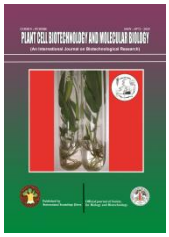

Plant Cell Biotechnology and Molecular Biology

Volume 25, Issue 9-10, Page 1-13, 2024; Article no.PCBMB.12164
ISSN: 0972-2025

Research on the Biochemical Alterations in Mungbean [*Vigna radiata* (L.) Wilczek] Caused by the Mungbean Yellow Mosaic India Virus (MYMIV)

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: https://doi.org/10.56557/pcbmb/2024/v25i9-108746

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://prh.ikprress.org/review-history/12164

Original Research Article

Received: 10/04/2024
Accepted: 13/06/2024
Published: 26/06/2024

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Cite as: *Kalaria, Rishee K., Jigar Bhabhor, Nilima Karmakar, and Samarth R. Patel. 2024. "Research on the Biochemical Alterations in Mungbean [Vigna Radiata (L.) Wilczek] Caused by the Mungbean Yellow Mosaic India Virus (MYMIV)". PLANT CELL BIOTECHNOLOGY AND MOLECULAR BIOLOGY 25 (9-10):1-13. https://doi.org/10.56557/pcbmb/2024/v25i9-108746.*

ABSTRACT

In the current study, the biochemical changes in mungbean caused by the yellow India virus were investigated. At 30 and 60 days after sowing (DAS), the biochemical contents were examined by the researchers. These included total phenol, total sugar, total protein, total chlorophyll, and enzyme activity of phenyl nine ammonia lyase (PAL), polyphenol oxidase (PPO), and peroxidase (PO). It was found that the phenol concentration of resistant genotypes was higher than that of extremely sensitive genotypes. A similar pattern was seen in the concentration of chlorophyll, total phenol, and the activity of the enzymes peroxidase, polyphenol oxidase, and phenyl nine ammonia lyase. Among resistant genotypes, NMS-21-95 exhibited the highest total chlorophyll content (0.27 mg/g) and phenol content (0.55 mg/g). Nonetheless, it was shown that the resistant genotypes NMS-21-01 had the highest total sugar content (2.93 mg/g). Likewise, NMS-21-22 had the greatest protein content (0.59 mg/g) at 30 DAS. The resistant genotype NMS-21-06 displayed a notably higher PO activity of 0.410 A/min/g in terms of enzyme activity. Meanwhile, PPO activity was found to be much higher (0.245 A/min/g) in resistant genotypes 40C. Moreover, the PAL enzyme-resistant genotype NMS-21-01's activity at 30 DAS was measured in 145.5 moles of trans-cinnamic acid min-1 g1. These extensive metabolic changes imply that defense mechanisms against host-pathogen interactions are strengthening essential components for plant survival under biotic stress. Thus, the study revealed that in the mungbean and MYMV interaction, the morphological and biochemical activities which are supportive to establish defence mechanism.

Keywords: Mungbean yellow mosaic India virus (MYMIV); Vigna radiata; PAL; PPO; PO.

1. INTRODUCTION

“Mungbean or green gram, scientifically known as [*Vigna radiata* (L.) Wilczek], is one of the most important short-duration pulse crops of India and an excellent source of good quality protein. It is also known as golden gram, mung or moong” [1]. “It is also important food source for human because of presence of essential amino acids andcontains prominent folate and iron” [2,3]. “It has a 2n=2x=22 chromosome number and its genome size are predicted to be 579 Mb” [4]. “Mungbean yellow mosaic virus (MYMV), Mungbean yellow mosaic India virus (MYMIV), Horsegram yellow mosaic virus (HYMV) and Dolichos yellow mosaic virus (DYMV) are the four most common types of YMD” [5,6]. “Because of Yellow mosaic disease (YMD) is the major constraint that causes great yield losses up to the extent of 85% in mungbean production” [7]. In India, about majorly growing region has been passes through the moderately to severely infections of YMV in the field of mung bean [8]. Therefore, the need of evaluation strategies of resistant genotypes for selections in crop improvement programme [9]. The assessment of the viruses affected many genotypes can be screened based on the biochemical analysis of particular factors. This can help to understand the metabolic reaction of genotype during the infection process of viruses. Thus, this could be a potential approaches to understand the resistance and segregating the plants that able to adapt the virus-like biotic stress and modify their cellular mechanisms to survive throughout the lifecycle [10,11].This was also described by the molecular level and gene expression mechanisms by different scientists followed by enzyme expressions and related RNA expressions [12,13]. Followed by this, various breeding strategies were developed for the breeding improvement in mung bean for such type of threats [14,15]. Yellow mosaic disease (YMD) is the most dangerous viral disease produced by the yellow mosaic virus [16]. The disease is mostly shown in India, Sri Lanka, Pakistan, and Bangladesh [17]. Yellow mosaic disease (YMD) is caused by a variety of whitefly-transmitted *Geminiviruses* from the genus *Begomovirus* and the family *Geminiviridae* [18]. MYMV-caused YMD outbreaks have been linked to the virus's capacity to undergo genetic recombination and transmission *via* the polyphagous pest whitefly *(Bemisia tabaci),* which serves as an efficient vector. In India, MYMV and MYMIV are the main pathogens that cause YMD in the mungbean. MYMIV causes yellow mosaic disease in Northern and Central India, while MYMV causes it in South and West India [19,20-22]. YMD infection can be managed *via* chemical, cultural and genetic methods. However, if host resistance alone is adequate to control the disease, it should be favoured above other approaches due to its cost-effectiveness and environmental friendliness [23]. Therefore, the present study was conducted to integrate the

biochemical basis of resistance to MYMIV infection in mungbean genotypes.

2. MATERIALS AND METHODS

For the estimation of biochemical parameters, a total of 12 genotypes were selected, 6 from resistant reactions and 6 from highly susceptible reactions screened during *Summer* 2021-22 at pulse farm, Navsari Agricultural University, Navsari. The evaluation of mungbean against YMD under hot-spot conditions are carried out using infector-row technique in certain standard statistical experimental design.

The biochemical constituents such as protein, total sugar content, total phenol and chlorophyll content and enzyme activity of PO, PPO and PAL were estimated. The Protein content was estimated by Lowry [24], total phenol by Sadasivam and Manickam [25], total sugar by Hedge and Hofreiter [26], chlorophyll content by Dubois [27], and peroxidase enzyme activity was estimated by the spectrophotometer method as described by Hartee [28], polyphenol oxidase and phenylalanine ammonia-lyase enzyme activity by Mayer [29].

3. RESULTS AND DISCUSSION

3.1 Variation of Biochemical Components in Mungbean Genotypes due to MYMIV Infection

3.1.1 The total phenol content in MYMIV-infected mungbean genotypes

In resistant genotypes, the phenol content ranged from 0.48 (NMS-21-22) to 0.55 (NMS-21-95) mg/g at 30 DAS. The least amount of phenol was found in highly susceptible genotypes, with values ranging from 0.10 (NMS-21-24) to 0.15 (NMS-21-68) mg/g. At 60 DAS, resistant genotypes also exhibited a similar pattern, with phenol contents ranging from 0.70 mg/g (NMS-21-22) to 0.77 mg/g (NMS-21-95). However, it varied between 0.19 (NMS-21-24) and 0.26 (NMS-21-68) mg/g in highly susceptible genotypes. In comparison to the highly susceptible genotypes, resistant genotypes were found to have higher phenol content (Table 1, Fig. 1, Fig. 2). “Resistant plants synthesis more of phenolic when they interact with the virus infection. Phenolic content enhances the metabolic activity of the host cell walls by the secretion of lignin and suberin which are the major components in the development of physical barriers that restrict the virus from spreading” [30]. This is assigned that the phenolic compounds have disease and pest-resistant properties due to the influenced biosynthesis of phenols in the host-pathogen interactions mechanism [31]. Total amount of phenol in infected MYMV In resistant genotypes, the phenol concentration ranged from 0.51 (PAU-911) to 0.72 (KKM-4) mg/g at 30 DAS. On the other hand, genotypes that were extremely sensitive showed the lowest phenol concentration, ranging from 0.11 (VC6372) to 0.12 (ML-1299) mg/g. Similar trends were also seen in resistant genotypes at 60 DAS, with phenol content being reported between 0.70 (MGG-1) and 0.92 (KKM-4) mg/g. In contrast, the range of extremely susceptible genotypes was found to be 0.20 (VC 6372) to 0.28 (ML-1299) mg/g, with susceptible BGS-9 having the lowest phenol concentration (0.18 mg/g) [32].

3.1.2 The total sugar content in MYMV-infected mungbean genotypes

There was a significant difference between the mungbean genotypes in the total sugar content brought on by virus infection at 30 DAS. Total sugar content in resistant genotypes ranged from 2.78 (NMS-21-22) to 2.93 (NMS-21-01) mg/g. However, the lowest sugar content was found in genotypes with high susceptibility, ranged from 1.90 (NMS-21-68) to 1.98 (NMS-21-23) mg/g. At 60 DAS, a similar pattern was also seen. The resistant genotypes had a higher sugar content, ranging from 2.37 (NMS-21-22) to 2.45 (NMS-21-01) mg/g. However, it varied between 1.54 (NMS-21-68) and 1.65 (NMS-21-23) mg/g in highly susceptible genotypes (Table 1, Fig. 1, Fig. 2). “The resistant genotypes recorded more sugar content than susceptible genotypes due to modification in the rate of synthesis and translocation of carbohydrates depends on the interactions of the virus with the plants and it cause hindrances in translocation of sugars in the plant system. The lower sugar content of infected plants was explained possible reduction or faster breakdown of carbohydrates due to accelerated respiration and escalated conversion of carbohydrates into amino acids” [30]. “Among the mungbean genotypes, there was a considerable variance in the total sugar content caused by virus infection at 30 DAS. The range of total sugar concentration in resistant genotypes was 2.78 (PAU-911) to 2.93 (KKM-4) mg/g. The least amount of sugar content, however, ranged from 1.95 (VC 6372) to 1.98 (ML-1299) mg/g in highly susceptible genotypes. At 60 DAS, a similar trend was also noted” [32].

Table 1. Variation of biochemical components of mungbean due to MYMV infection

Genotypes	Reactions	30 DAS				60 DAS			
		Phenol content	Sugar content	Chlorophyll content	Protein content	Phenol content	Sugar content	Chlorophyll content	Protein content
40 C	R	0.52	2.80	0.33	0.51	0.74	2.38	0.25	0.62
NMS-21-01	R	0.50	2.93	0.29	0.58	0.71	2.45	0.21	0.69
NMS-21-06	R	0.51	2.85	0.36	0.55	0.73	2.40	0.26	0.67
NMS-21-22	R	0.48	2.78	0.30	0.59	0.70	2.37	0.22	0.70
NMS-21-49	R	0.53	2.90	0.32	0.53	0.75	2.42	0.24	0.64
NMS-21-95	R	0.55	2.88	0.38	0.57	0.77	2.41	0.27	0.68
GM 4	HS	0.13	1.92	0.10	0.18	0.23	1.56	1.57	0.35
NMS-21-23	HS	0.14	1.98	0.13	0.22	0.25	1.65	1.61	0.41
NMS-21-24	HS	0.10	1.93	0.11	0.25	0.19	1.59	1.55	0.43
NMS-21-40	HS	0.12	1.95	0.15	0.20	0.21	1.62	1.60	0.37
NMS-21-68	HS	0.15	1.90	0.12	0.24	0.26	1.54	1.65	0.40
NMS-21-69	HS	0.11	1.96	0.14	0.27	0.20	1.61	1.64	0.44
SD		0.20	0.48	0.11	0.17	0.26	0.42	0.71	0.14
S.Em ±		0.05	0.13	0.03	0.05	0.07	0.12	0.20	0.04
CV%		5.68	10.14	4.68	3.97	4.23	7.56	4.10	3.68

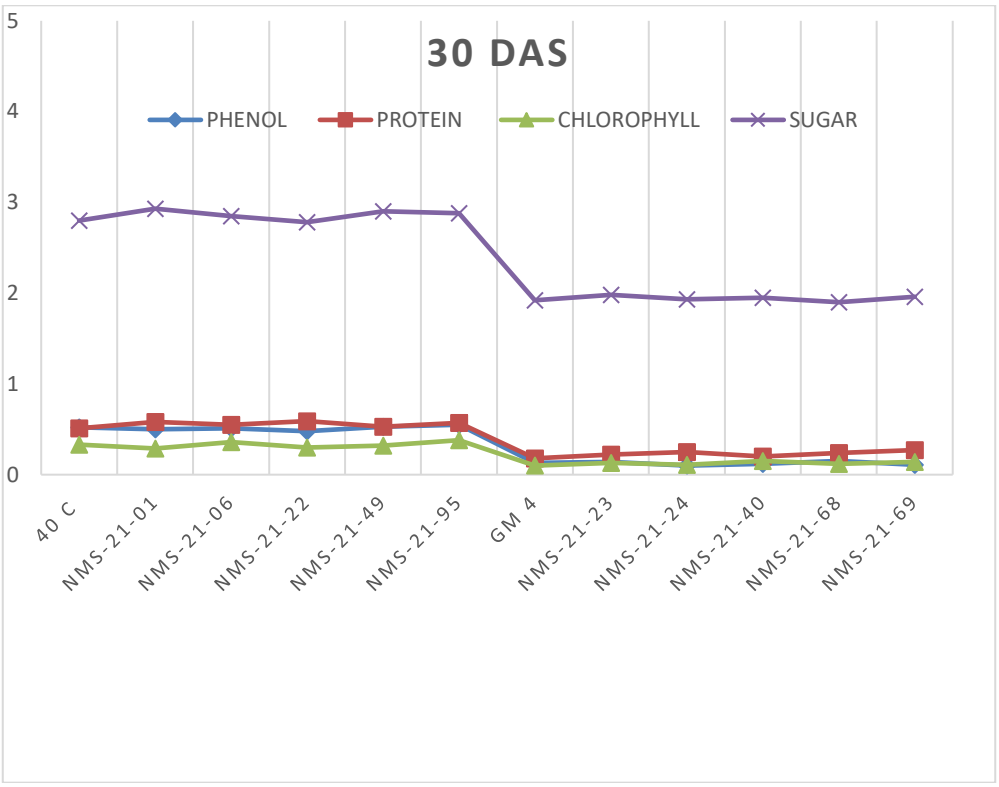

Fig. 1. Effect of phenol, protein, chlorophyll and sugar onmungbean yellow mosaic India virus disease incidence at 30 DAS

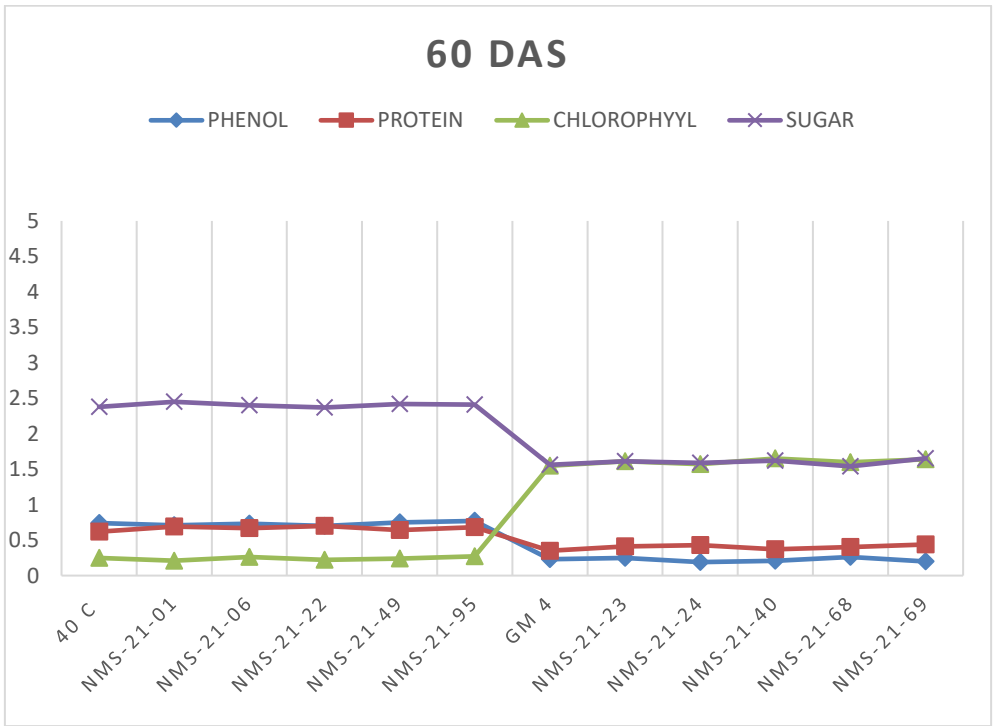

Fig. 2. Effect of phenol, protein, chlorophyll and sugar on mungbean yellow mosaic India virus disease incidence at 60 DAS

3.1.3 Total protein content in MYMV-infected genotypes

At 30 DAS, the resistant genotypes' protein content ranged from 0.51 (40C) to 0.59 (NMS-21-22) mg/g. However, the least amount of protein was found in highly susceptible genotypes, with values ranging from 0.18 (GM 4) to 0.27 (NMS-21-69) mg/g. At 60 DAS, a similar pattern was also seen, with resistant genotype protein contents ranging from 0.62 (40C) to 0.70 (NMS-21-22) mg/g. In contrast, highly susceptible genotypes were found in the range of 0.35 (GM 4) to 0.44 (NMS-21-69) mg/g. The least amount of protein was found in susceptible check BGS-9 (0.27 mg/g), while resistant genotypes had higher protein contents than susceptible genotypes (Table 1, Fig. 1, Fig. 2). The main cause of the lower synthesis of protein in susceptible genotypes might be due to disturbance in photosynthetic activity and their de novo synthesis [33]. Kundu and his coworker [34] confirmed through proteomics techniques and revealed that the photosynthesis-related proteins were severely damaged in the susceptible genotype leading to a lower rate of photosynthesis under MYMIV stress. In resistant genotypes, the protein level varied at 30 DAS, ranging from 0.52 (MGG-1) to 0.59 (KKM-4) mg/g. The least amount of protein, however, was found in highly sensitive genotypes, ranging from 0.23 (VC6372) to 0.25 (ML-1299) mg/g. At 60 DAS, a similar pattern was also noted, with resistant genotypes' protein contents ranging from 0.66 (MGG-1) to 0.69 (KKM-4) mg/g. In contrast, genotypes that were shown to be extremely sensitive ranged from 0.37 (VC 6372) to 0.41 (ML-1299) mg/g [32].

3.1.4 Total chlorophyll content in MYMV-infected genotypes

Chlorophyll content in resistant genotypes ranged from 0.29 (NMS-21-01) to 0.38 (NMS-21-95) mg/g at 30DAS. The chlorophyll content of highly susceptible genotypes, however, ranged from 0.10 (NMS-21-24) to 0.15 (NMS-21-68) mg/g. Similar to this, during 60 DAS, highly susceptible genotypes' chlorophyll content ranged from 1.55 (NMS-21-24) to 1.65 (NMS-21-68) mg/g, while resistant genotypes ranged from 0.21 (NMS-21-01) to 0.27 (NMS-21-95) mg/g. (Table 1, Figs. 1,2).Following that Kundu and his coworker [34] explained the differential intensities of chlorophyll fluorescence and chlorophyll contents by proteomics data. It was revealed that Photosystem II electron transports are the primary targets of MYMIV during pathogenesis. That is the major proof of the reduction in chlorophyll content as it was described by a gradual increase in chlorotic patches in susceptible genotypes [23]. “In resistant genotypes chlorophyll content at 30DAS was varied from 0.29 (MGG-1) to 0.35 (KKM-4) mg/g. However, highly susceptible genotypes recorded very low chlorophyll content from 0.12 (VC 6372) to 0.14 (ML-1299) mg/g. Similarly, during 60 DAS, resistant genotypes recorded chlorophyll content between 0.21(PAU-911) and 0.23 (KKM-4) mg/g and in highly susceptible genotypes, it was ranged from 0.08 (VC 6372) to 0.10 (ML-1299) mg/g” [32,35].

3.2 Variation of Different enzyme Activity in Mungbean Genotypes due to MYMV Infection

3.2.1 Peroxidase (PO) enzyme activity in MYMV infected genotypes

At 30DAS, PO activity significantly changed as a result of a virus infection (Table 2). The peroxidase activity ranged from 0.371 (NMS-21-01) to 0.410 (NMS-21-06) A/min/g in resistant genotypes, and from 0.136 (NMS-21-23) to 0.152 (NMS-21-40) A/min/g in highly susceptible genotypes. At 60 DAS, the same trend was seen in all genotypes. The range of PO activity in resistant genotypes was 0.460 (NMS-21-01) to 0.487 (NMS-21-06) A/min/g. However, genotypes with high susceptibility showed PO activity ranging from 0.211 (NMS-21-23) to 0.229 (NMS-21-40) A/min/g. (Table 2, Fig. 3, Fig. 4). The activity of the PO enzyme was increased in resistant genotypes. This is determined that significant changes in the activity of antioxidant enzymes occur in response to viral infection to prevent damage and spread of infection [36,37]. The least peroxidase activity was found in highly sensitive genotypes, ranging from 0.318 (VC 6372) to 0.159 (ML-1299) ΔA/min/g. In resistant genotypes, the range was 0.369 (PAU-911) to 0.411 (KKM-4) ΔA/min/g. At 60 DAS, the similar trend was seen in all genotypes. PO activity ranged from 0.440 (PAU-911) to 0.461 (KKM-4) ΔA/min/g in resistant genotypes [32].

3.2.2 Polyphenol oxidase enzyme (PPO) activity in MYMV infected genotypes

PPO activity significantly varied between mungbean genotypes at 30 DAS ranging from 0.232 (NMS-21-22) to 0.245 (40 C) A/min/g in resistant genotypes. However, PPO activity

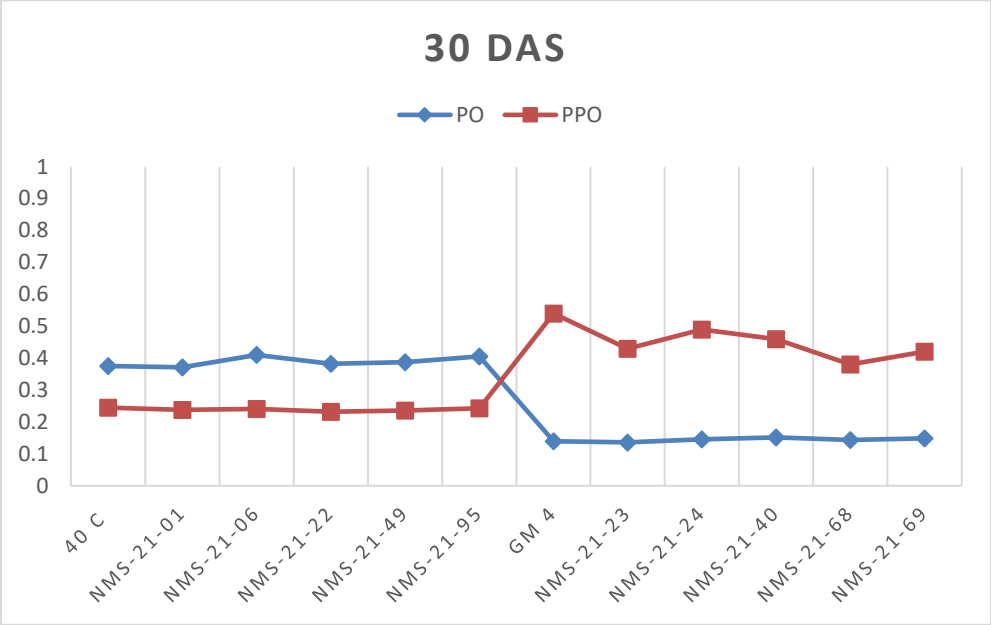

Fig. 3. Effect of Peroxidase (PO) and Polyphenol oxidase (PPO) on mungbean yellow mosaic India virus disease incidence at 30 DAS

ranged from 0.040 (VC 6372) to 0.055 (ML-1299) A/min/g in highly susceptible genotypes. At 60 DAS, the same pattern was seen. PPO activity was lowest in highly susceptible genotypes and ranged from 0.347 (PAU-911) to 0.405 (KKM-4) min/g in resistant genotypes. From 0.154 (VC 6372) to 0.169 (ML-1299), a /min/g was the range. The susceptible check showed the least PPO activity (0.146 A min/g). (Table 2, Fig. 3, Fig. 4). Overall in the resistant genotype the PPO activity was depicted as increasing.PPO activity has been reported to increase following infection by yellow mosaic disease in mungbean and it may be due to activation and solubilization of latent host enzyme ascribed to PPO which is usually due to denovo synthesis [32,37]. The PPO activity of the different mungbean genotypes varied substantially at 30 DAS. varied from 0.233 (PAU-911) to 0.291 (KKM-4) ΔA/min/g in resistant genotypes. On the other hand, genotypes that were extremely sensitive showed the lowest PPO activity, ranging from 0.040 (VC 6372) to 0.055 (ML-1299) ΔA/min/g. A comparable pattern was noted at 60 DAS. PPO activity varied from 0.347 (PAU-911) to 0.405 (KKM-4) min/g in resistant genotypes, with highly sensitive genotypes exhibiting the least PPO activity. The values varied between 0.154 (VC 6372) and 0.169 (ML-1299) a/min/g. The susceptibility check showed the lowest PPO activity (0.146 A min/g) [32].

3.2.3 Phenyl ammonia lyase (PAL) enzyme activity in MYMV infected genotypes

All mungbean genotypes showed significant variation in PAL enzyme activity 30 days after sowing. The resistant genotypes ranged in moles of trans-cinnamic acid min-1 g1 from 136.4 (NMS-21-68) to 145.5 (NMS-21-01). While low PAL activity was recorded by highly susceptible genotypes between 101.5 (NMS-21-23) and 109.3 (NMS-21-24) moles of trans-cinnamic acid min^{1} g^{1}. Similar to this, at 60 DAS, PAL activity in highly susceptible genotypes ranged from 103.6 (NMS-21-23) to 111.8 (NMS-21-24) moles of trans-cinnamic acid min^{-1} g^{-1} and between 140.6 (NMS-21-22) and 149.9 (NMS-21-01) moles of trans-cinnamic acid min-1 g-1 in resistant genotypes. The results showed that from 30 to 60 DAS, PAL activity was significantly higher in resistant genotypes than in susceptible genotypes. (Table 2, Fig. 5, Fig. 6). Throughout the infestation of the virus, overall pal enzyme expressivity was higher in the resistant genotypes. This is a key enzyme in phenylpropanoid metabolism and plays a significant role in the synthesis of various secondary metabolites that are involved in plant immunity and induce resistance by PGPR. These secondary metabolites are in the restriction and invasion of the virus [38,39]. Thirty days after sowing, PAL enzyme activity varied significantly across all mungbean genotypes. The range of resistance genotypes was 138.0 (PAU-911)

to 141.2 (KKM-4) min−1 g−1 µ moles of transcinnamic acid. Conversely, the genotypes that were most sensitive showed the lowest levels of PAL activity, ranging from 79.5 (VC6372) to 83.1 (ML-1299) µ moles of transcinnamic acid min−1 g−1. Similarly, after 60 DAS, PAL activity ranged from 132.9 (KM-17–197) to 134.9 km-14–214) µ moles of transcinnamic acid min−1 g−1, for resistant genotypes, and from 85.8 (VC6372) to 94.8 (ML-1299) µ moles for highly susceptible genotypes [32].

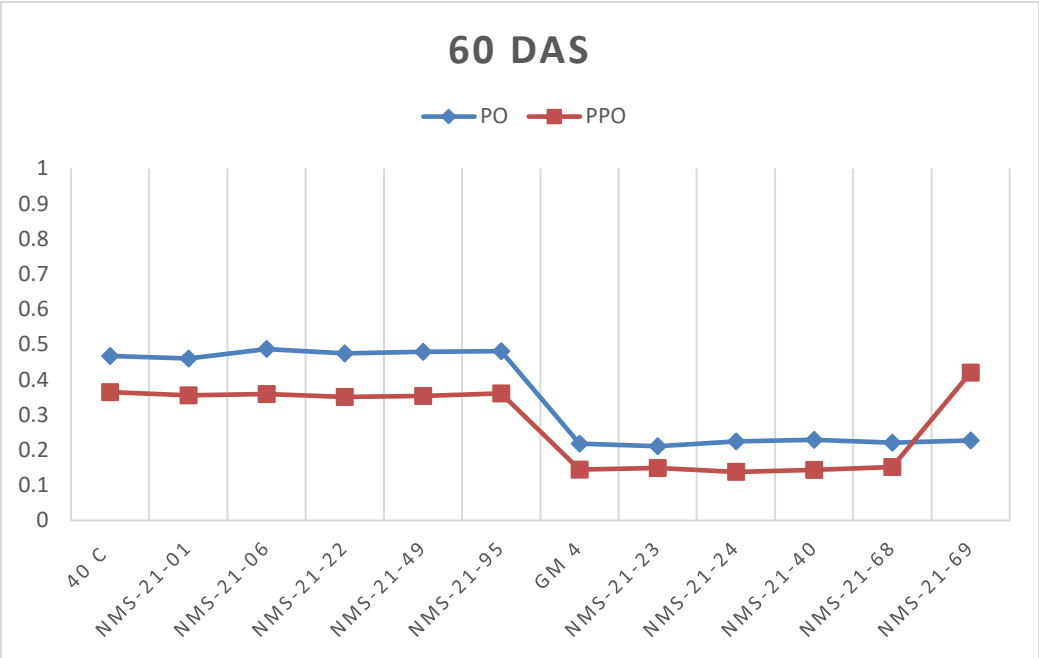

Fig. 4. Effect of Peroxidase (PO) and Polyphenol oxidase (PPO) on mungbean yellow mosaic India virus disease incidence at 60 DAS

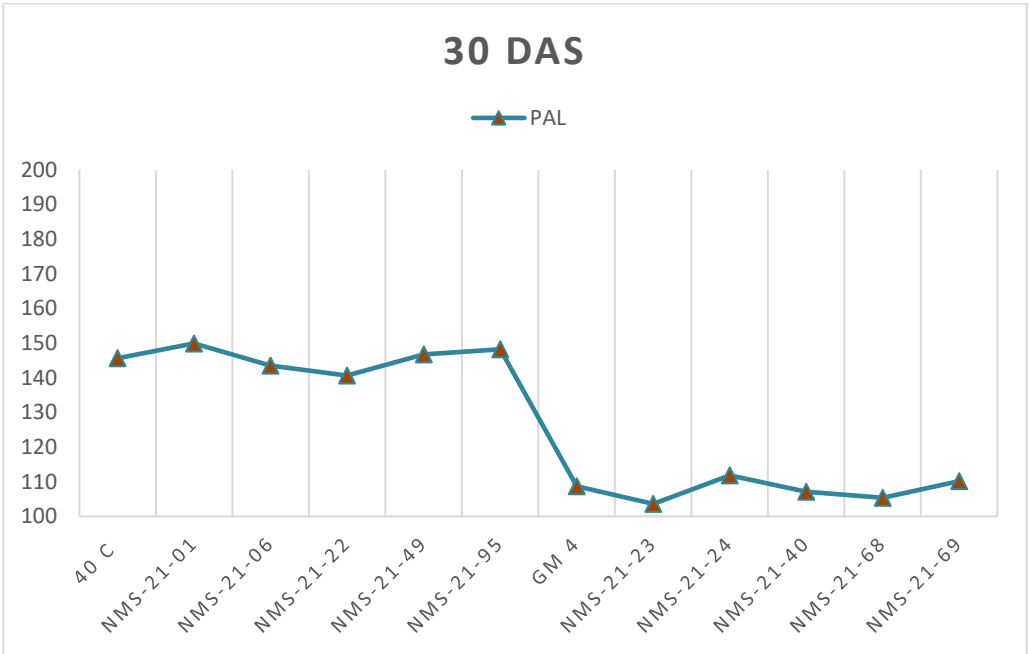

Fig. 5. Effect of Phenyl nine Ammonia Lyase (PAL) onmungbean yellow mosaic India virus disease incidence at 30 DAS

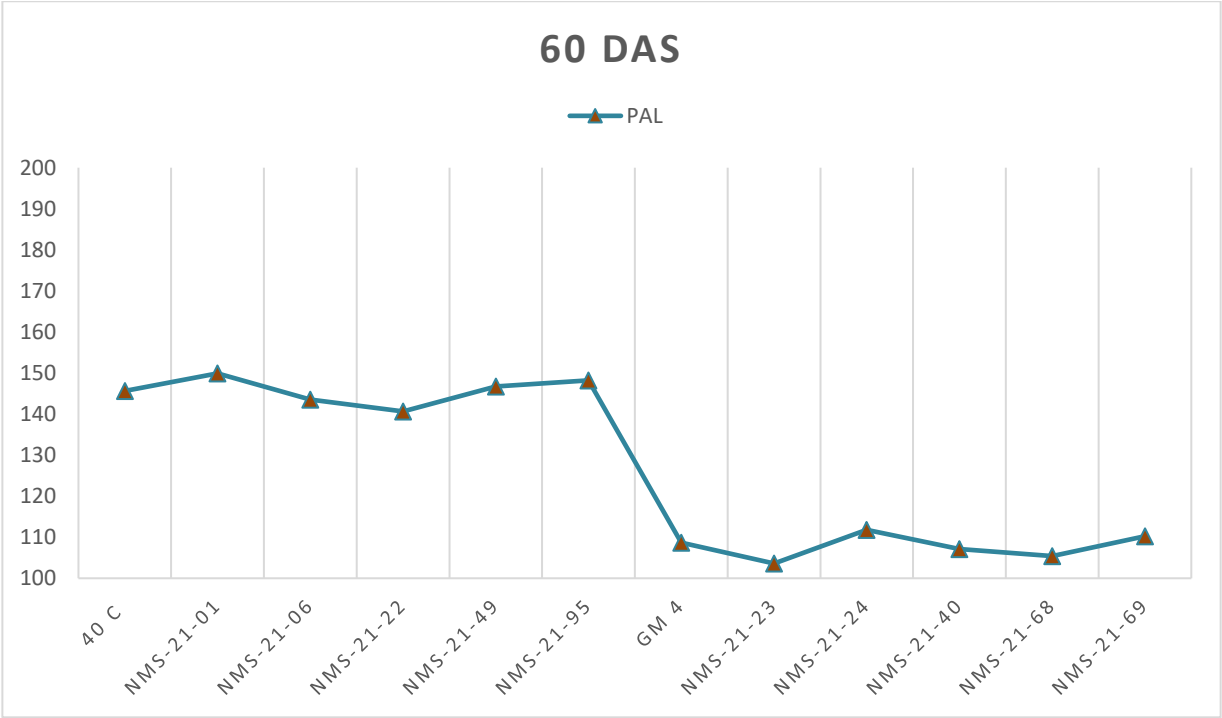

Fig. 6. Effect of Phenyl nine Ammonia Lyase (PAL) on mungbean yellow mosaic India virus disease incidence at 60 DAS

Table 2. Enzyme activity in mungbean genotypes with varying reactions to MYMV infection during summer 2021-22

Genotypes	Reactions	30 DAS			60 DAS		
		Peroxidase	Polyphenol oxidase	Phenyl nine Ammonia Lyase	Peroxidase	Polyphenol oxidase	Phenyl nine Ammonia Lyase
40 C	R	0.375	0.245	140.0	0.467	**0.365**	145.6
NMS-21-01	R	0.371	0.238	145.5	0.460	0.356	**149.9**
NMS-21-06	R	0.410	0.241	138.2	**0.487**	0.359	143.5
NMS-21-22	R	0.382	0.232	136.4	0.475	0.351	140.6
NMS-21-49	R	0.387	0.236	141.2	0.479	0.354	146.7
NMS-21-95	R	0.405	0.243	143.8	0.481	0.361	148.2
GM 4	HS	0.140	0.54	106.2	0.218	0.145	108.7
NMS-21-23	HS	0.136	0.43	101.5	0.211	0.149	103.6
NMS-21-24	HS	0.146	0.49	109.3	0.225	0.138	111.8
NMS-21-40	HS	0.152	0.46	105.9	0.229	0.144	107.1
NMS-21-68	HS	0.144	0.38	103.6	0.221	0.152	105.4
NMS-21-69	HS	0.149	0.42	108.8	0.227	0.156	110.2
SD		0.12	0.11	18.51	5.6	0.11	20.05
S.Em ±		0.03	0.03	5.34	1.41	0.03	5.78
CV%		3.32	3.49	35.36	3.31	3.11	39.67

4. CONCLUSION

Higher protein content was often seen in YMV resistant mungbean genotypes (*i.e.* NMS-21-22), followed by highly susceptible genotypes. When compared to healthy plants of all genotypes, infected plants showed an increased protein content. YMV resistant genotypes (*i.e.* NMS-21-95) of mungbean showed a higher phenol content than highly susceptible genotypes. When compared to healthy plants of all genotypes, infected plants showed an increased phenol content. Higher sugar content was found in mungbean genotypes (*i.e.* NMS-21-01) that were resistant to YMV, followed by those that were highly susceptible.YMV resistant genotypes of mungbean showed a higher phenol content than highly susceptible genotypes. When compared to healthy plants of all genotypes, infected plants showed an increased phenol content. Compared to susceptible cultivars, resistant cultivars (*i.e*. NMS-21-06 and 40 C) showed a sustained increase in peroxidase (PO) and polyphenol oxidase (PPO) up to 60 DAS. The resistant cultivar's (*i.e*. NMS-21-01) phenylalanine ammonia-lyase consistently increased up to 60 DAS compared to the susceptible variety. The findings unambiguously showed that, in comparison to susceptible cultivars, higher induction of PR proteins is positively connected with their resistance to mungbean yellow mosaic disease. Consequently, the study showed that the morphological and biochemical activities that support the establishment of the mungbean and MYMV interaction a defense system [40].

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:
The peer review history for this paper can be accessed here:
https://prh.ikprress.org/review-history/12164