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Biochemical analysis of chickpea genotypes against dry root rots (*Macrophomina phaseolina* (Tassi) Goid)

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Abstract
There was an increase in the activity of peroxidase, catalase, Phenylalanine ammonia lyasetotal phenols, and total free amino acids in all the genotypes when inoculated with *M. phaseolina*. Whereas, there was decrease in the activity of total sugar, reducing sugar and non-reducing sugar in all the chickpea genotypes upon inoculation with *M. phaseolina*. Highest enzymatic activity and sugar content was recorded in moderately resistant genotypes *viz*., Phule G 12107, NDG 13-21 and IPC 2010-112 compared to susceptible genotype GNG 2228and highly susceptible genotype L550. The enhanced biochemical activities during plant pathogen interaction triggers the defense related enzymes such as lignin, suberin, wall-bound phenolics, flavonoids, lignin, induction of hypersensitive reaction (HR) etc., which resulted in cell strengthening and enhances resistance to pathogen. The depletion of sugars during host-parasite interaction might be due to increased utilization of sugars by the fungi for energy and synthetic reactions involved in multiplication of the pathogen.

Keywords: Peroxidase, Catalase, Phenylalanine Ammonia Lyase, Total Phenols and Total free Amino Acids, Total Sugars, *Macrophominaphaseolina,* Chickpea

Introduction
Chickpea (*Cicer arietinum* L.), is the second most important pulse crop in the world, India accounting for 60 to 75 per cent of the world's chickpea production. The global area under chickpea is 14.80 million ha, with production of 14.23 million tonnes and productivity of 962 kg/ha. In India, it is grown in an area of about 10.74 million ha with production of 9.88 million tonnes and productivity of 920 kg/ha.(Anon., 2015) [4]. The per hectare production is low in spite of high yielding varieties and new agronomic practices due to incidence of diseases. The crop is known to be affected by number of pathogens *i.e*., fungi, bacteria, viruses and nematodes. Soil borne diseases such as wilt, dry root rot, black root rot, collar rot, and stem rot are important in reducing the yield of the crop. The foliar diseases *viz*., Ascochyta blight and grey mould are more severe in chickpea crop. Diseases with limited distribution are economically important because of continuous changes in cultural practices, human interventions and climate change. The dry root rot (*M. phaseolina*) is a major constraint in the chickpea production as it is emerging as a potential threat to chickpea cultivation in semi-arid regions due to moisture stress and high temperatures during the flowering to pod filling stage (Sharma *et al*., 2010) [38]. The annual yield loss due to this disease alone is 10-20 per cent (Vishwadhar and Chaudhary, 2001) [47].

The dry root rot disease generally appears around flowering and podding time. The disease may also appear at seedling stage, however, the susceptibility of the plant increases with age. The disease generally appears when day temperature is more than 30 ^{0}C and soil moisture content of 60 per cent. Drooping of petioles and leaflets is confined to those at the very top of the plant. Sometimes when rest of the plant is dry, the top most leaves are chlorotic. The leaves and stems of affected plants are usually straw colored. The lower portion of the tap root usually remains in the soil when plants are uprooted. The tap root is dark and is devoid of most of its lateral and finer roots. Dark, minute sclerotial bodies can be seen on the roots or inside the wood (Nene *et al.,* 2012) [27]. *M. phaseolina* is primarily seed and soil-borne fungal pathogen. Prolonged saprophytic survival ability of the pathogen in soil makes chemical control and crop rotation ineffective. Resistant cultivars is the most practicable, feasible, and economical approach for the management of dry root rot of chickpea, but only a few sources with low level of genetic resistance are available, so there is a need to identify the resistant sources in chickpea. Biochemical analysis is important to understand the resistant mechanism before and after infection of plant and to isolate the resistance sources.

Materials and Methods

Assay of peroxidase (PO)

The peroxidase activity was assayed spectrophotometerically (Hartee, 1955) [13]. The reaction mixture consisted of 1.5 ml of 0.05 M pyrogallol, 0.5 ml of the enzyme extract and 0.5 ml of one per cent H_2O_2. The reaction mixture was incubated at room temperature (28±1 °C). The change in absorbance was recorded at 470 ηm at a time interval of 30 sec upto 3 min in Hitachi U-2900 spectrophotometer. The boiled enzyme preparation served as blank. The enzyme activity was expressed as change in the absorbance at 420 ηm/min/g on fresh weight basis.

Assay of catalase

The activity of catalase was determined by the method of Aebi (1984) [1]. With slight modification by monitoring the disappearance of H_2O_2 at 240 ηm. 3 ml H_2O_2 phosphate buffer was taken in cuvette and 100 µl enzyme extract was added. The absorbance was taken in the spectrophotometer at 240 ηm. The absorbance was allowed to decrease from 0.45 to 0.40 from the time required in seconds (Δt) against diluted phosphate buffer as blank. Δt should be less than 60 seconds. The activity was calculated by using extinction coefficient 0.036 m/M/cm. One unit of the enzyme is the amount necessary to decompose 1 mmol of H_2O_2 per min at 25 °C.

Assay of phenylalanine ammonia lyase (PAL)

PAL activity was determined as the rate of conversion of L-phenyl alanine to trans-cinnamic acid at 290 ηm as per the method described by Ross and Seder off (1992) [34]. Samples containing 0.4 ml of enzyme extract were incubated with 0.5 ml of 0.1 M borate buffer, pH 8.8 and 0.5 ml of 12 mM L-phenylalanine in the same buffer for 30 min at 30 °C. The reaction was arrested by adding 0.5 ml of 1M TCA and incubated at 37 °C for 5 min. The blank contains 0.4 ml of crude enzyme extract and 2.7 ml of 0.1 M borate buffer (pH 8.8) and absorbance was measured at 290 ηm in Hitachi U-2900 spectrophotometer. Standard curve was drawn with graded amounts of cinnamic acid dissolved in acetone. The enzyme activity was expressed as µM of trans-cinnamic acid/min/g fresh weight of tissue.

Assay of total phenols

The total phenol content was estimated as per the procedure given by Sadasivam and Manickam (1996) [35]. Two hundred milligram of powdered root sample was grinded with pestle and mortar 10 times the volume of 80 per cent ethanol. The homogenate was centrifuged at 10000 rpm for 20 minutes. The supernatant was collected and the residue was re-extracted with 5 times the volume of 80 per cent ethanol, centrifuged and supernatant was collected. The supernatant was kept in hot water bath until it gets dried. The residue was dissolved in 5 ml of distilled water and used for estimation of total phenols.

Assay of total free amino acids

Total amino acids were determined by the ninhydrin method of Yemm and Cocking (1955) [49]. With some modifications. The incubation mixture (5±1 ml final volume) containing 100 µl of the ethanolic extract, 1 ml of 80 per cent ethanol, 1 ml of 0±2 ml citrate buffer pH 5, and 2 ml of an acetonicninhydrin solution (1% ninhydrin and 0±06 % KCN in acetone), was incubated for 15 min at 100 °C. The mixture was cooled for 5 min in tap water before 8 ml of distilled water was added. The absorbance was recorded at 570 ηm. Glycine equivalents were calculated from a standard curve obtained with pure analytical grade glycine.

Assay of total sugars

The total sugar content was estimated by Anthrone method and as per the procedure given by Hedge and Hofreiter (1962) [15]. One hundred mg of the powdered root sample was hydrolysed by keeping it in a boiling water bath for 3 hrs with 5 ml of 2.5 N HCl. The tube was cooled to room temperature and the acid was neutralised with solid sodium carbonate until effervescence ceases. The volume was made to 10 ml and this was centrifuged at 1000 rpm for 5 min. The supernatant was collected and used for estimation of total sugars.

Assay of reducing sugars

The reducing sugars of powdered plant root samples was estimated by Nelson-Somogyi method (Nelson, 1944) [24]. 10 ml of stock was diluted to 100 ml with distilled water (100 µg/ml). Extraction of plant root material (Loomis and Shull, 1957) [19]. One hundred mg of powdered root sample was grounded with 5 ml of 80 per cent boiling ethanol. The supernatant was collected and kept on hot water bath at 80 °C for complete evaporation of ethanol. The sugars in test tube was dissolved by adding 5 ml of distilled water. This extract was used for further estimation.

Results and Discussion

Peroxidase activity

There was an increase in the activity of peroxidase in all the genotypes when inoculated with *M. phaseolina*. Highest enzymatic activity was recorded at 48 hpi (hours after post inoculation) in moderately resistant genotypes, Phule G 12107 (3.80 min/g), NDG 13-21 (3.08 min/g) and IPC 2010-112 (2.93 min/g) compared to susceptible genotype GNG 2228(2.64 min/g) and highly susceptible genotype L550 (2.56 min/g). However peroxidase activity started declining after 48 hpi (Table 1). The mean total peroxidase activity was found to be more in Phule G 12107 (2.73 min/g) followed by NDG 13-21(2.33 min/g),IPC 2010-112 (2.12 min/g), GNG 2228(1.78 min/g) and L550 (1.67 min/g) at all stages of sampling.

During oxidative stress, the plant protects itself against reactive oxygen species by antioxidant enzymes as well as a wide array of non-enzymatic antioxidants (Das and Roy Choudhury, 2014 [8]. Superoxide dismutase was considered to be the first line of defense against reactive oxygen species (ROS) and was the major O_2^- scavenger. Its enzymatic action resulted in H_2O_2 and O_2 formation. The H_2O_2 produced was then scavenged by several classes of peroxidases. Peroxidases decomposed H_2O_2 by the oxidation of phenolic compounds. Increased peroxidase activity had often been studied in connection with the oxidation of phenolic substances in the diseased plants and resistance in host attributed to toxicity of these oxidation products (Fric, 1976) [9]. Moreover, enhanced peroxidase activity was linked with synthesis of lignin (Ride, 1975) [33].

Similarly there was an increase in the activity of peroxidase in all the genotypes when inoculated with peigonpea sterility mosaic virus. Highest enzymatic activity after 28 DAI (Days after inoculation) was recorded in resistant/moderately resistant genotypes, ICP 7035 (37.40 %) and BRG 1 (34.69 %) at all stages of sampling compared to susceptible genotypes maruthi (19.44 %) and TTB 7 (18.91 %). However in susceptible genotypes peroxidase activity slightly increase over healthy and was observed upon 21 DAI later on it was decreased (Manjunatha, 2012) [21]. The peroxidase was

increased in resistant cultivars than susceptible ones in *Fusariumoxysporum*f. sp. *ciceri* inoculated seedlings over control. In Vijay (wilt resistant), peroxidase activity was increased from 2.06 to 8.54 units (un-inoculated over inoculated) and in JG-62 (susceptible) activity was increased from 1.68 to 1.96 units (un-inoculated over inoculated) in seedlings (Dalvi *et al*., 2011) [7].

Catalase activity

There was an increase in the activity of catalase in all the genotypes when inoculated with *M. phaseolina*. Highest enzymatic activity was recorded at 144 hpi (hours after post inoculation) in moderately resistant genotypes, Phule G 12107 (5.42 µM/min), NDG 13-21 (4.66 µM/min) and IPC 2010-112 (4.01 µM/min) compared to susceptible genotype GNG 2228(1.30 µM/min) and highly susceptible genotype L550 (1.25 µM/min) which recorded highest activity at 96 hpi. Catalase activity started declining after 96 hpi in susceptible and highly susceptible genotypes whereas, in moderately resistant genotypes the catalase activity prolonged upto 144 hpi (Table 1). The mean catalase activity was found to be more in Phule G 12107 (2.04 µM/min) followed by NDG 13-21 (1.62 µM/min), IPC 2010-112 (1.45 µM/min), GNG 2228(0.60 µM/min) and L550 (0.49 µM/min).

Superoxide dismutase was considered as first line of defense against reactive oxygen species (ROS) and was the major O_2^- scavenger. Its enzymatic action resulted in H_2O_2 and O_2 formation. The H_2O_2 produced was then scavenged by catalase and several classes of peroxidases. The catalase was a tetrameric heme protein found in peroxisomes, cytosol and mitochondria (Krych *et al*., 2014) [18]. This enzyme had hyperoxidase activity which catalyzed the dismutation of hydrogen peroxide into water and oxygen. Catalase activity increased during infection as a mechanism to scavangefungitoxic H_2O_2. The major function of catalase within cells was to prevent the accumulation of toxic levels of hydrogen peroxide formed as a by-product of metabolic processes primarily that of the electron transport pathway (Montalbini, 1991) [4].

Similar results were obtained by Amoako (2015) [3] where he observed that infected leaf extract had 141.02 ± 3.536 mg/mL protein, apparent Michealis constant (Kmapp) of 26.7 µM and maximum rate of reaction (Vmax) of 54.50 µM/min compared to the uninfected leaf extract with 75.04±0.560 mg/mL protein, Kmapp of 39.61 µM and Vmax of 143.06 µM/min. The activation energy of the infected extract was 0.1578 J/mol compared to 0.2181 J/mol obtained for the uninfected extract. Activity of the crude catalase in the viral infected leaf extract was higher than that in the uninfected one.

Phenylalanine ammonia lyase (PAL) activity

The increase in the activity of PAL in all the genotypes was recorded when chickpea roots were inoculated with *M. phaseolina*. Highest enzymatic activity was recorded at 144 hpi (hours after post inoculation) in moderately resistant genotypes, Phule G 12107 (8.01 µl/ml), NDG 13-21 (7.47 µl/ml) and IPC 2010-112 (7.24 µl/ml) compared to susceptible genotype GNG 2228(1.09 µl/ml) and highly susceptible genotype L550 (1.06 µl/ml) which recorded highest activity at 96 hpi. PAL activity started declining after 96 hpi in susceptible and highly susceptible genotypes whereas in moderately resistant genotypes the PAL activity prolonged upto 144 hpi (Table 1). The mean PAL activity was found to be more in Phule G 12107 (3.37 µl/ml) followed by NDG 13-21 (3.07 µl/ml), IPC 2010-112 (2.88 µl/ml), GNG 2228(0.67 µl/ml) and L550 (0.52 µl/ml). Therefore, one can conclude that the plant defense mechanism started after the pathogen attack with higher synthesis of PAL compounds and defense related enzymes.

Phenylalanine ammonia-lyase (PAL) was the main enzyme of plant phenolics production including those under stress exposure. It was the key enzyme that catalyzed core reaction in phenylpropanoid metabolism leading to functionally diverse defense related products such as lignin, suberin, wall-bound phenolics, flavanoidsetc (Wen *et al.,* 2005) [48]. There was an increase in total polyphenols and activity of PAL in infected plants of banana against *Fusariumoxysporum*. Though the plant pathogen interaction had triggered the activities of defense enzymes initially but later the activities drastically declined when pathogen colonized the root tissues (Thakkar *et al*., 2007) [45]. Biochemical response of guar against *M. phaseolina* infection revealed that there was maximum accumulation of phenolic acids in infected plants *i.e*., 23 per cent higher than in control after 120 hours of infection in all the four cultivars. Similarly, PAL activity was also significantly increased by 37 per cent after 96 or 120 hours of infection depending upon the cultivar in comparison to control. Phenolic acid accumulation and enhanced PAL activity in the compatible host–pathogen combination presumes that both participated actively in the guar resistance to root rot (Sharma *et al*., 2011) [37].

Similarly biochemical changes in banded leaf and sheath blight affected maize plants caused by *Rhizoctoniasolani*f. sp. *sasakii* recorded increased phenylalanine ammonia lyase (PAL) activities in leaf sheaths when inoculated with the pathogen (Sivakumar and Sharma, 2003) [41]. Fungal elicitors 34 are known to induce the production of phenyl ammonia lyase (PAL) and peroxidase which were involved in the synthesis and depolymerization of lignin precursors. The rapid increase and higher levels of PAL and peroxidases activity was found in resistant cultivars as compared to the susceptible cultivars of chickpea against *Fusarium* wilt (Aguilar *et al*., 2000) [2].

Total phenol activity

The increase in the activity of phenol was recorded when chickpea roots were inoculated with *M. phaseolina*. Highest phenol activity was recorded at 96 hpi (hours after post inoculation) in moderately resistant genotypes, Phule G 12107 (1.43 µl/ml), NDG 13-21 (1.35 µl/ml) and IPC 2010-112 (1.03 µl/ml) compared to susceptible genotype GNG 2228(0.86 µl/ml) and highly susceptible genotype L550 (0.74 µl/ml) which recorded highest activity at 48 hpi. Phenol activity started declining after 48 hpi in susceptible and highly susceptible genotypes whereas in moderately resistant genotypes the phenol activity prolonged upto 96 hpi (Table 1). The mean phenol activity was found to be more in Phule G 12107 (0.97 µl/ml) followed by NDG 13-21 (0.93 µl/ml), IPC 2010-112 (0.83 µl/ml), GNG 2228(0.67 µl/ml) and L550 (0.54 µl/ml).

Phenolic compounds were found to be most important group of secondary plant products that played an important role in reducing the susceptibility of a plant to pathogen (Jyosthna *et al*., 2004) [16]. During pathogen infection, deposition of phenolics into the cell wall was an important defense mechanism, either because of a hypersensitive reaction (HR) of entire cells or due to local wall reinforcement (Congeicao *et al*., 2006) [6]. A distinct correlation between the degree of plant resistance and phenolics was reported in plant tissues.

phenolic compounds such as cinnamic and ferulic acids released into plant cell walls was a common and early response to fungal attack, which resulted in cell strengthening and then enhanced resistance to pathogen penetration (Stadnik and Buchenauer, 2000) [42]. This showed that when plant cells were recruited into infection, it switches from normal primary metabolism to a multitude of secondary metabolism defense pathway and activation of novel defense enzymes and genes takes place (Tan *et al*., 2004) [44].

The results obtained are in conformity with Shreenivas (2000) [39]. reported that resistant varieties (ICPL 8863 and ICPL 87119) showed higher levels of phenolics contents for *Fusarium* in both the control and inoculated pigeonpea seedlings over the susceptible (TTB 7 and AKT 9221) at all the growth stage. Similarly Mandavia *et al.* (2002) [20]. Collected root exudates from ten day old seedlings and were analyzed for total phenol content. A significant inverse relationship was found between wilt susceptibility and total phenol content in root exudates of chickpea seedlings. Resistant genotypes (0-30 % wilt) had the highest amount of phenol, followed by moderately resistant (31-70 % wilt) and susceptible (71-100 % wilt) genotypes.

Manjunatha (2012) [21]. Reported that total phenol content of healthy leaves and resistant/moderately susceptible varieties, ICP 7035 (4.416 mg) and BRG 1 (2.981 mg), exhibited highly increase in phenolic content than susceptible variety maruthi (2.241 mg) and TTB 7 (2.251 mg). However phenol content was increased upon inoculation with peigonpea sterility mosaic virus upto 28 days in resistant genotypes, whereas it stared declining after 21 days in susceptible genotypes.

Variety ICP 8863 recorded total phenol content of 0.49, 1.03, 2.36 and 3.45 mg/g of fresh roots at 30, 60, 90 and 120 days respectively upon inoculation with pigeon pea sterility mosaic virus. WRP 1 had total phenol content of 0.32, 0.79, 1.56 and 2.02 mg/g of fresh roots at 30, 60, 90 and 120 days respectively. Similarly, TTB 7 registered total phenol content of 0.20, 0.57, 1.02 and 1.98 mg/g of fresh roots at 30, 60, 90 and 120 days respectively. ICP 8863 a wilt resistant variety had highest total phenol content compared to WRP 1, whereas, TTB 7 wilt susceptible variety had lowest total phenol content. As the age of the crop progressed, consequently there was increase in the total phenol content (Asha, 2012) [5]. In the present study also phenol content increased after infection and it was high in resistant genotypes than susceptible genotypes.

Total free amino acid activity

There was an increase in the activity of total free amino acids in all the genotypes when chickpea roots were inoculated with *M. phaseolina*. Highest amino acid activity was recorded at 48 hours after inoculation (hpi) and continued to be constant upto 96 hpi and started declining after 96 hpi. At 48 hpi enzymatic activity was highest in resistant genotypes Phule G 12107 (2.45 mg/ml), NDG 13-21 (2.26 mg/ml) and IPC 2010-112 (2.18 mg/ml) compared to susceptible genotype GNG 2228(1.17 mg/ml) and highly susceptible genotype L550 (1.09 mg/ml). The mean amino acid activity was found to be more in Phule G 12107 (2.06 mg/ml), followed by NDG 13-21 (1.88 mg/ml),IPC 2010-112 (1.79 mg/ml), GNG 2228(0.93 mg/ml) and L550 (0.81 mg/ml) data presented in Table 2.

Involvement of amino acids in the relationship between host and parasite was established (Omokolo *et al*., 2002; Omokolo and Boudjeko, 2005) [28, 29], indicating an accumulation during the development of infections. Amino acids might act directly to inhibit fungal development, or indirectly by their implication in the metabolic ways associated with resistance to diseases (Graham *et al.,* 1990) [10]. After infection of resistant genotypes of some host species, accumulation of certain specific amino acids such as glutamine, histidine, glycine and arginine were observed in tomatoes (Hassan *et al.,* 1994; Starrat and Lazarovits, 1996) [14, 43], tyrosine and alanine in wheat (Tyuterev and Tarlakovskii, 1994) [46] and asparagin, glutamic acid, proline, glycine and arginine in citrus (Nemec, 1995) [26]. Free amino acids were important indicators of the plant conditions, arising as a consequence of protein degradation in tissues under programmed cell death (Scarpari, *et al*. 2005) [36]. The increase in amino acids under infection conditions may also indicate pathogen effects on molecular transportation via phloem tissues. Indeed, inhibition of metabolite translocation due to the presence of pathogen in infected plants was described by Guthrie *et al*. (2001) [12] in papaya and by Maust *et al*. (2003) [22] in coconut. These results are in accordance with Gupta and Khare (1992) [11] who carried out biochemical analysis of chickpea resistant and susceptible cultivars to vascular wilt, caused by *F. oxysporum*f. sp. *ciceris.* They established that the quantities of total polysaccharides, total carbohydrates, total amino acids, total phenols and mineral contents were greater in the roots of resistant cultivars. Similarly amino acids content increased in 70 per cent of genotypes after injury or infection by *Phytopthoramegakarya* in cocoa. A significant positive relationship was observed between amino acid contents and the severity of necrosis (Pierr *et al*., 2011) [30]. In the present study also amino acid activity was recorded high in resistant genotypes compare to susceptible genotypes and the activity increased after infection.

Total sugar content

There was a decrease in the activity of total sugar content in all the genotypes when chickpea roots were inoculated with *M. phaseolina*. The mean total sugar content was found to be more in resistant genotypes Phule G 12107 (3.37 mg/ml), NDG 13-21 (3.04 mg/ml) and IPC 2010-112 (2.50 mg/ml) compared to susceptible genotype GNG 2228(1.74 mg/ml) and highly susceptible genotype L550 (1.64 mg/ml) data presented in Table 2.

Reducing sugar content and non-reducing sugar content

The decrease in the activity of reducing sugar content was observed in all the genotypes when chickpea roots were inoculated with *M. phaseolina*. The mean reducing sugar content was found to be more in resistant genotypes Phule G 12107 (1.71 mg/ml), NDG 13-21 (1.57 mg/ml) and IPC 2010-112 (1.46 mg/ml) compared to susceptible genotype GNG 2228(0.54 mg/ml) and highly susceptible genotype L550 (0.47 mg/ml) data presented in Table (2). The mean non-reducing sugar content was found to be more in resistant genotypes Phule G 12107 (1.66 mg/ml), NDG 13-21 (1.48 mg/ml) and IPC 2010-112 (1.04 mg/ml) compared to susceptible genotype GNG 2228(1.21 mg/ml) and highly susceptible genotype L550 (1.17 mg/ml) data presented in (Table 2). A similar trend of decreased sugar levels in diseased plants was observed by Prasad *et al.* (1976) [31]. The depletion of sugars during host-parasite interaction might be due to increased respiration or utilization of sugars by the fungi which depends on the capability of fungi to secrete carbohydrate degrading enzyme. Similarly Nema (1989) [25] suggested that reduction in sugars during disease development might be due to utilization of sugars probably for energy and synthetic reactions involved in multiplication of the pathogen.

These results are in agreement with Kaur and Dhillon (1989) [17] they also noticed susceptible cultivars of groundnut showed a rapid decrease of sugars but the decrease was relatively slow in resistant cultivars. Similarly, Sindhan *et al.* (1999) [40] screened two hundred and sixty genotypes of mungbean against *Cercospora* leaf spot reported that total sugar, reducing sugar and non reducing sugar content was decreased in diseased leaves of susceptible varieties than resistant varieties. The *Fusarium* infected plants resistant varieties ICP 8863 had highest total sugar content compared to BDN 2010 (moderately resistant), whereas, TTB 7 (susceptible) has lowest total sugar content (Prasad, 2011) [31]. The present study also correlates with decrease in total sugars, reducing sugars and non-reducing sugars after infection with *M. phaseolina*. The sugar content was found to be more in resistant genotypes compare to susceptible genotypes.

Conclusion

There was an increase in the activity of peroxidase, catalase, total phenols, Phenylalanine ammonia lyase and total free amino acids in all the genotypes when inoculated with *M. phaseolina*. Highest enzymatic activity was recorded in moderately resistant genotypes compared to susceptible genotype and highly susceptible genotype. The enhanced activity of Peroxidase, Catalase, Phenylalanine Ammonia Lyase, Total Phenols and Total free Amino Acids is linked with production of defense related products such as lignin, suberin, wall-bound phenolics, flavonoids, lignin, induction of hypersensitive reaction (HR) etc., which resulted in cell strengthening and then enhanced resistance to pathogen penetration. This shows that when plant cells were subjected into infection, it switches from normal primary metabolism to secondary metabolism defense pathway and activation of novel defense enzymes and genes takes place which inhibit fungal development, or indirectly by their implication in the metabolic ways associated with resistance to diseases. Whereas, there was decrease in the activity of total sugar, reducing sugar and non-reducing sugar in all the chickpea genotypes upon inoculation with *M. phaseolina*. The depletion of sugars during host-parasite interaction might be due to increased respiration or utilization of sugars by the fungi which depends on the capability of fungi to secrete carbohydrate degrading enzyme.

Table 1: Peroxidase activity (min/g), Catalase activity (µM/min), Total Phenol activity (µl/ml) and Phenylalanine Ammonia Lyaseactivity in chickpea genotype

	Peroxidase activity (min/g)						**Catalase activity (µM/min)**					
Genotypes	**Duration of inoculation**						**Duration of inoculation**					
	Healthy	**24 hpi**	**48 hpi**	**96 hpi**	**144 hpi**	**Mean**	**Healthy**	**24 hpi**	**48 hpi**	**96hpi**	**144 hpi**	**Mean**
IPC 2010-112 (MR)	2.52	2.54	2.93	2.14	0.45	2.12	0.22	0.33	0.54	2.16	4.01	1.45
Phule G 12107 (MR)	3.24	3.54	3.80	2.57	0.48	2.73	0.30	0.64	0.69	3.18	5.42	2.04
NDG 13-21 (MR)	2.82	2.91	3.08	2.36	0.46	2.33	0.26	0.38	0.54	2.29	4.66	1.62
GNG 2228 (S)	1.98	2.14	2.64	1.87	0.26	1.78	0.10	0.28	0.49	1.30	0.83	0.60
L550 (HS)	1.75	1.98	2.56	1.80	0.23	1.67	0.08	0.16	0.36	1.25	0.62	0.49
S. Em ±	Genotypes	Hours		Genotypes × Hours			Genotypes		Hours	Genotypes × Hours		
	0.01	0.014		0.031			0.018		0.018	0.039		
CD at 1 %	0.053	0.053		0.118			0.067		0.067	0.149		
	Total Phenol activity(µl/ml)						**Phenylalanine Ammonia Lyaseactivity (µl/ml)**					
Genotypes	**Duration of inoculation**						**Duration of inoculation**					
	Healthy	**24 hpi**	**48 hpi**	**96 hpi**	**144 hpi**	**Mean**	**Healthy**	**24 hpi**	**48 hpi**	**96hpi**	**144 hpi**	**Mean**
IPC 2010-112 (MR)	0.63	0.71	0.93	1.03	0.86	0.83	0.39	0.64	1.14	5.01	7.24	2.88
Phule G 12107 (MR)	0.69	0.79	1.00	1.43	0.94	0.97	0.66	0.84	1.36	6.00	8.01	3.37
NDG 13-21 (MR)	0.68	0.76	0.95	1.35	0.92	0.93	0.41	0.64	1.23	5.63	7.47	3.07
GNG 2228 (S)	0.63	0.84	0.86	0.68	0.32	0.67	0.08	0.63	0.92	1.09	0.64	0.67
L550 (HS)	0.59	0.63	0.74	0.52	0.20	0.54	0.02	0.57	0.63	1.06	0.32	0.52
	Genotypes	Hours		Genotypes × Hours			Genotypes		Hours	Genotypes × Hours		
S. Em ±	0.018	0.018		0.040			0.016		0.016	0.037		
CD at 1 %	0.067	0.067		0.150			0.062		0.062	0.138		

*hpi – Hours after post inoculation, MR – Moderately resistant, S – Susceptible, HS – Highly susceptible

Table 2: Total Free Amino Acids content (mg/ml), Total Sugar content (mg/ml), Reducing Sugar (mg/ml) and Non-reducing sugar (mg/ml)in chickpea genotype

	Total Free Amino Acids content (mg/ml)						**Total Sugar content (mg/ml)**					
Genotypes	**Duration of inoculation**						**Duration of inoculation**					
	Healthy	**24 hpi**	**48 hpi**	**96hpi**	**144 hpi**	**Mean**	**Healthy**	**24 hpi**	**48 hpi**	**96hpi**	**144 hpi**	**Mean**
IPC 2010-112 (MR)	1.15	1.69	2.18	2.18	1.75	1.79	5.09	3.12	2.55	0.99	0.73	2.50
Phule G 12107 (MR)	1.40	2.00	2.45	2.45	2.01	2.06	5.96	4.48	3.64	1.95	0.82	3.37
NDG 13-21 (MR)	1.20	1.80	2.26	2.26	1.86	1.88	5.63	4.21	2.71	1.89	0.78	3.04
GNG 2228 (S)	0.40	0.92	1.17	1.17	1.01	0.93	3.05	2.37	1.92	0.90	0.48	1.74
L550 (HS)	0.32	0.73	1.09	1.08	0.83	0.81	3.01	2.01	1.89	0.82	0.46	1.64
S. Em ±	Genotypes	Hours		Genotypes × Hours			Genotypes		Hours		Genotypes × Hours	
	0.007	0.007		0.016			0.079		0.079		0.177	
CD at 1 %	0.027	0.027		0.061			0.299		0.299		0.669	
	Reducing Sugar (mg/ml)						**Non-reducing sugar(mg/ml)**					
Genotypes	**Duration of inoculation**						**Total sugars (mg/ml)**		**Reducing sugars (mg/ml)**		**Non-reducing sugars (mg/ml)**	
	Healthy	**24 hpi**	**48 hpi**	**96 hpi**	**144 hpi**	**Mean**						

IPC 2010-112 (MR)	2.55	2.1	1.53	0.81	0.29	1.46	2.50	1.46	1.04
Phule G 12107 (MR)	2.70	2.52	1.87	0.94	0.51	1.71	3.37	1.71	1.66
NDG 13-21 (MR)	2.56	2.28	1.67	0.9	0.42	1.57	3.04	1.57	1.48
GNG 2228 (S)	1.21	0.99	0.3	0.10	0.08	0.54	1.74	0.54	1.21
L550 (HS)	1.05	0.81	0.22	0.19	0.06	0.47	1.64	0.47	1.17
	Genotypes	Hours		Genotypes × Hours			--	--	--
S. Em ±	0.005	0.005		0.010			--	--	--
CD at 1 %	0.017	0.017		0.039			--	--	--

*hpi – Hours after post inoculation, MR – Moderately resistant, S – Susceptible, HS – Highly susceptible

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