

Original Research Article

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

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, superoxide dismutase (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.

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.

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), *Acyrtosiphon 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.

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 (H₂O₂), superoxide (O₂⁻), 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 superoxide dismutase (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].

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 trifoliolate 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 trifoliolate leaves after inoculation in 15 to 20 days. These plants were maintained as virus inoculum for further studies in the insect-proof cages [32].

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.

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 H₂O₂. 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 H₂O₂. 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].

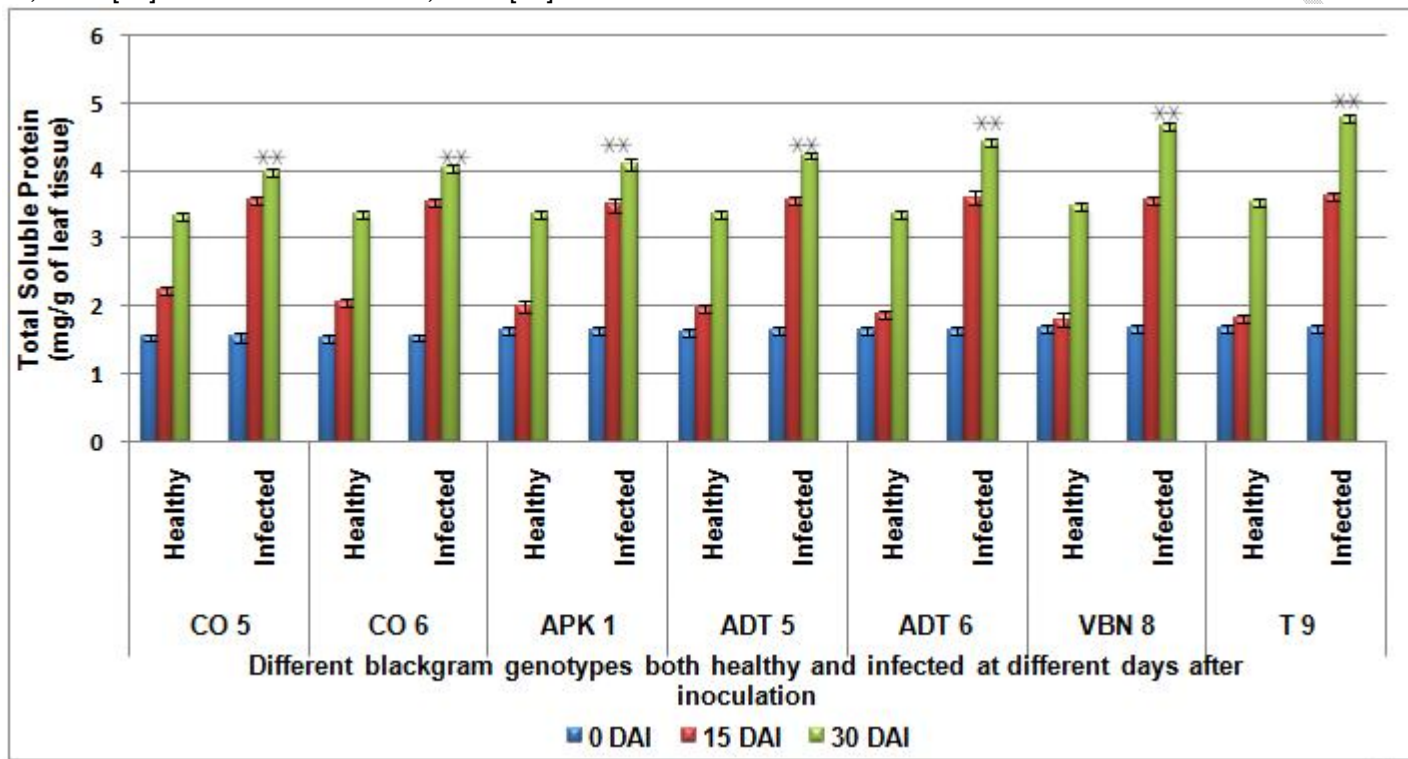


Fig. 1. Accumulation of total protein in different blackgram genotypes at different days post inoculation

Results are the mean \pm 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 by 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.

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 Buonauro 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.

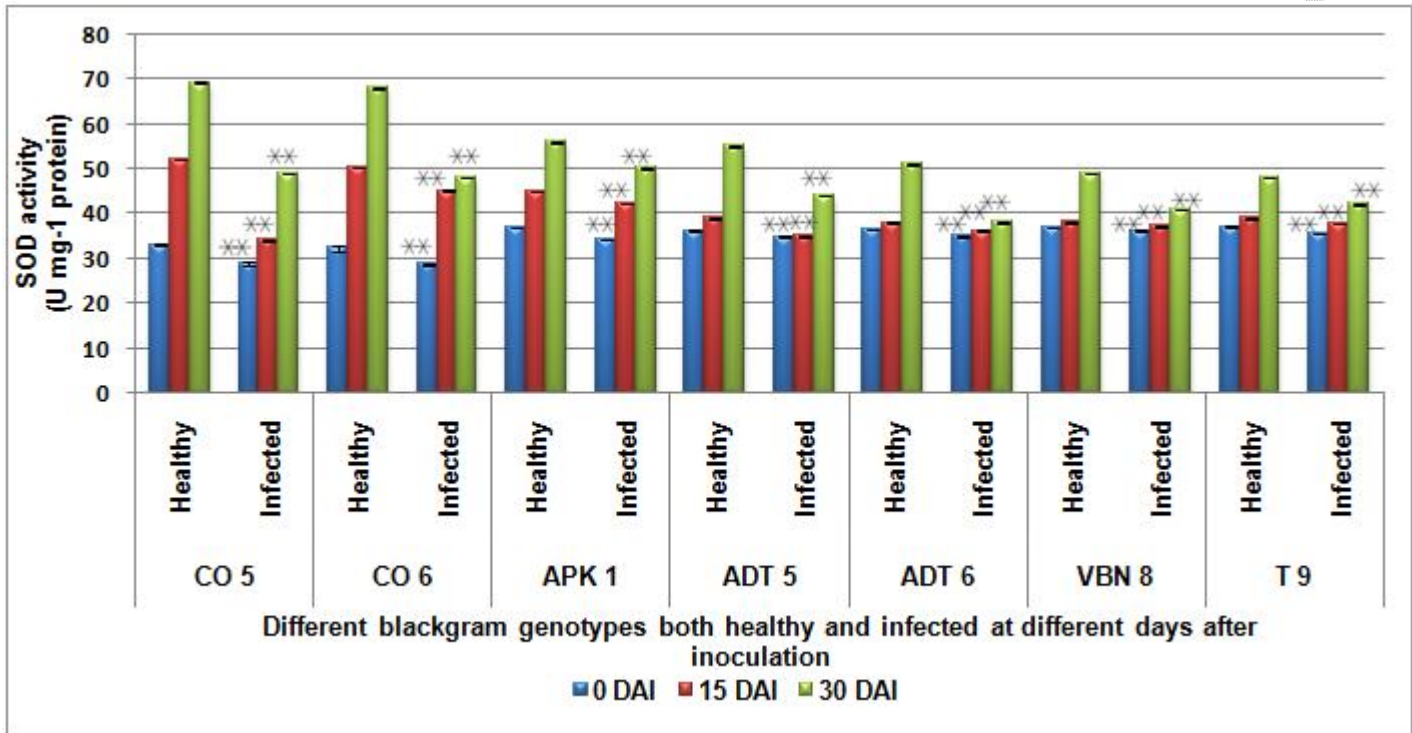


Fig. 2. Specific Activity of SOD in different blackgram genotypes at different days post inoculation

Results are the mean \pm 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 $\text{mg}^{-1} \text{min}^{-1}$ protein at 15 and 30 DAI) and CO 6 (170.22 ± 0.01 and 250.19 ± 0.06 $\text{mg}^{-1} \text{min}^{-1}$ 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 $\text{mg}^{-1} \text{min}^{-1}$ protein at 15 and 30 DAI) genotype, whereas the susceptible genotypes ADT 5 (170.45 ± 0.02 and 210.46 ± 0.01 $\text{mg}^{-1} \text{min}^{-1}$ protein at 15 and 30 DAI) and ADT 6 (170.52 ± 0.01 and 210.51 ± 0.01 $\text{mg}^{-1} \text{min}^{-1}$ protein at 15 and 30 DAI) and highly susceptible VBN 8 (150.26 ± 0.06 and 200.13 ± 0.02 $\text{mg}^{-1} \text{min}^{-1}$ protein at 15 and 30 DAI) and T 9 (150.33 ± 0.10 and 200.02 ± 0.02 $\text{mg}^{-1} \text{min}^{-1}$ 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 H_2O_2 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].

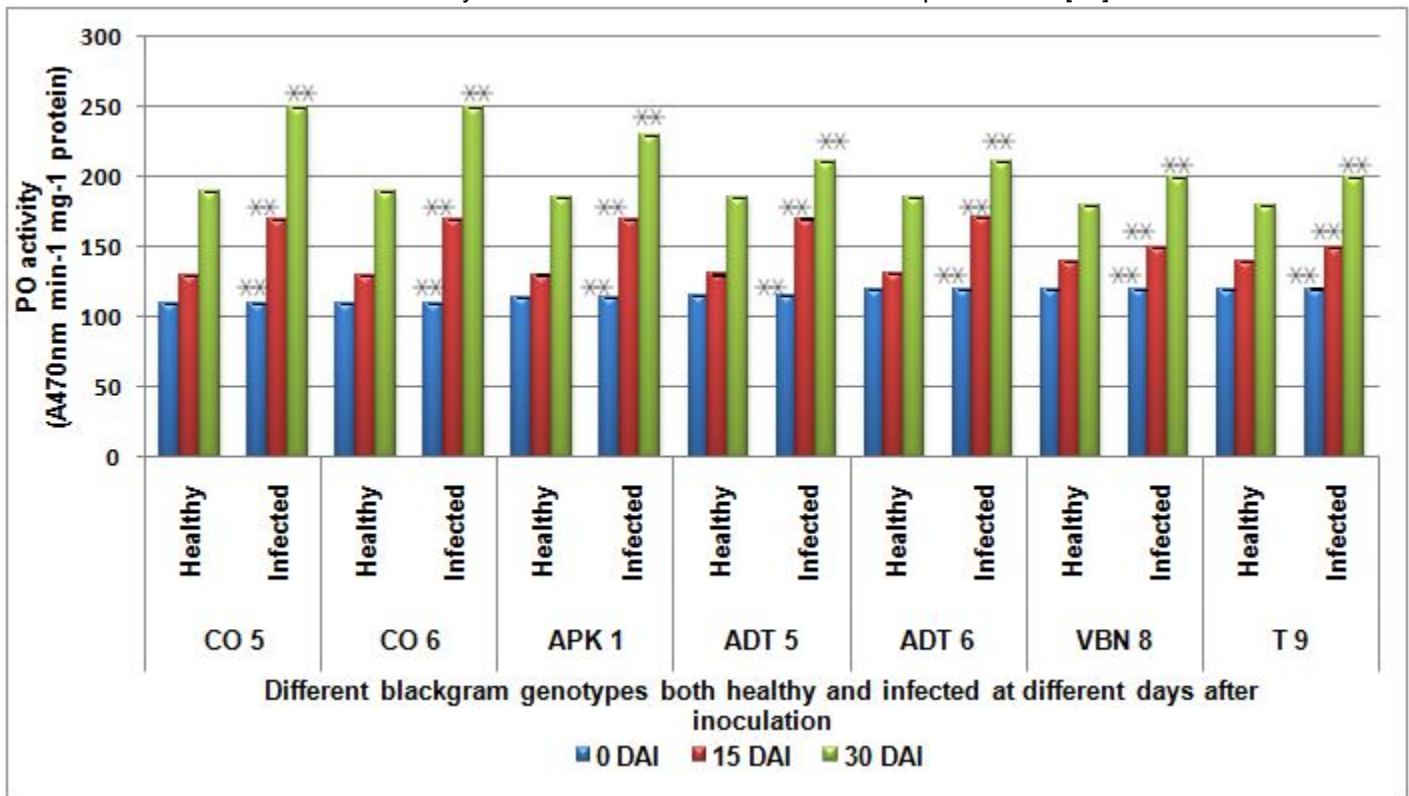


Fig. 3. Specific activity of peroxidase in different blackgram germplasms at different days after inoculation

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 $\text{mg}^{-1} \text{min}^{-1}$ protein at 15 and 30 DAI) and CO 6 (2.51 ± 0.01 and 3.74 ± 0.10 $\text{mg}^{-1} \text{min}^{-1}$ protein at 15 and 30 DAI) and susceptible cultivars VBN 8 (2.24 ± 0.02 and 2.41 ± 0.01 $\text{mg}^{-1} \text{min}^{-1}$ protein at 15 and 30 DAI) and T 9 (2.24 ± 0.01 and 2.44 ± 0.10 $\text{mg}^{-1} \text{min}^{-1}$ 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 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.

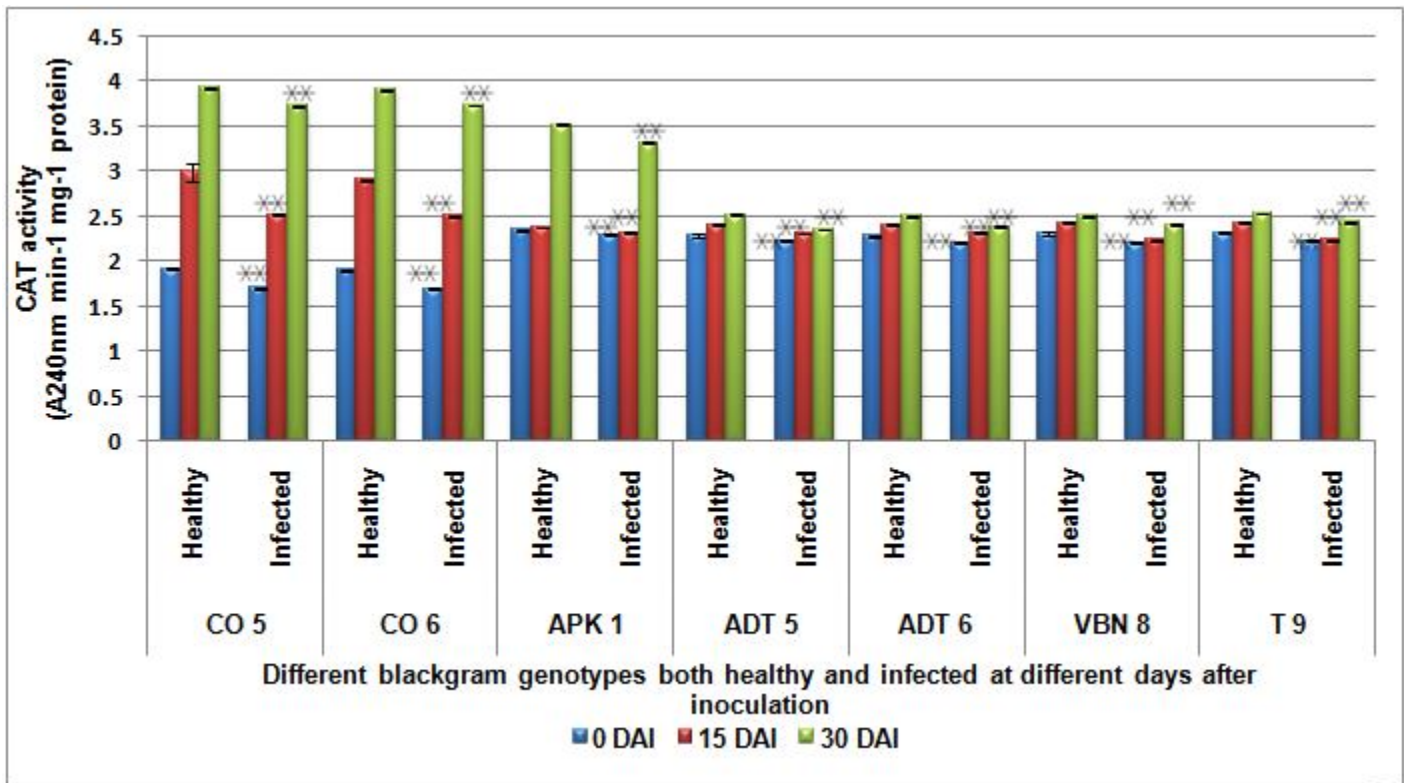


Fig. 4. Specific activity of catalase in different blackgram genotypes at different days after inoculation

Results are the mean \pm 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.

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