Correlation Analysis for Leaf Pubescence, Leaf Micro-macro Nutrient Content and MYMV Disease Index in Blackgram genotypes (Vigna mungo (L.) Hepper)

B. Rajitha, V. Rajarajeswari and P. Sudhakar

Department of Crop Physiology, S. V. Agricultural College, Tirupati-517502, A.P, India.

Authors’ contributions
This work was carried out in collaboration among all authors. Author BR designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors VR and PS managed the analyses of the study. Author PS managed the literature searches. All authors read and approved the final manuscript.

ABSTRACT
The present field study was conducted to characterize the blackgram genotypes based on morpho and nutrient content under yellow mosaic virus disease infection. The present investigation was carried out with ten blackgram genotypes (5 known tolerant + 5 known susceptible) in Randomized Block Design (RBD) during summer 2018. Experimental crop was affected by yellow mosaic virus (YMV) naturally at 30 DAS. No management practices were taken for controlling of whitefly population during crop season. Correlation studies were done at 50 DAS which helps to find out the most resistant and most susceptible genotypes among ten backgram genotypes based on leaf pubescence and nutrient content in leaf. Leaf pubescence is one of the most important resistant factors in a number of crops and Minerals, apart from being a vital part of the plant nutrition, may manifest certain maladies in the plants either through disturbing normal metabolism and physiology of the plants or by favouring or by discouraging the plant pathogens, if in excess or otherwise deficient.

Original Research Article

Received 01 March 2021
Accepted 06 May 2021
Published 10 May 2021

*Corresponding author: E-mail: rajagr26@gmail.com, madhusudan454@gmail.com;
Correlation analysis of present field investigation revealed that blackgram genotypes showed strong negative correlation for leaf pubescence, leaf Phosphorous (P) and leaf potassium (K) content with percentage disease index (PDI%) as well as strong positive correlation for leaf Nitrogen (N), leaf Iron (Fe) and leaf Zinc (Zn) content under YMV.

**Keywords:** Blackgram; YMV; leaf pubescence; micro and macro nutrients; correlation.

**1. INTRODUCTION**

Blackgram [Vigna mungo (L.) Hepper] is an important grain legumes short duration crop and widely cultivated in India. In the developed countries, grain legumes are an important indirect source of protein. It gives us an excellent source of easily digestible good quality protein and ability to restore the fertility of soil through symbiotic nitrogen fixation. The seeds are highly nutritious with protein (24-26%), carbohydrates (60%), fat (1.5%), minerals, amino acids and vitamins. The biological value improves greatly, when wheat or rice is combined with blackgram because of the complementary relationship of the essential amino acids such as arginine, leucine, lysine, isoleucine, valine and phenylalanine etc [1]. In India, the area comes under blackgram is about 4.50 million hectares with the production of 3.23 million tonnes [2]. Yellow Mosaic Disease (YMD) is as significant biotic stress causing profound yield loss in blackgram. Yellow Mosaic Virus (YMV) belongs to the genus Begomo virus and transmitted by the vector whitefly, Bemisia tabaci. Yield loss due to this disease varies from 5 to 100 per cent depending upon disease severity, susceptibility of cultivars and population of whitefly [3]. Research on epidemiological aspects indicates that MYMV disease incidence depends upon the host genotypes, growing seasons and prevailing environmental conditions. Certain resistant genotypes are now available to the breeders and farmers [4,5] but no information is available on the mechanism of disease resistance in these germplasms. Being dependent on metabolic system, plant viruses cause disturbances in the physiology and anatomy of infected plants [6]. No generalization appears justified concerning the metabolic effects following infection with plant viruses. Most of the metabolic changes observed are probably indirect effects of viral infection as a result of interference with various physiological processes, besides the transport of water, nutrients, and other substances.

Minerals, apart from being a vital part of the plant nutrition, may manifest certain maladies in the plants either through disturbing normal metabolism and physiology of the plants or by favouring or by discouraging the plant pathogens, if in excess or otherwise deficient. Disturbance in growth regulation results in morphological abnormalities, ranging from a mosaic pattern on leaves and flowers to necrotic spots and streaks to leaf enation and tumours [7]. However, correlation studies for micro and macro nutrients and leaf pubescence helps to screening the blackgram genotypes for YMV tolerance and moreover needed for further evaluation of nutrients efficacy in improving YMV tolerance through foliar nutrition.

**2. MATERIALS AND METHODS**

The present field experiment was conducted at S.V. Agricultural college farm, Tirupati campus of Acharya N.G. Ranga Agricultural University, during summer 2018 which is graphically situated at 13.5°N latitude and 79.5°E longitude, with an altitude of 182.9 m above the mean sea level in the southern Agro-Climatic Zone of Andhra Pradesh. YMV infection was high and uniform during Summer compared to Rabi [8]. Hence the present evaluation was taken up during summer 2018 only where disease pressure is high and reliable data can be generated regarding nutrient interventions in YMV tolerance.

The experiment was laid out in a Randomized Block Design (RBD) with ten genotypes and replicated thrice as per the details given below. Treatments consist of totally ten blackgram genotypes [6] known tolerant (LBG 752, PU 31, GBG 1, LBG 645, LBG 623, LBG 685, PBG 32 and PBG 1) obtained from Regional Agricultural Research Station (Lam) and Regional Agricultural Research Station (Tirupati). Need based plant protection measures were taken. Experimental crop was affected by yellow mosaic virus (YMV) naturally at 30 DAS. No management practices were taken for controlling of whitefly population during crop season. Sampling was done at 50 DAS.

**2.1 Scoring of YMV Infection %**

It was calculated by counting number of plants infected and total number of plants in a plot.
Per cent disease incidence = \[
\frac{\text{Number of plants infected in a row}}{\text{Total number of plants in a row}} \times 100
\]

The readings were recorded at 50 DAS and the data were analysed statistically.

2.2 Leaf Pubescence (No. of trichomes per sq. cm) (Zeiss, model: Zeiss Stemi 2000)

Leaf pubescence was measured based on trichome density by using stereomicroscope (40X). The leaflet was cut into bits of 0.25 cm² and number of trichomes present on the upper and lower surface was counted under stereo zoom trinocular microscope and expressed as number of trichomes per square centimetre leaf area.

2.3 Nutrient Content in Leaf

2.3.1 Nitrogen (mg 100 mg⁻¹)

Nitrogen content was determined by using the Microkjeldhal distillation apparatus (AOAC, 1970) and expressed as mg 100 mg⁻¹.

2.3.2 Phosphorus (mg 100 mg⁻¹)

Vanadomolybdo phosphoric yellow colour method [9] was used for estimating Phosphorus content in leaf samples. The intensity of the yellow colour was measured at a wave length of 420 nm in spectrophotometer and expressed as mg 100 mg⁻¹.

2.3.3 Potassium (mg 100 mg⁻¹)

Potassium content was determined by using the flame photometer [9] from the diacid extract and expressed as mg 100 mg⁻¹.

2.3.4 Micronutrients (Fe and Zn) (ppm)

Micronutrients viz., Fe and Zn were estimated in diacid extract using an Atomic Absorption Spectrophotometer [10] and expressed as ppm.

2.4 Calculation

The uptake of Nitrogen, Phosphorus, Potassium, Iron and Zinc in plant was calculated as follows.

Uptake of nutrients = Nutrient Concentration × Wt. of drymatter

100

2.5 Statistical Analysis

The experimental data were analyzed statistically by following standard procedure outlined by Panse and Sukhatme [11]. Significance was tested by comparing 'F' value at 5 per cent level of probability. Correlation studies were undertaken for biochemical parameters according to the method proposed by Fisher and Yates [12].

3. RESULTS

Correlation analysis of leaf pubescence and micro- macro nutrient content against percentage disease index (PDI%) for screening of blackgram susceptible and tolerant genotypes under yellow mosaic virus condition (YMV) at 50 DAS was investigated in present field study.

3.1 Scoring of YMV Infection %

Significant differences were observed in both susceptible and tolerant genotypes during the crop growth. Susceptible genotypes showed higher percent of YMV infection of 12.63 per cent compared to tolerant genotypes 0.77 per cent at 50 DAS during summer 2018. Among all the genotypes, LBG-623 resulted higher YMV infection of 16.56 per cent followed by PBG-32 and PBG-1. Similarly Devi (2016) also found higher disease severity (PDI) in genotype LBG-623. Among the tolerant genotypes higher PDI was recorded in LBG-792 as well as lower in TBG-104 whereas in susceptible genotypes LBG-623 recorded higher PDI and lower PDI was recorded in LBG-685.

3.2 Leaf Pubescence (No. of trichomes per sq. cm)

Trichomes play a major role by providing a first line defence in reducing whitefly infestation, with a subsequent beneficial effect of reducing the incidence of virus. The variation in trichome density of YMV susceptible and tolerant blackgram genotypes has been depicted in Plate 1. Among the tolerant and susceptible group of genotypes, tolerant genotypes recorded higher mean leaf pubescence of 23.53 per one sq cm compared to susceptible mean value of 16.20 per one sq cm.
Leaf pubescence showed strong negative correlation with percentage disease index ($R^2 = 0.690$) as depicted in Fig. 1, which denotes lower trichome density is favourable to whitefly infestation.

**Table 1. Mean data of PDI%, leaf pubescence, leaf N, P, K, Fe and Zn content for YMV tolerant and susceptible blackgram genotypes during summer 2018**

| S. No | Genotypes | PDI% | leaf pubescence (No. of trichomes per sq.cm) | Nutrient content (50 DAS) (mg 100 mg$^{-1}$) | ppm |
|-------|------------|------|-----------------------------------------------|-----------------------------------------------|-----|
|       |            |      |                                               | Tolerant                                      |     |
| 1     | LBG-752    | 0.88 | 25.00                                         | 2.55                                         | 0.60 | 1.00 | 620.67 | 53.14 |
| 2     | PU-31      | 0.67 | 23.00                                         | 2.58                                         | 0.54 | 0.98 | 545.80 | 63.21 |
| 3     | GBG-1      | 1.33 | 20.67                                         | 2.87                                         | 0.51 | 0.90 | 809.67 | 87.33 |
| 4     | LBG-792    | 0.86 | 20.00                                         | 3.08                                         | 0.39 | 0.93 | 847.80 | 52.87 |
| 5     | TBG-104    | 0.10 | 29.00                                         | 2.65                                         | 0.62 | 1.17 | 562.80 | 49.33 |
| Mean  |            | 0.77 | 23.53                                         | 2.75                                         | 0.53 | 1.00 | 677.30 | 61.20 |
|       |            |      |                                               | Susceptible                                   |     |
| 6     | LBG-645    | 10.92| 18.00                                         | 3.69                                         | 0.40 | 0.89 | 747.13 | 66.35 |
| 7     | LBG-623    | 16.56| 15.33                                         | 4.26                                         | 0.20 | 0.57 | 848.80 | 74.87 |
| 8     | LBG-685    | 7.97 | 16.00                                         | 3.79                                         | 0.31 | 0.72 | 750.47 | 71.33 |
| 9     | PBG-32     | 14.04| 16.33                                         | 3.98                                         | 0.23 | 0.68 | 894.47 | 79.33 |
| 10    | PBG-1      | 13.67| 15.33                                         | 4.18                                         | 0.24 | 0.53 | 980.80 | 78.79 |
| Mean  |            | 12.63| 16.20                                         | 3.89                                         | 0.27 | 0.69 | 844.30 | 74.10 |
| Grand Mean | 6.70 | 19.87|                                               | 3.36                                         | 0.40 | 0.84 | 760.84 | 67.66 |
| SE m ±|            | 0.385| 1.1205                                         | 0.0065                                       | 0.01 | 0.0132 | 2.8872 | 0.5204 |
| CD (P=0.05) | 1.145 | 3.3293|                                               | 0.0193                                       | 0.0297 | 0.0392 | 8.5786 | 1.5462 |

**Fig. 1. Correlation between leaf pubescence and PDI % in blackgram genotypes during summer 2018 at 50 DAS**

$$y = -1.1918x + 30.375$$

$R^2 = 0.6902$

**Fig. 2. Correlation between Nitrogen content (mg 100 mg$^{-1}$) and PDI % in blackgram genotypes during summer 2018 at 50 DAS**

$$y = 9.2873x - 24.533$$

$R^2 = 0.9256$
3.3 Nutrient Content in Leaf

3.3.1 Nitrogen (mg 100 mg⁻¹)

Higher nitrogen content was observed in susceptible genotypes of 2.75 mg 100 mg⁻¹ compared to tolerant genotypes of 2.77 mg 100 mg⁻¹.

Nitrogen content of blackgram genotypes showed strong positive correlation with percentage disease index ($R^2 = +0.925$) as depicted in Fig. 2.

3.3.2 Phosphorus (mg 100 mg⁻¹)

At 50 DAS higher phosphorous content was observed in tolerant genotypes of 0.53 mg 100 mg⁻¹ compared to susceptible genotypes of 0.27 mg 100 mg⁻¹.
Plate 1. Trichome density of YMV tolerant and susceptible blackgram genotypes at 50 DAS during summer 2018

The correlation results revealed that strong negative correlation ($R^2=-0.808$) as depicted in Fig. 3 existed between phosphorous content and disease severity.

3.3.3 Potassium (mg 100 mg\(^{-1}\))

Potassium content was decreased from 30 DAS to 50 DAS irrespective of genotypes. But reduction percent was high in susceptible genotypes compared to tolerant genotypes so due to this disease severity was more pronounced in susceptible genotype can be explained by the factor that it is a mobile nutrient and is transported through the phloem from older to younger leaves.

Higher mean potassium content was observed in tolerant genotypes of 1.00 mg 100 mg\(^{-1}\) compared to susceptible genotypes of 0.69 mg 100 mg\(^{-1}\).

Potassium content of blackgram genotypes showed strong negative correlation with percentage disease index ($R^2=-0.785$) as depicted in Fig. 4.

3.3.4 Micronutrients (Fe and Zn) (ppm)

The mean iron content was reduced by 42.96 per cent in tolerant genotypes compared to susceptible genotypes. The correlation results revealed that strong positive correlation ($R^2=+0.604$) as depicted in Fig. 5 existed between iron content and disease severity.

The mean zinc content was reduced by 47.50 per cent in tolerant genotypes compared to susceptible genotypes. Zinc content of blackgram genotypes showed positive correlation with percentage disease index ($R^2=+0.589$) as depicted in Fig. 6.

4. DISCUSSION

Lower trichome density is favourable to whitefly infestation. Lower density attains more vectors, thus higher YMV infection due to whitefly adults
prefer to oviposit near trichomes is because of the selection pressure exerted by the natural enemies or the improved microhabitat on the blackgram leaves. Similar results were reported by Subedi et al. [13] in blackgram. A general increase of the total N in virus infected plants have been reported for a number of host virus combination by Selman and Grant, [14]. The main component of total N was probable insoluble protein, free amino acids, nucleic acids and amides. Alteration in any one of the components may disturb the nitrogen metabolism. Virus protein contributes significantly to the total protein of the plant and as a consequence there is an increase in total N.

The higher percentage seems due to virus multiplication which entails the synthesis of virus specific abnormal protein that accumulates an ultimately raises the percentage over healthy. Increase in protein contents observed in infected blackgram plant leaves may also be correlated with respiration. Increased nitrogen uptake by diseased plants associated with rapid respiration probably helps in the synthesis of more amino acids [15]. Strong negative correlation of potassium might be due to elements of phloem in virus infected plants get blocked due to cell multiplication in parenchymatous tissues. Since the transportation of potassium takes place via phloem from aged to new young leaves, therefore, decrease of potassium concentration in leaves of virus- infected conditions [16]. The positive correlation of iron against PDI% which is similarly reported by Ashfaq et al., 2010 who opined iron as a component of various flavoproteins (Metalloflavoproteins) active in biological oxidation, which may increase as result of inoculation with the virus.

5. CONCLUSION

From the present findings based on the results and correlation analysis of blackgram genotypes showed strong negative correlation for leaf pubescence, leaf Phosphorous (P) and leaf potassium (K) content with percentage disease index (PDI%) as well as strong positive correlation for leaf Nitrogen (N), leaf Iron (Fe) and leaf Zinc (Zn) content under YMV. The experiment has to be conducted on long term basis to find the standard reasons for fluctuations in micro and macro nutrients as well as to recommend the present findings.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Vadicel K, Manivannan N, Mahalingam A, Satya V, Vanniarajan C, Saminathan VR. Correlation analysis for yield, yield components and MYMV disease scores in blackgram (Vigna mungo (L.) Hepper). Electronic Journal of Plant Breeding. 2019;10(2):712-716.
2. Annonymous. Project Coordinator’s Reports. (Mungbean and Urdbean) 2017-18. All India; 2018.
3. Nene YL. A survey of the viral diseases of pulse cropsin Uttar Pradesh, G.B. Pant University of Agriculture and Technology, Pantnagar, Res. Bull. 1972:4:191.
4. Bashir M, Ahmad Z, Ghafoor A. Sources of genetic resistance in mungbean and blackgram against Urdbean leaf crinkle virus (ULCV). Pakistan Journal of Botany. 2005;37:47–51.
5. Ashfaq M, Khan MA, Mughal SM, Javed N, Mukhtar T, Bashir M. Evaluation of Urdbeangermplasm for resistance against urdbean leaf crinkle virus. Pakistan Journal of Botany. 2007;39:2103–2111.
6. Ashfaq M, Aslam KM, Javed N, Mughal SM, Shahid M, Sahi ST. Effect of urdbean leaf crinkle disease infection on total soluble protein and antioxidant enzymes in blackgram plants. Pakistan Journal of Botany. 2010;42:447–454.
7. Luria SE, Darnell JR, Baltimore D, Campbell A. General Virology. John Wiley and Sons, New York, USA; 1978.
8. Rajitha B. Characterization of blackgram genotypes for tolerance to YMV and nutritional intervention o enhance YMV tolerance. Ph.D. Thesis; 2018.
9. Jackson MC. Soil chemical analysis. Prentice Hall of India Private Limited, New Delhi; 1973.
10. Vogel Al. A text book of quantitative inorganic analysis. Richard clay (The Chancer Press) Limited, Britain; 1978.
11. Panse VG, Sukhatme PV. Statistical methods for agricultural workers. Edition-2 pp. 205. Indian Council of Agricultural Research, New Delhi; 1985.
12. Fisher and Yates. Statistical tables for biology, agriculture and medical research. Oliver and Boyd, Edinburgh; 1963.

53
13. Subedi S, Neupane S, Ghimire TN. Screening of mungbean and black gram genotypes as sources of genetic resistance against Mungbean Yellow Mosaic Disease. Nepalese Journal of Agricultural Sciences. 2016;148-155.

14. Selman IW, Grant SA. Some effects of nitrogen supply on the infection of tomato plants with tomato spotted wilt virus. Ann. Appl. Biol. 2008;45:448-455.

15. Szczepanski M, Redolfi P. Changes in the proteins of bean leaves infected with tobacco necrosis or Alfalfa mosaic viruses. J. Phytopathol. 2008;113:57-65.

16. Ashraf M, Zafar ZU, McNeilly T, Veltakamp CJ. Some morphoanatomical characteristics of cotton (Gossypium hirsutum L.) in relation to resistance to cotton leaf curl virus (CLCuV). Journal of Applied Botany. 1999;73:76–82.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sdiarticle4.com/review-history/68233