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## Study on YMV resistance and biochemical factors responsible for resistance in Blackgram (*Vigna mungo* L. Hepper)

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### Abstract

Blackgram (*Vigna mungo* L. Hepper) is an important pulse crop widely cultivated in Asian countries like India which is the highest producer of blackgram in the world. It is rich in protein (23g/100 g) content and is more commonly cultivated in South India. The yield of black gram is mainly affected by Mung bean yellow mosaic disease (MYMV) accounting for up to 100% yield loss under severe infection. Hence screening for MYMV resistance and studying the factors responsible for MYMV resistance is essential for the selection of genotypes resistant to MYMV. Secondary metabolites produced by the plants like phenols and tannins are associated with biotic resistance. The present investigation was carried out to study the effect of biochemical factors like tannins and phenols on MYMV disease resistance in black gram. In this study, the RILs developed from the cross ADT3 X KKB 15 052, where, ADT3 is a high yielding but susceptible variety to MYMV and KKB 15 052 is resistant to MYMV were evaluated for MYMV resistance by infector row method and scoring was done by the modified scale of All India Coordinated Research Project on MuLLaRP (Alice and Nadarajan, 2007). Biochemical factors viz. phenol and tannin were estimated by following Folin ciocalteau technique and folin-Denis method respectively. The varieties Co 5 and VBN 8 were used as susceptible and resistant checks respectively. The results showed that the RILs 8-21NH, 7-13, 7-24H, and 8-32H were resistant to MYMV with a resistance score of 0 to 2 and showed high content of tannin and phenols over the susceptible RILs which showed disease rating between 5.1-9.0.

**Keywords:** Black gram, YMV, tannin, phenol and resistance

### Introduction

Black gram is one of the most widely cultivated and consumed pulse crop in India due to its high nutritional index. Black gram occupies over 23% of India's overall pulse production, as well as about 19% of its entire pulse acreage. India is the largest producer of Black gram in the world followed by Pakistan and Myanmar. The production of black gram in India during 2020-2021 was 24.19 lakh tonnes. The area under black gram cultivation in Tamil Nadu was 4.02 lakh ha with 2.25 lakh tonnes production and 560 kg/ha yield rate in 2020-21 making Tamil Nadu as the highest black gram producing state in India. (Season and crop report, 2020) <sup>[12]</sup>. Yellow mosaic disease (YMD) is the most common disease in Black gram which is detrimental to the crop. It also causes yield loss to other leguminous crops like Mung bean (*Vigna radiata*), Horse gram (*Macrotyloma uniflorum*), moth bean (*Vigna aconitifolia*), pigeon pea (*Cajanus cajan*), French bean (*Phaseolus vulgaris*), Dolichos (*Lablab purpureus*) etc. (Ramesh *et al.*, 2017; Dikshit *et al.*, 2020) <sup>[10, 4]</sup>. It is transmitted by whitefly (*Bemisia tabaci*). Yellow mosaic virus belongs to the family geminiviridae and genus Begomovirus. The symptom is characterized by the presence of small yellow patches on the leaf that start with a small speck and gradually increase in number and size depending on the severity of infection and resistance of the plant. It causes up to 85-100% yield loss under severe conditions in blackgram. Hence it is important to develop Blackgram varieties resistant to MYMV either by conventional or molecular breeding approaches.

Phenols and tannins are important phytochemical and secondary metabolites of plants which play an important role in plant protection function which involves resistance to pests and diseases. Selection of resistant genotypes based on mere phenotypic screening may not be effective as the resistance might be due to escape or avoidance. Hence, screening of genotypes based on biochemical factors associated with MYMV resistance helps in identification of genotypes with true resistance. Phenols are secondary metabolites produced by the plant which have an aromatic ring and one phenol component hydroxyl substitute, if more than one phenol

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component is present to the hydroxyl substitutes it is termed as polyphenol. Phenols show radical scavenging activity i.e they have the ability to convert free radicals like O<sub>3</sub> and H<sub>2</sub>O<sub>2</sub> that have the potential to cause DNA damage into bonded forms which do not cause any cellular damage in addition to plant protection against biotic stress. Hence efforts are made to study the total phenol content (TPC) in plants by many researchers. Tannins are poly phenolic metabolites that have the ability to bind with proteins and precipitate them, they are characterized by the presence of astringency and acidity besides the presence of several phenyl groups. Tannins play a key role in plant protection by protecting the plant from predation by pests and other animals like birds, which help in plant resistance but they are also anti nutritional in nature i.e presence of high amount of tannins in crop will cause digestive issues or poisoning. There are two types of tannins, condensed tannins and hydrolysable tannins. Hydrolysable tannins have the ability to hydrolyse with acids or enzymes whereas condensed tannins do not have the ability to hydrolyse even under the action of acids and enzymes due to the absence of glucose moiety. Condensed tannins provide dietary forage protein, However high condensed tannins i.e more than 55 g / kg of dry matter causes digestibility issues in grazing animals. Hydrolysable tannins (HT) when present in high concentrations can cause poisoning but they do not show any effect on protein digestibility (Piluzza *et al.*,2013) [8]. Hence estimating total tannin content helps not only in determining whether the crop has the potential resistance to a disease but also if the crop is fit for consumption. The present study was conducted with the objectives to determine the MYMV resistant genotypes with associated biochemical factors for MYMV resistance in the RIL population of ADT3 & KKB15052.

## Materials and methods

Scale	Description
0	No visible symptoms on leaves
1	Very minute yellow specks on leaves
2	Small yellow specks with restricted spread covering 0.1 – 5.0% leaf area of plant
3	Yellow mottling of leaves covering 5.1-10.0% leaf area of plant
4	Yellow mottling of leaves covering 10.1-15.0% leaf area of plant
5	Yellow mottling and discoloration of 15.1-30.0% leaf area of plant
6	Yellow discoloration of 30.1-50.0% leaf area of plant
7	Pronounced yellow mottling and discoloration of leaves and pods, reduction in leaf size and stunting of plants covering 50.1-75.0% foliage of plant
8	Severe yellow discoloration of leaves covering 75.1 – 90.0% foliage, stunting of plants and reduction of pod size
9	Severe discoloration of leaves covering above 90.1% of foliage of plants, stunting of plants and no pod formation

**Table 2:** Modified MUL Lar P scale

## Estimation of total phenols

In the present study total phenol was estimated by following folin ciocalteu method, the principle involved in this method is that Folin ciocalteu reagent is reduced by the phenol hydroxyl group and the blue color developed is read at 660 nm by using a colorimeter. Ethanol extraction was followed since phenols are highly soluble in alcohols.

## Estimation of tannins

In the present study Folin – Denis method was used for estimation of tannin which is based on the principle that

The present study was carried out at Agricultural College and Research Institute, Killikulam during kharif 2021.

## Plant materials

The experimental materials consisted of the RILs developed from the cross ADT3 X KKB 15 052 (ADT3 is a high yielding susceptible to MYMV for MYMV and KKB 15 052 is a resistant culture for MYMV) which were used for the screening of MYMV resistance. Co5 was used as susceptible check. One row of Co5 was raised for every three rows of RIL. The variety VBN8 was used as the resistant check.

## Screening for MYMV

Screening for MYMV resistance was performed by following infector row method in the field with three rows of the RIL and one row of susceptible check in natural epidemic conditions. Disease infestation was estimated according to the formula of Chandrajini Devi *et al.*,2016 [3].

$$\text{Percent of disease incidence (PI)} = \frac{\text{No of infected plants in the plot}}{\text{Total number of plants in the plot}} \times 100$$

The severity of disease was recorded by modified (0-9 scale) of All India co-ordinated Research Project on MULLARP (Alice and Nadarajan 2007) [1] and categorization was done based on (Gantait and Kantidas, 2009) [5]. (Table 2).

**Table 1:** The severity of disease was recorded by modified (0-9 scale) of All India

Disease severity percent	Rating	Reaction
0.1-5	1.0-2.0	Resistant (R)
5.1-15	2.1-4	Moderately Resistant (MR)
15.1-30	4.1-5	Moderately susceptible (MS)
30.1-75	5.1-7	Susceptible (S)
75.1-100	7.1-9	Highly susceptible (HS)

tannin reduces phospho tungsto molybdic acid in alkaline solution to produce a blue solution where the intensity is measured in 700nm.

## Results and discussion

In this study 23 RILs were screened for MYMV resistance by infector row method using Co5 as susceptible check and categorization was done (Gantait and Kantidas, 2009) [5]. The screening of MYMV resistance in blackgram genotypes is close to the findings of Bandi 2017. The results showed that the RILs 8-21NH, 7-13, 7-24H and 8-32H were resistant to

MYMV with resistance score between 0 to 2. The RILs 7-23, 7-25, 8-12, 8-19, 8-32 NH, 8- 35, 8-36 and 8-39 were found to be moderately resistant with disease rating score between to 4. The RILs 7-21, 7-24NH, 8-18 and 8-26 were identified as moderately susceptible to MYMV with disease rating score between 4.1 to 5.0. The RILs 8-21H, 8-30, 8-37, 8-38H and 8-38NH were found to be susceptible with disease rating score between 5.1 to 9.0. The data on total phenols, tannins and resistance to MYMV was presented in Table 3, from the data on phenol and tannin content it was found that the resistant RILs 7-13,8-32,8-21NH have either high content of phenol or tannin or both, 7-13 has high total phenol and tannin content with 208 mg/100g and 870mg/100g. However VBN8 has 190mg/100g and 845mg /100g of total phenol and tannin, respectively. The RILs with high phenol content i.e 8-21NH, 7-13, 7-24H and 8-32H showed resistance over the RILs with low phenol which were either moderately susceptible or susceptible to MYMV and the results are in accordance to the studies of Ranjitha *et al.*, (2018) in blackgram and Prabu and Warade (2009)<sup>[9]</sup> in wild okra inter specific hybrids.

Moderately resistant RILs like 8-12, 7-25 and 8-32 NH showed higher phenol content over resistant RILs and moderately susceptible RIL 8-36 with 176 mg/100 g of total phenol content (TPC) showed similar amount of phenol to that of a resistant RIL 7-24H and these results are in accordance to the findings of Suhail *et al.*, (2020)<sup>[14]</sup> in mung bean. It was also evident that apart from phenols and tannins other genetic and morphological factors play an important

role in resistance since the RIL 8-21NH (187.90 mg/100 g & 808mg/100g of phenol and tannin respectively) had comparatively lower phenol and tannin content over the resistant check VBN8 but it was observed to have 0 scale rating with higher degree of resistance. The susceptible RILs, 8-37, 8-38H, 8-38 NH,8-21 H and 8-15 had comparatively lower phenol or tannin content or both. Hence, it was evident that closely related RILs like 8-38H and 8- 38NH had 127mg/100g &719mg/100 g:129 mg/100 g & 7-32 mg/100 g of total phenol and tannin respectively. Similarly, 8-32H and 8-32 NH had 211.32 mg/100 g and 798.09 mg/100 g; 201 mg/100 g and 730 mg/100g of phenol and tannin content respectively. It was also observed that hairiness which is a morphological trait which imparts resistance to MYMV was not associated with phenol and tannin content as the susceptible RIL 8-21 H had low phenol content while 8-21 NH being highly resistant and non-hairy had high phenol and tannin content, but the vector whiteflies showed more preference to non-hairy genotypes over hairy genotypes which is in conformity with the research of Taggar *et al.*, (2012)<sup>[15]</sup>. Hence it was found that the correlation between morphological and biochemical factors for MYMV disease resistance is negligible. Even though high phenol and tannin content is preferred for resistance, in terms of nutritional values low phenol and tannin content is preferred. (Suneja *et al.*, (2011)<sup>[13]</sup>. The tannin content in the RIL population studied here was found to be similar to the analysis performed by (Kanth *et al.*,2021)<sup>[6]</sup>.

**Table 3:** Total phenol and tannin content and MYMV disease resistance scale of RILs

Genotype	Total phenol content (mg/100g)	Total tannin content (mg /100g)	Category of disease severity	Scale
7-13	208.7	870.18	Resistant	1
7-21	121.58	781.80	Moderately susceptible	5
7-23	111.89	823.69	Moderately susceptible	3
7-24 H	178	781.38	Resistant	2
7-24 NH	176.49	779.09	Moderately susceptible	5
7-25	188.01	784.09	Moderately resistant	4
8-12	190.76	777.34	Moderately resistant	3
8-15	134.65	706.09	Susceptible	6
8-18	167.98	734.59	Moderately susceptible	5
8-19	179.28	777.97	Moderately resistant	4
8-21 H	165.98	802.6	Susceptible	7
8-21 NH	187.90	808.12	Resistant	0
8-26	155.76	765.93	Moderately susceptible	5
8-30	164.33	767.98	Moderately Susceptible	6
8-32 H	211.32	798.09	Resistant	2
8-32 NH	201.98	790.33	Moderately resistant	3
8-35	202.22	787.43	Moderately resistant	3
8-36	176.45	735.82	Moderately susceptible	4
8-37	133.98	729.68	Susceptible	8
8-38 H	127.68	719.97	Susceptible	7
8-38 NH	129.79	732.89	Susceptible	7
8-39	198.76	769.08	Moderately resistant	3
8-41	191.32	810.97	Moderately resistant	3
Co 5	143.79	727.39	Susceptible	8
VBN 8	190.01	845.42	Resistant	1

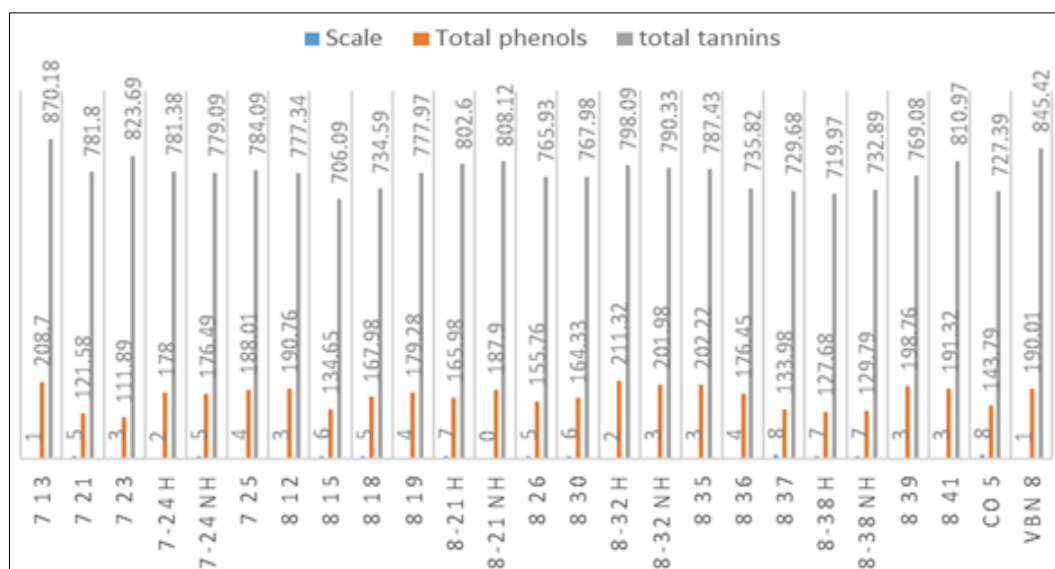


Fig 1: Association between biochemical factors and MYMV resistance

## Conclusion

As MYMV is the most devastating disease in Blackgram screening of genotypes in the field and molecular methods is essential so as to develop new Blackgram varieties using resistant sources. It is also essential to identify the factors responsible for disease resistance to carry out further breeding programs. MYMV resistance is mainly contributed by the genetic make-up of the particular RIL along with biochemical, morphological and environmental factors. Molecular breeding is highly recommended over conventional breeding due to its high specificity in marker identification and less time consumption. From the present study it is evident that the RILs 8-21 NH, 7-23 and 8-32 H are highly resistant and have high amount of plant protecting biochemical factors. Hence it is suggested to carry out further molecular analysis in the RILs for improvement and new varietal development programs. The susceptible RILs 8-37, 8-38H, 8-38 NH, 8-21 H and 8-15 can be backcrossed or subjected to mutations for imparting resistance to MYMV.

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