Original Research Article

Biochemical Impact Inflicted by Aphid Induced Urdbean Leaf Crinkle Virus Infection on Different Blackgram Genotypes

Comment [PB1]: Add (ULCD)

ABSTRACT

Aims: To investigate the variations in total protein content and anti-oxidative enzymes in blackgram genotypes showing various levels of resistance against aphid-mediated Urdbean leaf crinkle disease (ULCD).

Study design: Completely Randomized Design

Place and Duration of Study: The studies were carried out at the Insectary belonging to the Department of Agricultural Entomology and the Centre of Innovation at Agricultural College and Research Institute, Madurai, Tamil Nadu, India.
in January 2021

Methodology: The research was conducted on seven blackgram genotypes exhibiting different levels of resistance to ULCD. A pot culture study was established and the test plants were subjected for inoculation of urdbean leaf crinkle virus (ULCV) via feeding by 10 viruliferous *Aphis craccivora* adults/plant. The alterations in the total protein content, superdioxide mutase (SOD), catalase (CAT) and peroxidase (PO) activity was estimated using spectrophotometric methods in comparison to healthy genotypes on 0, 15 and 30 days after inoculation (DAI).

Results: No discernible increase in the levels of protein content was seen in either of ULCV inoculated or un-inoculated genotypes on the day of the inoculation. At post inoculation, leaf protein content of ULCV highly susceptible genotypes, VBN 8 and T 9 was only marginally higher than that of resistant genotypes, CO 5 and CO 6, whereas in moderately resistant genotype, APK 1 it had only slightly increased. The SOD activity showed only a non-significant drop between inoculated versus un-inoculated plant leaves in highly susceptible VBN 8 and T 9 genotypes. At 15 and 30 days post inoculation, a significantly less SOD activity, but considerably increased PO under ULCV inoculated versus un-inoculated conditions was observed in ULCV resistant CO 5 and CO 6 genotypes. While the susceptible ADT 5 and ADT 6 and highly susceptible VBN 8 and T 9 genotypes did not show any significant increase in PO levels between inoculated versus un-inoculated conditions. Interestingly, ULCV inoculation considerably decreased the reduced CAT activity both in resistant and susceptible genotypes, when compared to the un-inoculated healthy ones.

Conclusion: Fluctuations in the levels of antioxidant enzymes as well as the total protein content was significant only at 15 Days post inoculation. The ULCV infection tends to increase the total protein content in inoculated plants compared to un-inoculated plants. The aphid transmission of ULCV infection in different blackgram genotypes inflicted considerable increase in PO while decrease in SOD and CAT activities in resistant CO 5 and CO 6 genotypes.

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Keywords: Plant defense, Urdbean Leaf crinkle virus (ULCV)*, blackgram, anti-oxidative enzymes, plant resistance, biotic stress*

1. INTRODUCTION

Blackgram (*Vigna mungo* (Linn.) Hepper) is one of the Asiatic species of the pantropical genus *Vigna* and a member of the family Leguminosae, subfamily Papilionaceae. Blackgram's carbonized seeds, which were discovered at the ancient sites of Navdotoli and Maheshwar, show that it was first cultivated on the Indian Subcontinent [1]. It has

extensive adaptations to semi-arid and subtropical areas [2]. It is grown in 23 nations, with India ranking as one of the world's top producers and users of blackgram with a 54.39 lakh acres of area and annual production of 35.62 lakh tonnes [3]. It is the primary pulse crop in Myanmar, Thailand, Bangladesh, Pakistan, and India [4, 5]. Because of its industrial and nutritional benefits, it is highly prized and blackgram seeds are rich with 23.4 percent protein, 60.4 percent carbohydrate, lysine, and phosphoric acid [6]. The production of blackgram is impaired by many yield constraints, mainly insects and diseases, and when compared to other pulses, blackgram's vulnerability to Urdbean Leaf Crinkle Disease (ULCD) is the main obstacle to its successful production [7] in recent decades.

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The debilitating and economically significant ULCD that infects blackgram may cause excessive crinkling, puckering, rugosity in the leaves, and infertility in the pollen grains [8], resulting in 35 to 81 percent loss of seed output [9]. Factors such as cropping season, infection timing, and cultivars' disease resistance all had played a role in the yield loss caused by ULCD [6]. Transmission of ULCD in pulse plants is reported as possible by grafting, seed, or sap transmission [7] and through a few insect vectors [8] such as *Aphis craccivora* Koch (Hemiptera: Aphididae), *Myzus persicae* Sulzer (Hemiptera: Aphididae), *Acyrthosiphon pisum* Harris (Hemiptera: Aphididae), *Henosepilachna dodecastigma* Wiedemann (Coleoptera: Coccinellidae) [10,11] and whiteflies [12]. The transmission by different insect species is still under different steps of further investigation and confirmation.

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Plants tend to use a wide range of physio-chemical mechanisms against biotic stresses induced by insect pests [13-15] and the expression of prior and raised enzyme levels is a significant feature of resistance against pathogens [16]. These resistance strategies may be either independent of herbivore attack as constitutive event [17] or triggered when plants are attacked as inducible response [18]. The most crucial line of protection in plants against insect attack is the accumulation of protective chemicals through physiological, morphological, and chemical changes [15, 19, 20]. By increasing the formation of ROS [i.e., hydrogen peroxide (H_2O_2), superoxide (O_2), and hydroxyl (OH) radicals] by greater electron leakage to molecular oxygen, it has been discovered that the plant immunological response to the viral infection increases [21]. In addition to causing irreversible DNA damage and cell death, ROS serve as significant signaling molecules that control plants to grow normally and react to stress. However, high levels of ROS production result in oxidative stress, which weakens the structure of the plant. Therefore, plants activate the enzymatic antioxidant system, which consists of the enzymes ascorbate peroxidase (APX), superoxide dismutase (SOD), peroxidase (PO), and catalase (CAT), in order to maintain the ROS at the ideal level [22]. The superdioxide mutase (SOD), is a crucial scavenging enzyme that catalyses the dismutation of superoxide radicals into active oxygen species hydrogen peroxide [23,24] and it involved in first line of defense against plant pathogens.

The production and buildup of oxidative enzymes like peroxidase (PO) and catalase (CAT) is one of the most common plant responses to insect herbivore attack [15, 25-30]. These enzymes have been linked to plant resistance against insect herbivores because of their putative functions in plant signaling, the manufacture of defensive chemicals, and/or the tolerance to oxidative stress [31].

The goal of this study was to compare the enzymatic reactions of blackgram genotypes with different resistant responses to *A. craccivora* feeding and ULCV infection in order to elucidate processes that will aid in development of blackgram variants with long-lasting aphid and disease resistance. The current investigation focuses on the induction of defensive compounds especially protein accumulation and enzymes such as PO, SOD and CAT in seven genotypes blackgram genotypes that showed distinguishable reactions in resistance screening studies against challenged aphid feeding and ULCV infection.

2. MATERIAL AND METHODS

2.1 Insect culture

Field collections of pulse aphids (*A. craccivora*) were made from healthy cowpea and blackgram crops in the Theni and Madurai districts of Tamil Nadu. To prevent desiccation of the sample, the stalks of aphids that were blooming with apterous adults were carefully cut from the plant and moved to the research area in small plastic boxes with ventilation. Each apterous matriarchal aphid was separated from the others using a camel brush and reared in individual insect-proof cages (wooden frames with dimensions of 150 cm x 150 cm x 75 cm designed with nylon mesh of 100-micron mesh size covered in three sides, a wooden platform, and a glass top and door) containing a healthy potted local variety of cowpea (*Vigna unguiculata*) plant aged 7 days after sowing (DAS) [32].

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2.2 Virus culture maintenance

Blackgram plants exhibiting the signs of ULCD were marked in the field at the National Pulses Research Centre (NPRC), TNAU, Vamban, Tamil Nadu, and used for the collection of fresh trifoliate leaves contaminated with ULCV. Fresh blackgram leaves with the ULCV infection that were taken from the field and kept there under -20°C for use in sap transmission [33].To generate abrasion on the leaf lamina, the extracted sap was applied with a pinch of carborundum to healthy potted blackgram plants that were 7 days old (variety T 9; susceptibility check acquired from local market) [34]. The treated leaves were hand sprayed with distilled water after 5 to 10 minutes. The symptoms appeared in the successive trifoliate leaves after inoculation in 15 to 20 days. These plants were maintained as virus inoculum for further studies in the insect-proof cages [32].

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Chemicals source:
The chemicals used in this experiment for analysis of enzymes were purchased from Sigma- Aldrich Chemicals Pvt. Limited, Bengaluru, India.

2.1 Preparation of Plant samples

The seeds of the blackgram genotypes that showed different reaction responses to ULCV based on Per cent Disease Index (PDI) assessed in the resistant screening technique *viz*., ULCV resistant genotypes CO 5 and CO 6, moderately resistant APK 1, ADT 5, and ADT 6 and highly susceptible VBN 8 and T 9 genotypes were used in the study. The pre-washed seeds were sown in pots (28 cm dia.) filled with soil and healthy seedlings were thinned to one per pot 5 days post sowing. On 7day-old seedlings ULCV inoculation was done with the release and confinement of ten numbers of viruliferous aphids per seedling of each genotype by subjecting aphids for 10 min acquisition feeding period in source T 9 blackgram ULCV infected plants mentioned above in virus culture maintenance. After allowing 10 min. inoculation feeding period on test seedlings, aphids were killed by insecticide spray. For the mock test plants, non- viruliferous aphids were allowed to feed on the test entries. This trial was replicated thrice. Leaves were collected from all the entries on 0, 15, and 30 days post inoculation and were stored in a deep freezer (Cryo Scientific- Model: LF-V-550S (Chennai) 0.5 watts; 2.1 amp; 550L capacity) at -20˚C for further biochemical analysis. The samples were subjected to the estimation of antioxidant enzyme assays such as total protein content, SOD, CAT, and PO analysis.

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2.2 Preparation of plant homogenates

One 100 mg of leaf sample from each entry was homogenized in cold phosphate buffer using a pestle and mortar (with desired pH and M as mentioned below in each assay). The end product was filtered before refrigerated centrifugation for 15 min. at 10,000 rpm and 4°C. Enzymatic studies were performed on the obtained supernatant. For each enzyme and entry, three separate biological replicates were used for all biochemical assays. Each enzyme's activity was expressed in terms of protein basis.

2.2.1 Estimation of Total Protein content

Around 1.0 ml of Bradford reagent was added to ten µl of each sample in a sterile test tube [35]. The samples were incubated for 10 to 20 min at 37˚C along with the blank. The standard curve was constructed using Bovine serum albumin (BSA) and the protein in the test samples was calculated from the standard curve. The absorbance was taken at 595 nm with the aid of a UV- Vis spectrophotometer (Agilent Technologies- Cary Series). The total protein content in each sample was expressed in mg/g of leaf tissue.

2.2.2 Estimation of Superoxide dismutase (SOD)

The reaction solution (3ml) contained 100 µl enzyme extract, 50 mM potassium phosphate buffer (pH 7.8), 13 mM methionine, 75 mM EDTA, 50 µM NBT and 1.3 µM riboflavin. The assay for SOD activity was performed at 560 nm in the UV- Vis spectrophotometer (Agilent Technologies- Cary Series) [36] and the reaction solution were exposed under a light bank (15 fluorescent lamps) for 15 min. 50 % inhibition of the reaction between the NBT and riboflavin in the presence of methionine is defined as one unit of SOD activity. One unit of SOD activity was expressed as units/ mg of protein.

2.2.3 Estimation of Catalase (CAT)

The estimation of CAT activity was determined by the method suggested by Chance and Maehly (1955) [37]. The reaction solution (3 ml) contained 0.1 ml enzyme extract, 50 mM potassium phosphate buffer (pH: 7.0) and 5.9 mM H_2O_2. The reaction was initiated by adding the enzyme extract. At 240 nm the change in absorbance was observed every 20s in UV- Vis spectrophotometer. One unit CAT activity was defined as an absorbance change of 0.01 units/ min.

2.2.4 Estimation of Peroxidase (PO)

The PO reaction solution (3 ml) contained 0.1 ml enzyme extract 50 mM potassium phosphate buffer (pH 5.0), 20 mM guaiacol (240 mg guaiacol dissolved in water and made up to 100 ml), and 40 mM H_2O_2. At 470 nm, the changes in absorbance were determined every 20 s. One unit PO activity was defined as an absorbance change of 0.01 units/min [38].

2.3 Statistical Analysis

The pooled replication data and means and standard errors of means were calculated. The data were subjected using square root transformation. The significance level was set as p=0.05. The data were subjected to analysis of variance (ANOVA) among genotypes using SPSS (IBMCorp, 2013). The experiment was conducted under Completely Randomized Block Design (CRBD). Grouping of data was done using Tukey's HSD (Honestly Significant Difference) test [39].

3. RESULTS AND DISCUSSION

3.1 TOTAL PROTEIN CONTENT

Accumulation of protein was recorded in highly susceptible to resistant genotypes of blackgram plants infected by ULCV at 15 and 30 days post inoculation when compared to the healthy plants that were ULCV un-inoculated (Fig.1.). No discernible increase in the levels of protein content was seen at the start of the inoculation in either the inoculated or uninoculated genotypes on the day of the inoculation. The protein content of the leaves of the highly ULCV susceptible genotypes VBN 8 (4.7±0.06 mg/g of leaf tissue) and T 9 (4.8±0.05 mg/g of leaf tissue) was marginally higher than that of the resistant genotypes CO 5 (3.9±0.05 mg/g of leaf tissue) and CO 6 (4.0±0.06 mg/g of leaf tissue). Comparing the moderately resistant genotype APK 1 (4.1±0.10 mg/g of leaf tissue) to the extremely susceptible genotypes, it was found that the protein content had only slightly increased at 30 DAI (das after inoculation)

This result is in consistency with studies, which had that shown that after 10, 20, and 30 days of ULCV inoculation, the amount of viral proteins in the infected plant tends to increase the total protein content [40-43]. On the other hand, numerous studies have documented how viral infection causes the total protein content to decrease [44, 45]. Furthermore, Brar and Rataul (1990) [46] have noted a decreasing phase in the total protein content and chlorophyll content in immature blackgram plants infected with ULCV. Karthikeyan *et al.* (2022) [47] has reported that VBN 6 (moderately resistant to ULCV) shown a marginal increase of 7.07 % of total soluble protein and the susceptible cultivar CO 5 shown a substantial decrease of 26.96 % of total soluble protein after viral inoculation when compared to the un-inoculated VBN 6 and CO 5 cultivars. The latter report was also in agreement with the studies conducted by Siddique *et al.,* 2014 [16] and Madhumitha *et al.,* 2020 [48].

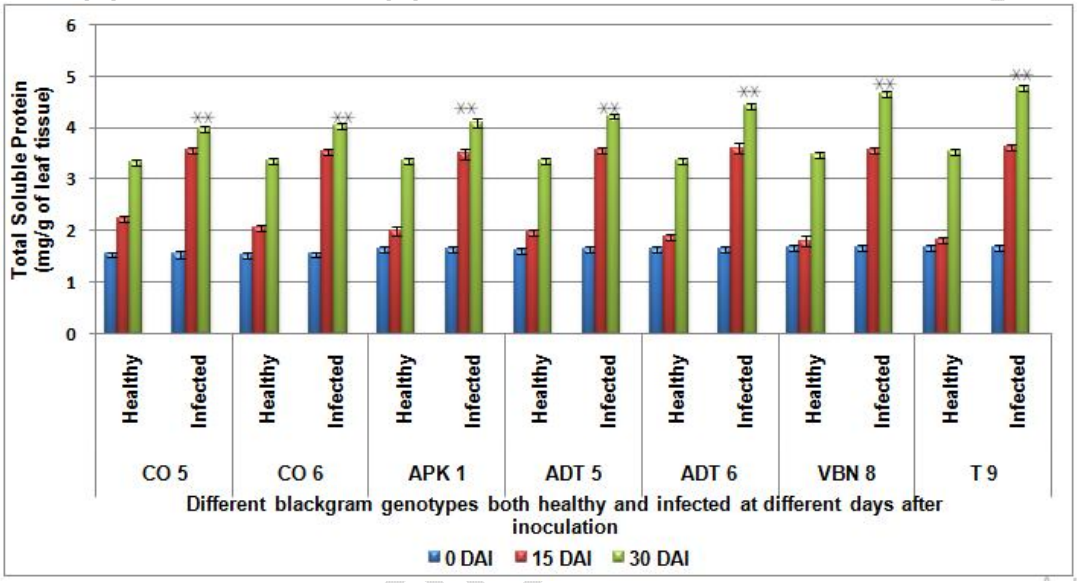

Fig. 1. Accumulation of total protein in different blackgram genotypes at different days post inoculation

Results are the mean±SE of three plants

*Test entries: significant from normal control, *P < 0.05; **P < 0.001*

3.2 Fluctuation in antioxidant enzymes

In an early stage of infection, the moderately resistant cultivars produce H2O2 in abundance than susceptible cultivars. Thus, an early ROS burst is witnessed in resistant cultivars. To avoid intracellular damage b the pathogen, H2O2 is produced on the site of infection. This first line of defense is achieved by microbial activity at the invasion site or by oxidative crosslinking of hydroproline rich protein or by phytoalexins or by reinforcing processes of cell wall [49]. Such evidence of induced ROS bursts have been reported in various pathosystems such as *mungbean yellow mosaic India virus* and *Tobacco mosaic virus* (TMV) in blackgram and tomato, respectively [50,51]. To ensure the protection of cells from ROS toxicity, the cells tend to synthesize enzymes such as SOD, PO and CAT.

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3.2.1 Superoxide dismutase

The SOD activity of the susceptible and resistant genotypes showed different trends. Up to 30 days post inoculation, the leaves of the healthy plants (un-inoculated) exhibited a rising trend in SOD activity. When compared to the un-inoculated highly susceptible VBN 8 and T 9 genotypes, the inoculated VBN 8 (37.5±0.20 and 41±0.13 mg^{-1} protein at 15 and 30 DAI, respectively) and T9 (38±0.02 and 42±0.22 mg^{-1} protein at 15 and 30 DAI, respectively) showed a non-significant drop in SOD activity at 15 and 30 DAI *i.e.* the un-inoculated VBN 8 and T9 at 15 and 30 DAI was observed with 38.28±0.19 mg^{-1} protein and 49.13±0.15 mg^{-1} protein; and 39.14±0.16 mg^{-1} protein and 48.17±0.15 mg^{-1} protein , respectively (Fig.2.). At 15 and 30 days post inoculation, the resistant CO 5 (34.15±0.17 and 49.08±0.12 mg^{-1} protein at 15 and 30 DAI, respectively) and CO 6 (45.23±0.18 and 48.05±0.19 mg^{-1} protein at 15 and 30 DAI, respectively) showed

significantly less SOD activity than the un-inoculated CO 5 (52.22±0.07 and 69.29±0.25 mg^{-1} protein at 15 and 30 DAI, respectively) and CO 6 (50.52±0.03 and 68.09±0.13 mg^{-1} protein at 15 and 30 DAI, respectively) genotypes. The findings of Ashfaq *et al*. (2010) [42] and the above result are comparable. Similar responses were noted by Buonaurio and Montalbini (1993) [52] in *Potato Virus Y*-infected tobacco, Zhuang *et al*. (1993) [53] in soybean mosaic virus-infected soybean, Riedle-Bauer (1998) [54] in *cauliflower mosaic virus*-infected cucumber plant, in *White Clover Mosaic Potexvirus* (WCIMV) infected *Phaseolus vulgaris* by Clarke *et al*. (2002) [55] and Hernandez *et al*. (2004) [56] in *plum pox virus*-infected peaches. The above findings were also in accordance with the reports of Karthikeyan *et al*. (2022) [47] who reported that there was an upsurge of SOD activity on 3 DPI (days post inoculation) in ULCV inoculated resistant cultivar VBN 6 than the inoculated CO 5 cultivar.

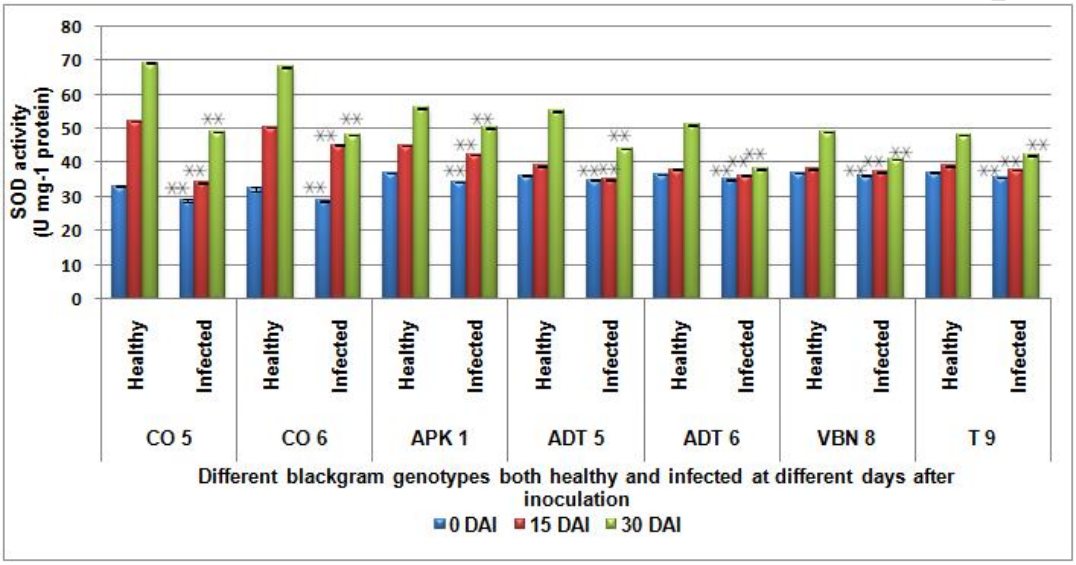

Fig. 2. Specific Acitivity of SOD in different blackgram genotypes at different days post inoculation

Results are the mean±SE of three plants

*Test entries: significant from normal control, *P < 0.05; **P < 0.001*

3.2.2 Peroxidase

After 15 and 30 days post inoculation, a significant increase in the level of PO activity was evinced in the resistant genotypes CO 5 (170.11±0.12 and 250.04±0.05 mg^{-1} min^{-1} protein at 15 and 30 DAI) and CO 6 (170.22±0.01 and 250.19±0.06 mg^{-1} min^{-1} protein at 15 and 30 DAI) (Fig. 3). Similar increasing trend of PO activity was observed in moderately resistant APK 1 (170.37±0.01 and 230.33±0.02 mg^{-1} min^{-1} protein at 15 and 30 DAI) genotype, whereas the susceptible genotypes ADT 5 (170.45±0.02 and 210.46±0.01 mg^{-1} min^{-1} protein at 15 and 30 DAI) and ADT 6 (170.52±0.01 and 210.51±0.01 mg^{-1} min^{-1} protein at 15 and 30 DAI) and highly susceptible VBN 8 (150.26±0.06 and 200.13±0.02 mg^{-1} min^{-1} protein at 15 and 30 DAI) and T 9 (150.33±0.10 and 200.02±0.02 mg^{-1} min^{-1} protein at 15 and 30 DAI) genotypes, witnessed a non-significant increase in PO level. This increasing level of peroxidase in the ULCV inoculated resistant cultivars might be the underpinning reason in creating resistance against the ULCV. The results of this study was supported by the reports of Ashfaq *et al.* (2010) [42] who observed a similar trend in increasing peroxidase level in resistant cultivars than in susceptible cultivars when ULCV was inoculated. The findings were also in agreement with the reports of Clarke *et al*. (2002) [55] and Karthikeyan *et al*. (2009) [57] who observed the rise in PO activity in WCIMV infected *P. vulgaris* and ULCV infected blackgram, respectively.

Induced resistance cannot be attributed to increased PO activity since, as noted by Van Loon (1976) [58] and Nadlong and Sequeira (1980) [59], it may be caused by a virus infection that results in physiological changes in the host. Unregulated or unchecked POs may be responsible for virus-infected plants' reduced growth and incurred deformity [60]. PO participates in a variety of plant defense mechanisms where H2O2 is released by an oxidative burst, a common defense response [61,62]. According to studies by Hammerschmidt and Kuc (1982) [63] and Espelie *et al*. (1986) [64], the cell wall appears to be the primary site for PO polymer chemical processes such as suberization and lignification as well as the cross-linking of cell wall structural proteins [65]. According to Ashfaq *et al*. (2010) [42], elevated PO activities

interfere with the signals created as a result of elevated ROS, which may be giving blackgram the ability of resistance to ULCV. Potato plants infected by *Potato virus* PVX and PVY was accompanied by an upsurge of 39.4 % proline content and 2.4 times increase in the total peroxidase activity when compared to healthy plants [66]. *Telfairia mosaic virus* infection in *P. vulgaris*. engendered increase in peroxidase activity of 57% on 8 weeks after inoculation [67]. A significant increase in PO was observed in both ULCV susceptible cultivar CO 5 and moderately resistant cultivar VBN 6 on 14 DPI. PO was found to increase in moderately resistant cultivar more than the susceptible CO 5 [47].

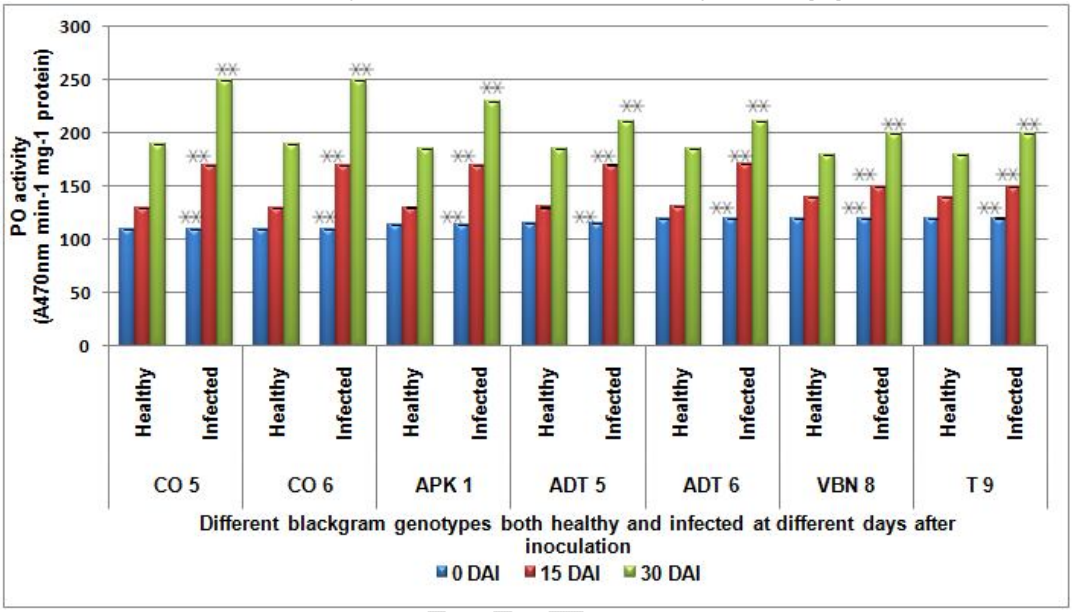

Fig. 3. Specific activity of peroxidase in different blackgram germplasms at different days after inoculation

Results are the mean±SE of three plants

*Test entries: significant from normal control, *P < 0.05; **P < 0.001*

3.2.3 Catalase activity

There are conflicting reports on the CAT activity in the ULCV infected blackgram plants. A significant decrease in level of CAT activity was recorded in ULCV infected resistant cultivars CO 5 (2.52±0.02 and 3.73±0.01 mg^{-1} min^{-1} protein at 15 and 30 DAI) and CO 6 (2.51±0.01 and 3.74±0.10 mg^{-1} min^{-1} protein at 15 and 30 DAI) and susceptible cultivars VBN 8 (2.24±0.02 and 2.41±0.01mg^{-1} min^{-1} protein at 15 and 30 DAI) and T 9 (2.24±0.01 and 2.44±0.10 mg^{-1} min^{-1} protein at 15 and 30 DAI) when compared to the healthy cultivars (Fig 4). This study was also supported by the reports of several researchers [43,55,68,69]. Similar declining trend of CAT activity was observed in other crops such as French bean infected by *white clover mosaic virus* [55] and in *Tobacco Mosaic Virus* (TMV) infected tobacco plants [68,69]. However, there was no significant decrease in CAT level in both the ULCV susceptible and resistant cultivars of blackgram as reported by Ashfaq *et al.,* (2010) [42] and this report was in agreement with the findings of Hernandez *et al.* (2001) [70] and Riedle- Bauer (1998) [54] who didn't observe any substantial decrease in CAT level in plum pox virus affected apricots and Cucumber Mosaic Virus (CMV) affected cucumber plants, respectively. Contrast reports to the latter were reported by Karthikeyan *et al.* (2022) [47] were the researcher witnessed suppression of CAT in ULCV inoculated resistant cultivar VBN 6 than CO 5 cultivar. Substantial increase in CAT as observed in ULCV inoculated VBN 6 in the later stages of development *i.e* on 14 DPI.

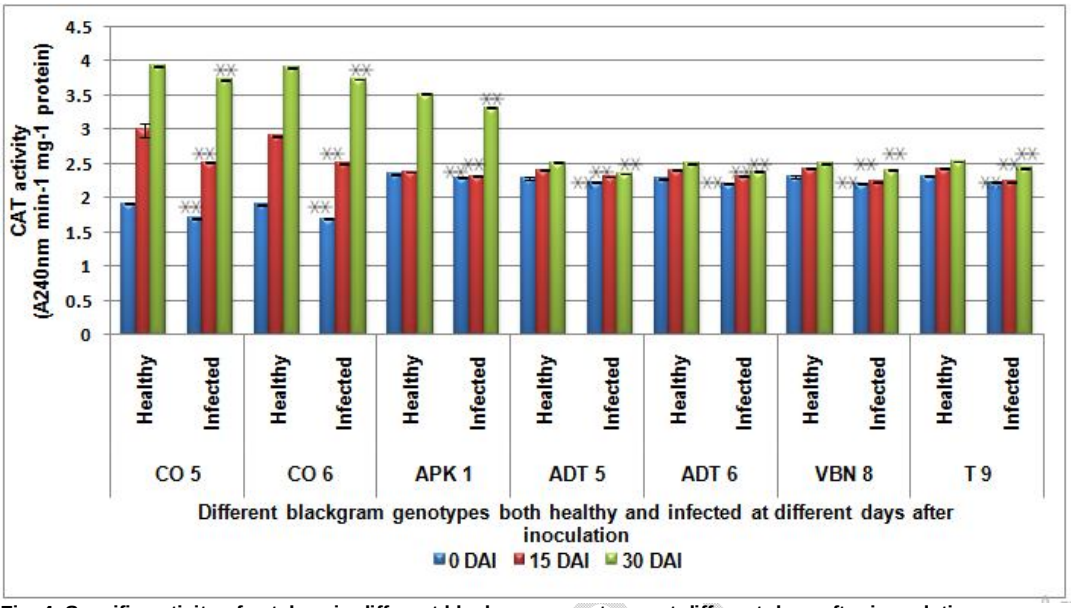

Fig. 4. Specific activity of catalase in different blackgram genotypes at different days after inoculation

Results are the mean±SE of three plants

*Test entries: significant from normal control, *P < 0.05; **P < 0.001*

4. CONCLUSION

The findings of the current experiments are consistent with earlier research that found POs were induced by viral infections spread by insect vectors. Insect infestation or viral infection in plants causes an increase in PO activity, which detoxifies the peroxides and lessens plant tissue damage. Plants create a variety of defense-related enzymes and other protein-based defensive chemicals to counteract the biotic and abiotic stressors. The variation in SOD, CAT, and total protein levels has been demonstrated as a line of defense assisting in the establishment of resistance in plants. These findings shed light on the seven genotypes of blackgram's diverse defensive responses to vector-mediated ULCV and provide insight into plant resistance in virus-plant interactions. The development of enzyme markers for determining insect vector mediated resistance and/or susceptibility in blackgram genotype may also be facilitated by the use of such knowledge. By comparing the gene expression of resistant and susceptible genotypes, this research will enable comparisons of the biological pathways that contribute to plant resistance to viruses and insects as well as aid to identify the genes causing the resistance. In order to screen additional blackgram genotype for the existence of these genes, it is necessary to identify genes that are specific to the resistant blackgram genotypes. These genes may then serve as valuable markers for tolerance.

REFERENCES

1. Vavilov, NI. Studies on the origin of cultivated plants. Bulletin Appl. Botany Plant Breeding. 1926; 16:1-248.
2. Ganguly PR, Bhat KV. Study of the pattern of variation for microsatellite markers in black gram, *Vigna mungo* (L.) Hepper germplasm. Double Helix Research-International Journal of Biomedical and Life Sciences. 2013; 3(1): 168-173.
3. Singh JM, Kaur A, Chopra S, Kumar R, Sidhu MS, Kataria P. Dynamics of Production Profile of Pulses in India. Legume Research-An International Journal. 2022; 45(5):565-72.
4. Lawn RJ, Ahn CS. Mung bean (*Vigna radiata* (L.) Wilczek. In: RJ Summerfield and EH Roberts (eds.), Grain legume crops. William Collins Sons & Co. Ltd, London. 1985; 584-623.
5. Rubatzky VE, Yamaguchi M. World vegetables principles, production, and nutritive values. Fruits. 1997; 5(51):381.

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6. Ravinder Reddy C, Tonapi VA, Varanavasiappan S, Navi SS, Jayarajan R. Influence of plant age on infection and symptomatological studies on urd bean leaf crinkle virus in urd bean (*Vigna mungo*). International Journal of Agriculture Sciences. 2005; 1(1):1-6.
7. Bashir MU, Ahmad ZA, Ghafoor AB. Sources of genetic resistance in mungbean and blackgram against Urdbean leaf crinkle virus (ULCV). Pak. J. Bot. 2005; 37(1):47-51.
8. Nene YL. A survey of viral diseases of pulse crops in Uttar Pradesh. GB Pant University of Agriculture and Technology. Res. Bull. 1972; 4:191.
9. Gautam NK, Kumar K, Prasad M. Leaf crinkle disease in urdbean (*Vigna mungo* L. Hepper): An overview on causal agent, vector and host. Protoplasma. 2016; 253(3):729-746.
10. Dhingra KL, Chenula VV. Studies on the transmission of urdbean leaf crinkle and chickpea leaf reduction viruses by Aphis crassivora Koch. Indian Phytopathology. 1981; 34:38–42
11. Bhardwaj SV, Dubey GS. Studies on the relationship of urdbean leaf crinkle virus and its vectors, Aphis craccivora and Acyrthosiphon pisum. Journal of Phytopathology. 1986;115(1):83-8.
12. Narayansamy P, Jaganathan T. Vector transmission of blackgram leaf crinkle virus. Madras Agric J. 1973; 6(7):651–652
13. Rasmann S, Agrawal AA. Plant defense against herbivory: progress in identifying synergism, redundancy, and antagonism between resistance traits. Curr Opin Plant Biol. 2009; 12:473-8
14. Sharma HC, Sujana G, Rao DM. Morphological and chemical components of resistance to pod borer, *Helicoverpa armigera* in wild relatives of pigeonpea. Arthropod-Plant Interact. 2009; 3:151-61
15. Usha Rani P, Jyothsna Y. Biochemical and enzymatic changes in rice as a mechanism of defense. Acta Physiol Plant. 2010; 32:695-701
16. Siddique Z, Akhtar KP, Hameed A, Sarwar N, Imran-Ul-Haq, Khan SA. Biochemical alterations in leaves of resistant and susceptible cotton genotypes infected systemically by cotton leaf curl *Burewala* virus. Journal of Plant Interactions. 2014; 9(1):702-11.
17. Franceschi VR, Krokene P, Christiansen E, Krekling T. Anatomical and chemical defenses of conifer bark against bark beetles and other pests. New Phytol. 2005; 167:353-75
18. Kessler A, Baldwin IT. Plant responses to insect herbivory: the emerging molecular analysis. Annu Rev Plant Biol. 2002; 53:299-328
19. Agrawal AA, Fishbein M, Jetter R, Salminen JP, Goldstein JB, Freitag AE, *et al*. Phylogenetic ecology of leaf surface traits in the milkweeds (*Asclepias* spp.): chemistry, ecophysiology, and insect behavior. New Phytol. 2009; 183:848-677.
20. Broz AK, Broeckling CD, De-la-Peña C, Lewis MR, Greene E, Callaway RM, *et al*. Plant neighbor identity influences plant biochemistry and physiology related to defense. BMC Plant Biol. 2010; 10:115
21. Arora A, Sairam RK, Srivastava GC. Oxidative stress and antioxidative system in plants. Current science. 2002; 25:1227-38.
22. Van Loon LC. Rep., MY, Pieterse, CM. Significance of inducible defense-related proteins in infected plants. Ann Rev Phytopathol. 2006; 44:135-62.
23. Ehsani-Moghaddam B, Charles MT, Carisse O, Khanizadeh S. Superoxide dismutase responses of strawberry cultivars to infection by Mycosphaerella fragariae. Journal of Plant Physiology. 2006;163(2):147-53.
24. Hameed A, Iqbal N. Chemo-priming with mannose, mannitol and H2O2 mitigate drought stress in wheat. Cereal Research Communications. 2014; 42(3):450-62.
25. Green TR, Ryan CA. Wound-induced proteinase inhibitor in plant leaves: a possible defense mechanism against insects. Science. 1972; 175:776-7
26. Hildebrand DF, Rodriguez JG, Brown GC, Luu KT, Volden CS. Peroxidase responses of leaves in two soybean genotypes injured by two-spotted spider mites (Acari: Tetranychidae). J econ Ent 1986; 79:1459-65
27. Felton GW, Summers CB, Mueller AJ. Oxidative responses in soybean foliage to herbivory by bean leaf beetle and three-corned alfalfa leafhopper. J Chem Ecol. 1994; 20:639-50
28. Stout MJ, Fidantsef AL, Duffey SS, Bostock RM. Signal interactions in pathogen and insect attack: systemic plant mediated interactions between pathogens and herbivores of the tomato, *Lycopersicon esculentum.* Physiol Mol Plant Pathol. 1999; 54:97-114
29. Constabel CP, Yip L, Patton JJ, Christopher ME, Christopher ME. Polyphenol oxidase from hybrid poplar. Cloning and expression in response to wounding and herbivory. Plant Physiol. 2000; 124:285-95
30. Zhang LL, Wen DZ. Photosynthesis, chlorophyll fluorescence, and antioxidant enzyme responses of invasive weed *Mikania micrantha* to *Bemisia tabaci* infestation. Photosynthetica. 2008; 46:457-62
31. Taggar GK, Gill RS, Gupta AK, Sandhu JS. Fluctuations in peroxidase and catalase activities of resistant and susceptible black gram (*Vigna mungo* (L.) Hepper) genotypes elicited by *Bemisia tabaci* (Gennadius) feeding. Plant signaling & behavior. 2012 Oct 1;7(10):1321-9.
32. Bala K, Shanthi M, Murugan M, Srinivasan G, Kalpana K, Mini ML. *Aphis craccivora*: A potent transmitter of urdbean leaf crinkle virus in blackgram [*Vigna mungo (*L.) Hepper]. Biological Forum- An International Journal. 2022; 14(2a): 220-226.

33. Teja TR, Latha TKS, Priyanga T, Prabakar K, Karthikeyan G, Raveendran M, Balasubramani V. Standardization of inoculation method for the maintenance of pure inoculum of leaf crinkle virus in Urdbean (*Vigna mungo* L. Hepper). The Pharma Innovation Journal. 2021; 10(10): 2282-2285
34. Sharma PN, Sharma A, Sharma OP, Sharma SK, Garg ID. Association of an unusual filamentous virus with leaf crinkle disease of urdbean in Himachal Pradesh. Journal of Mycology and Plant Pathology. 2014; 44(3): 257-263
35. Bradford MM. A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical Biochemistry. 1926; 72: 248-254.
36. Giannopolitis CN, Ries SK. Superoxide dismutases occurrence in higher plants. Plant Physiolology. 1977; 59: 309-314.
37. Chance M, Maehly AC. Assay of catalases and peroxidases. Methods in Enzymology. 1955; 2: 764-817.
38. Liu X, Huang B. Heat stress injury in relation to membrane lipid peroxidation in creeping bentgrass. Crop Science. 2000; 40: 503-510.
39. Tukey JW. Exploratory data analysis. 1977; 2: 131-160.
40. Langham M, Glover K. Effects of *Wheat streak mosaic* virus (genus: Tritimovirus, family: *Potyviridae*) on spring wheat. Phytopathology. 2005; 95: 556.
41. Yardimci N, Eryigit H, Erdal I. Effect of *Alfalfa mosaic virus* (AMV) on the content of some macro-and micronutrients in alfalfa. Journal of Culture Collections. 2007; 5: 90-93.
42. Ashfaq M, Khan MA, Javed N, Mughal SM, Shahid M, Sahi ST. Effect of urdbean leaf crinkle virus infection on total soluble protein and antioxidant enzymes in blackgram plants. Pakistan journal of Botany. 2010 1;42(1):447-54.
43. Srivastava S, Singh AK. Changes in catalase activity and total protein content in Urdbean [Vigna mungo (L.) Hepper] plants as a result of UL CV Infection. Indian Journal of Scientific Research. 2010;1(2):67-9.
44. Thind SK, Monga PK, Kaur N, Cheema SS. Analysis of some biochemical and micro-nutrient constituents of yellow mosaic virus infected moong. Indian Journal of Virology.1996; 12(2): 157-159.
45. Taiwo MA, Akinjogunla J. Cowpea viruses: Quantitative and qualitative effects of single and mixed viral infections. African Journal of Biotechnology. 2006; 5(19): 1749-1756.
46. Brar JS, Rataul HS. Leaf crinkle virus induced biochemical changes in mash bean (*Vigna mungo*) and its effect on Aphis craccivora Koch. Journal of Insect Science. 1990; 3(1):62-6.
47. Karthikeyan A, Akilan M, Samyuktha SM, Ariharasutharsan G, Shobhana VG, Veni K, Tamilzharasi M, Keerthivarman K, Sudha M, Pandiyan M, Senthil N. Untangling the Physio-Chemical and Transcriptional Changes of Black Gram Cultivars After Infection With Urdbean Leaf Crinkle Virus. Frontiers in Sustainable Food Systems. 2022; 266.
48. Madhumitha B, Karthikeyan A, Devi GP, Aiyanathan KE, Sudha M. Comparative evaluation of biochemical changes in the leaves of resistant and susceptible mungbean plants infected by Mungbean Yellow Mosaic Virus. Research Journal of Biotechnology Vol. 2020; 15:2.
49. Shetty NP, Mehrabi R, Lütken H, Haldrup A, Kema GH, Collinge DB, Jørgensen HJ. Role of hydrogen peroxide during the interaction between the hemibiotrophic fungal pathogen Septoria tritici and wheat. New Phytologist. 2007; 174(3):637-47.
50. Chakraborty N, Basak J. Molecular and biochemical characterization of mungbean yellow mosaic India virus resistance in leguminous host Vigna mungo. Journal of plant biochemistry and biotechnology. 2018; 27(3):318-30.
51. Zhang XN, Wang XR, Zhang L, Ahammed GJ, Li QY, Li X. Epigallocatechin-3-gallate enhances tomato resistance to tobacco mosaic virus by modulating RBOH1-dependent H2O2 signaling. Plant Physiology and Biochemistry. 2020; 150:263-9.
52. Buonaurio R, Montalbini P. Peroxidase, superoxide dismutase and catalase activities in tobacco plants protected against *Erysiphe cichoracearum* by a necrotic strain of *Potato virus Y*. Rivewe of Patologia Vegetale. 1993; 3: 23-31.
53. Zhuang BC, Xu B, Liao L. Change of superoxide dismutase, peroxidase and storage protein in soyabean leaves after infection with *Soyabean mosaic virus*. *Acta* Phytopathol Sinica. 1993; 23: 261-355.
54. Riedle BM. Activities of antioxidant enzymes in cucumber plants infected with *Cucumber mosaic virus*. Phyton. 1998; 38: 149-157.
55. Clarke SF, Guy PL, Burrit DJ, Jameson PE. Changes in the activities of antioxidant enzymes in response to virus infection and hormone treatment. Physiologia Plantarum. 2002; 114(2): 157-164.
56. Hernandez JA, Olmos JE, Portillo B, Rubio M, Dicenta F, Martinez-Gomez P. Effect of *Plum pox virus* infection on photosynthesis and antioxidant enzyme activity in peach 'GF305'. Acta Hort. (ISHS). 2004; 657: 349-353.
57. Karthikeyan G, Doraisamy S, Rabindran R. Induction of systemic resistance in blackgram (*Vigna mungo*) against urdbean leaf crinkle virus by chemicals. Archives of Phytopathology and Plant Protection. 2009; 40(1): 1-15.
58. Van Loon LC. Systemic acquired resistance, peroxidase activity and lesion size in tobacco reacting hypersensitively to *Tobacco mosaic virus*. *Physiological Plant Pathology*. 1976; 8: 231-242.
59. Nadlong L, Sequeira L. Increases in peroxidase activities are not directly involved in induced resistance to *Tobacco mosaic virus*. *Physiological Plant Pathology*. 1980; 16: 1-8.

60. Riedle-Bauer M. Role of reactive oxygen species and antioxidant enzymes in systemic virus infections of plants. Journal of Phytopathology. 2000; 148(5):297-302.
61. Mareschbacher BM, Kogel KH, Noll U, Reisener HJ. An elicitor of the hypersensitivity lignification response in wheat leaves isolated from the rust fungus *Puccinia graminis f.sp. tritici*. I. Partial purification and characterization. Z. Naturforschung.1986; 41: 830-838.
62. Dixon RA, Lamb CJ. Molecular communication in interactions between plants and microbial pathogens. Annual Review of Plant Molecular Biology. 1990; 41: 339-367.
63. Hammerschmidt R, Kuc J. Lignification as a mechanism for induced systemic resistance in cucumber. Physiology & Plant Pathology. 1982; 20: 61-71.
64. Espelie KE, Franceshi VR, Kolattukudy PE. Immunocytochemical localization and time course of appearance of an anionic peroxidase associated with suberization in wound healing potato tuber tissue. Plant Physiology. 1986; 81: 487-492.
65. Fry SC. Cross-linking of matrix polymers in the growing cell walls of angiosperms. Annual Review of Plant Physiology. 1986; 37: 165-183.
66. Baliuk NV, Laman NA, Nedved HL, Yarullina LG, Kalatskaja JN. Effect of potato plant treatment with bacillus subtilis and salicylic acid on their viral infection at water deficiency conditions. Plants stress and adaptation. 2021; 183-184.
67. Mofunanya AA, Effa EA, Ngele BA, Akomaye FA, Damian AO. Physiological interactions of Phaseolus vulgaris L. with Telfairia mosaic virus. Acta Scientific AGRICULTURE (ISSN: 2581-365X). 2021; 5(6).
68. Chen Z, Silva H, Klessig DF. Active oxygen species in the induction of plant systemic acquired resistance by salicylic acid. Science. 1993; 262: 1883-1886.
69. Neuenschwander U, Verooj L, Friedich S, Uknes H, Kessmann RJ. Is hydrogen peroxide a second messenger of salicylic acid in systemic acquired resistance. The Plant Journal. 1995; 8: 227-233.
70. Hernandez JA, Talavera JM, Martinez-Gomez P, Dicenta F, Sivella F. Response of antioxidative enzymes to *Plum pox virus* in two apricot cultivars. Physiologia Plantarum . 2001; 111: 313-321.