



Role of Antioxidative Defense in Yellow Mosaic Disease Resistance in Black Gram [*Vigna mungo* (L.) Hepper]

Yuviana J. Singh¹ · Satvir Kaur Grewal¹ · Ranjit Kaur Gill²

Received: 14 August 2020 / Accepted: 19 June 2021 / Published online: 6 July 2021
© The Author(s), under exclusive licence to Springer Science+Business Media, LLC, part of Springer Nature 2021

Abstract

Yellow mosaic virus (YMV) transmitted by whitefly (*Bemisia tabaci*) causes yellow mosaic disease (YMD) in blackgram cultivars only during *kharif* season but disease was not observed in the same cultivars sown during spring season. The main objective of the present investigation was to study the cooperative role of various antioxidative enzymes such as superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), guaiacol peroxidase (GPOX), glutathione reductase (GR), monodehydro ascorbate reductase (MDHAR), dehydroascorbate reductase (DHAR) along with non- enzymic antioxidants and free radical scavenging activities under natural epiphytotic disease conditions in black gram cultivars during *kharif* and spring season. Three black gram cultivars-KUG253 (highly susceptible to YMD), MASH114 (resistant) and Cross (KUG253 × MASH114) at F5 generation were sown during both the seasons. During the *kharif* season, YMV caused severe chlorosis only in KUG253 cultivar at 40 days after sowing (DAS) which affected its quantum efficiency by 58% as compared to the spring season. Higher specific activities of MDHAR, DHAR, and GR in Cross and MASH114 cultivars during *kharif* season at 20DAS might have helped these cultivars to fight off YMV right from the beginning of the infection. Cross cultivar showed the highest superoxide anion scavenging activity (SASA), ferric reducing antioxidant power (FRAP), 2,2-diphenyl-1-picryl hydrazyl (DPPH) free radical scavenging activity and total reducing power (TRP). On the other hand, KUG253 cultivar struggled to maintain the specific activity of CAT, GR, and DHAR as the disease progressed further. GPOX and APX enzymes might have tried to compensate for the loss of CAT enzyme but high specific activity of NADPH Oxidase promoted the production of harmful ROS which ultimately lead to higher oxidative damage in KUG253 cultivar.

Keywords Black gram · Yellow mosaic disease (YMD) · Yellow mosaic virus (YMV) · Antioxidative defense mechanism · Oxidative stress · Reactive oxygen species (ROS)

Abbreviations

YMV yellow mosaic virus
YMD yellow mosaic disease
DAS days after sowing
ROS reactive oxygen species
CAT catalase
SOD superoxide dismutase
GPOX guaiacol peroxidase
APX ascorbate peroxidase

MDHAR monodehydroascorbate reductase
DHAR dehydroascorbate reductase
GR glutathione reductase
AA ascorbic acid
DHA dehydroascorbic acid
HRSA hydroxyl radical scavenging activity
SASA superoxide anion scavenging activity
FRAP ferric reducing antioxidant power
DPPH 2,2-Diphenyl-1-picryl hydrazyl (DPPH) free radical scavenging activity
TRP total reducing power

Handling Editor: Paloma Sanchez.

✉ Satvir Kaur Grewal
satvir_pau@pau.edu

¹ Department of Biochemistry, Punjab Agricultural University, Ludhiana 141004, India

² Department of Plant Breeding and Genetics, Punjab Agricultural University, Ludhiana 141004, India

Introduction

Black gram (*Vigna mungo*) is an essential component of the vegetarian diet as it contains copious amounts of proteins, carbohydrates, soluble and insoluble fibers along with the

critical amount of folic acid and iron. Apart from the various environmental challenges such as drought, salinity, temperature variations, unpredictable weather conditions, black gram production is suffering from a substantial decrease in its productivity due to various diseases. Several diseases such as mung bean yellow mosaic virus (MYMV), powdery mildew (PM), *Cercospora* leaf spot, rust, urd bean leaf crinkle virus (UCLV) and wilt (*Fusarium oxysporum*) are responsible for severe loss in the productivity of blackgram (Pratap et al. 2018). However, the most destructive disease of the black gram crop is the yellow plague of pulses known as the yellow mosaic disease (YMD) of black gram (Archana et al. 2018). It is caused by the mung bean yellow mosaic India virus (MYMIV) which is also called the yellow mosaic virus (YMV) as it is also capable of infecting other pulse crops apart from the mungbean.

The incidence of YMD is higher in the black gram if sown as a *kharif* crop i.e. sown during the beginning of the summer rains and then harvested in the autumn; as compared to the spring season in North India because of the favorable climatic conditions for building up of white fly population during that period. Weather parameters like temperature, relative humidity and directly affect the whitefly population and incidence of yellow mosaic disease in the *kharif* season (Parihar et al. 2017). Singh and Sandhu (2019) observed that population dynamics of white fly and yellow mosaic disease incidence are governed by weather parameters based on the experiments conducted at Punjab Agricultural University (PAU), Ludhiana. They reported that the weather conditions during the *kharif* season are naturally conducive for the YMV infection because temperature, relative humidity and rainfall are naturally favourable for the growth of white fly during *kharif* season. On the other hand, during the spring season, lower relative humidity, temperature and rainfall negatively affects the whitefly population and ultimately the spread of the YMV. Garg and Patel (2018) have reported that during this period when the whitefly population is highest on the black gram, the maximum temperature, minimum temperature, rainfall, wind speed, relative humidity, and sunshine hours are 29.07 °C, 23.15 °C, 71.10 mm, 6.50 km/h, 66.57% and 13.00, respectively.

This disease is characterized by typical symptoms; mild yellow spots on young leaves at the initial stage which subsequently expand covering the whole leaf causing chlorosis, reduced plant size, decrease in the number of flowers and pods and ultimately leading to considerable yield losses. About 80% loss in yield has been reported under optimal environmental conditions due to the above-mentioned characteristic manifestations of this disease (Gupta et al. 2013). Biotic stress causes many physiological and biochemical changes in the plant due to the production and accumulation of many cytotoxic reactive oxygen species (ROS) in the plant cell. This accumulation of free radicals is called oxidative

stress which renders the plant vulnerable to apoptosis or cell death (Ighodaro and Akinloye 2018). ROS are normally formed during photosynthesis in the chloroplasts, oxidative phosphorylation inside the mitochondria, and breakdown of hydrogen peroxide inside the peroxisomes but during the biotic stress, plants start accumulating ROS to protect them against various pathogenic infections (Diebold and Chandel 2016). This phenomenon is known as the first line of defense in the plant. The process of accumulation of ROS is almost similar in case of any biotic stress in plants irrespective of the type of microorganism and the host involved (Ighodaro and Akinloye 2018). These partially reduced species of oxygen are highly reactive and have a very short half-life. These reactive species of oxygen are superoxide radical ($O_2^{\cdot-}$), singlet oxygen (O_2^1), hydrogen peroxide (H_2O_2), and hydroxyl radical (OH^{\cdot}).

Low pH results in the inevitable dismutation of the oxygen molecules. This dismutation is either excitation or reduction giving rise to singlet oxygen (O_2^1) and superoxide radical ($O_2^{\cdot-}$) respectively. The superoxide radical again dismutates to give rise to another reactive molecule called hydrogen peroxide (H_2O_2). Hydrogen peroxide (H_2O_2) is the initiator of the Fenton reaction or the Haber Weiss process as it reacts with the ferrous ions to give rise to the most harmful species of ROS i.e. hydroxyl radical (OH^{\cdot}). The high concentration of these harmful byproducts damages the protein molecules, DNA, and cause peroxidation of the lipids in the cell membrane. Apart from causing damage to the cell, they also act as signaling molecules to induce enzymatic and non-enzymatic components of the antioxidative defense mechanism in plants (Foyer et al. 2018).

Various plant organelles directly involved in the scavenging of the free radicals are mitochondria, chloroplasts, peroxisomes, endoplasmic reticulum and apoplasts (Mignolet-Spruyt et al. 2016). The aberrant changes caused by this virus in the leaves are variation in chlorophyll fluorescence, decreased levels of chlorophyll pigments, reduced efficiency of photosynthesis, and reduction in the number of stomata (Murray et al. 2016, Otulak et al. 2015 and Lehto et al. 2003). Therefore, the chloroplasts are one of the essential organelles that integrate the viral signals and transmit the pre defense signals for amplification based on ROS accumulation (Serrano et al. 2016).

The major components of the antioxidative defense mechanism are superoxide dismutase (SOD), catalase (CAT), peroxidase (POX), ascorbate peroxidase (APX), monodehydroascorbate reductase (MDHAR), dehydroascorbate reductase (DHAR) and glutathione reductase (GR), ascorbic acid and dehydroascorbic acid (DHA) (Gill et al. 2011). NADPH Oxidase produces ROS in the apoplast which acts as a signal to the other parts of the cell to resist the biotic stress caused by the pathogen. This type of resistance is known as systemic acquired resistance in the plants (Durrant and

Dong 2004). The coordinated action of these components in the plant cell forms the ROS quenching mechanism during biotic stress and hence, they are known as the antioxidative defense system of the plants.

Punjab Agricultural University, Ludhiana falls in the Indo-Gangetic plain and is situated at 36°–54'N latitude, 25°–48'E longitude and at a mean height of 247 m above sea level. PAU, Ludhiana is a hotspot location for the incidence of YMD during *kharif* season but the incidence of disease was not observed in cultivars during the spring season. YMV causing YMD in mungbean and blackgram under natural conditions at PAU, Ludhiana has been characterized (Urdbean: New Delhi: 2011) Accession number JQ 398669.1 under climatic conditions of Punjab (Ludhar et al. 2019). Two blackgram cultivars KUG253 (highly susceptible to YMV), MASH114 (resistant) and their cross (KUG253 × MASH114) showing consistent results to YMD incidence for the last five years were chosen for the present study. These cultivars were sown both during *kharif* and spring seasons. Until now, several reports have been published on the role of antioxidative defense mechanism during abiotic stress but very limited research articles have been published regarding YMD resistance in plants. A comprehensive study in black gram cultivars differing in their behavior towards incidence of yellow mosaic disease in different seasons will help in comprehending the role of antioxidative defense mechanism in imparting resistance against YMD in black gram.

Materials and Methods

Plant Material, Sowing, and Collection of Samples

The seeds of two black gram cultivars, KUG253 (highly susceptible to YMV), MASH114 (resistant) and their Cross (KUG253 × MASH114) in the F5 generation were obtained from the Department of Plant Breeding and Genetics, Punjab Agricultural University, India and were sown during *kharif* (1st week of July) and spring season (2nd week of March) in three plots of equal sizes, having four rows of 5 m each, under natural epiphytotic disease conditions. Weather conditions significantly contributed to build up of white fly population during *kharif* season. The high-relative humidity of 73%, maximum temperature of about 33 to 35° C and abundant rainfall favored the YMV infection during the *kharif* season whereas the weather conditions during the spring season such as low rainfall, mild to low temperature, and little or low rainfall hindered the growth of the whitefly population and the spread of the YMV in blackgram (Supplementary Table 1). Therefore the epidemic of YMD occurred during *kharif* season only on KUG253, but not in the spring season, which was consistently observed

for the last five years. KUG253, MASH114, and Cross (KUG253 × MASH114) were graded by a rating scale (1–9) for YMD (Singh and Bhan 1998). MASH114, KUG 253, and Cross (KUG253 × MASH114) were characterized as resistant, highly susceptible, and resistant based on rating scale of 1, 9 and 1 respectively. The leaves of the three cultivars were plucked at 20 and 40 days after sowing (DAS) corresponding to appearance of disease and severity of the disease, respectively, in the *kharif* season and also at same stages in spring season for biochemical analysis in triplicate. The leaves were green during the spring season in all the cultivars whereas during *kharif* season leaves of MASH114 and Cross (KUG253 × MASH114) were green in contrary to KUG253 leaves which were having both green and yellow colored patches at 20 DAS and were completely yellow and feeble at 40 DAS. After 40 DAS, the severity of the disease was so high during the *kharif* season that most of the leaf tissue of the susceptible cultivar (KUG253) just withered away (Fig. 1).

All the biochemical parameters were done on freshly plucked leaf samples with an equal interval between the two stages i.e. 20 DAS and 40 DAS in each season. After bringing the leaf samples into the laboratory, they were carefully washed with distilled water so that their surface was devoid of any kind of debris and soil particles. After washing, the leaves were completely dried by pressing the surface of the leaves against the folds of the filter paper. After drying, the leaves were weighed and homogenized in suitable buffers for various biochemical tests.

Quantum Efficiency by Chlorophyll Fluorescence

Quantum efficiency by chlorophyll fluorescence of the three cultivars was measured in both the *kharif* and spring season with Junior PAM (Pulse amplitude modulated) chlorophyll fluorometer (Walz). It contains WinControl-3 System Control and Data acquisition Program for data acquisition and analysis. The readings for PAR and Fv/Fm were taken with the help of Leaf-Clip Junior BD by placing it on both sides of the central vein. The efficiency of dark-adapted PSII was calculated by $Fv/Fm = (Fm - Fo)/Fm$. Fo and Fm represent the maximum and minimum fluorescence of the leaf samples in the dark.

Extraction and Estimation of Superoxide Dismutase (SOD), Catalase (CAT), Guaiacol Peroxidase (GPOX), Ascorbate Peroxidase (APX) and Glutathione Reductase (GR)

Leaf samples (100 mg) were homogenized in 2 mL of chilled 0.1 M Potassium phosphate buffer (pH 7.5) containing 1 mM EDTA, 10 mM β-mercaptoethanol and 1% (w/v) polyvinylpyrrolidone (PVP) using cold pestle and mortar.

Fig. 1 Leaves of the black gram cultivars during the *khariif* season under natural epiphytotic disease conditions at 20DAS and 40DAS



The homogenate was centrifuged at $10,000 \times g$ for 25 min at 4 °C. The supernatant after centrifugation was used in the assay of antioxidant enzymes. The same extraction procedure was followed for the estimation of catalase (CAT), guaiacol peroxidase (GPOX), ascorbate peroxidase (APX), and glutathione reductase (GR).

Estimation of Superoxide Dismutase (SOD) (EC 1.15.1.1)

Estimation of SOD was done by following the method of Marklund and Marklund (1974). 1 mL of the reaction mixture contained 0.1 M Tris HCl buffer (pH 8.2), 6 mM EDTA, and 0.1 mL of enzyme extract and reaction was initiated by adding 1.0 mL of pyrogallol solution (0.6 mM). The above components of the reaction mixture were added directly into the cuvette. Autoxidation of pyrogallol was recorded at 420 nm using spectrophotometer after an interval of 30 s up to 3 min in the absence and presence of enzyme. The reaction mixture having buffer instead of enzyme extract serve as control. One unit of enzyme activity has been defined as that amount of enzyme which causes 50% inhibition of autoxidation of pyrogallol and specific activity was calculated as units/min/mg protein.

Estimation of Catalase (CAT) (EC 1.11.1.6)

Estimation of CAT was done by following the method of Chance and Maehly (1955). The reaction mixture consisted of 1 mL of H_2O_2 solution (0.2 ml of 30% H_2O_2 was diluted to 25 mL with 50 mM sodium phosphate buffer (pH 7.5), 1.8 mL of 50 mM sodium phosphate buffer (pH 7.5) and

0.2 mL of enzyme extract was added. The hydrogen peroxide solution was added in the end to initiate the reaction. Estimation was done by utilization of hydrogen peroxide at an interval of 30 s up to 3 min by noting the decrease in absorbance at 240 nm. Catalase specific activity was measured as $\mu\text{moles of } H_2O_2 \text{ decomposed/min/mg protein}$ by using $0.0394 \text{ mM}^{-1} \text{ cm}^{-1}$ as the molar extinction coefficient.

Estimation of Guaiacol Peroxidase (GPOX)(EC 1.11.1.7)

The estimation of GPOX was done by following the method of Rao et al. (1966). The reaction mixture contained 0.05 M guaiacol and enzyme extract and the reaction was initiated with the addition of 0.8 M H_2O_2 . The rate of change in absorbance per minute was recorded at 470 nm using the UV–Visible spectrophotometer. Peroxidase specific activity has been defined as a change in absorbance/min/mg protein.

Estimation of Ascorbate Peroxidase (APX)(EC 1.11.1.1)

Estimation of APX was done by following the method of Nakano and Asada (1981). The reaction mixture (3 mL) consisted of 1 mL of 50 mM potassium phosphate buffer (pH 7.0), 0.8 mL of 0.5 mM ascorbate, 0.2 mL of enzyme extract and 1 mL of 0.4 mM H_2O_2 solution. The enzyme activity was estimated by recording the decrease in absorbance at 290 nm using a UV spectrophotometer. APX specific activity was measured in $\mu\text{moles of mono dehydro ascorbate formed/min/mg protein}$. The molar extinction coefficient

used for calculating the specific activity of ascorbate peroxidase was $2.8 \text{ mM}^{-1} \text{ cm}^{-1}$.

Estimation of Glutathione Reductase (GR) (EC 1.6.4.2)

Estimation of GR was done by following the method of Smith et al. (1988). The assay mixture contained 0.2 mL of 0.1 M potassium phosphate buffer (pH 7.5), 0.1 mL of 1.5 mM MgCl_2 , 0.1 mL of 2 mM EDTA, 0.2 mL of 0.025 mM NADPH and 0.2 mL of enzyme extract. In the end, 0.2 mL of 0.25 mM oxidized glutathione was added to start the reaction. Contents were gently mixed and the enzyme activity was estimated by observing the decrease in absorbance at 340 nm after an interval of 30 s till 3 min. Glutathione reductase specific activity was expressed as the number of μmoles of NADP^+ formed/min/mg protein by using the molar extinction coefficient of $6.22 \text{ mM}^{-1} \text{ cm}^{-1}$.

Extraction and Estimation of Monodehydroascorbate Reductase (MDHAR)(EC 1.6.5.4) and Dehydroascorbate Reductase (DHAR) (EC 1.8.5.1)

Extraction and assay of MDHAR and DHAR were done by following the method of Hossain and Asada (1984). 200 mg of the leaf tissue was homogenized with 2 mL of 100 mM phosphate buffer (pH 7.5) containing 2% PVP, 12 mM ascorbic acid and 1 mM EDTA. After homogenizing, centrifugation was carried out at 4°C at $10,000\times\text{g}$ for 25 min and the supernatant was utilized for estimating the activity of MDHAR and DHAR enzymes.

For the assay of MDHAR, 2 mL of the reaction mixture contained 50 mM Tris HCl buffer, 0.2 mM NADPH, 2.5 mM ascorbate, 0.2 mL of enzyme extract and one unit of ascorbate oxidase. The decrease in absorbance was measured at 340 nm for 3 min. The specific activity of MDHAR was measured as μmoles of NADP^+ formed /min/ mg protein.

DHAR activity was estimated by the formation of ascorbic acid at 340 nm. 1 mL of the assay mixture contained 50 mM potassium phosphate buffer (pH 7.0), 0.5 mM dehydroascorbic acid (DHA), 2.5 mM reduced glutathione (GSH) and enzyme extract. The decrease in the absorbance values was recorded at 340 nm for 3 min. Its specific activity was expressed as μmol of ascorbic acid formed/min/mg protein.

Extraction and Estimation of NADPH Oxidase (EC 1.6.3.1)

The extraction and assay of the NADPH oxidase enzyme was done by following the method of Sarath et al. (2007). 200 mg of the leaf tissue was homogenized with 2 mL of chilled 10 mM sodium phosphate buffer. Tissue extract was

made clear by centrifuging the mixture at $16,000\times\text{g}$ for 25 min at 4°C . The leaf homogenates were precipitated with cold acetone at -20°C for 20 min. Recovery of precipitated proteins was done by centrifuging the contents at $13,000\times\text{g}$ for 25 min at 4°C . Protein pellets that were obtained after centrifugation were resuspended in buffer 50 mM Tris–HCl buffer (pH 8.0) containing 0.1 mM MgCl_2 , 0.25 M sucrose and 0.1% Triton-X 100. Later it was used for the assay of NADPH oxidase.

NADPH oxidase activity was determined by the reduction of nitroblue tetrazolium (NBT). The reaction mixture contained 0.5 mL enzyme extract and 0.5 mL of 730 μM NBT in addition to 1 mL 100 μM NADPH to initiate the reaction. NBT was converted into monoformazan with the two molecules of superoxide. Absorbance was measured by UV–Visible spectrophotometer at 530 nm. Final monoformazan concentration was calculated using an extinction coefficient of $12.8 \text{ mM}^{-1} \text{ cm}^{-1}$ and the specific activity was measured in $\mu\text{moles min}^{-1}\text{mg}^{-1}$ protein.

Extraction and Estimation of Ascorbic Acid (AA) and Dehydroascorbic Acid (DHA)

Two-hundred milligram of leaf tissue was homogenized in 2 ml of 5% chilled metaphosphoric acid and centrifugation was done at $10,000\times\text{g}$ for 10 min. The clear supernatant was used for the estimation of the content of ascorbic acid and dehydroascorbic acid.

The ascorbic acid content was measured by following the method of Law et al. (1983). The reaction mixture contained supernatant, 5 mM EDTA, 1.7% TCA, 16 mM FeCl_3 prepared in 100 mM potassium phosphate buffer (pH 7.5), 7.6% o-phosphoric acid and 44 mM bipyridyl. After the incubation of 40 min at 40°C , absorbance was measured at 525 nm. The standard curve was used for calibration, taking different concentrations of ascorbic acid (10–40 μmoles). The ascorbic acid content was measured in $\mu\text{moles g}^{-1}$ FW.

The dehydroascorbic acid content was measured by following the method of Joseph et al. (1990). The reaction mixture contained supernatant and 2,4-dinitrophenylhydrazine (2%) in 9 N H_2SO_4 . The tubes were kept at 37°C for 1 h and then cooled in an ice bath. While keeping them in the ice bath, 1 mL of 85% of H_2SO_4 was added dropwise into each test tube. The yellow color compound formed was read spectrophotometrically at 540 nm after 30 min. A standard curve was used for calibration in the range of 10–50 μmoles of dehydroascorbic acid. The dehydroascorbic acid content was measured in $\mu\text{moles/ g}$ FW.

Preparation of Methanolic Extract

Four-hundred milligram of the leaf sample was refluxed with 5 ml of 80% methanol for 1 h. The refluxed material

was filtered and the volume was made to 10 mL by washing repeatedly with methanol. This extract was used for estimation of Hydroxyl Radical Scavenging Activity (HRSA), Superoxide anion scavenging activity (SASA), Ferric reducing antioxidant power (FRAP), 2,2-diphenyl-1-picryl hydrazyl (DPPH) free radical scavenging activity and Total Reducing Power (TRP).

Hydroxyl Radical Scavenging Activity (HRSA)

Hydroxyl ion radical scavenging was measured based on the Fenton reaction system which is responsible for producing hydroxyl radicals and assayed by the method of Li et al. (2008). The reaction mixture consisted of 1 mL of 0.75 mM 1,10-phenanthroline and 1 mL of 0.75 mM ferrous sulfate, 0.5 mL of the sample was added along with 1.5 mL of 0.2 M phosphate buffer (pH 7.4). The reaction mixture without sample was termed as control. The reaction was started by adding 1 mL of 0.1% hydrogen peroxide. The absorbance was measured at 536 nm after incubating the reaction mixture at 37 °C for 60 min to calculate the percentage scavenging activity.

Superoxide Anion Scavenging Activity (SASA)

Superoxide radical scavenging activity was estimated using the spectrophotometric monitoring of the inhibition of pyrogallol autoxidation by the method of Marklund and Marklund (1974). Pyrogallol solution (6 mM) was added into a tube containing sample mixture pre-dissolved in 0.1 mM Tris–HCl buffer (pH 8.2) and EDTA (6 mM). The absorbance was measured at 420 nm using a UV–Visible spectrophotometer. The antioxidative activity was determined as the percentage of inhibition by autoxidation of pyrogallol, which was calculated from the absorbance measured in the presence and the absence of pyrogallol and reaction mixture.

Ferric Reducing Antioxidant Power (FRAP)

Ferric reducing antioxidant power (FRAP) was estimated using the method of Benzie and Strain (1996). This method is based on the ability of the sample to reduce Fe^{3+} ions into Fe^{2+} ions in the presence of TPTZ (2,4,6-Tri-(2-pyridyl)-S-triazine). FRAP reagent was formed by preparing 0.2 M Sodium acetate buffer (pH 3.6), 10 mM TPTZ in 40 mM HCl and 20 mM $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$. A working FRAP reagent was prepared before use by mixing the above solutions in the ratio of 10:1:1 respectively. 1.9 mL of FRAP reagent was incubated at 37 °C for 10 min after that 100 μL of the sample was added to it. The absorbance of the reaction mixture was measured at 593 nm after half an hour of incubation at 37 °C. FRAP activity was calculated from $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ standards (5–30 μg) run simultaneously.

2,2-diphenyl-1-picryl Hydrazyl (DPPH) Free Radical Scavenging Activity

2,2-diphenyl-1-picryl hydrazyl (DPPH) free radical scavenging activity was assessed by the method of Blois (1958). This method is based on the ability of the antioxidant to scavenge the DPPH cation radical. Into 1 mL of the methanolic extract in amber cloured glass tubes, 3 mL of the 0.1 mM DPPH reagent was added before vortexing. The reaction mixture was incubated in dark for half an hour and discoloration of DPPH was measured against reagent blank at 517 nm. The reaction mixture containing methanol instead of extract serve as control.

Total Reducing Power (TRP)

Total reducing power was calculated by the method of Sreeramulu et al. (2009). To 500 μL of methanolic extract, 0.2 M sodium phosphate buffer (pH 6.6), 1% potassium ferricyanide was added to it and the mixture was incubated at 50 °C for 20 min. Then 10% TCA was added to the reaction mixture, vortexed and centrifuged at 11,000 $\times g$. The upper layer was pipetted and mixed with the same volume of the double distilled water and finally, 0.1 mL solution of 0.1% ferric chloride was added into it. The absorbance of the reaction mixture was measured spectrophotometrically at 700 nm against reagent blank. Ascorbic acid standard (5–40 μg) was run simultaneously along with the sample.

Estimation of Total Soluble Proteins

Total soluble proteins were estimated by following the method of Lowry et al. (1951). To 0.5 mL of the sample, 2.5 mL of Lowry C (Lowry A: 2% sodium carbonate prepared in 0.1 N sodium hydroxide; Lowry B: 0.5% $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ prepared in 1% sodium potassium tartrate and Lowry C: Reagent A and Reagent B mixed in the ratio of 50:1) solution was added. The contents were then mixed properly and incubated at room temperature for 10 min. After incubation, 0.25 mL of diluted Lowry D (Folin–Ciocalteu's phenol reagent and water in 1:1 ratio) reagent was added. The intensity of the blue color developed was read after 30 min at 520 nm. The standard curve was prepared by taking varying concentrations of BSA (20–200 μg) to find the amount of protein in the samples. The specific activity of the enzyme was expressed in terms of units/min/mg protein.

Statistical Analysis

The data obtained for different biochemical parameters were presented as Mean \pm SD. The data were subjected to analysis of variance (ANOVA) using the CPCS-I package (Cheema and Singh, 1993) where the mean values were compared

with the critical difference values (CD) at 5% level of significance. Post hoc analysis was performed by the Tukey test using SPSS 16.0 software ($p \leq 0.05$). Correlation analysis was carried out by using Pearson's correlation coefficient. Different letters presented in tables and figures as superscript depict significant differences among the cultivars at different days of sowing during *kharif* and spring season at $p \leq 0.05$.

Results and Discussion

Effect of YMV Infection on Photosynthetic Efficiency in Black Gram

PAR and F_v/F_m values are important in determining photosynthetic efficiency in plants especially in stress conditions (Murchie and Lawson 2013). F_v/F_m value which represents quantum efficiency decreased significantly in the MASH114 and KUG253 in *kharif* season indicating that YMV infection affected photosynthetic efficiency in these cultivars drastically, but somehow Cross cultivar maintained almost the same quantum efficiency at 20 and 40 DAS despite the severity of the disease (Fig. 2A). Synkova et al. (2006) also reported that potato virus Y infected (PVY) plants showed photoinhibition, which in turn is responsible for reduced F_v/F_m ratios. Repression of photosynthesis related genes in susceptible blackgram cultivar after infection resulted in chlorosis of leaves (Kundu et al. 2015).

On comparing, F_v/F_m values at 20 DAS in the *kharif* season with the spring season, a 70% increase in MASH114, a 58% increase in KUG253 and 4-fold increase in cross cultivar was observed during the spring season (Fig. 2A). Similarly at 40 DAS, a significant difference in the quantum

efficiency values was observed in both the seasons. High quantum efficiency during spring season exerts upon the fact that the presence of YMV severely affected the photosynthetic efficiency of the plant. A high increase in F_v/F_m ratios significantly increased the photosynthetically active radiation (PAR) values, reflecting high photosynthetic efficiency in the absence of YMV disease in the spring season. The F_v/F_m ratio and PAR values were found to be significantly correlated ($r=0.509$) (Fig. 2B and Table 1). According to Monteith and Moss (1977), PAR value and efficient conversion of this radiation into dry matter played an indispensable role in biomass accumulation. Campoe et al. (2013) also gave a linear function between PAR and biomass accumulation. Therefore, high PAR values in the spring season at both 20 and 40 DAS in all three cultivars might suggest a high yield in the spring season than in the *kharif* season.

Effect of YMV Infection on Different Antioxidative Defense Parameters in Leaves of Black Gram

Superoxide Dismutase (SOD)

In the *kharif* season at 20 DAS, the specific activity of SOD was significantly higher in all the cultivars but it decreased significantly by 49.15-fold in MASH114, 68.8-fold in Cross cultivar and 1.7-fold in KUG253 at 40 DAS (Fig. 3A). In the spring season, specific activity significantly decreased in KUG253 and cross cultivars by 18.2 and 2.6-fold, respectively at 40 DAS (Fig. 3A). The higher specific activity of KUG253 cultivar in the *kharif* season might be responsible for the activation of the antioxidative defense system to remove the superoxide ions and convert them into hydrogen

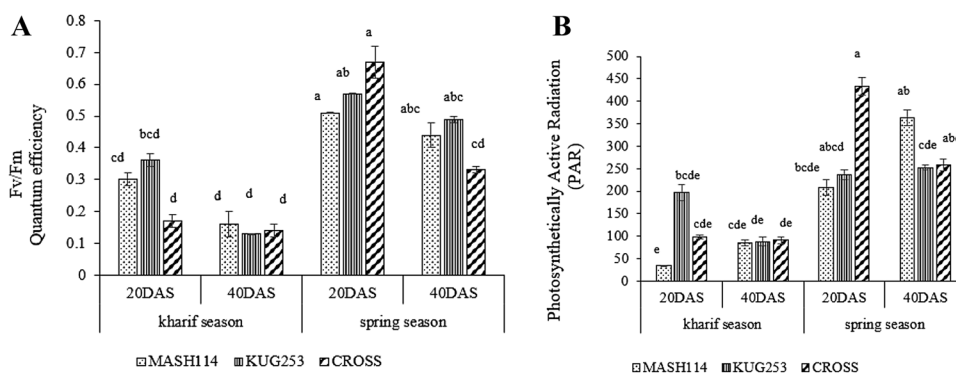


Fig. 2 A and B represent quantum efficiency and photosynthetically active radiation in leaves of black gram cultivars at different DAS in *kharif* and spring season respectively. Data represent the mean of triplicate values; error bars represent SD of triplicate values. The data were subjected to analysis of variance (ANOVA) according to which values are significant at 5% level of significance where A-NS, B-0.53, AB-NS, C-0.53, AC-NS, BC-NS, ABC-0.13 represent CD values for cultivars, stages, seasons, and interaction among each

other respectively for quantum efficiency and A-NS, B-40, AB-69.28, C-NS, AC-69.28, BC-NS, ABC-NS represent CD values for cultivars, stages, seasons and interaction among each other respectively for photosynthetically active radiation (PAR). Different letters explain the significant differences obtained by Tukey's post hoc test, among the cultivars at 20DAS and 40DAS during *kharif* and spring season at $p \leq 0.05$

Table 1 Pearson's correlation coefficient (r) between different biochemical parameters in black gram cultivars

	SOD	CAT	GPOX	APX	GR	MDHAR	DHAR	NADPHO	AA	DHA	HRSA	SASA	FRAP	DPPH	TRP	Fv/Fm	PAR
SOD	1	0.024	0.629**	-0.143	0.394*	0.393*	0.609**	-0.067	-0.107	-0.443**	-0.240	0.281	-0.246	0.385*	-0.030	0.013	0.112
CAT		1	-0.114	0.252	0.525**	-0.219	-0.205	0.010	0.553**	-0.198	-0.097	0.045	0.492**	0.098	0.540**	0.075	-0.205
GPOX			1	0.270	0.136	0.181	0.699**	0.186	-0.057	-0.305	-0.320	0.168	0.005	0.367*	0.265	-0.297	-0.055
APX				1	0.185	-0.716**	-0.284	0.721**	0.317	0.163	-0.445**	-0.327	0.573**	0.154	0.757**	-0.141	0.257
GR					1	-0.058	0.022	0.111	0.355*	-0.482**	-0.110	0.047	0.370*	0.467**	0.410*	0.158	0.110
MDHAR						1	0.722**	-0.689**	-0.184	-0.541**	0.234	0.565**	-0.348*	0.222	-0.534*	-0.258	-0.364*
DHAR							1	-0.373*	-0.053	-0.569**	-0.008	0.507**	-0.190	0.342*	-0.124	-0.504**	-0.366*
NADPHO								1	0.091	0.270	-0.346*	-0.365*	0.114	0.115	0.454**	0.131	0.447**
AA									1	-0.363*	0.147	0.085	0.692**	0.264	0.607**	-0.205	-0.137
DHA										1	-0.056	-0.216	-0.088	-0.323	0.008	0.303	0.313
HRSA											1	-0.064	-0.214	-0.249	-0.090	-0.386*	-0.247
SASA												1	-0.031	0.524**	-0.084	-0.137	-0.065
FRAP													1	0.359*	0.772**	-0.175	-0.065
DPPH														1	0.397*	0.098	0.208
TRP															1	-0.143	-0.007
Fv/Fm																1	0.509**
PAR																	1

SOD superoxide dismutase, CAT catalase, GPOX guaiacol peroxidase, APX ascorbate peroxidase, MDHAR monodehydro ascorbate reductase, DHAR dehydro ascorbate reductase, GR glutathione reductase, NADPHO NADPH oxidase, AA ascorbic acid, DHA dehydro ascorbic acid, HRSA hydroxyl radical scavenging activity, SASA superoxide anion scavenging activity, FRAP ferric reducing antioxidant power, DPPH 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activity, TRP total reducing power, Fv/Fm efficiency of dark-adapted PSII, PAR photo-synthetically active radiation

*Correlation is significant at the 0.05 level (2-tailed)

**Correlation is significant at the 0.01 level (2-tailed)

peroxide and oxygen molecules (Ehsani-Moghaddam et al. 2006).

As the infection progressed at 40 DAS in the *kharif* season, oxidative stress also increased, in order to quench the high accumulation of superoxide free radicals, the specific activity of SOD remained on the higher side in KUG253 as compared to the other two cultivars at 40 DAS. This indicated a higher accumulation of superoxide ions and their removal by the SOD enzyme during severe YMD infection. Similar results were obtained in the mesta plants infected melon fly (*Bacterocera cucurbitae*), in cucumber (*Cucumis sativus*) and Chayote (*Sechium edule*) (Somegowda et al. 2017). In the spring season, lower SOD activity at 40 DAS signified the absence of the disease and the oxidative stress in the three cultivars. High activity of superoxide dismutase was also recorded in the plants of *Hibiscus cannabinus* L. infected with *mesta yellow vein mosaic virus* as compared to the resistant variety. High SOD activity levels indicated the participation of the antioxidative defense system to surmount the biotic stress caused by the virus (Chatterjee and Ghosh 2008). Kundu et al. (2015) reported that transcripts coding for ROS homeostasis such as superoxide dismutase and ascorbate peroxidase were upregulated after MYMIV infection, with their higher expression in resistant cultivar as compared to susceptible cultivar of blackgram. Late induction and lower magnitude of these enzymes in susceptible cultivar might be responsible for oxidative stress.

Catalase (CAT)

In the *kharif* season, no significant difference was observed in the specific activity of CAT among the cultivars. The high specific activity of CAT in MASH114 and cross cultivar at 20 and 40 DAS was responsible for the efficient removal of the hydrogen peroxide molecules produced by the SOD during the infection (Wu et al. 1997).

The low-specific activity of CAT in the KUG253 cultivar as compared to the other two cultivars at 40 DAS was in agreement with the low CAT activity observed in the Urdbean leaf crinkle virus (ULCV)-infected urdbean cultivars (Srivastava and Singh 2010). Low-specific activity in the infected cultivar could result from induced oxidative stress in the plant cell which might have resulted in the production of peroxisomal endopeptidases and its proteolysis (Palma et al. 2002). According to Mittler (2002) induction of NADPH oxidase and suppression of CAT was necessary for inducing programmed cell death in the plants. Magbanua et al. (2007) also reported higher CAT activity in the maize leaves which were resistant to *Aspergillus flavus* infection. Hence, high CAT-specific activity at 20 and 40 DAS in the MASH114 and Cross cultivar during *kharif* season might be responsible for efficient removal of hydrogen peroxide molecules produced by SOD enzyme (Fig. 3B). As the production of

hydrogen peroxide molecule was not allowed to reach a high value during infection due to the proper working of the CAT enzyme, this phenomenon might be responsible for providing resistance to the cultivars against the disease.

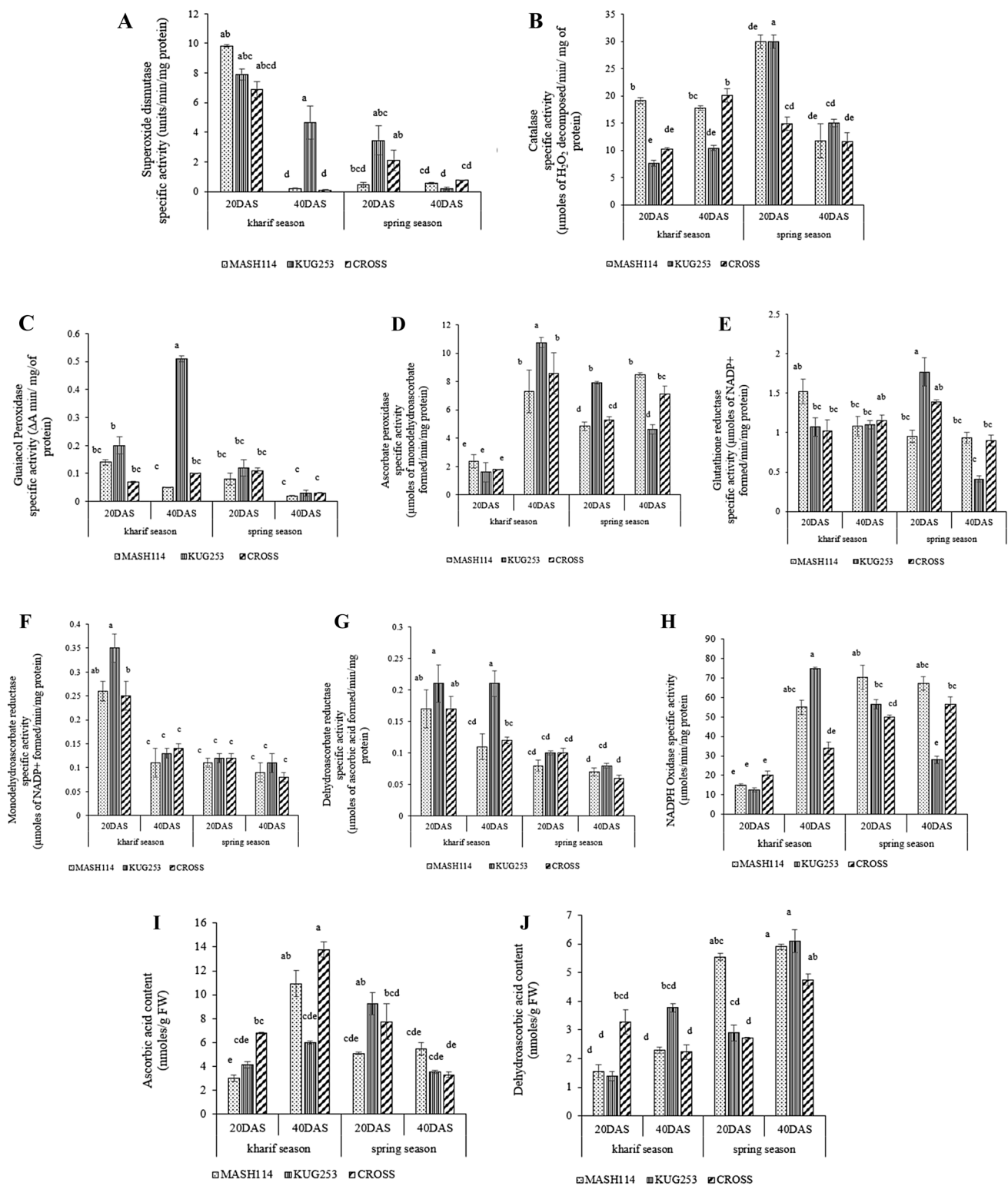
Guaiacol Peroxidase (GPOX)

In the *kharif* season, specific activity of GPOX in KUG253 and cross cultivars increased by 2.5-fold at 40 DAS (Fig. 3C). MASH114 cultivar showed a 2.26-fold decrease in value at 40 DAS. In the spring season, a non significant decrease in specific activity in all the three cultivars was seen at 40 DAS. GPOX plays an important role in curbing oxidative stress and providing resistance against infection in the plants. An increase in GPOX activity was due to the acquired resistance in the cultivar as it catalyzes the monoglignol polymerization and oxidizes phenols to toxic quinones (Brisson et al. 1994). An increase in GPOX activity in KUG253 cultivar might be due to the improper functioning of the CAT enzyme during infection as well as its induction due to the accumulation of hydrogen peroxide in the cell. Data suggested that the specific activity of SOD remained high in KUG253 cultivar at 40 DAS in the *kharif* season to remove superoxide free radicals, but CAT failed to upregulate itself, due to which the accumulation of hydrogen peroxide molecules continued in the cell. Hydrogen peroxide is a signaling molecule that upregulated GPOX enzyme to convert it into nontoxic products i.e. water and oxygen molecule (Fig. 4).

Similar results were obtained by Papaiah and Narasimha (2014) as they observed higher GPOX activity in the sunflower infected by *sunflower necrosis virus*. The higher POX activity was also observed in the leaves of wheat plants during the late stages of *P. oryzae* infection suggesting that GPOX plays a vital role in plant defense (Xavier et al. 2011).

Ascorbate Peroxidase (APX)

The specific activity of APX increased at 40 DAS as compared to 20 DAS in the *kharif* season. At 40 DAS each cultivar showed an increase in the specific activity of APX enzyme as it reduced hydrogen peroxide to water and DHA with the help of Ascorbic acid (AA) as a reducing agent. The highest increase of 6.7-fold in specific activity was observed in the KUG253 cultivar (Fig. 3D). A higher increase in the KUG253 cultivar might be due to the compensatory effect of the lower CAT activity due to the damaging effects of oxidative stress. It was observed that APX specific activity increased up to 40 DAS in both MASH114 and Cross during spring season but in KUG253, APX specific activity decreased at 40DAS. APX reduces hydrogen peroxide to water and DHA with the help of Ascorbic acid (AA) as a reducing agent. Monodehydroascorbate reductase (MDHAR)



in turn is responsible for regenerating ascorbic acid from the short-lived monodehydroascorbate (MDHA) with the help of NADPH. Dehydroascorbate reductase (DHAR) performs the reduction of dehydroascorbate (DHA) to AA with the help of reduced glutathione (GSH) as an electron

donor. Thus, DHAR in addition to MDHAR restores the AA pool and maintains the redox state of the cell. Due to this coordinated action of MDHAR and DHAR in restoring AA, both were found to be positively correlated to each other ($r=0.722$) and they both exhibited almost similar correlation

Fig. 3 **A** and **B** represent superoxide dismutase specific activity and catalase specific activity in leaves of black gram cultivars at different DAS in *kharif* and spring season respectively. Data represent the mean of triplicate values; error bars represent SD of triplicate values. The data were subjected to analysis of variance (ANOVA) according to which values are significant at 5% level of significance where A-NS, B-1.11, AB-1.94, C- 1.11, AC-NS, BC-1.58, ABC-2.74 represent CD values for cultivars, stages, seasons, and interaction among each other respectively for superoxide dismutase and A-NS, B-NS, AB-2.95, C-NS, AC-NS, BC-NS, ABC-NS represent CD values for cultivars, stages, seasons and interaction among each other respectively for catalase. Different letters explain the significant differences obtained by Tukey's post hoc test, among the cultivars at 20DAS and 40DAS during *kharif* and spring season at $p \leq 0.05$. **C, D** and **E** represent guaiacol peroxidase specific activity, ascorbate peroxidase specific activity, and glutathione reductase specific activity in leaves of black gram cultivars at different DAS in *kharif* and spring season respectively. Data represent the mean of triplicate values; error bars represent SD of triplicate values. The data were subjected to analysis of variance (ANOVA) according to which values are significant at 5% level of significance where A-0.40, B-0.32, AB-0.56, C-NS, AC-0.56, BC-0.46, ABC-0.80 represent CD values for cultivars, stages, seasons, and interaction among each other respectively for guaiacol peroxidase; A-NS, B-0.48, AB-0.83, C-0.48, AC-0.83, BC-0.67, ABC-1.17 represent CD values for cultivars, stages, seasons and interaction among each other respectively for ascorbate peroxidase and A-NS, B-NS, AB-0.NS, C-0.17, AC-0.29, BC-0.24, ABC-0.42 represent CD values for cultivars, stages, seasons, and interaction among each other respectively for glutathione reductase. Different letters explain the significant differences obtained by Tukey's post hoc test, among the cultivars at 20DAS and 40DAS during *kharif* and spring season at $p \leq 0.05$. **F, G,** and **H** represent monodehydroascorbate reductase specific activity, dehydroascorbate reductase specific activity, and NADPH oxidase specific activity in leaves of black gram cultivars at different DAS in *kharif* and spring season respectively. Data represent the mean of triplicate values; error bars represent SD of triplicate values. The data were subjected to analysis of variance (ANOVA) according to which values are significant at 5% level of significance where A-0.26, B-0.21, AB-NS, C-0.21, AC-NS, BC-0.30, ABC-0.52 represent CD values for cultivars, stages, seasons, and interaction among each other respectively for monodehydroascorbate reductase; A-0.15, B-0.12, AB-0.21, C-0.12, AC-0.21, BC-NS, ABC-NS represent CD values for cultivars, stages, seasons and interaction among each other respectively for dehydroascorbate reductase and A-5.36, B-4.37, AB-7.60, C-4.37, AC-NS, BC-6.19, ABC-10.72 represent CD values for cultivars, stages, seasons and interaction among each other respectively for NADPH oxidase. Different letters explain the significant differences obtained by Tukey's post hoc test, among the cultivars at 20DAS and 40DAS during *kharif* and spring season at $p \leq 0.05$. **I** and **J** represent ascorbic acid content and dehydroascorbic acid content in leaves of black gram cultivars at different DAS in *kharif* and spring season respectively. Data represent the mean of triplicate values; error bars represent SD of triplicate values. The data were subjected to analysis of variance (ANOVA) according to which values are significant at 5% level of significance where A-1.13, B-0.923, AB-1.59, C- NS, AC-1.59, BC-1.30, ABC-NS represent CD values for cultivars, stages, seasons, and interaction among each other respectively for ascorbic acid content and A-NS, B-0.49, AB-0.84, C-0.49, AC-0.84, BC-0.69, ABC-NS represent CD values for cultivars, stages, seasons and interaction among each other respectively for dehydroascorbic acid content. Different letters explain the significant differences obtained by Tukey's post hoc test, among the cultivars at 20DAS and 40DAS during *kharif* and spring season at $p \leq 0.05$

patterns with NADPH Oxidase, SOD, DHA and SASA (Table 1). This also explains the significant positive correlation of FRAP and TRP with AA ($r=0.692$, $r=0.607$) and APX ($r=0.573$, $r=0.757$), respectively.

Hence according to Apel and Hirt (2004) and Cuypers et al. (2011) to upregulate the antioxidative defense mechanism, APX-specific activity increased to detoxify accumulating hydrogen peroxide molecules inside the plant cell, as shown by KUG253 cultivar in the *kharif* season. Gullner et al. (1997) also observed an increase in APX activity when the leaves of *N. benthamiana* were infected with TNV and of *N. tabacum* were infected with TMV. This increase in the APX activity in the *kharif* season was also in accordance with the results of Vanacker et al. (1998) who reported that APX level increased in the barley leaves during biotrophic pathogen infection.

As from the data, it can be concluded that in the *kharif* season during YMV infection, KUG253 exhibited lower CAT activity at 40 DAS which resulted in a high amount of hydrogen peroxide molecule in the cell but on the other hand, the specific activity of APX was higher in KUG253 at 40 DAS which tried to boost the antioxidative defense mechanism in this cultivar (Fig. 5). Singh et al. (2010) have suggested that this trend may occur due to the presence of APX enzyme and absence of CAT enzyme in the chloroplasts. During severe viral infection, the damage gets localized in the chloroplasts, signaling the activation of the APX enzyme whereas the peroxisomes may get damaged at the earlier stages which in turn destroys CAT activity as well.

Glutathione Reductase (GR)

The higher specific activity of GR in MASH114 might have caused early activation of GR in MASH114 at 20 DAS which might be responsible for providing resistance against the YMD in the *kharif* season (Fig. 3E). This trend signified that APX and GR cycle was efficient in MASH114 during the *kharif* season at 20 DAS i.e. during initiation of the infection. At 40 DAS, no increase in GR activity was observed in the MASH114 cultivar indicating no oxidative stress. Higher specific activity of GR also maintained high GSH levels which increased antioxidative capacity in plants. A significant positive correlation of GR with different antioxidative enzymes such as SOD ($r=0.394$), CAT ($r=0.525$), FRAP ($r=0.370$), DPPH free radical scavenging activity ($r=0.467$) and TRP ($r=0.410$) was observed from the data (Table 1). Reduced glutathione (GSH) is required to regenerate AA from MDHA and DHA and it gets converted to its oxidized form (GSSG). GR is an integral enzyme of the ASC-GSH cycle as it helps in the formation of disulfide bonds in the glutathione to maintain equilibrium between cellular GSH/GSSG ratio during stress conditions (Moller et al. 2007). On the other hand, no significant difference in

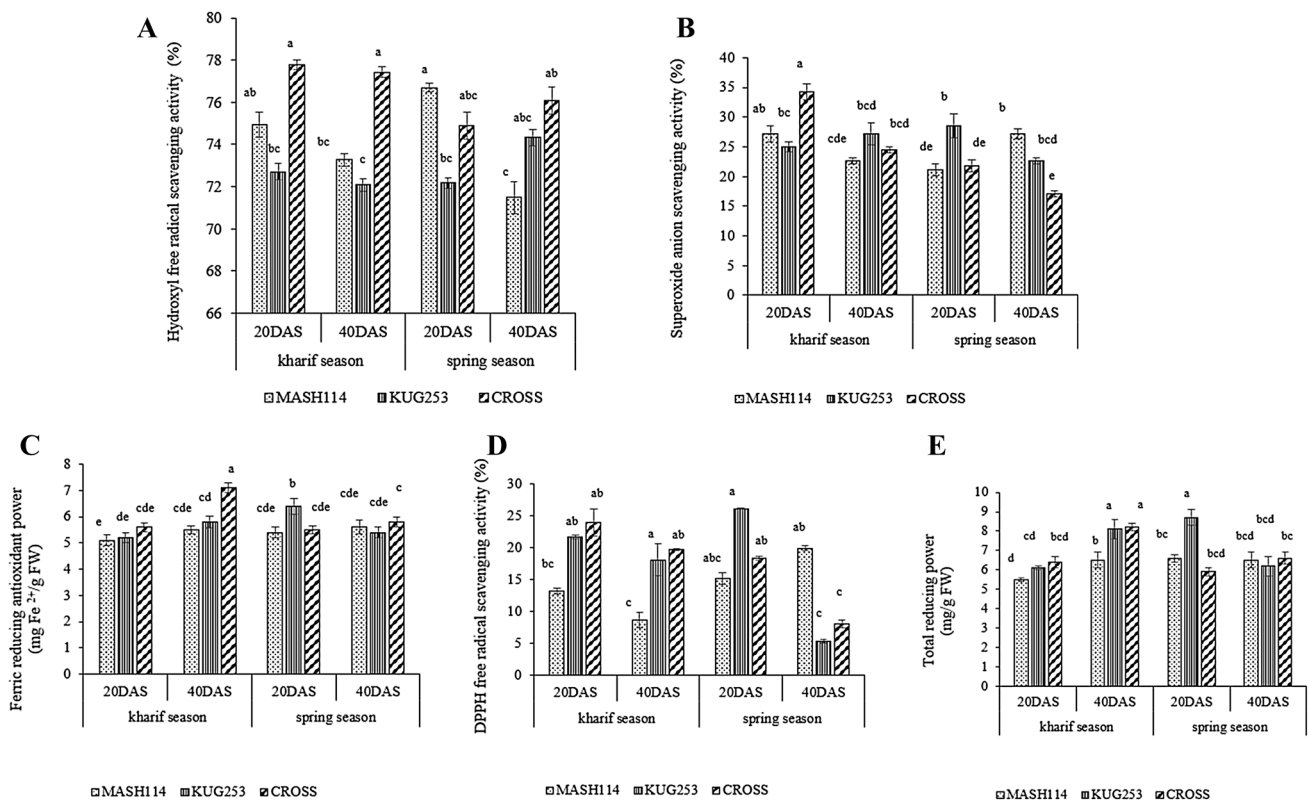


Fig. 4 **A** and **B** represent hydroxyl free radical scavenging activity and superoxide anion scavenging activity in leaves of black gram cultivars at different DAS in *kharif* and spring season respectively. Data represent the mean of triplicate values; error bars represent SD of triplicate values. The data were subjected to analysis of variance (ANOVA) according to which values are significant at 5% level of significance where A-0.97, B-NS, AB-1.40, C-0.80, AC-1.40, BC-NS, ABC-1.96 represent CD values for cultivars, stages, seasons, and interaction among each other respectively for hydroxyl free radical scavenging activity and A-1.51, B-1.23, AB-2.13, C-1.23, AC-2.13, BC-1.74, ABC-3.02 represent CD values for cultivars, stages, seasons and interaction among each other respectively for superoxide anion scavenging activity. Different letters explain the significant differences obtained by Tukey's post hoc test, among the cultivars at 20DAS and 40DAS during *kharif* and spring season at $p \leq 0.05$. **C**, **D**, and **E** represent ferric reducing antioxidant power, DPPH free radical scavenging activity, and total reducing

power in leaves of black gram cultivars at different DAS in *kharif* and spring season respectively. Data represent the mean of triplicate values; error bars represent SD of triplicate values. The data were subjected to analysis of variance (ANOVA) according to which values are significant at 5% level of significance where A-0.17, B-NS, AB-0.24, C-0.14, AC-0.24, BC-0.19, ABC-0.34 represent CD values for cultivars, stages, seasons, and interaction among each other respectively for ferric reducing antioxidant power; A-0.65, B-NS, AB-0.92, C-0.53, AC-0.92, BC-0.75, ABC-0.13 represent CD values for cultivars, stages, seasons and interaction among each other respectively for DPPH free radical scavenging activity and A-0.34, B-NS, AB-0.49, C-0.28, AC-0.49, BC-0.40, ABC-0.69 represent CD values for cultivars, stages, seasons and interaction among each other respectively for total reducing power. Different letters explain the significant differences obtained by Tukey's post hoc test, among the cultivars at 20DAS and 40DAS during *kharif* and spring season at $p \leq 0.05$

specific activities of GR was seen in KUG253 and Cross cultivars indicating the failure to maintain reduced glutathione levels in the glutathione pool under severe infection (Pandey et al. 2015).

In the spring season, high-GR specific activity was seen in the KUG253 and cross cultivar at 20 DAS which contributed towards better growth as high GR maintained good concentrations of reduced glutathione which was responsible for connecting various antioxidative and detoxification pathways in the plant cell (Noctor et al. 2002). Similarly, Hernandez et al. (2001) reported that the resistant apricot cultivars exhibited higher GR activity upon infection with

the *Plum pox virus*. Kumar et al. (2009) also found that GR activity is suppressed in maize plants infected with *Piriformospora indica* in the initial stages. Sharma et al. (2012) have also reported that GR activity decreased in the diseased tissue of *Withania somnifera* when it was infected with the leaf spot disease.

Monodehydroascorbate Reductase (MDHAR)

During the *kharif* season, the high-specific activity was observed in all three cultivars at 20DAS, which decreased by 2.7-fold at 40 DAS in the *kharif* season (Fig. 3F). As

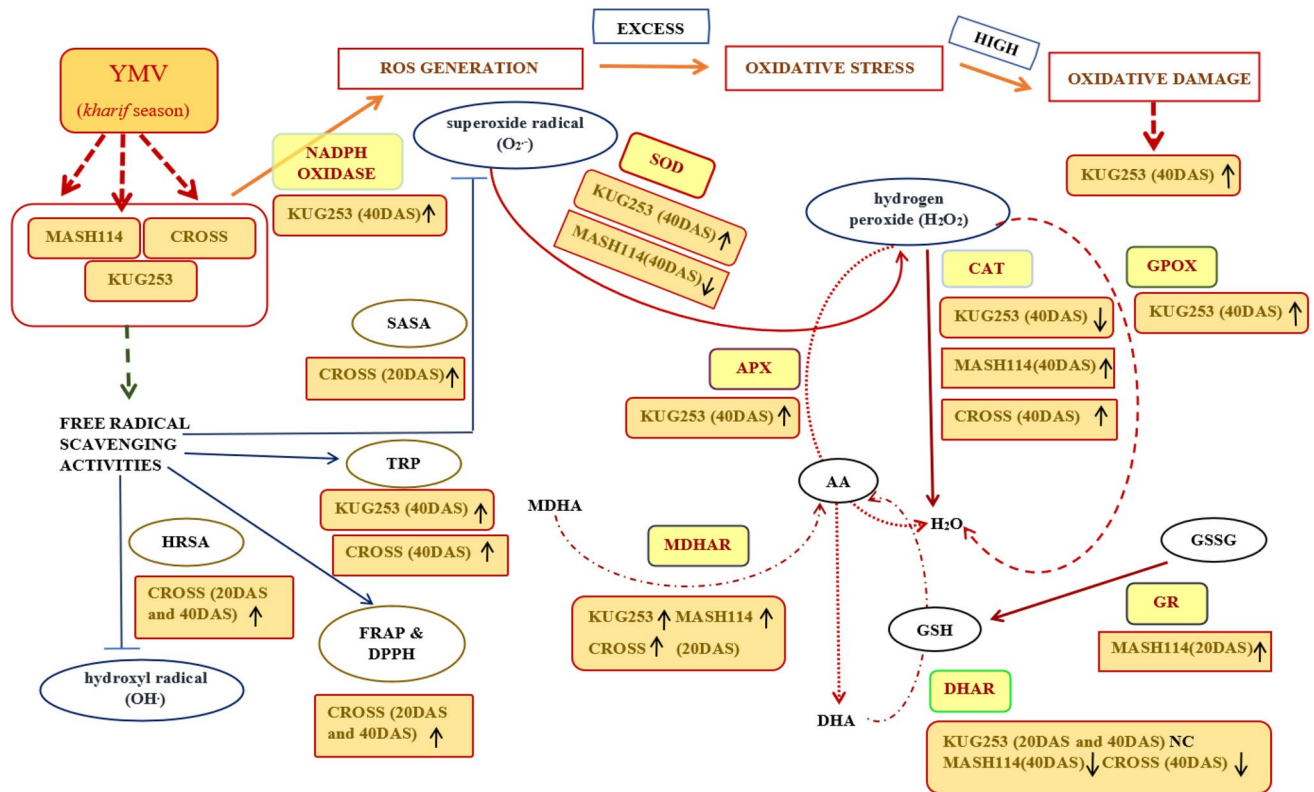


Fig. 5 Coordinated interplay of different antioxidative enzymes in providing yellow mosaic virus (YMV) resistance to different black gram cultivars. The coordinated action of this defense system provided dynamic stability to the cultivars during the YMV infection in the *khariif* season. NADPH oxidase resulted in the production of ROS which in turn signalled various antioxidative enzymes to start their functioning. The higher specific activity of SOD in the KUG253 cultivar in the *khariif* season might be responsible for the activation of the antioxidative defense system to remove the superoxide ions and convert them into hydrogen peroxide and an oxygen molecule. High CAT specific activity at 20 DAS and 40 DAS in the MASH114 and Cross cultivar during *khariif* season might be responsible for efficient

removal of hydrogen peroxide molecules produced by SOD enzyme. KUG253 exhibited low CAT activity at 40 DAS which resulted in a high amount of hydrogen peroxide molecule in the cell but on the other hand, APX and GPOX specific activity was higher in KUG253 at 40 DAS which might have tried to compensate for the loss of CAT enzyme. The higher specific activity of GR and MDHAR at 20 DAS signified the constant effort of MDHAR to control the formation of MDHA, a free radical intermediate produced by the APX enzyme. The Cross cultivar showed the highest specific activity for SASA, FRAP, DPPH, and TRP which might be responsible for providing resistance to this cultivar

MDHAR was responsible for regenerating ascorbic acid from monodehydroascorbate (MDHA), higher specific activity of MDHAR in MASH114, KUG253, and cross corresponded with the high values of ascorbic acid content at 40 DAS. The same correlation was reported by Li et al. (2008) while studying ascorbic acid accumulation in the apple fruit. Eltayeb et al. (2007) reported that overexpression of MDHAR in *A. thaliana* provided resistance against salinity, ozone, and dehydration; in the same way, it might also be responsible for providing resistance against the YMD infection.

The high MDHAR-specific activity at 20 DAS signified the constant effort of MDHAR to control the formation of MDHA, a free radical intermediate produced by the APX enzyme (Ding et al. 2011). The specific activity trend of MDHAR at 40DAS in the above cultivars during the spring season coincided with the accumulation of dehydroascorbic acid (DHA), which supported the hypothesis given by

Arrigoni (1994). Arrigoni (1994) stated that low-specific activities of MDHAR and DHAR are related to the accumulation of DHA despite replenishing the ascorbate pool.

Dehydroascorbate Reductase (DHAR)

A significant difference was seen in the DHAR-specific activity among the cultivars at 20 DAS during the *khariif* season. In the *khariif* season at 40 DAS, KUG253 maintained DHAR specific activity as compared to MASH114 and Cross cultivar (Fig. 2G). The higher DHAR specific activity might be due to low ascorbic acid content as a result of low MDHAR activity and insufficient GSH levels needed by DHAR to regenerate ascorbic acid in the KUG253 at 40 DAS in the *khariif* season. The decrease in specific activity of DHAR in MASH114 and Cross at 40DAS might be due

to the decrease in oxidative stress and efficient quenching of the ROS by other antioxidative enzymes.

Lu et al. (2008) found that DHAR activity increased in *E. odoratum* with both heat and cold stress. In the spring season, all three cultivars showed a decrease in the specific activity at 40 DAS due to developmental and seasonal changes (Chen and Gallie 2006).

NADPH Oxidase

Apart from the chloroplasts, mitochondria, and peroxisomes, NADPH Oxidase produced ROS in the apoplast which acted as a signal to the other parts of the cell to resist the biotic stress caused by the pathogen. This type of defense is known as systemic acquired resistance in the plants. A significant difference was seen among the cultivars in the *kharif* season and spring season, but the highest increase was seen in the specific activity of KUG253 cultivar (6-fold) at 40 DAS followed by a 1.3-fold increase in the cross-cultivar (Fig. 3H). On the other hand, a 28% decrease in NADPH oxidase was observed in the KUG253 during the spring season at 40DAS (Fig. 3H). Vivancos et al. (2006) reported that *Prunus* species susceptible to plum pox virus exhibited higher oxidative stress in the apoplastic space due to the high activity of NADPH oxidase and PPO. Higher NADPH oxidase activity increased ROS production inside the plant cell to resist pathogen's attack. Hence as the YMV infection grew severe during *kharif* season, NADPH oxidase specific activity increased in all the cultivars. Chakraborty and Basak (2018) observed that late increase in expression of ROS regulatory transcripts as well as their lower expression in susceptible cultivar of blackgram after MYMIV infection might be insufficient to prevent the disease development.

Ascorbic Acid (AA) and Dehydroascorbic Acid (DHA)

A significant difference was seen in the ascorbic acid content among the cultivars in the *kharif* season and spring season, but the highest increase was seen in MASH114 and Cross cultivar i.e. 3-fold at 40 DAS followed by a 2-fold increase in the cross cultivar in the *kharif* season (Fig. 3I). In the spring season, no significant difference was observed in contents at 20 and 40 DAS in MASH114, whereas a 3-fold decrease in KUG253 and a 2-fold decrease in cross-cultivars was seen at 40 DAS.

Upon infection, a significant AA regeneration, as indicated by a marked increase in AsA/DHA ratio of 4.78 and 6.18 in MASH114 and KUG253 suggested an efficient ascorbic acid rate of regeneration. This regeneration was necessary for the proper scavenging of accumulated ROS in the plants under pathogen infection (Apel and Hirt 2004). As MDHAR was responsible for regenerating ascorbic acid from monodehydroascorbate (MDHA), higher activity of

MDHAR in MASH114 and cross cultivar corresponded with the high values of ascorbic acid content at 40 DAS.

The non-significant difference in dehydroascorbic content was seen among the cultivars in the *kharif* season and spring season, but the highest increase was seen in KUG253 cultivar i.e. three-fold at 40 DAS followed by a 1.5-fold increase in the MASH114 cultivar in the *kharif* season. In the spring season, no significant difference was observed in the values at 20 and 40 DAS by MASH114, whereas a three-fold increase in KUG253 and a two-fold increase in cross cultivars was seen at 40 DAS (Fig. 3J). The suppressed activity of MDHAR in KUG253 during *kharif* season coincided with the accumulation of dehydroascorbic acid (DHA).

Fujiwara et al. (2013) studied the effect of ascorbic acid and dehydroascorbic acid accumulation in the *B. rapa* and *A. thaliana* infected with *Turnip mosaic virus*. It was reported that ascorbic acid content increased in the resistant cultivars to boost the total ascorbic acid content in the plants. Their results also suggested that ascorbic acid and dehydroascorbic acid, both have an antiviral effect on the plants under biotic stress.

Significant production and accumulation of ROS occurred during the attack of the pathogen, susceptible plants exhibited a high amount of dehydroascorbic acid content due to the continuous oxidation of ascorbic acid. This resulted in the higher DHA content in KUG253 cultivar at 40 DAS under severe YMD. Similar results were reported in tomato (*Solanum lycopersicum*) after infecting it with a weak strain of the *Cucumber mosaic virus* by Tsuda et al. (2005).

Role of Free Radical Scavenging Activities Against YMV Infection in Leaves of Black Gram

Reactive species of oxygen that are produced in nominal amounts during various metabolic processes and in excess during stress conditions are superoxide radical ($O_2^{\cdot-}$), singlet oxygen (O_2^1), hydrogen peroxide (H_2O_2) and hydroxyl radical (OH \cdot). Apart from ROS, the reactive nitrogen species (RNS) which are nitric oxide (NO), its oxidized derivatives like peroxynitrite (ONOO), S nitrosothiols, and S nitroso-glutathione (GSNO) are also produced under stressed conditions (Cheng et al. 2016). Due to the accumulation of harmful, reactive species in the cell, various defense mechanisms come into play to protect against oxidative stress by detoxifying the harmful products (Usha Rani and Jyothsna 2010).

Hydroxyl Free Radical Scavenging Activity (HRSA)

In the *kharif* season, the highest value (77%) of hydroxyl radical scavenging activity was observed in cross-cultivar at 20 and 40 DAS whereas the lowest scavenging activity (74%) was observed in MASH114 cultivar. In the spring season, the highest scavenging activity (76.7%) was observed

in MASH114 cultivar at 20 DAS followed by cross-cultivar at 40 DAS (Fig. 4A).

The higher scavenging activity of cross-cultivar was responsible for providing it better antioxidative defense capability. High hydroxyl radical scavenging activity in the Cross cultivar at 40 DAS in the *kharif* and spring season corresponded with the low NADPH oxidase activity, which signified that high hydroxyl free radical caused an increase in the NADPH oxidase activity under stress conditions as observed in the KUG253 cultivar in the *kharif* season but high hydroxyl scavenging activity decreased the production of ROS by efficiently removing the free hydroxyl species. This was also observed by its significantly negative correlation with APX ($r = -0.445$) and NADPH oxidase ($r = -0.363$) (Table 1).

Superoxide Anion Scavenging Activity (SASA)

In the *kharif* season, at 20 DAS highest value (34%) of scavenging activity was observed in the Cross cultivar whereas the lowest scavenging activity (25%) was observed in KUG253 cultivar. At 40 DAS in the *kharif* season, KUG253 showed an increase in the superoxide anion scavenging activity whereas the other two cultivars showed a decrease in activity. In the spring season, the highest scavenging activity was observed in KUG253 (28.52%) and MASH114 (27.22%) at 20 and 40 DAS, respectively (Fig. 4B).

The initial rise in the superoxide anion scavenging activity in MASH114 and cross cultivar at the initiation of the disease provided a prolonged defense to these cultivars throughout the season. On the contrary, KUG253 failed to initiate the response to scavenge the superoxide anions, as a result, the concentration of the superoxide anions might have increased in the cell which caused severe damage to its components.

Thus, the increase in scavenging activity in KUG253 at 40 DAS might be due to the excess superoxide anions generated due to the YMD infection in the cell. The superoxide anion might have produced hydrogen peroxide molecules, which further generated hydroxyl free radicals in the presence of metals. These hydroxyl free radicals might further cause lipid peroxidation and damage to the cell membrane (Mittler 2002).

Ferric Reducing Antioxidant Power (FRAP)

In the *kharif* season, the highest value of FRAP was seen in the cross cultivar at 40 DAS whereas lowest scavenging activity was seen in the MASH114 cultivar at 20 DAS, though similar scavenging activity was seen in MASH114 and KUG253 at 20 DAS. In the spring season, the highest scavenging activity was seen in KUG253 cultivar and cross-cultivar at 20 and 40 DAS, respectively (Fig. 4C). Several

plants have been reported which possessed a high amount of antioxidative activity or power, their high antioxidative power was measured in terms of high FRAP values by Aparth et al. (2012), Shantabi et al. (2014), Lalrinzuali et al. (2015) in *Cleome*, *Croton caudatus* and *Oroxylum indicum*. Cross cultivar exhibited the highest antioxidant power at 40 DAS in the *kharif* season. High antioxidant power might be responsible for imparting resistance against YMD disease. Pearson's correlation coefficient analysis also suggested a significantly positive correlation of FRAP with CAT ($r = 0.492$), APX ($r = 0.573$), AA ($r = 0.692$) and a significantly negative relation with MDHAR ($r = -0.348$).

2,2-diphenyl-1-picryl Hydrazyl (DPPH) Free Radical Scavenging Activity

In the *kharif* season, the highest value of 23% of scavenging was observed in Cross-cultivar at 20 DAS whereas the lowest scavenging activity of 8% was observed in MASH114 cultivar at 40 DAS, though KUG253 also showed 21% of scavenging at 20 DAS. In the spring season, the highest scavenging activity was observed in KUG253 cultivar (26.06%) at 20 DAS and the lowest scavenging activity was observed in the KUG253 cultivar at 40 DAS (Fig. 4D).

Somegowda et al. (2017) reported that *Melon fly (Bacterocera cucurbitae)* infected cucurbits possessed higher DPPH free radical scavenging activity which provided a good defense mechanism to the plants. Similarly, the high value of DPPH in the Cross and KUG253 cultivar might be responsible for providing resistance against the YMD. Similar to the FRAP, high DPPH values have been reported by Aparth et al. (2012), Shantabi et al. (2014), Lalrinzuali et al. (2015) in *Cleome*, *Croton caudatus* and *Oroxylum Indicum*. FRAP was also found to be significantly correlated with SOD ($r = 0.385$), CAT ($r = 0.367$), GR ($r = 0.467$), SASA ($r = 0.524$) and FRAP ($r = 0.359$).

Total Reducing Power (TRP)

The reducing capacity or power is an indicator of antioxidative capacity in the plants. Antioxidative capacity depends on several mechanisms including inhibition of chain initiation, binding with transition metal ions, peroxide decomposition, and reducing capacity (Gulcin et al. 2003).

In the *kharif* season, the similar value of scavenging activity was observed in cross cultivar and KUG253 at 20 and 40 DAS, whereas the lowest scavenging activity was observed in MASH114 cultivar at 20 and 40 DAS (Fig. 4E). The highest scavenging activity was observed in KUG253 cultivar at 20 DAS in the spring season, and similar scavenging activity was observed in all the three cultivars at 40 DAS. Several plants have been known to possess high antioxidative capacity by increasing ferric ion

reducing power such as *Cleome* by Aparadh et al. (2012). Dikilitas et al. (2011) reported high total reducing power in the *Capsicum annum*, infected with pepper mild mottle virus (PMMV). The management of the YMD is one of the major challenges in the field of agriculture. Incessant efforts of the various scientists have led to the compilation of significant data that directly points to the genetics of the YMD. Still, the biggest challenge in YMD management is comprehending and studying the effects of the YMV on different resistant cultivars (Mishra et al. 2020). YMD resistance in black gram is reported to be governed by one or two major genes and the nature of gene action varies in different genetic backgrounds, for instance, Rambabu et al. (2018) studied the genetics of YMD in an F₂ population developed from the cross between LBG759 (susceptible) and T9 (resistant) in black gram. The results indicated that the monogenic recessive gene governs the YMD resistance. Singh et al. (2020) have studied that the previously studied marker-trait association in the legumes can be similarly associated with the genes that control the YMV resistance. Therefore, several genes/QTLs are responsible for providing resistance against this disease.

Kundu et al. (2015) compared the gene expression in resistant VMR84 and susceptible T9 cultivar of *Vigna mungo* after MYMIV infection. They explored the dynamics of 345 candidate genes expressed differentially in both the cultivars. They observed that over representation of defence related genes timely in resistant cultivar VMR84 are responsible for induced expression of transcripts coding for ROS homeostasis and phenylpropanoid pathways. However, poor execution of these transcript modulations exhibiting remarkable repression of photosynthesis related genes in T9 resulted in chlorosis of leaves. RNA-Seq profiles of virus-responsive transcripts in both resistant and susceptible black gram genotypes revealed that in the resistant genotypes pathogen recognition transcripts are more promptly expressed than in the susceptible cultivars. Expression profiles of several pattern triggered immunity (PTI) related genes including receptor like kinases (RLKs), serine threonine protein kinase (STPK), cysteine rich receptor like protein kinase, BED finger in MYMIV resistant background were up-regulated relative to a susceptible host (Kundu et al. 2019). Dasgupta et al. (2021) have also reported that the resistance to MYMIV exhibits a complicated gene network that includes the production of PAMPs (Pathogen associated molecular patterns) and activation of different types of signaling cascades which in turn are responsible for the expression of specific resistance providing genes such as PR

proteins, virus resistance, and R gene proteins. A set of novel stress related EST-SSRs have also been identified from the RNA-Seq data which may be used to find the linked genes/QTLs with the YMD resistance. Recent studies indicate that the characterization of YMD using various molecular and biochemical approaches is a pressing matter which needs to be dealt with in the coming years (Nair et al. 2019).

Conclusion

The results obtained from the present investigation indicated that the antioxidative defense mechanism played a vital role in providing resistance against the YMD. The coordinated action of this defense system provided dynamic stability to the cultivars during the YMV infection in the *kharif* season (Fig. 5). NADPH oxidase resulted in the production of ROS which in turn signaled various antioxidative enzymes to control the oxidative stress. Moreover, the antioxidative scavenging activity of HRSA, SASA, FRAP, DPPH, and TRP further improved the functioning of CAT, SOD, APX, MDHAR, and DHAR enzymes. The close-knit pattern of the functions performed by these enzymes helped the cross cultivar to resist the YMV infection during the *kharif* season. SOD, GPOX, APX, MDHAR, and DHAR tried to compensate for the low specific activity of CAT to remove the ROS but the high NADPH oxidase resulted in high oxidative stress which could not be handled at a right time due to the severity of the infection (Fig. 5). During the spring season, the YMV did not infect the leaves of the susceptible cultivar (KUG253) and the resistant cultivar (MASH14 and Cross cultivar). The lower specific activity of NADPH oxidase, SOD, APX, GPOX, MDHAR, DHAR at 20 and 40 DAS suggest the absence of oxidative damage in the KUG253 cultivar in the spring season (Fig. 6). MASH14 and Cross cultivars also exhibited normal specific activity for the above-mentioned antioxidative defence enzymes, which implies that during stress-free conditions the cultivars efficiently maintained the production as well as quenching of the free radicals. These results also highlight the role of the current weather conditions in providing a better yield of the black gram. The comparison of the functioning of the antioxidative defense mechanism during the *kharif* and the spring season underlines the role of exigent weather changes, prevailing today or which might change in the future, in determining better agricultural yields.

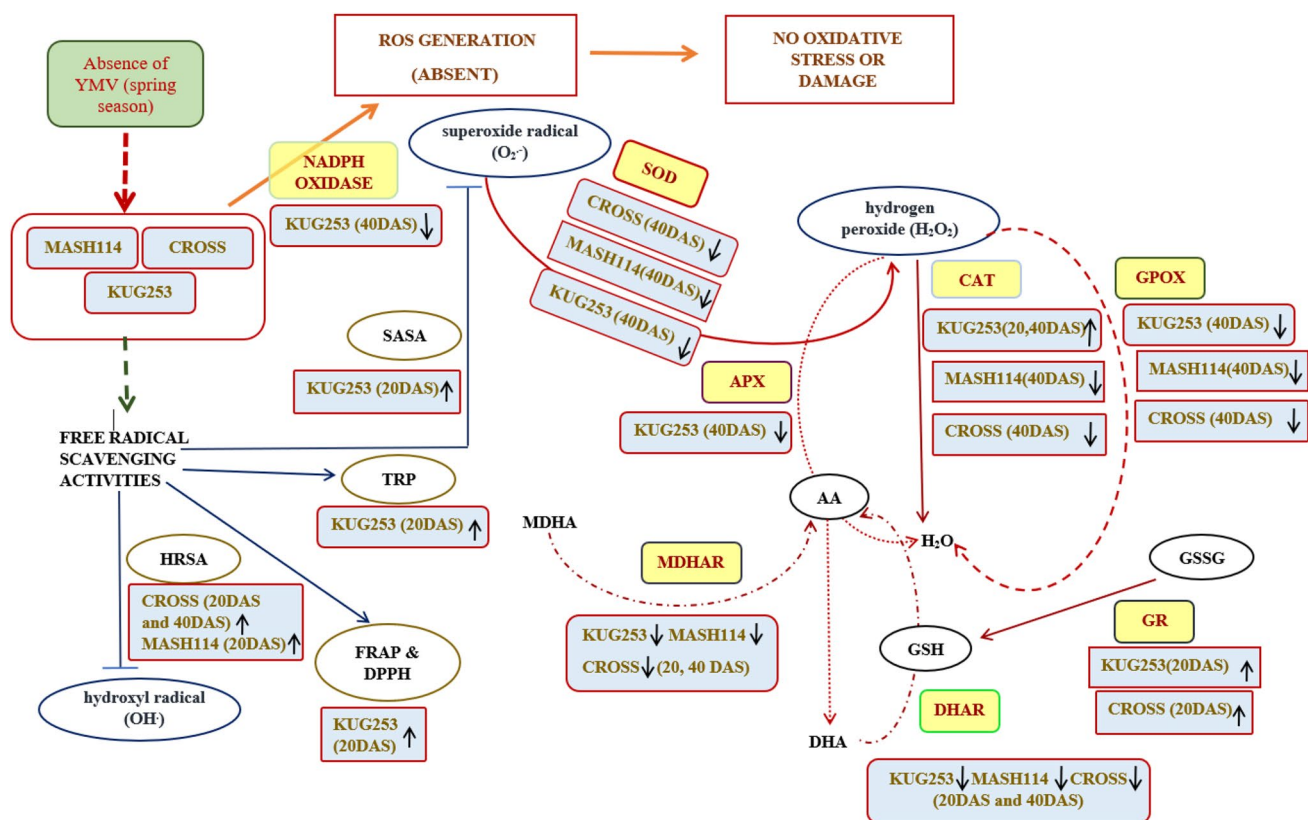


Fig. 6 Coordinated interplay of different antioxidative enzymes during the spring season. During the spring season, the YMV did not infect the leaves of the susceptible cultivar (KUG253) or the resistant cultivar (MASH114). The lower specific activity of NADPH oxidase, SOD, APX, GPOX, MDHAR, DHAR at 20 and 40 DAS suggest the absence of oxidative damage in the KUG253 cultivar in

the spring season. MASH114 and cross-cultivars also exhibited normal specific activity for the above-mentioned antioxidative defence enzymes, which implies that during stress-free conditions the cultivars efficiently maintained the production as well as quenching of the free radicals during this period

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s00344-021-10431-1>.

Acknowledgements The authors extend sincere gratitude to the Department of Plant Breeding and Genetics, Punjab Agricultural University for providing seeds of different black gram cultivars and maintaining the field for our current investigation.

Author Contributions SKG and RSG designed the experiments; YJS conducted the experiment, analyzed the data and wrote the first draft of the manuscript; SKG supervised the analysis and finalized the manuscript; RSG contributed research materials and helped in manuscript editing.

Declarations

Conflict of interest The authors declare that they have no conflicts of interest.

References

- Apardh VT, Naik VV, Karadge BA (2012) Antioxidative properties (TPC, DPPH, FRAP, metal chelating ability, reducing power and TAC) within some Cleome species. *Annali Di Botanica* 2:49–56
- Apel K, Hirt H (2004) Reactive oxygen species: metabolism, oxidative stress, and signal transduction. *Annu Rev Plant Biol* 55:373–399
- Archana S, Venkatesh PAS, Nagaraju N, Manjunatha N (2018) Management of yellow mosaic disease (YMD) of blackgram (*Vigna mungo* L.) in Southern dry zone of Karnataka. *J Entomol Zool Stud* 6:860–863
- Arrigoni O (1994) Ascorbate system in plant development. *J Bioenerg Biomembr* 26:407–419
- Benzie IF, Strain JJ (1996) The ferric reducing ability of plasma (FRAP) as a measure of antioxidant power: The FRAP assay. *Anal Biochem* 239:70–76
- Blois MS (1958) Antioxidant determination by the use of stable free radical. *Nature* 181:1199–1200
- Brisson LF, Tenhaken R, Lamb C (1994) Function of oxidative cross-linking of cell wall structural proteins in plant disease resistance. *Plant Cell* 6:1703–1712
- Campoe OC, Stape JL, Nouvellon Y, Laclau JP, Bauerle WL, Binkley D (2013) Stem production, light absorption and light use efficiency between dominant and non-dominant trees of *Eucalyptus*

- grandis* across a productivity gradient in Brazil. *Forest Ecol Manag* 288:14–20
- Chakraborty N, Basak J (2018) Comparative transcriptome profiling of a resistant vs. susceptible *Vigna mungo* cultivar in response to Mungbean yellow mosaic India virus infection reveals new insight into MYMIV resistance. *Curr Plant Biol* 15:8–24
- Chance B, Maehly AC (1955) Assay of catalases and peroxidases. *Methods Enzymol* 2:764–775
- Chatterjee A, Ghosh SK (2008) Alterations in biochemical components in mesta plants infected with yellow vein mosaic disease. *Braz J Plant Physiol* 20:267–275
- Cheema HS, Singh B (1993) CPCS 1: a programme package for the analysis of commonly used experimental designs. Punjab Agricultural University, Ludhiana
- Chen Z, Gallie DR (2006) Dehydroascorbate reductase affects leaf growth, development, and function. *Plant Physiol* 142:775–787
- Cheng F, Yin LL, Zhou J, Xia-Jian X, Shi K, Yu JQ, Zhou YH, Foyer CH (2016) Interactions between 2-cys peroxidoredoxins and ascorbate in autophagosome formation during the heat stress response in *Solanum lycopersicum*. *J Exp Biol* 67:1919–1933
- Cuypers A, Smeets K, Ruytinx J, Opendakker K, Keunen E, Remans T, Van Belleghem F (2011) The cellular redox state as a modulator in cadmium and copper responses in *Arabidopsis thaliana* seedlings. *J Plant Physiol* 168:309–316
- Dasgupta U, Mishra GP, Dikshit HK, Mishra DC, Roy A (2021) Comparative RNA-Seq analysis unfolds a complex regulatory network imparting yellow mosaic disease resistance in mungbean [*Vigna radiata* (L.) R. Wilczek]. *PLoS ONE* 16(1):e0244593
- Diebold L, Chandel NS (2016) Mitochondrial ROS regulation of proliferating cells. *Free Radic Biol Med* 100:86–93
- Dikilitas M, Guldur ME, Erel O (2011) Antioxidant and oxidant levels of pepper (*Capsicum annuum* cv. ‘Charlee’) infected with pepper mild mottle virus. *Not Bot Horti Agrobo* 39:58–63
- Ding L, Charles MT, Tsao R, Khanizadeh S (2011) Changes in ascorbate–glutathione pathway enzymes in response to *Mycosphaerella fragariae* infection in selected strawberry genotypes. *Arch Phytopathol Plant Protect* 44:712–725
- Durrant WE, Dong X (2004) Systemic acquired resistance. *Annu Rev Phytopathol* 42:185–209
- Ehsani-Moghaddam B, Charles MT, Carisse O, Khanizadeh S (2006) Superoxide dismutase responses of strawberry cultivars to infection by *Mycosphaerella fragariae*. *J Plant Physiol* 163:147–153
- Eltayeb AE, Kawano N, Badawi GH, Kaminaka H, Sanekata T, Shibahara T (2007) Overexpression of monodehydroascorbate reductase in transgenic tobacco confers enhanced tolerance to ozone, salt and polyethylene glycol stresses. *Planta* 225:1255–1264
- Foyer CH, Wilson MH, Wright MH (2018) Redox regulation of cell proliferation: bioinformatics and redox proteomics approaches to identify redox-sensitive cell cycle regulators. *Free Radic Biol Med* 122:137–149
- Fujiwara A, Shimura H, Masuta C, Sano S, Inukai T (2013) Exogenous ascorbic acid derivatives and dehydroascorbic acid are effective antiviral agents against Turnip mosaic virus in *Brassica rapa*. *J Gen Plant Pathol* 79:198–204
- Garg VK, Patel Y (2018) Influence of weather parameters on population dynamics in kharif legumes. *Ann Plant Soil Res* 20(4):371–374
- Gill SS, Khan NA, Anjum NA, Tuteja N (2011) Amelioration of cadmium stress in crop plants by nutrients management: morphological, physiological and biochemical aspects. *Plant Stress* 5:1–23
- Gulcin I, Buyukokuroglu ME, Oktay M, Kufrevioglu OI (2003) Antioxidant and analgesic activities of turpentine of *Pinus nigra* Arn. subsp. *pallsiana* (Lamb) Holmboe. *J Ethnopharmacol* 86:51–58
- Gullner G, Adam AL, Barna B, Komives T (1997) Local and systemic responses of antioxidants to tobacco mosaic virus infection and to salicylic acid in tobacco. *Plant Physiol* 114:1443–1451
- Gupta SD, Gupta S, Anjum KT, Pratap A, Kumar J (2013) Inheritance and molecular tagging of MYMIV resistant gene in black gram. *Euphytica* 193:27–37
- Hernandez JA, Talavera JM, Martínez-Gómez P, Dicenta SF (2001) Response of antioxidative enzymes to plum pox virus in two apricot cultivars. *Physiol Plant* 111:313–321
- Hossain M, Nakano Y, Asada K (1984) Monodehydroascorbate reductase in spinach chloroplasts and its participation in the regeneration of ascorbate for scavenging hydrogen peroxide. *Plant Cell Physiol* 25:385–395
- Ighodaro OM, Akinloye OA (2018) First line defence antioxidants-superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX): their fundamental role in the entire antioxidant defence grid. *Alexandria J Med* 54:287–293
- Joseph T, Vanderslice J, Gladys B (1990) Ascorbic acid and dehydroascorbic acid content of foods-as-eaten. *J Food Compos Anal* 3:105–106
- Kumar M, Yadav V, Tuteja N, Kumar AJ (2009) Antioxidant enzyme activities in maize plants colonized with *Piriformospora indica*. *Microbiol* 155:780–790
- Kundu A, Patel A, Paul S, Pal A (2015) Transcript dynamics at early stages of molecular interactions of MYMIV with resistant and susceptible genotypes of the leguminous host *Vigna mungo*. *PLoS ONE* 10(4):e0124624
- Kundu A, Singh PK, Dey A, Ganguli S, Pal A (2019) Complex molecular mechanisms underlying MYMIV resistance in *Vigna mungo* revealed by comparative transcriptome profiling. *Sci Rep* 9:8858
- Lalrinzuali K, Vabeiryureilai M, Jagetia GC, Lalawmpui PC (2015) Free radical scavenging and antioxidant potential of different extracts of *Oroxylum indicum* in vitro. *Adv Biomed Pharm* 2:120–130
- Law MY, Charles SA, Halliwell B (1983) Glutathione and ascorbic acid in spinach (*Spinacea oleracea*) chloroplasts. *Biochem J* 210:899–903
- Lehto K, Tikkanen M, Hiriart JB, Paakkanen V, Aro EM (2003) Depletion of the photosystem II core complex in mature tobacco leaves infected by the flavum strain of tobacco mosaic virus. *Mol Plant Microbe Interact* 16:1135–1144
- Li Y, Jiang B, Zhang T, Mu Z, Liu J (2008) Antioxidant and free radical scavenging activities of chickpea protein hydrolysate (CPH). *Food Chem* 106:444–450
- Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1951) Protein measurement with folin phenol reagent. *J Biol Chem* 193:265–275
- Lu H, Shen J, Sang W, Zhang X, Lin J (2008) Pollen viability, pollination, seed set and germination of crofton weed (*Eupatorium adenophorum*) in China. *Weed Sci* 56:42–51
- Ludhar NK, Sirari A, Bains TS, Sharma A, Patel SAH, Sharma P, Rani U, Cheema HK (2019) Deployment of yellow mosaic disease resistance in mungbean [*Vigna radiata* (L.) Wilczek] through interspecific hybridization. *Legume Res* 44:152–157
- Magbanua ZV, De Moraes CM, Brooks TD, Williams WP, Luthe DS (2007) Is catalase activity one of the factors associated with maize resistance to *Aspergillus flavus*? *Mol Plant Microbe Interact* 20:697–706
- Marklund SL, Marklund G (1974) Involvement of the superoxide anion radical in the autooxidation of pyrogallol and a convenient assay for superoxide dismutase. *Eur J Biochem* 47:469–474
- Mignolet-Spruyt L, Xu E, Idanheimo N, Hoerberichts FA, Muhlenbock P, Brosche M, Van Breusegem F, Kangasjarvi J (2016) Spreading the news: subcellular and organellar reactive oxygen species production and signaling. *J Exp Bot* 67:3831–3844
- Mishra GP, Dikshit HK, Tripathi K (2020) Yellow mosaic disease (YMD) of mungbean (*Vigna radiata* (L.) Wilczek): current status and management opportunities. *Front Plant Sci* 11:918
- Mittler R (2002) Oxidative stress, antioxidants and stress tolerance. *Trends Plant Sci* 7:405–410

- Moller IM, Jensen PE, Hansson A (2007) Oxidative modifications to cellular components in plants. *Annu Rev Plant Biol* 58:459–481
- Monteith JL, Moss CJ (1977) Climate and the efficiency of crop production in Britain. *Philos Trans Royal Soc B* 281:277–294
- Murchie EH, Lawson T (2013) Chlorophyll fluorescence analysis: a guide to good practice and understanding some new applications. *J Exp Bot* 64:3983–3998
- Murray RR, Emblow MS, Hetherington AM, Foster GD (2016) Plant virus infections control stomatal development. *Sci Rep* 6:34507
- Nair RM, Pandey AK, War AR, Hanumantharao B, Shwe T, Alam A (2019) Biotic and abiotic constraints in mungbean production progress in genetic improvement. *Front Plant Sci* 10:1340
- Nakano Y, Asada K (1981) Hydrogen peroxide is scavenged by ascorbate specific peroxidase in spinach chloroplasts. *Plant Cell Physiol* 22:867–880
- Noctor G, Gomez L, Vanacker H, Foyer CH (2002) Interactions between biosynthesis, compartmentation, and transport in the control of glutathione homeostasis and signaling. *J Exp Bot* 53:1283–1304
- Otulak K, Chouda M, Bujarski J, Garbaczewska G (2015) The evidence of tobacco rattle virus impact on host plant organelles ultrastructure. *Micron* 70:7–20
- Palma JM, Sandalio LM, Corpas FJ, Romero-Puertas C, McCarthy I, Del Río LA (2002) Plant proteases, protein degradation, and oxidative stress: role of peroxisomes. *Plant Physiol Biochem* 40:521–530
- Pandey P, Singh J, Achary VMM, Reddy MK (2015) Redox homeostasis via gene families of ascorbate-glutathione pathway. *Front Environ Sci* 3:25
- Papaiah S, Narasimha G (2014) Peroxidase and polyphenol oxidase activities in healthy and viral infected sunflower (*Helianthus annuus* L.) leaves. *Biotechnol An Indian J* 9:01–05
- Parihar AK, Basandrai AK, Dinakaran D, Singh D, Kannan K, Kushawaha KPS, Adinarayan M, Akram M, Latha TKS, Paranidharan V (2017) Assessment of mungbean genotypes for durable resistance to yellow mosaic disease: genotype 3 environment interactions. *Plant Breed* 136(1):94–100
- Pratap A, Prajapati U, Singh CM, Gupta S, Rathore M, Malviya N (2018) Potential, constraints and applications of in vitro methods in improving grain legumes. *Plant Breed* 137:235–249
- Rambabu E, Anuradha C, Sridhar V, Reddy SS (2018) Genetics of mungbean yellow mosaic virus (MYMV) resistance in blackgram (*Vigna mungo* (L) Hepper). *Int J Curr Microbiol App Sci* 7(4):3869–3872
- Rao PS, Sarma KG, Seshadri TR (1966) Chemical components of *Lobaria subsidiosa* L. *retigera* and *L. subretigera* from Western Himalayas. *Curr Sci* 35:147–148
- Sarath G, Hou G, Baird LM, Mitchell RB (2007) Reactive oxygen species, ABA and nitric oxide interactions on the germination of warm-season C4-grasses. *Planta* 226:697–708
- Serrano I, Audran C, Rivas S (2016) Chloroplasts at work during plant innate immunity. *J Exp Bot* 67:3845–3854
- Shantabi L, Jagetia GC, Ali MA, Singh TT, Devi SV (2014) Antioxidant potential of croton caudatus leaf extract in vitro. *Transl Med Biotechnol* 2:1–15
- Sharma P, Jha AB, Dubey RS, Pessarakli M (2012) Reactive oxygen species, oxidative damage and antioxidative defense mechanism in plants under stressful conditions. *J Bot* 2012:1–26
- Singh G, Bhan LK (1998) Diseases of mungbean and urdbean and their management. In: Upadhyay RK, Mukerji KG, Rajak RL (eds) IPM system in agriculture. Aditya Book Pvt. Ltd., New Delhi, pp 311–371
- Singh K, Sandhu SK (2019) Whitefly dynamics and disease incidence as influenced by growing environment and weather parameters in *kharif* mungbean. *Agric Res J* 56(2):340–344
- Singh BK, Sharmab SR, Singh B (2010) Antioxidant enzymes in cabbage: variability and inheritance of superoxide dismutase, peroxidase and catalase. *Sci Hort* 124:9–13
- Singh CM, Pratap A, Gupta S, Singh NP (2020) Association mapping for mungbean yellow mosaic India virus resistance in mungbean (*Vigna radiata* L Wilczek). *3 Biotech* 10:33
- Smith IK, Vierhaller TL, Thorne CA (1988) Assay of glutathione reductase in crude tissue homogenates using 5,5'-dithiobis (2-nitrobenzoic acid). *Anal Biochem* 175:408–413
- Somegowda M, Vigneshwaran V, Rajeshwar AN, Shivashankar S, Siddanakoppalu NP (2017) Biotic stress induced by bacterocera cucurbitae (melon fly) triggers defense related phenylpropanoid pathway (PPP) and ROS detoxifying enzymes in cucurbits. *Asian J Plant Sci Res* 7(4):18–29
- Sreeramulu D, Reddy CVK, Raghunath M (2009) Antioxidant activity of commonly consumed cereals, millets, pulses and legumes in India. *Indian J Biochem Biophys* 46:112–115
- Srivastava S, Singh AK (2010) Changes in catalase activity and total protein content in urd bean plants as a result of ULCV infection. *Indian J Sci Res* 2:67–69
- Synkova H, Semorádová S, Schnablová R (2006) Effects of biotic stress caused by potato virus Y on photosynthesis in transgenic and control *Bacterocera cucurbitae* L. *Plant Sci* 171:607–616
- Tsuda K, Kosaka Y, Kobori T, Kataoka M (2005) Effects of fertilizers applied on yield and vitamin C content of tomato inoculated with the attenuated isolate CM95 of cucumber mosaic virus. *Jpn J Phytopathol* 71:1–5
- Usha Rani P, Jyothsna Y (2010) Biochemical and enzymatic changes in rice as a mechanism of defense. *Acta Physiol Plant* 32:695–701
- Vanacker H, Carver TLW, Foyer CH (1998) Pathogen-induced changes in the antioxidant status of the apoplast in barley leaves. *Plant Physiol* 117:1103–1114
- Vivancos PD, Rubio M, Mesonero V, Martínez-Gómez P (2006) The apoplastic antioxidant system in *Prunus*: response to long-term plum pox virus infection. *J Exp Bot* 57:3813–3824
- Wu GS, Shortt BJ, Lawrence EB, Leon J, Fitzsimmons KC, Shah DM (1997) Activation of host defense mechanisms by elevated production of hydrogen peroxide in transgenic plants. *Plant Physiol* 115:427–435
- Xavier F, Rodrigues MS, Domiciano FA, Oliveira GP, Moreira WR (2011) Wheat resistance to leaf blast mediated by silicon. *Aust Plant Pathol* 40:28–38

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.