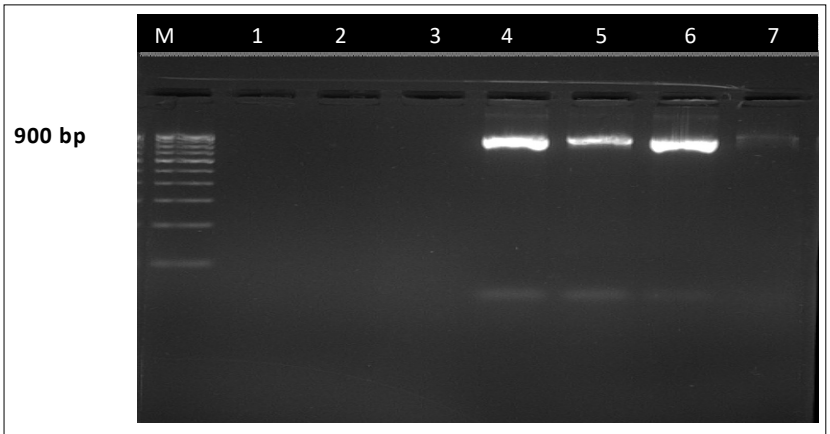
To confirm the presence of MYMV in infected and healthy mungbean and urdbean plants, the leaf samples were collected from the field at 30 DAS from each treatment for virus detection. Total genomic DNA was extracted from all the samples by using the CTAB method and subjected for polymerase chain reaction and amplified by using Coat-protein mediated MYMV specific primer. A band was visualized approximately at 900 bp when exposed to UV-rays through gel documentation unit indicating the conformity of virus (Plate 1).

Leaf thickness

Significant difference was recorded between mungbean and urdbean leaf thickness with age of the crop. The leaf thickness of mungbean and urdbean ranged from 81.10 to 112.00 and 95.13 to 132.00 µm respectively from 15 to 90 DAS. The maximum leaf thickness was recorded in urdbean (132 µm), whereas in mungbean it was 112 µm, comparatively thinner than urdbean at 90 DAS. The significantly lowest leaf thickness was recorded in mungbean leaves (81.00 µm), whereas urdbean plants showed 95.13 µm at 15 DAS. Leaf thickness act as one of the important



Fig 3: The cuticular thickness measurement in urdbean and mungbean by using a digital vernier caliper.



Lane 1: Healthy mungbean plants; Lane 2: Healthy urdbean plant; Lane 3: Non-viruliferous whiteflies; M: 100 bp ladder; Lane 4: MYMV infected mungbean; Lane 5: MYMV infected urdbean; Lane 6: Viruliferous whiteflies.

Plate 1: Molecular detection of MYMV in mungbean, urdbean and whiteflies.

morphological factor which cause impact on the vector feeding activity, there by indirectly affect the virus transmission to the host which may render the MYMV infection. Devi *et al.* (2019) reported that MYMV susceptible genotypes were thinner than resistant genotypes and observed Significant negative correlation existed between leaf thickness and disease severity.

Trichome density

Significant variation was recorded between number of trichomes in urdbean and mungbean leaves and also with respect to upper and lower surface of the leaves. The number of trichomes of upper surface of mungbean and urdbean leaves ranged from 12.00 to 36.00 per sq cm and 19.00 to 47.33 per sq cm, respectively at 15 to 90 DAS. Similarly, the number of trichomes on the lower surface of mungbean and urdbean leaves ranged from 6.33 to 18.33 and 14.33 to 28.33 per sq cm. The results indictaed that maximum number of trichomes on upper and lower surface was recorded in urdbean (47.33 and 18.33 per sq cm) than the mungbean leaves (36.00 and 18.33 per sq cm). The least number of trichomes was recorded in mungbean leaves in upper and lower surface (12.00 and 6.33) at 15 DAS. The results revealed that the disease incidence of MYMV and preference of *B. tabaci* was negatively correlated to higher number of trichomes on the lower surface of the leaves. The present studies corroborated by Sanchez-Pena *et al*. (2006) who reported that the density of leaf trichomes has a defensive reliability that prevents whitefly infestation by deterring or restricting their establishment. As a result, locomotion, feeding and ovipositor activity has become even more difficult (Table 1).

Epicuticular wax

The results revealed that significant variation of epicuticular wax content in urdbean and mungbean leaves was observed from 15 to 90 DAS. The maximum epicuticular wax content was recorded in urdbean (0.18 to 0.44 mg dm^{-2}) when compared to mungbean (0.11 to 0.36 mg dm^{-2}) leaves. Further, the results also indicated that the epicuticular wax content was increased with age of the crop in both the crops indicating increased in wax content leads to more MYMV resistance in both the crops. The present studies were confirmed by Chand and Verma (1983) who reported that MYMV resistant mungbean and urdbean plants recorded thicker cuticular wax content than the susceptible ones (Table 1).

Total phenol

Significant highest amount of phenol content was recorded in urdbean healthy and diseased leaves (0.42 and 0.57 mg/g/DW) than the mungbean healthy and diseased (0.31 to 0.52 mg/g/DW) leaves. Further, the phenol content of urdbean leaves were higher than the mungbean leaves and also total phenol content was increased with increase of disease severity. The results of the present study are on par with the results of Mantesh *et al.* (2020) who reported that increased phenolic content was observed in mungbean MYMV resistant genotypes than the susceptible genotypes (Table 2).

Tannin content

The maximum tannin content was recorded in MYMV infected urdbean leaves (0.51 mg/g) than the mungbean leaves (0.49 mg/g). Similarly, healthy urdbean leaves (0.45 mg/g) showed maximum tannin content than the mungbean leaves (0.37 mg/g) at 90 DAS (Table 2). The results also revealed that minimum tannin content was present in the healthy young leaves of urdbean and mungbean than the older leaves. Significantly higher tannins and flavonoids contents were observed in the MYMV resistant varieties of blackgram (Taggar *et al*., 2012).

Protein content

Results were revealed that the protein content of both crops was highest in diseased plants than the healthy plants. The highest protein content was recorded in diseased urdbean (0.43 mg/g) than the mungbean (0.41 mg/g) leaves and the maximum amount of protein content was observed in 90 days after sowing than 15 days after sowing. The current findings were in agreement with Shivaprasad *et al.* (2005),

Table 1: Morphological variation in between mungbean and urdbean at different intervals.

Intervals	Morphological characteristics							
	Leaf thickness (µm)		Trichome density (Number of trichomes per square cm)				Epicuticular wax content (mg/dm^2)	
DAS	Mungbean	Urdbean	Mungbean		Urdbean		Mungbean	Urdbean
			Upper	Lower	Upper	Lower		
15 DAS	81.10	95.13	12.00	6.33	19.00	14.33	0.11	0.18
30 DAS	92.10	109.10	16.33	11.00	22.00	17.67	0.17	0.30
45 DAS	98.00	124.33	23.33	14.00	36.00	25.33	0.23	0.36
60 DAS	104.00	128.73	33.00	16.33	46.00	26.00	0.32	0.42
75 DAS	110.2	130.87	33.67	17.00	46.33	26.67	0.34	0.43
90 DAS	112.00	132.00	36.00	18.33	47.33	28.33	0.36	0.4
SEm±	1.58	1.58	0.78	1.83	0.78	2.04	0.06	0.06
CD @ 5%	4.63	4.63	2.28	5.37	228	5.99	0.17	0.17

Table 2: Biochemical variation in between mungbean and urdbean at different intervals.

	Biochemical parameters											
Intervals	Phenol (mg/g/DW)				Tannin (mg/g/DW)				Protein (mg/g/DW)			
DAS	Mungbean		Urdbean		Mungbean		Urdbean		Mungbean		Urdbean	
	Healthy	Diseased	Healthy	Diseased	Healthy	Diseased	Healthy	Diseased	Healthy	Diseased	Healthy	Diseased
15 DAS	0.12	0.17	0.29	0.34	0.11	0.16	0.30	0.28	0.21	0.24	0.25	0.28
30 DAS	0.20	0.28	0.33	0.44	0.22	0.29	0.33	0.34	0.26	0.27	0.29	0.33
45 DAS	0.24	0.43	0.36	0.47	0.26	0.35	0.35	0.43	0.32	0.34	0.34	0.37
60 DAS	0.28	0.47	0.39	0.52	0.31	0.43	0.42	0.49	0.36	0.37	0.37	0.40
75 DAS	0.29	0.51	0.41	0.55	0.35	0.47	0.43	0.50	0.38	0.40	0.39	0.42
90 DAS	0.31	0.52	0.42	0.57	0.37	0.49	0.45	0.51	0.39	0.41	0.40	0.43
SEm±CD	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.07	0.14	0.06	0.14
@ 5%	0.04	0.3	0.04	0.3	0.03	0.03	0.03	0.03	0.21	0.42	0.36	0.40

Table 3: Biochemical variation in between mungbean and urdbean at different intervals.

	Biochemical parameters											
Intervals	Total soluble sugar (mg/g/DW)				Peroxidase (ΔA/min/g)				Polyphenol oxidase (ΔA/min/g)			
DAS	Mungbean		Urdbean		Mungbean		Urdbean		Mungbean		Urdbean	
	Healthy	Diseased	Healthy	Diseased	Healthy	Diseased	Healthy	Diseased	Healthy	Diseased	Healthy	Diseased
15 DAS	2.77	2.20	2.85	2.30	0.12	0.20	0.19	0.35	0.16	0.21	0.24	0.34
30 DAS	3.31	2.87	3.25	2.92	0.24	0.29	0.34	0.48	0.23	0.30	0.28	0.56
45 DAS	3.50	3.31	3.55	3.38	0.31	0.35	0.43	0.55	0.3	0.37	0.35	0.89
60 DAS	3.63	3.40	3.76	3.47	0.48	0.44	0.49	0.70	0.44	0.71	0.40	0.95
75 DAS	3.71	3.46	3.89	3.59	0.54	0.58	0.52	0.77	0.46	0.82	0.50	1.03
90 DAS	3.85	3.57	3.94	3.60	0.57	0.62	0.61	0.79	0.48	0.87	0.58	1.08
SEm±CD	0.06	0.08	0.06	0.08	0.08	0.06	0.08	0.06	0.09	0.12	0.05	0.14
@ 5%	0.18	0.25	0.18	0.25	0.22	0.20	0.22	0.20	0.27	0.36	0.16	0.40

who they reported that higher percentages of protein are produced as a result of virus multiplication, which involves the synthesis of virus-specific protein, which accumulates in infected leaves and eventually raises the percentage over healthy leaves (Table 2).

Total soluble sugar

The highest amount of total soluble sugar content was recorded in diseased urdbean leaves (3.60 mg/g/DW) as compared to diseased mungbean and recorded as 3.57 mg/g/DW, similarly, the total soluble sugar content in healthy urdbean and mungbean was recorded as 3.94 and 3.81 mg/g/DW respectively at 90 DAS. Present studies revealed that the totals soluble sugar content were maximum in healthy urdbean plants than the mungbean plants. The total soluble content was increased over a period of time and TSS showed a significant difference between healthy and diseased urdbean and mungbean plants. However, TSS content was decreased with increased disease severity over the period. Our studies were confirmed by Ramrao *et al.* (2020) they reported that all of the greengram genotypes evaluated had lower leaf total sugar content as they progressed from vegetative to reproductive stages (Table 3).

Peroxidase enzyme activity

The maximum peroxidase activity was recorded in MYMV infected urdbean leaves (0.79 ΔA/min/g) than the infected mungbean leaves (0.62 ΔA/min/g) and also healthy urdbean leaves (0.61ÄA/min/g) showed highest peroxidase content than compared to healthy mungbean leaves (0.57 ΔA/min/g). The results confirmed that the peroxidase activity of healthy and diseased leaves was maximum in urdbean leaves during older stage of the crop than the healthy and diseased mungbean older stage crop. present study results are in agree with studiesof Singh *et al.* (2003) reported that levels of peroxidase activity in muskmelon leaves infected with the downy mildew pathogen was showed found increased peroxidase activity in resistant leaves than in susceptible leaves (Table 3).

Polyphenol oxidase activity

The PPO in healthy mungbean and urdbean ranged from 0.16 to 0.48 and 0.24 to 0.58 respectively, similarly in diseased mungbean and urdbean leaves it ranged from 0.21 to 0.87 and 0.34 to 1.08 respectively from 15 to 90 DAS. The significantly highest amount of polyphenol oxidase activity of 1.08 and 0.87 ΔA/min/g was recorded in diseased urdbean and mungbean leaves respectively, in case of healthy urdbean and mungbean leaves showed polyphenol activity of 0.58 and 0.48 ΔA/min/g respectively at 90 DAS. The results were confirmed that polyphenol oxidase activity of healthy and diseased leaves was maximum in urdbean leaves at an older stage of the crop than the healthy and diseased mungbean older stage crop. Studies were agreed by Mantesh *et al.* (2020) reported that higher polyphenol oxidase content was observed on MYMV resistant genotypes than the susceptible genotypes (Table 3).

CONCLUSION

The present investigation results revealed that among the mungbean and urdbean crop, urdbean showed maximum resistant behavior to MYMV and *B. tabaci* than mungbean plants. All morphological characters *viz*., leaf thickness, trichome density and epicuticular wax content was maximum in urdbean plants than mungbean plants, similarly biochemical parameters *viz*., total phenol, tannin, protein, total soluble sugars and enzyme activity (Peroxidase activity and Polyphenol oxidase activity) was maximum in urdbean than the mungbean. All these morphological and biochemical parameters supports the resistant behavior of urdbean. Due to the presence of maximum leaf thickness and trichome density and epicuticular wax content avoid the probing activity of whiteflies is prevented and the secondary metabolites inhibits the multiplication of MYMV in the resistant host plants. The present study confirms that these factors are more congenial for the disease development in mungbean than compared with urdbean, hence it is proved that mungbean is most preferable host for the MYMV. The Insight of this experiment help to develop resistant varieties against MYMV by using the urdbean traits by conducting molecular experiment in future studies.

Conflict of interest

All authors declared that there is no conflict of interest.

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