The Pharma Innovation Journal 2022; 11(11): 1289-1298

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2022; 11(11): 1289-1298

www.thepharmajournal.com
Received: 21-08-2022
Accepted: 22-09-2022

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Variations of the biochemical response in cowpea genotypes infected by rust pathogen *Uromyces phaseoli* var. *Vignae* (Barcl.) Arth.

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Abstract
The cowpea genotypes showing immune, resistant, moderately resistant, moderately susceptible, susceptible and highly susceptible reaction to the rust pathogen (*Uromyces phaseoli* var. *vignae* (Barcl.) Arth.) were used to study the biochemical variations due to its infection. Various biochemical parameters viz., total phenols, total sugars, protein content, total chlorophyll, peroxidase, phenylalanine ammonia lyase and β1, 3-glucanase activity were assessed. The results revealed that the amount of these biochemical components were significantly higher in leaves of diseased and healthy plants of resistant genotypes compared to other genotypes. However, excepting total sugars and total chlorophyll content all the other parameters were high in infected genotypes compared to healthy genotypes. Further, among the resistant genotypes the total phenols, total sugars, proteins, total chlorophyll, peroxidase, PAL and β 1, 3-glucanse content was highest in the resistant genotypes *viz.*, EC-17058-1-1 (6.341 mg/g of fresh wt.), MFC-08-14 (3.887 mg/g of fresh wt.), MFC-08-14 (5.547mg/g of fresh wt.), KBC-2(1.733 mg/g of fresh weight),MFC-08-14(1.379 $Absmin^{-1}g^{-1}$), EC-17058-1-1 (152 µmol of trans cinnamic acid min^{-1} g^{-1}) and EC-17058-1-1 (31 µg of glucanase released g^{-1} fresh wt.) respectively. Whereas, least amount of these components was recorded in highly susceptible genotype C-152. Correlation study showed that all the biochemical parameters *viz*., total phenols, proteins, total sugars, total chlorophyll content, peroxidase, PAL and β 1, 3- glucanase enzyme activities were negatively correlated with disease severity of cowpea rust.

Keywords: Cowpea, resistant genotypes, biochemical parameters, correlation and rust

Introduction
Pulses are one of the best sources of vegetable protein. Important pulse crop grown in India are Redgram, Bengalgram, greengram, Blackgram, cowpea and peas. Among them cowpea (*Vigna unguiculata* L.) is an important crop which is widely grown in the arid and semi-arid tropical regions. It is basically grown for grain purpose; however, it is also used as vegetable and nutritious fodder (Giridhar *et al.*, 2020) [11].

Cowpea is cultivated all over India as *Kharif* and warm season pulse crop. It is been grown over 0.5 million ha area in India and is easily adapted to wide range of soils and rainfall situations and fits easily in multiple and intercropping systems, however under rainfed conditions farmers grow it as a sole crop (Rajpoot and Rana, 2016) [30]. In marginal, drought-prone places where there are low rainfall situations and less developed irrigation systems, this crop is an attractive alternative to farmers as the crop of drought tolerant with short rowing period (Martin *et al*., 2009) [25].

The major constraints for cowpea cultivation includes pests and diseases, among them rust disease caused by *Uromyces phaseoli* var. *vignae* (Barcl.) Arth. is one of the most important diseases that cause huge economic loss. It is one of the foliar diseases occurring in all parts of the world wherever cowpea is cultivated (Deshpand *et al*., 2010) [8]. Usually, the foliage of the host is infected producing numerous urediospores which are easily airborne (Uma *et al*., 2016) [38] there by assisting rapid spread of the pathogen in short duration. About 2,000 urediniospores of the pathogen are released per day during the dry season making it the most severe and devastating disease (Souza *et al.*, 2013) [37]. Because of this foliage infection, rust interferes in the photosynthetic activity by reducing the foliage area considerably (Honnur *et al*., 2016) [18].

The most likely and preferred control measures taken up to combat this disease is the use of fungicides.

However, indiscriminate use during the growing season within small sized land holdings is uneconomical besides being deleterious to both environment and its user. Further, such huge application of fungicides leads to persistent residues both in the food and environment (Petit *et al*., 2008) [29].

Development of resistant varieties is one of the best and very effective means to manage the disease. Understanding the host pathogen interaction becomes a key point in selection of resistant source. Whenever plants are attacked by the pathogens, various physiological and biochemical events set in to defend themselves against them is evident. Following ion flux and oxidative burst production of phenolics, phytoalexins and pathogenesis-related proteins are produced in host plants to slow down the pathogen invasion apart from lignification, suberization and callose deposition leading to strengthening of cell walls (Bowels, 1990) [5]. β-1,3-glucanase, chitinase, polyphenol oxidase and phenylalanine ammonia lyase are the important pathogenesis-related proteins having broad spectrum defense activity (Deborah *et al*., 2001; Kumari and Vengadaramana, 2017) [7, 21]. However, this host pathogen interaction studies are very much lacking in cowpea infected with rust pathogen. Therefore, investigation was conducted on the quantitative estimations of various biochemical components like total phenols, total sugars, protein content, total chlorophyll, peroxidase, phenylalanine ammonia lyase and β1, 3-glucanase activity, determining their role in rust disease and healthy plants of resistant, moderately resistant, moderately susceptible, susceptible and highly susceptible cowpea genotypes.

Materials and methods:

The cowpea genotypes showing immune, resistant, moderately resistant, moderately susceptible, susceptible and highly susceptible reaction upon screening were selected for this study. Two healthy and infected plants from each genotype were selected. for biochemical analysis.

Collection of leaves

Cowpea leaves were collected from the field at 50, 65 and 80 DAS for biochemical analysis. Different Biochemical parameters such as chlorophyll, sugars, phenols and proteins concentrations were determined. Enzymes activities such as peroxidase, phenylalanine ammonia lyase and β 1,3-*glucanase* were also determined according previously published protocols.

Drying and grinding of leaves

Both healthy and infected leaves were washed in tap water and dried under shade. The dry weight of leaves were recorded and powdered by using an electric grinder. The powdered leaf samples were used for analysis of various biochemical parameters.

Extraction of phenols from leaves

200mg of powdered leaf samples was homogenated in 80% ethanol. The homogenate was centrifuged at 10000 rpm for 20 minutes. The supernatant was collected and the pellet was resuspended in 80% ethanol and centrifuged again and pooled. The resuspended sample was concentrated to dryness in hot water bath. The concentrated sample was dissolved in 5 ml of distilled water and used for further analysis.

Determination of phenol concentration in leaves

The concentration of phenol leaf sample was determined according to Singleton and Rossi (1965) [34] with slight modifications. 0.5 ml of sample was taken in a test tube and 0.5 ml of FCR reagent was added and mixed thoroughly. The final volume of the reaction mixture was made up to 4.0 ml with distilled water. The test tubes were incubated for 3 minutes at room temperature and 1 ml of saturated sodium carbonate solution was added. The contents were boiled in water bath for a minute and cooled the tubes. The absorbance was measured at 625 nm against the blank in a Cary-60 UV spectrophotometer. Varied concentration of catechol was used to prepare standard curve. The concentration of phenols in the leaf sample was calculated and expressed as µg g^{-1}.

Extraction of sugar from the leaf sample

Powdered leaf sample (100 mg)was hydrolyzed with 5.0 ml of 2.5 N HCl in a boiling water bath for 3 hours. The tubes were cooled to room temperature and the acid was neutralized with solid sodium carbonate until effervescence ceases. The volume was made to 10 ml and this was centrifuged at 1000 rpm for 5 minutes. The supernatant was collected and used for the estimation of sugars.

Determination of sugar concentration the leaf sample

The concentration of sugar in the leaf sample was determined according to Hedge et. al, with slight modifications Hedge and Hofreiter (1962) [16]. The 0.5 ml of extract was taken in test tubes and 2.0 ml of anthrone reagent was slowly added. The contents of the tubes was mixed and placed in boiling water bath for 15 minutes. The tubes were cooled and color intensity was measured at 620 nm in Cary-60 UV spectrophotometer. Standard curve was developed using glucose. The concentration of sugar in the sample was calculated and expressed as µg $g^{-1}$of leaf sample and leaf sample extract.

Extraction of protein from the leaf sample

Crude proteins were extracted from the fresh leaves (0.5 g) by homogenization in 100 mM phosphate buffered saline pH, 7.4 at 4°C. The homogenate was centrifuged at 10000 rpm for 10 minutes at 4°C and the obtained supernatant was used for protein estimation and determination of enzymes activity.

Determination of protein concentration in leaf sample

The concentration of protein in the leaf sample extract was determined according to Lowry *et al*. (1951) [24]. The leaf sample (0.5 ml) was taken in the test tube and volume was made up to 1.0 ml with distilled water. Add 5.0 ml of Lowry's reagent and incubate the tubes for 10 minutes at room temperature. 0.5 ml of 1:1 diluted FC reagent was added and mixed the tubes properly and incubate at room temperature for 30 minutes. The intensity of color was measured at 660 nm in Cary-60 UV spectrophotometer. Bovine serum albumin was used to generate the standard curve of the protein. The concentration of protein in the leaf sample was calculated and expressed as µg g^{-1}.

Extraction of chlorophyll from the leaf sample

Fresh leaves (1 g) was homogenized in of 80% acetone and centrifuged at 2000 rpm for 20 minutes at room temperature. The final volume of the homogenate was made up to 100 ml using 80% acetone.

Determination of chlorophyll concentration in leaf sample
The color intensity of leaf samples were read at 645 nm and 663 nm in Cary-60 UV spectrophotometer, where 80% of acetone was used as a blank. The obtained absorbance values at different wavelengths were substituted to Arnon's equation to determine the chlorophyll a, chlorophyll b and total chlorophyll (ref).

Determination of peroxidase enzyme activity
The peroxidase enzyme activity was determined according to the method as described by Hartee, 1955 [15] (ref). 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 1% $H_2O_2$was incubated at room temperature (28±10 °C). The change in absorbance was recorded at 470 nm at a time interval of 30 secup to 3 min in Cary-60 UV spectrophotometer. The boiled enzyme preparation served as blank. The enzyme activity was expressed as the change in the absorbance at 420 nm min^{-1} g^{-1}.

Determination of phenylalanine ammonia lyase (PAL):
PAL activity was determined as the rate of conversion of L-phenyl alanine to trans-cinnamic acid at 290 nm as per the method described by Ross and Senderoff (1992) [32] (ref). 0.4 ml of leaf samples were incubated with 0.5ml of 0.1 M borate buffer at pH 8.8. Later 0.5 ml of 12 mM L-phenylalanine was added and incubated 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 was prepared with 0.4 ml of extract and 2.7 ml of 0.1 M borate buffer (pH 8.8). The absorbance was measured at 290 nm in a Cary-60 UV spectrophotometer. A standard curve was drawn using cinnamic and the enzyme activity was expressed as µM of trans-cinnamic acid min^{-1} g^{-1} fresh weight of leaf sample.

Determination of β 1, 3-*glucanase*
The assay of β 1, 3-*glucanase* was carried out as per the method described by Rakshit *et al.* (2000) [31]. One ml reaction mixture contained 95 µl of laminarin and 50 µl of crude enzyme extract was incubated at 37 °C for 30 min. The reducing sugar released into the solution at the end of the reaction was estimated by Nelson-Somogyi's method (Somogyi, 1952) [36] (ref). The protein content in the crude enzyme extract was estimated by Lowry's method (ref). The β-1, 3-glucanase activity was expressed as µg of glucanase released g^{-1} of fresh weight.

Results and Discussion
Total phenols
Plant phenolics are secondary metabolites that encompass several classes of structurally diverse products arising from the shikimate–phenylpropanoid pathways. Plants use phenolic compounds for pigmentation, growth, reproduction, resistance to pathogens and many other functions. Phenolic compounds possess antimicrobial properties against fungi, bacteria and viruses (Martin *et al*., 2009) [25].
The results of the present experiment revealed that the total phenol content was significantly higher in healthy and infected resistant genotypes ranging from 4.207 (KBC-6) to 5.100 (EC-17058-1-1) and 5.064 (KBC-6) to 6.341 (EC-17058-1-1) mg/g of fresh weight respectively and lower in highly susceptible genotype C-152 (2.167 and 1.630mg/g of fresh weight, respectively) at 80 DAS. In moderately resistant genotypes and moderately susceptible and susceptible genotypetotal phenol content in infected leaves ranged from4.124 (Plant-loob 3) to 4.731(IC-402154), 2.082(NBC-40) to 3.063(CP-17) and 2.081 (C-325) to 2.430 (PGCP-11) mg/g of fresh weight respectively. The above results revealed that the total phenol content was high as the infection progresses and it was high in resistant genotypes than the susceptible ones (Table 1).
The results were in close conformity with Harde *et al*. (2019) [14], who also reported that the total phenol content of resistant genotypes was significantly higher (50 per cent) than the susceptible genotypes at all the stages of observations. Similar observations were made by Ammajamma and Patil (2008) [1] where the resistant soyabean genotypes had more total phenol content (21.25 and 32.19% at 45 and 75 DAS, respectively) than susceptible ones infected by rust caused by *Phakosporapachyrhizi.* Thus, if pre-formed antifungal phenolics are not sufficient to stop the development of the infection process, plant cells usually respond by increasing the level of antifungal phenols at the infection site. It has been shown that some hydroxycinnamic acids, flavan-3-ols (epicatechin, procyanidin B1, catechin) and dihydrochalcones may be involved in the defence mechanism in crop like apple leaves against the scab fungus *Venturia inaequalis* (Mikulic Petkovsek *et al*., 2009; Slantar *et al.*,2012) [26, 35]. Therefore, the resistant types did show a higher amount of total phenols in resistant types than the susceptible ones.

Proteins
John (1963) [20] reported that infection by different pathogens interferes in the protein metabolism of host**.** During the current study it was found that in infected leaves the protein content was significantly high compared to healthy leaves in all genotypes. The resistant genotype MFC-08-14 recorded highest 3.887 mg/g of fresh weight in infected leaves compared to all the other genotypes which was significantly high (Table 2). The protein content was least in the highly susceptible genotype C-152 (1.607 mg/g of fresh weight.). In moderately resistant genotypes and moderately susceptible genotype proteins varied from 2.968(Plant-loob 3)to3.175 (KBC-4) and 2.273(NBC-40) to2.767 (CP-17) mg/g of fresh weight respectively (Table 2).Similar results were obtained by Mishra *et al*.(2011) [27], where they reported maximum soluble protein content of 31.52, 30.79, 29.73 and 29.50 mg g^{-1} of fresh leaf at seedling, flowering, dough and hard dough stage in the variety K 0708, respectively. Where they indicated that the increased amount of protein content might due to defense response of wheat plant infected by Alternaria blight. Further, there are reports indicating that there was an increase in protein content in plants such as rice (Kumavat *et al*., 2008) [22], which are involved in defending the plants against plant pathogens. Further, Antifungal compounds like Pathogenesis-Related proteins which function in restricting the pathogen invasion and multiplication are one produced due to activation of defense response after pathogen infection(Sels *et al*., 2008) [33].

Total sugars
Contrasting to total phenols and proteins, total sugar content was significantly high in healthy leaves compared to infected ones of all the genotypes and tend to decrease in its content in the infected leaves of all the genotypes. In resistant genotypes at 80 DAS, significantly highest total sugar content ranging from 5.239 (KBC-6) to 5.547 (MFC-08-14) mg/g of fresh

weight was recorded in infected leaves than other genotypes. However, it was less (5.737 (KBC-6) to 5.873 (KBC-2) mg/g of fresh weight compared to the healthy leaves (Table 3).
The results were supported by findings of Ammajamma and Patil (2008) [1] where the total sugar content was 1.5 and 14.42 per cent more in resistant genotypes compared to susceptible genotypes at 45 and 75 DAS, respectively in soybean rust. The higher concentration of total sugars which is present in the resistant host, might inhibit the pathogen by blocking its enzyme synthesis (Batema and Miller, 1966). Also, that soluble sugars such as sucrose, glucose and fructose in plant host cells not only play the role as donors of carbon skeletons, but they may also induce metabolic signals influencing the expression of defense genes. These metabolites function in a complex network with many bioactive molecules, which independently or in dialogue, induce successive defense mechanisms (Formela-Luboińska *et al*., 2020) [9].

Total chlorophyll:
The results on total chlorophyll content showed a similar trend as that of total sugars where it was significantly high in healthy leaves compared to infected ones in all the genotypes. The resistant genotypes showed a higher total chlorophyll (1.537 (EC-458480) to 1.733(KBC-2) mg/g of fresh weight) in infected leaves, whereas, it was least in the infected leaves of highly susceptible genotype C-152 (0.350) mg/g of fresh weight. and lower in the susceptible genotype (0.374 (C-325) to 0.769 (PGCP-6) mg/g of fresh weight). The above results revealed that the total chlorophyll content reduces as the infection progresses and it was high in resistant genotypes and least in highly susceptible genotype (C-152 variety- 0.350) (Table 4).
The results are in accordance with Harde *et al*. (2019) [14] who reported that rust inoculations decreased the total chlorophyll content drastically in susceptible lines and moderately in the resistant lines. Further, De Jesus *et al*. (2001) [6] reported that the photosynthetic rate in *Colletotrichum lindemuthianum* inoculated plants reduced, indicating that the pathogen caused strong negative effects and caused reduction in photosynthesis rate and photosynthetic pigment. Pigment reduction and the consequent lower capacity to absorb light promotes a decrease in the photosynthesis rate.

Peroxidase (POX) activity
Peroxidase is one of the important enzymes which are produced in plants upon pathogen infection. POX have important roles during pathogenesis, involved in the production of reactive oxygen species leading to oxidative burst and thereby offering resistance to pathogen infection (Bindschedler *et al*., 2006) [4]. It is evident that peroxidase activity is higher in infected leaves of resistant genotype ranging from 1.151 (COFC-8) to 1.379 (MFC-08-14) **b** Abs min^{-1} $g^{-1}$and lower in the susceptible genotype (0.592 (C-325) to 0.643 (IVTC-4) b Abs min^{-1} g^{-1}). However, least peroxidase content was recorded in highly susceptible genotype C-152 (0.457) b Abs $min^{-1}g^{-1}$ (Table 5).
The results were similar with the findings of Harde *et al*. (2019) [14], who reported high peroxidase activity in the resistant genotypesin comparision with the susceptible groundnut genotypes. It was more than two times in resistant lines in the susceptible genotypes. Similarly infection of cowpea and broadbean plants by rust pathogens *Uromyces vignae* and *Uromyces fabae* respectively lead to the production of POX enzyme (Mould *et al.* (2003) [28] and Jakupovic *et al.* (2006) [19]. Thus, in resistant genotypes, the increased POX activity is correlated to host protection against pathogen infection (Kuvalekarand Gandhe, 2010) [23].

PAL activity
The activation of PAL is stated in many cases during the initial disease resistance reactions of plants which further leads to synthesis of many defence-related compounds such as antimicrobial phytoalexins and lignin (Hahlbrock and Scheel, 1989, Hemm *et al.*, 2004) [13, 17].
In the present experiment PAL activity was recorded high in infected leaves which ranged from 150 (KBC-2) to 152 (EC-17058-1-1) µmol of trans cinnamic acid min^{-1} g^{-1}than healthy leaves 145 (KBC-2) to 147 of resistant genotype. Also, it was highest in the resistant genotype compared to the infected leaves of highly susceptible C-152(95 µmol of trans cinnamic acid min^{-1} g^{-1}) and susceptible genotypes 105 (PGCP-6) to 108 (PGCP-11) µmol of trans cinnamic acid min^{-1} g^{-1}(Table 6). Similarly, Geetha *et al*. (2005) [10] reported the defensive role of PAL enzyme in pearl millet against *Sclerospora graminicola*, by Wang *et al.*, 2004 [39] in paddy against paddy blast pathogen *Pyricularia oryzae*, by Xu *et al*. (2011) [40] in cotton against wilt pathogen *Verticillium dahlia*, thus emphasizing its role in disease resistance.

β -1, 3-glucanse
β -1,3-glucanase, being an important member of Pathogenesis Protein 2 family, has the ability to hydrolyze fungal cell wall and thereby preventing pathogen infection. These endoglucanases catalyze the hydrolytic cleavage of the (1,3)-β -D-glucosidic linkages in (1,3)- β-glucans and act primarily on glucans present in the fungal cell wall (Gupta *et al.*, 2013) [12].
The infected leaves of all the genotypes showed higher amounts of β - 1, 3-glucanseenzyme than its healthy genotypes (Table 7). Significantly maximum amount of this enzyme was recorded in infected leaves of resistant genotypes viz., KBC-6 (29) to EC-17058-1-1 (31) µg of glucanase released g^{-1} fresh wt. The highly susceptible genotype C-152 recorded least β -1,3-glucanase (13 µg of glucanase released g^{-1} fresh wt) (Table 7).
These results are in confirmity with Rakshit *et al*. (2000) [31] who reported that β 1, 3- glucanase activity in powdery mildew resistant lines (1.87 ± 0.20 µ mole glucose eq min1mg-1 protein) was 2.03 times more than powdery mildew susceptible lines (0.92 ± 0.20 µ mole glucose eq min-1 mg-1 protein). Thus, its activity clearly indicates its role in defense in resistant genotypes.

Correlation between the rust disease severity and biochemical parameters.
Correlation study showed that all the biochemical parameters *viz*., total phenols, proteins, total sugars, total chlorophyll content, peroxidase, PAL and β 1, 3- glucanase enzyme activities were negatively correlated with disease severity as represented in Table 8.
Data from the table revealed that there is significant negative associationbetween total phenols (-0.291), total sugars(-0.313), protein(-0.298), total chlorophyll (-0.299), Peroxidase enzyme activity(-0.282), β 1, 3- glucanase (-0.231) and phenylalanine ammonia lyase (-0.225)with disease severity.These results are line with Mishra *et al*. (2011) [27]

who also observed negative correlation co-efficient between total soluble protein and disease severity. This suggests the involvement of total phenols, proteins, peroxidase, phenylalanine ammonia lyase and β 1, 3- glucanase enzyme activities in defense to protect plants against pathogen infection.

Table 1: Influence of rust disease on total phenol content of cowpea genotypes at different days interval

Sl. No.	Genotypes	Reaction	Total phenol content (mg/g of fresh wt.)					
			50 DAS		65 DAS		80 DAS	
			Healthy	Infected	Healthy	Infected	Healthy	Infected
1	MFC-08-14	R	0.837	0.544	3.133	4.175	4.167	5.161
2	KBC-6		0.723	0.567	3.167	4.082	4.207	5.064
3	KBC-2		0.920	0.722	4.133	4.594	4.933	6.029
4	COFC-8		0.690	0.428	3.800	4.486	4.620	5.853
5	EC-458480		0.717	0.657	3.800	4.742	4.767	6.165
6	EC-17058- 1-1		0.817	0.756	3.843	4.575	5.100	6.341
7	IC-402154	MR	0.660	0.493	3.143	3.834	4.217	4.731
8	NBC-16		0.557	0.388	2.943	3.424	4.113	4.646
9	KBC-4		0.617	0.558	3.103	3.945	3.893	4.786
10	NBC-41		0.493	0.347	2.797	3.284	3.720	4.147
11	V-578(C)		0.567	0.415	2.900	3.735	3.767	4.293
12	IC-402104		0.627	0.498	3.183	3.967	3.693	4.377
13	C-24-1		0.680	0.455	3.024	3.744	3.560	4.172
14	Plant-loob 3		0.597	0.409	2.910	3.690	3.443	4.124
15	CP-17	MS	0.520	0.413	3.317	3.080	3.200	3.063
16	KM-5		0.617	0.478	3.105	2.568	3.500	3.123
17	IC-202804		0.473	0.316	3.067	2.438	3.267	2.658
18	IC-402181		0.527	0.416	2.947	2.331	3.200	2.421
19	NBC-47		0.610	0.476	3.033	2.737	3.037	2.398
20	PGCP-12		0.563	0.424	2.857	2.083	3.167	2.161
21	NBC-40		0.657	0.349	2.830	2.108	3.033	2.082
22	IC-202722		0.563	0.423	2.917	2.338	3.100	2.502
23	C-157		0.610	0.417	3.070	2.465	2.933	2.259
24	PGCP-6	S	0.393	0.218	2.070	1.459	3.000	2.326
25	PGCP-11		0.460	0.279	1.920	1.334	2.867	2.430
26	IVTC-4		0.430	0.218	2.067	1.355	2.567	2.167
27	IVTC-5		0.493	0.398	2.167	1.455	2.767	2.088
28	PGCP-5		0.477	0.224	1.840	1.289	2.700	2.170
29	C-325		0.360	0.221	1.943	1.125	2.667	2.081
30	C-152	HS	0.250	0.124	1.643	1.063	2.167	1.630
	F		**	**	**	**	NS	**
	S.Em±		0.0030	0.0036	0.0377	0.0232	1.4734	0.0305
	CD @ 5%		0.0849	0.0101	0.1067	0.0657	4.1680	0.0862

**- Significant NS- Non significant

R-Resistant, MR-Moderately resistant, MS-Moderately susceptible, S- Susceptible, HS-Highly susceptible

Table 2: Variation in the protein content of cowpea genotypes at different days interval

Sl. No.	Genotypes	Reaction	Protein content (mg/g of fresh wt.)					
			50 DAS		65 DAS		80 DAS	
			Healthy	Infected	Healthy	Infected	Healthy	Infected
1	MFC-08-14	R	1.600	1.679	1.800	2.356	1.933	3.887
2	KBC-6		1.500	1.567	1.700	2.277	1.867	3.682
3	KBC-2		1.600	1.765	1.733	2.163	1.967	3.478
4	COFC-8		1.467	1.471	1.600	2.270	1.900	3.377
5	EC-458480		1.733	1.711	1.833	2.362	2.033	3.276
6	EC-17058-1- 1		1.700	1.554	1.800	2.171	2.067	3.377
7	IC-402154	MR	1.400	1.400	1.600	1.974	1.900	3.173
8	NBC-16		1.533	1.501	1.667	2.067	1.933	3.080
9	KBC-4		1.533	1.352	1.600	2.178	1.800	3.175
10	NBC-41		1.433	1.267	1.533	2.076	1.700	2.966
11	V-578(C)		1.400	1.367	1.533	1.954	1.767	2.965
12	IC-402104		1.567	1.487	1.600	1.954	1.733	3.083
13	C-24-1		1.500	1.350	1.567	1.867	1.833	3.085
14	Plant-loob 3		1.400	1.273	1.500	1.875	1.700	2.968
15	CP-17		1.167	1.167	1.267	1.655	1.433	2.767
16	KM-5		1.367	1.271	1.467	1.860	1.633	2.664
17	IC-202804		1.300	1.070	1.400	1.655	1.600	2.560
18	IC-402181		1.333	1.179	1.433	1.759	1.667	2.380

19	NBC-47	MS	1.233	1.096	1.400	1.553	1.700	2.480
20	PGCP-12		1.433	1.203	1.533	1.669	1.767	2.374
21	NBC-40		1.300	1.177	1.433	1.765	1.800	2.273
22	IC-202722		1.267	1.099	1.333	1.838	1.633	2.473
23	C-157		1.300	1.128	1.400	1.762	1.700	2.274
24	PGCP-6	S	1.133	1.098	1.200	1.454	1.433	2.075
25	PGCP-11		0.867	0.876	1.033	1.270	1.233	1.867
26	IVTC-4		0.900	0.963	0.967	1.390	1.100	1.778
27	IVTC-5		0.967	1.090	1.000	1.555	1.667	2.062
28	PGCP-5		1.000	0.974	1.233	1.479	1.400	1.964
29	C-325		0.967	0.841	1.133	1.377	1.433	1.869
30	C-152	HS	0.833	0.717	1.067	1.174	1.500	1.607
	F		**	**	**	**	**	**
	S.Em±		0.0467	0.0230	0.0544	0.0128	0.0789	0.0273
	CD @ 5%		0.1322	0.0650	0.1540	0.0363	0.2231	0.0771

**- Significant

R-Resistant, MR-Moderately resistant, MS-Moderately susceptible, S- Susceptible, HS-Highly susceptible

Table 3: Total sugars in the cowpea genotypes of healthy and infected leaves

Sl. No.	Genotypes	Reaction	Total sugars (mg/g of fresh wt.)					
			50 DAS		65 DAS		80 DAS	
			Healthy	Infected	Healthy	Infected	Healthy	Infected
1	MFC-08-14	R	1.930	1.393	3.743	3.579	5.830	5.547
2	KBC-6		1.800	1.468	3.483	3.356	5.737	5.239
3	KBC-2		1.837	1.354	3.622	3.374	5.873	5.360
4	COFC-8		1.910	1.551	3.507	3.392	5.663	5.290
5	EC-458480		1.733	1.339	3.577	3.465	5.790	5.373
6	EC-17058-1- 1		1.833	1.235	3.550	3.481	5.833	5.406
7	IC-402154	MR	1.733	1.255	3.437	3.079	5.367	5.101
8	NBC-16		1.700	1.246	3.530	3.165	5.413	5.140
9	KBC-4		1.837	1.406	3.767	3.355	5.437	5.161
10	NBC-41		1.850	1.346	3.450	3.081	5.623	5.152
11	V-578(C)		1.370	1.037	3.307	3.084	5.263	4.779
12	IC-402104		1.557	1.249	3.303	2.875	5.187	4.727
13	C-24-1		1.557	1.210	3.163	2.925	5.340	4.905
14	Plant-loob 3		1.573	1.277	3.307	2.956	5.283	4.777
15	CP-17		1.353	0.944	3.073	2.434	5.113	4.643
16	KM-5		1.357	0.972	3.150	2.317	4.967	4.350
17	IC-202804		1.363	0.984	3.197	2.452	4.830	4.239
18	IC-402181	MS	1.273	0.829	3.183	2.571	4.933	4.384
19	NBC-47		1.163	0.748	3.100	2.669	4.787	4.284
20	PGCP-12		1.233	0.652	3.193	2.507	4.860	4.280
21	NBC-40		1.280	0.734	3.080	2.433	4.747	4.176
22	IC-202722		1.287	0.707	3.170	2.448	4.710	4.177
23	C-157		1.310	0.959	3.053	2.358	4.627	4.037
24	PGCP-6	S	1.170	0.773	2.880	2.128	5.043	3.960
25	PGCP-11		1.093	0.668	2.877	2.056	4.470	3.767
26	IVTC-4		1.163	0.531	3.130	2.047	4.273	3.777
27	IVTC-5		1.117	0.579	2.747	2.058	4.287	3.534
28	PGCP-5		1.100	0.608	2.877	2.041	4.453	3.647
29	C-325		1.000	0.423	2.700	2.073	4.353	3.499
30	C-152	HS	0.900	0.329	2.500	1.543	4.160	3.123
	F		**	**	**	**	**	**
	S.Em±		0.0297	0.0209	0.0344	0.0311	0.0912	0.0258
	CD @ 5%		0.0841	0.0591	0.0973	0.0879	0.2579	0.0730

**- Significant

R-Resistant, MR-Moderately resistant, MS-Moderately susceptible, S- Susceptible, HS-Highly susceptible

Table 4: Divergence in the total chlorophyll content in the cowpea genotypes due to pathogen infection

Sl. No.	Genotypes	Reaction	Total chlorophyll (mg/g of fresh wt.)					
			50 DAS		65 DAS		80 DAS	
			Healthy	Infected	Healthy	Infected	Healthy	Infected
1	MFC-08-14	R	1.133	1.083	1.400	1.200	1.833	1.600
2	KBC-6		1.067	1.033	1.600	1.347	1.867	1.570
3	KBC-2		1.000	0.980	1.500	1.400	1.900	1.733
4	COFC-8		1.033	0.960	1.567	1.333	1.767	1.600
5	EC-458480		1.000	0.967	1.367	1.133	1.800	1.537
6	EC-17058-1- 1		0.900	0.853	1.300	1.210	1.867	1.700
7	IC-402154	MR	0.867	0.823	1.200	1.040	1.567	1.500
8	NBC-16		0.933	0.907	1.400	1.133	1.700	1.333
9	KBC-4		0.733	0.690	1.133	0.867	1.700	1.400
10	NBC-41		0.733	0.700	1.200	0.967	1.603	1.300
11	V-578(C)		0.900	0.877	1.333	1.100	1.767	1.300
12	IC-402104		0.933	0.863	1.300	1.037	1.733	1.500
13	C-24-1		0.530	0.520	0.933	0.700	1.400	1.300
14	Plant-loob 3		0.767	0.730	1.000	0.900	1.433	1.200
15	CP-17		0.800	0.770	1.100	0.800	1.500	1.203
16	KM-5		0.667	0.653	0.967	0.867	1.400	1.280
17	IC-202804		0.600	0.573	1.033	0.800	1.500	1.183
18	IC-402181	MS	0.433	0.410	0.800	0.667	1.433	1.085
19	NBC-47		0.400	0.390	0.667	0.600	1.200	0.976
20	PGCP-12		0.500	0.480	0.933	0.900	1.400	0.865
21	NBC-40		0.533	0.513	1.000	0.903	1.300	1.103
22	IC-202722		0.600	0.557	0.900	0.807	1.367	1.192
23	C-157		0.400	0.390	0.833	0.793	1.300	0.965
24	PGCP-6	S	0.300	0.288	0.800	0.700	1.100	0.769
25	PGCP-11		0.367	0.357	0.800	0.600	1.067	0.671
26	IVTC-4		0.433	0.417	0.667	0.590	0.967	0.487
27	IVTC-5		0.300	0.303	0.633	0.537	0.933	0.377
28	PGCP-5		0.367	0.350	0.767	0.500	1.033	0.484
29	C-325		0.300	0.290	0.767	0.400	1.200	0.374
30	C-152	HS	0.233	0.200	0.800	0.300	1.233	0.350
	F		**	**	**	**	**	**
	S.Em±		0.0599	0.0291	0.0584	0.0258	0.0447	0.0583
	CD @ 5%		0.1581	0.0824	0.1651	0.0730	0.1265	0.1649

**- Significant

R-Resistant, MR-Moderately resistant, MS-Moderately susceptible, S- Susceptible, HS-Highly susceptible

Table 5: Divergence in the total peroxidase content in the cowpea genotypes due to pathogen infection

Sl. No.	Genotypes	Reaction	Total chlorophyll (mg/g of fresh wt.)					
			50 DAS		65 DAS		80 DAS	
			Healthy	Infected	Healthy	Infected	Healthy	Infected
1	MFC-08-14	R	0.789	0.955	0.827	1.2309	0.923	1.379
2	KBC-6		0.822	0.942	0.843	1.055	0.937	1.237
3	KBC-2		0.781	0.884	0.800	1.045	1.033	1.185
4	COFC-8		0.568	0.941	0.720	1.108	0.820	1.151
5	EC-458480		0.799	0.914	0.833	1.017	0.840	1.265
6	EC-17058-1- 1		0.833	0.936	0.850	1.064	0.870	1.222
7	IC-402154	MR	0.727	0.847	0.753	1.014	0.760	1.081
8	NBC-16		0.720	0.801	0.763	1.006	0.780	1.170
9	KBC-4		0.730	0.832	0.750	1.002	0.767	1.196
10	NBC-41		0.712	0.834	0.740	1.014	0.757	1.090
11	V-578(C)		0.696	0.810	0.723	1.033	0.743	1.148
12	IC-402104		0.737	0.827	0.753	0.952	0.760	1.092
13	C-24-1		0.733	0.824	0.757	0.974	0.773	1.099
14	Plant-loob 3		0.760	0.841	0.780	0.968	0.790	1.099
15	CP-17	MS	0.530	0.634	0.557	0.730	0.563	0.815
16	KM-5		0.540	0.629	0.560	0.722	0.566	0.820
17	IC-202804		0.550	0.661	0.573	0.747	0.586	0.829
18	IC-402181		0.520	0.662	0.543	0.757	0.551	0.833
19	NBC-47		0.530	0.657	0.553	0.752	0.563	0.834
20	PGCP-12		0.560	0.651	0.570	0.763	0.580	0.867
21	NBC-40		0.550	0.642	0.573	0.743	0.580	0.844

22	IC-202722		0.523	0.647	0.540	0.735	0.555	0.834
23	C-157		0.550	0.637	0.570	0.721	0.580	0.842
24	PGCP-6		0.413	0.454	0.430	0.474	0.450	0.618
25	PGCP-11		0.387	0.433	0.405	0.516	0.410	0.639
26	IVTC-4	S	0.373	0.430	0.383	0.520	0.403	0.643
27	IVTC-5		0.350	0.424	0.360	0.506	0.370	0.601
28	PGCP-5		0.320	0.399	0.343	0.441	0.360	0.597
29	C-325		0.333	0.444	0.340	0.491	0.350	0.592
30	C-152	HS	0.270	0.327	0.280	0.394	0.293	0.457
	F		**	**	**	**	**	**
	S.Em±		0.0447	0.0169	0.0039	0.0223	0.0071	0.0255
	CD @ 5%		0.1264	0.0478	0.0110	0.0629	0.0202	0.959

**- Significant
R-Resistant, MR-Moderately resistant, MS-Moderately susceptible, S- Susceptible, HS-Highly susceptible

Table 6: Changes in the phenylalanine ammonia lyase enzyme activity in cowpea genotypes of healthy and infected leaves

Sl. No.	Genotypes	Reaction	PAL (µ moles of trans cinnamic acid min^{-1} g^{-1})					
			50 DAS		65 DAS		80 DAS	
			Healthy	Infected	Healthy	Infected	Healthy	Infected
1	MFC-08-14		145	147	145	149	146	152
2	KBC-6		144	147	145	149	146	152
3	KBC-2	R	143	145	144	147	145	150
4	COFC-8		144	146	145	148	145	151
5	EC-458480		143	146	144	149	145	152
6	EC-17058-1- 1		145	147	146	149	147	152
7	IC-402154		129	131	130	134	130	138
8	NBC-16		130	133	131	135	132	138
9	KBC-4		132	134	132	136	133	137
10	NBC-41	MR	133	135	133	137	134	139
11	V-578(C)		134	137	134	139	134	140
12	IC-402104		135	138	136	139	137	141
13	C-24-1		133	136	134	137	134	140
14	Plant-loob 3		131	133	132	135	133	138
15	CP-17		120	122	121	126	122	128
16	KM-5		121	123	122	126	123	128
17	IC-202804		123	125	124	127	125	128
18	IC-402181		121	123	121	128	122	129
19	NBC-47		122	126	123	129	124	131
20	PGCP-12	MS	123	125	124	128	125	130
21	NBC-40		124	126	124	127	125	129
22	IC-202722		122	126	123	127	124	128
23	C-157		123	124	124	126	125	128
24	PGCP-6		99	102	99	103	100	105
25	PGCP-11		101	104	102	107	103	109
26	IVTC-4		101	105	102	106	103	108
27	IVTC-5	S	99	106	101	107	101	108
28	PGCP-5		102	107	103	108	104	109
29	C-325		99	105	100	107	101	108
30	C-152	HS	85	92	86	93	86	95
	F		**	**	**	**	**	**
	S.Em±		0.3702	0.08255	0.2108	0.7912	0.2277	0.9661
	CD @ 5%		1.0472	2.3353	0.5964	2.2381	0.6442	2.7329

**- Significant
R-Resistant, MR-Moderately resistant, MS-Moderately susceptible, S- Susceptible, HS-Highly susceptible

Table 7: Variation in the β 1, 3 -glucanase enzyme activity in cowpea genotypes at different days interval due to pathogen infection

Sl. No.	Genotypes	Reaction	β1, 3-glucanase (µg of glucanase released g^{-1} fresh wt)					
			50 DAS		65 DAS		80 DAS	
			Healthy	Infected	Healthy	Infected	Healthy	Infected
1	MFC-08-14		27	29	27	29	28	31
2	KBC-6		26	27	26	27	27	29
3	KBC-2	R	25	27	27	28	28	30
4	COFC-8		26	26	28	29	28	30
5	EC-458480		26	26	26	28	29	29
6	EC-17058-1- 1		25	27	27	28	29	30
7	IC-402154	MR	21	22	22	24	23	24

8	NBC-16		21	21	21	23	23	23
9	KBC-4		20	22	22	23	22	24
10	NBC-41		21	21	21	22	22	23
11	V-578(C)		21	22	22	23	22	24
12	IC-402104		20	21	21	22	21	22
13	C-24-1		22	22	22	23	24	24
14	Plant-loob 3		21	23	23	24	24	26
15	CP-17		17	19	18	19	19	20
16	KM-5		17	21	17	19	18	19
17	IC-202804		15	19	16	17	16	18
18	IC-402181		16	16	17	18	18	19
19	NBC-47	MS	16	18	17	18	17	18
20	PGCP-12		17	18	18	19	18	19
21	NBC-40		16	18	17	18	18	18
22	IC-202722		17	19	17	18	18	18
23	C-157		16	16	17	18	18	19
24	PGCP-6		14	15	15	16	15	16
25	PGCP-11		14	16	15	15	14	16
26	IVTC-4	S	13	15	14	16	14	16
27	IVTC-5		13	14	14	14	14	15
28	PGCP-5		14	15	15	16	15	16
29	C-325		14	16	14	15	14	16
30	C-152	HS	11	12	12	13	12	13
	F		**	**	**	**	**	**
	S.Em±		0.3333	0.5805	0.2018	0.6086	0.3162	0.4907
	CD @ 5%		0.9429	1.6423	0.5710	1.7216	0.8946	1.3880

**- Significant

R-Resistant, MR-Moderately resistant, MS-Moderately susceptible, S- Susceptible, HS-Highly susceptible

Table 8: Correlation between rust disease severity and biochemical parameters in cowpea genotypes

Sl. No.	Parameters	Disease severity
1	Phenols	-0.291**
2	Total sugars	-0.313**
3	Proteins	-0.298**
4	Total chlorophyll	-0.299**
5	Peroxidase	-0.282**
6	Phenylalanine ammonia lyase	-0.225**
7	β1, 3-glucanase	-0.231**

**- level of significance ($p \leq 0.01$)

Conclusion

The biochemical parameters viz., total phenols, total sugars, protein content, total chlorophyll, peroxidase, PAL and β 1, 3-glucanase activity was higher in resistant genotypes followed by moderately resistant genotype and was least in highly susceptible genotypes in both healthy and infected plants. Further, total phenols, protein content, peroxidase, PAL, β 1, 3-glucanase activity increased in both infected and healthy plants with increase in age of the cropand was highest in resistant genotypes compared to others while highly resistant genotypes recorded the least. Whereas, biochemical components like total sugars and chlorophyll decreased in infected leaves with the increase in age of the crop in all genotypes. However, total sugars and chlorophyll content was highest in resistant genotypes compared to the susceptible genotypes and least being in highly susceptible ones.

Acknowledgements

The author thanks the scientist of ZARS, V. C. Farm, Mandya, for providing the cowpea genotypes to conduct this study.

Conflict of Interest: On behalf of all the authors, the corresponding author wish to state that there is no conflict of interest.

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