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Bintu Dagar
Department of Plant Pathology, CCS Haryana Agricultural University, Hisar, Haryana, India

Vinod Kumar Malik
Department of Plant Pathology, CCS Haryana Agricultural University, Hisar, Haryana, India

Rakesh Kumar
Department of Plant Pathology, CCS Haryana Agricultural University, Hisar, Haryana, India

Tarun Verma
Department of Entomology, CCS Haryana Agricultural University, Hisar, Haryana, India

KS Ahlawat
Department of Forestry, CCS Haryana Agricultural University, Hisar, Haryana, India

Pavitra Kumari
Department of Plant Pathology, CCS Haryana Agricultural University, Hisar, Haryana, India

RS Chauhan
Department of Plant Pathology, CCS Haryana Agricultural University, Hisar, Haryana, India

Mamta Khaiper
Department of Forestry, CCS Haryana Agricultural University, Hisar, Haryana, India

Preety Verma
Department of Plant Pathology, CCS Haryana Agricultural University, Hisar, Haryana, India

Corresponding Author:
Vinod Kumar Malik
Department of Plant Pathology, CCS Haryana Agricultural University, Hisar, Haryana, India

Biochemical changes induced after challenging inoculation of *Magnaporthe grisea* in different genotypes of pearl millet

Bintu Dagar, Vinod Kumar Malik, Rakesh Kumar, Tarun Verma, KS Ahlawat, Pavitra Kumari, RS Chauhan, Mamta Khaiper and Preety Verma

Abstract
The present study entitled "Studies on *Magnaporthe grisea* incitant of blast disease of pearl millet [*Pennisetum glaucum* (L.) R. Br.]" was conducted during the *kharif* 2019 at research farm, Department of Plant Pathology, CCS Haryana Agricultural University, Hisar. Pearl millet is a rainfed crop which. Can survive well in the rainfall as 250 mm on relatively poor soils. Pearl millet blast disease is a devastating. Fungal disease causing considerable yield losses. Blast of pearl millet incited by *Magnaporthe grisea* is the most widespread and destructive disease of pearl millet in India and other pearl millet growing area of the world. Host physiology particularly host metabolites get. Disturbed due to attack of *Magnaporthe grisea*. Present study indicated significant. Change in phenolic compounds, chlorophyll compounds and sugars in resistant, moderately resistant and susceptible genotypes. The chlorophyll a, chlorophyll b, total chlorophyll and flavanol content were. Decrease at each stage in all the 3 genotypes continuously. Carotenoid and total soluble sugar content was higher in all the 3 genotype at 25 DAS but decrease upto 35 DAS but further increase at 45 DAS in all the genotypes. Maximum phenol content was recorded in (18-0426) genotype at 45 DAS stage and orthodihydroxy phenol was in (118-0035) genotype at 45 DAS.

Keywords: Carotenoid, flavanol, host metabolites and orthodihydroxy phenol

1. Introduction
Pearl millet [*Pennisetum glaucum* (L.) R. Br)] is one of the assured *Kharif* that can be grown. in the areas having adverse agr –climatic conditions *viz.*, hot, dry. weather and less fertile sandy soils with low moisture, hence called nutritious poor man's crop belonging to family *Poaceae*. Pearl millet is a rainfed. Crop which can survive well in the rainfall as 250 mm on relatively poor soils. Which is a highly cross-pollinated. Small-seeded cereal crop which is protogynous in nature. Bajra is cultivated in over 30 countries of Africa, America and Asia. Pearl millet ranks sixth among. Cereal crops based on world production and also more tolerant to harsh and water scarcity conditions.

The crop is best suited for areas with high temperature, low soil fertility, high salinity or low pH. In comparison to. Maize. And sorghum, pearl millet having more capacity of heat tolerance and more efficient. Utilization of soil moisture. More than 95 % pearl millet is grown for grain purpose only. The grain is also used as feed for swine, poultry and cat fish diets (Andrews and Kumar, 1992) and it is a good source of nutrient and energy for ruminant diet like dairy, cattle, beef and goats (Dove and Myer, 1995). It produces highly. Nutritious and palatable forage. It is a more abundant source of nutrients than other. Cereal crops. Pearl millet grains contain about 67.5% carbohydrates, 11.6% protein, 5% fat and 2.3% mineral (Yadav *et al.*, 2016). In pearl millet many. Economically. Important diseases are only a few that include blast, downy mildew, ergot, smut and rust (Thakur *et al*., 2011).

The data from 2002-2018 of blast disease incidence in pearl millet indicates that the disease is becoming more severe and. widespread (AICPMIP, 2002-2016). The pathogen has potential to damage the crop quantitatively as well as qualitatively. Its Green foliage yield can be reduced by 19-27% in plants with. Moderately severe and severe infections (Wilson & Gates, 1993). The plant responds both non-specifically and specifically to the invading pathogen and its virulence factors. After establishment of the relationship plants under go several physical and Change in level of biochemicals.

The importance of phenolic compounds in disease resistance has been recognized since the findings of Walker (1923 & 1926) who demonstrated the protective role of performed phenolics in the onion against smudge pathogen (*Colletotrichum circinans*). Kumar *et al.*, (2013) measured the change in concentration of biochemical compounds viz., total phenol, flavanol and ortho-dihydric phenol in the resistant (H00-256, HC-3) and susceptible (HC-1, HC-5 & L-550) chickpea genotypes at six different intervals, i.e. 0, 2, 4, 6, 8 and 10 days of the inoculation. The total phenols, flavanols and ortho-dihydric phenols were higher in resistant as compared to susceptible genotypes at all the comparable intervals. The concentrations of total phenols and flavanol showed an increase between two to four days of inoculation, while ortho-dihydric phenol increased up to sixth day followed by decline

Many infected plant tissues and resistant tissues show a common shift in the metabolism that results in the accumulation of an assay of secondary. Substances comprising of flavonoids and other compounds. Flavonoids are fairly well distributed in plant kingdom and possess insecticidal and antimicrobial activity. Chlorophyll are the pigments present in the plant cell imparting colour to the plant parts which may or may not have any role after pathogens contact and establishment. Mahatma *et al.*, (2009) reported that total chlorophyll content did not show any clear cut difference in resistant and susceptible genotypes at pre-infection stage, but at post-infection stage large decrease was observed in susceptible genotypes as compared to pre-infection.

Rathore *et al.*, 2001 also reported decrease in chlorophyll content in Plantago ovata infected by *Peronospora alta.* Ghose *et al.*, (2010) reported a drastic reduction of about 53.24% in total chlorophyll in blight infected. Mulberry leaves. The chlorophyll content was found to be lesser in the infected leaves than the healthy ones. Total chlorophyll contents reduced drastically in downy mildew infected leaves and in the ear heads showing tufting and complete malformation. Total chlorophyll was low when compared to healthy and diseased leaves of pearl millet.

2. Material and Methods

2.1 Level of biochemical

The following biochemical parameters. for six inbred namely18 – 0035, 18 – 0109, 18 – 0426, 18 – 0549, 18 – 0060, 18 – 0114 were carried out, repeated twice at 25, 35 and 45 DAS and results were recorded for following parameters.

(a) Total Phenols
(b) Orthodihydroxy phenols
(c) Flavanols
(d) Total soluble sugars
(e) Chlorophyll content

2.1.1 Extraction and estimation of total phenols

The collected leaves were dried and total phenols were extracted from dry leaves using 80% hot alcohol. Dry powdered sample (100 mg) of all. cultivars was homogenized with the 80% ethanol (making final total of 10 ml) and centrifuged at 10000 rpm for the 10 minutes, supernatant was taken and then final. Volume was made 10 ml with 80% ethanol. Total phenolic content was estimated by method of Swain and Hillis (1959) using Folin-Ciocalteau reagent, diluted 1:1(v/v), and saturated sodium carbonate. Solution (Dissolved anhydrous sodium carbonate (35 g) in 500 ml of distilled water by heating on a water bath at 70-80°C and then cooled the contents overnight and used the supernatant).

Thus, prepared alcohol extract (0.5 ml) was taken in the test tube and diluted with 3.5 ml of distilled water. Then 0.5 ml Folin- Ciocalteau reagent. Was added which was followed by 2 ml saturated sodium. Carbonate solution and then test tubes were heated in water for 2-3 minutes. The test tubes were cooled and the absorbance reading at 650 nm using spectrophotometer was taken. The blank was prepared. By using ethanol in the place of extract and remaining procedure was same. The amount of phenol in the sample was estimated. From the standard. Curve prepared gradually by taking Catechol as the standard phenol content and them data was expressed (mg/g dry weight).

2.1.2 Extraction and estimation of orthodihydroxy phenols

The orthodihydroxy phenols were determined by the method of Johnson and Schaal (1952). Two ml of extract. was taken in test tube and added 2 ml of 0.5 N HCl, 1 ml of Arrow's reagent (sodium nitrite 10 g and sodium molybdate 10 g in distilled water and made volume to 100 ml with distilled water) and 4 ml of distilled water in succession. After this 2 ml of 0.5 N NaOH was added and the solution was shaken till, pink colour appeared. Blank was prepared without the addition of Arrow's reagent. The amount of orthodihydroxy phenol in the sample was determined from the standard curve prepared simultaneously by taking Catechol as the standard phenol and the data was expressed as mg/g dry weight.

2.1.3 Extraction and estimation flavanols

The flavanols were determined by method of the Balbaa *et al*., (1974) with some slight modification. One ml of the alcohol extract was taken in. the test tube and then added 5ml of 0.1 M aluminium chloride solution (24.143 g of aluminium chloride was dissolved. in one litre of distilled water) in to it and thereafter, the absorbance of the solution was read at 420 nm using spectrophotometer. A blank was prepared and calculate the amount of flavanols by taking Catechol as standard and. the data was expressed (mg/g dry weight).

2.1.4 Extraction and estimation of total soluble sugars

The soluble sugars were estimated using the reagents H2SO4 and anthrone reagent (0.2 g in 100 ml conc. H2SO4). An amount of 0.5 ml of the alcohol extract was taken in the test tube and 1.5 ml of distilled water was added. Then 4 ml of anthrone reagent (0.2 g anthrone in 100 ml conc. H2SO4) was added. The tubes were shaken and then allowed to cool for 30 minutes and taken reading of the absorbance at 625 nm. The concentration of total sugars was calculated from standard curve of glucose prepared. Simultaneously and the data was expressed as mg glucose equi.g^{-1}.

2.1.5 Extraction and estimation of chlorophyll

The Chlorophyll content was calculated by using the method of Arnon (1956) at 25, 35 and 45 DAS. An amount of 100 mg of fresh leaf material from healthy and diseased leaves of different cultivars was homogenized in 80% acetone and centrifuged at 10,000 rpm for 10 minutes. The supernatant was taken and remaining residue was again extracted with 5ml 80% Acetone till the supernatant became color-less. All the supernatant samples were pooled together and the final. volume was made to 15 ml with 80% acetone. The optical

density. was taken at 450, 645 and 663 nm against 80% acetone (blank). Calculation was made as under:

Chlorophyll a mg/g tissue = 12.7 A663 – 2.69 A645 × V/1000×W 19

Chlorophyll. b mg/g tissue = 22.9 A645- 4.68 A663 × V/1000×W

Total chlorophyll mg/g tissue = 20.2 A645+ 8.02 (A663) × V/1000×W

Carotenoid (mg/g) = 10× A450 × V/ 2500× W

Where, A = Absorbance, V = final volume, W = Weight of the sample

3. Results and Discussions

Biochemical characters in resistant, moderately. Resistant and susceptible genotype of pearl millet

3.1 Phenols

The data revealed that at 25 DAS higher content of phenols were observed in resistant (9.21 mg catechol equi./g) in comparison. to moderate resistant (7.33 mg catechol equi./g) and susceptible line (4.65mg catechol equi./g) but with the advancement of disease total phenols decreased in resistant genotype (7.69 mg catechol equi./g) while slight hike in moderately resistant genotype (8.63 mg catechol equi./g) and. in susceptible (5.55mg catechol equi./g) at 35 DAS (Table 1). At 45 DAS, total phenol decrease again in resistant genotype (6.24 mg catechol equi./g) and increase in moderately resistant genotype (10.34 mg catechol equi./g) and in susceptible genotype (7.14mg catechol equi./g).

3.2 Orthodihydroxy Phenols

The data revealed that Orthodihydroxy phenol was maximum in moderately resistant genotype (2.26 mg catechol equi. /g) in comparison to resistant genotype (1.68 mg catechol equi./g) and in susceptible (1.65mg catechol equi. /g) at 25 DAS (Table: 1). At 35 DAS, slight hike in the resistant genotype (1.94 mg catechol equi./g) and in moderately resistant genotype (2.40mg catechol equi. /g) but slight decrease in susceptible genotype (1.27mg catechol equi. /g). At 45 DAS, sharp increase in all the three genotypes, the resistant genotype. (4.40 mg catechol equi./g), moderately resistant genotype (3.37mg catechol equi./g) and in the susceptible genotype (2.38 mg catechol equi./g).

Table 1: Phenolic contents in blast resistant (R), moderately resistant (MR) and susceptible (S) pearl millet genotypes at different stages of growth

Days after sowing (DAS)	Genotype	Total phenolic content (mg catechol equi./g)	Orthodihydroxy phenol (mg catechol equi./g)	Flavanol (mg catechol equi./g)
25	118-0035(R)	9.21	1.68	12.96
	18-0426 (MR)	7.33	2.26	9.83
	18-0060 (S)	4.65	1.65	7.81
35	118-0035 (R)	7.69	1.94	9.06
	18-0426 (MR)	8.63	2.40	8.91
	18-0060 (S)	5.55	1.27	5.56
45	118-0035 (R)	6.24	4.40	6.96
	18-0426(MR)	10.34	3.37	5.64
	18-0060 (S)	7.14	2.38	4.14
CD at 5%		D = 0.17	D = 0.33	D = 0.13
		G = 0.17	G = 0.33	G = 0.13
		D×G = 0.29	D×G =0.58	D×G = 0.23

Where D – Days after sowing G – Genotype

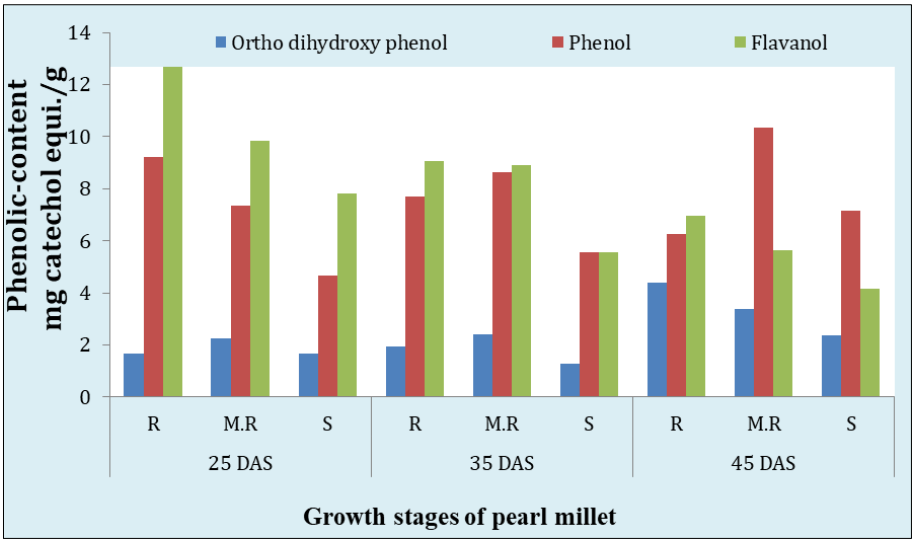

Fig 1: Phenolic content in pearl millet blast resistant (R), moderately resistant (MR) and susceptible (S) genotypes at different growth stages

3.3 Flavanol

The data of flavanol content revealed that higher flavanols were observed in resistant (12.96 mg catechol equi./g) in comparison to moderately resistant genotype (9.83 mg catechol equi./g) and in susceptible genotype (7.81mg catechol equi./g) at 25 DAS (Table 1). At 35 DAS, higher reduction in flavanol content was observed in all the three genotypes. But with the advancement of the disease. it further

decreased in resistant (6.96 mg catechol equi./g), moderately resistant genotype (5.64 mg catechol. equi./g) and in susceptible genotype (4.14 mg catechol equi./g) at 45 DAS.

3.4 Chlorophyll a, Chlorophyll b and total Chlorophyll

At 25 DAS, chlorophyll a (16.40 mg/g), chlorophyll b (15.08 mg/g) and total chlorophyll (14.43mg/g) was higher in resistant. Genotypes in comparison to moderately resistant and susceptible genotypes respectively. At every stage of crop development *viz*., 25, 35 and 45 DAS the chlorophyll content was higher in resistant genotype in comparison to moderately resistant and susceptible genotypes.

Table 2: Chlorophyll a, chlorophyll b, total chlorophyll, carotenoids and total soluble sugar content in pearl millet genotypes at different stages of growth

Days after sowing (DAS)	Genotype	Chlorophyll a (mg/g)	Chlorophyll b (mg/g)	Total chlorophyll (mg/g)	Carotenoids (mg/g)	Total soluble sugars (mg glucose equi./g)
25	118-0035 (R)	16.40	15.08	14.43	1.19	10.92
	18-0426 (MR)	15.56	14.26	13.28	1.09	6.37
	18-0060 (S)	14.92	13.44	11.72	1.00	2.28
35	118-0035 (R)	13.90	12.77	12.21	0.77	9.33
	18-0426 (MR)	12.68	10.86	9.96	0.61	4.94
	18-0060 (S)	9.81	10.34	8.61	0.51	2.15
45	118-0035 (R)	12.15	10.98	11.32	1.09	11.58
	18-0426 (MR)	11.02	9.14	7.74	0.96	6.93
	18-0060 (S)	7.79	7.19	6.06	0.69	1.35
CD at 5%		D = 0.69	D = 0.59	D = 0.53	D = 0.15	D = 0.18
		G = 0.69	G = 0.59	G = 0.53	G = 0.15	G = 0.18
		D×G = 1.20	D×G = 1.03	D×G = 0.92	D×G = N/S	D×G = 0.31

Where D – Days after sowing G – Genotype

3.5 Carotenoids and total soluble sugars

At 25 DAS, Carotenoid (1.19 mg/g) was higher in resistant genotypes in comparison to moderately resistant (1.09 mg/g) and susceptible genotypes (1.00mg/g) (Table 2). With the advancement of disease from 25 DAS to 45 DAS, there was reduction of carotenoid in resistant (0.77mg/g), moderately resistant (0.61mg/g) and susceptible genotypes (0.51mg/g) but shifting towards the last stage of crop severity, there was. Further increase in resistant (1.09mg/g), moderately resistant (0.96mg/g) and susceptible genotypes (0.69mg/g).

On the other hand, total soluble sugar at 25 DAS was high in resistant (10.92 mg glucose equi./g) in comparison to moderately resistant (6.37 mg glucose equi./g) and susceptible (2.28 mg glucose equi./g) genotype. With the advancement of disease at 35 DAS, the total soluble sugar content slight decrease in resistant genotype (9.33 mg glucose equi./g), moderately resistant (4.94 mg glucose equi./g) and susceptible genotypes (2.15 mg glucose equi./g).

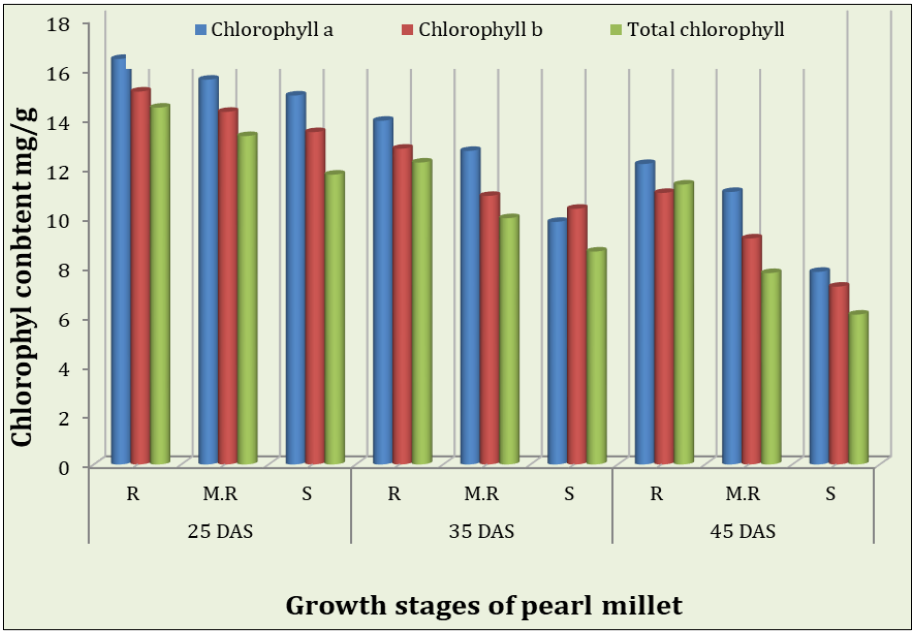

Fig 2: Chlorophyll a, chlorophyll b and total. Chlorophyll content in pearl millet blast resistant (R), moderately resistant (MR) and susceptible (S) genotypes at different growth stages**.**

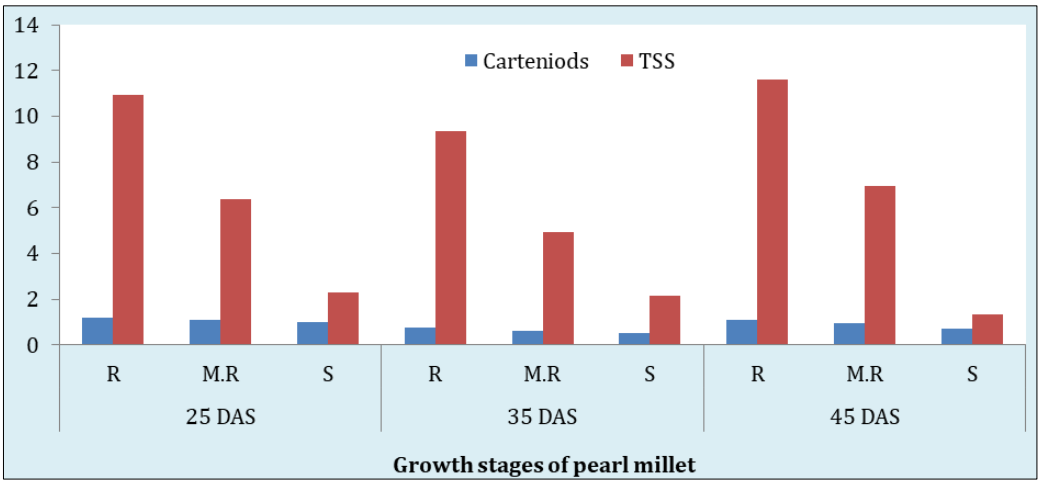

Fig 3: TSS and carotenoid content in pearl millet blast resistant (R), moderately resistant (MR) and susceptible (S) genotypes at different growth stages

Host physiology particularly host metabolites get disturbed due to attack of *Magnaporthe grisea*. Present study indicated significant change in phenolic compounds, chlorophyll compounds and sugars in resistant, moderately resistant and susceptible genotypes. Phenolic compounds are secondary metabolites of plant which constitute most widespread and common groups of substances in plants. The importance of phenolic compounds in resistance of disease has been recognized since the works of Walker (1923 and 1926) who demonstrated the protective role of performed. phenolics in onion against smudge pathogen *Colletotrichum circinans*. Total phenols were higher in resistant genotype at 25 DAS (9.21 mg catechol equi./g) in comparison to moderately resistant (7.33 mg catechol equi./g) and susceptible genotype (4.65 mg catechol equi./g) but with the progression of disease total phenols decreased in resistant genotype at 35 and 45 DAS but in moderately resistant and susceptible genotype they increased (Table 1). The results were found similar to the findings of Yadav *et al*., (1998) that total phenols increased during the early stages of plant. Growth (25 DAS) but decreased with plant age and increase in infection in moderately resistant and susceptible genotypes. The results of 35 and 45 DAS stage were similar to the finding of Shekhawat and Arya, (1979) that higher phenol level was registered in highly susceptible and susceptible entries of pearl millet. Similarly, Mahatma *et al.*, (2011) also reported that total phenols increased in susceptible genotypes and decreased in resistant genotypes of pearl millet after infection with downy mildew pathogen.

In present study, Orthodihydroxy phenol were higher (2.26-3.37 mg catechol equi./g) in moderately resistant genotype. at all the stages in comparison to resistant genotype (1.68-4.40 mg catechol equi./g) and susceptible genotype (1.65-2.38 mg catechol equi./g) except at 45 DAS stage (Table 2). These results are in contradiction with the findings of Kumar *et al*., 2013 that Orthodihydroxy phenols were higher in resistant genotype as compared to susceptible genotypes of chickpea in response to *Ascochyta* blight attack.

Many infected plant tissues particularly locally infected and resistant tissues show a common shift in metabolism that includes accumulation of an assay of secondary substances comprising of flavonoids and other compounds. Participation of flavanols in disease resistant reaction has been demonstrated in pigeon pea (Murthy and Bagyaraj, 1980). Present study revealed that flavanols were found to be higher (12.96 mg catechol equi./g) in resistant genotypes at 25 DAS in comparison to moderately resistant (9.83 mg catechol equi./g) and susceptible genotype (7.81 mg catechol equi./g). But with the progression of the disease, flavanol content decreased in all the three genotypes but more reduction was observed from 25 DAS to 45 DAS (12.96 to 6.96 mg catechol equi./g) (Table 12) in resistant genotype. Dhingra *et al*., (2013) reported higher total flavonoid content in moderately resistant genotype as compared to susceptible genotype of cauliflower against *Alternaria blight*.

In all the three genotypes there was reduction in chlorophyll a, chlorophyll b and total chlorophyll content from 25 to 45 DAS. (Table: 2, Fig. 2). Present study revealed that chlorophyll a, chlorophyll b and total chlorophyll content were found to be higher in resistant genotypes at 25 to 45 DAS in comparison to moderately resistant and susceptible genotype. In resistant genotype, carotenoid was more (1.19 mg/g) in comparison to moderately resistant (1.09 mg/g) and susceptible genotype (1.00 mg/g) at 25 DAS. At 35 DAS it decreased in resistant (0.77 mg/g) as well as in moderately resistant (0.61 mg/g) and susceptible genotype (0.51 mg/g). There was increase in carotenoid content in resistant genotype (1.09 mg/g), moderately resistant (0.96 mg/g) and slight in susceptible genotype (0.69 mg/g) at 45 DAS (Table 2, Fig. 2). Our results are similar to those of Mahatma *et al*., (2009) that at post-infection stage a significant decrease in chlorophyll and carotenoids content occurred in susceptible genotypes. Similarly, Ghose *et al.*, (2010) reported a drastic reduction of about 53.24% and 58.04% in total chlorophyll and carotene respectively in blight infected mulberry leaves.

Present study revealed that TSS were found to be higher (10.92 mg glucose equi./g) in resistant genotypes at 25 DAS in comparison to moderately resistant (6.37 mg glucose equi./g) and susceptible genotype (2.28 glucose mg equi./g). At 35 DAS it decreased in resistant (9.33 glucose mg equi./g) as well as in moderately resistant (4.94 mg glucose equi./g) and susceptible genotype (2.15 glucose mg equi./g). There was increase in TSS content in resistant genotype and moderately resistant at 45 DAS but in susceptible it further decreased (1.35 glucose mg equi./g). Results are in consistent with the finding of Dhingra *et al*., (2013) that moderately resistant genotypes had higher total soluble sugars than the susceptible genotypes against *Alternaria blight* in cauliflower

genotypes. A significant reduction in the total soluble sugar level was observed in all the genotypes. after infection. Sunkad and Kulkarni (2006) also reported the higher levels of sugars in resistant and moderately resistant groundnut genotypes as compared to susceptible genotypes in response to *Puccinia arachidis* infection.

4. Conclusion

Higher content of phenols were observed in resistant (9.21 mg catechol equi./g) in comparison to moderate resistant. (7.33 mg catechol equi./g) and susceptible line (4.65mg catechol equi./g). Orthodihydroxy phenol was maximum in moderately resistant genotype (2.26 mg catechol equi. /g) in comparison. to resistant genotype (1.68 mg catechol equi./g). Flavanol content revealed that higher flavanols were observed in resistant (in comparison to moderately resistant genotype and in susceptible genotype. Chlorophyll a, chlorophyll b and total chlorophyll was higher in resistant genotypes in comparison to moderately resistant and susceptible genotypes respectively. At every stage of crop development *viz*., 25, 35 and 45 DAS the chlorophyll content was higher in resistant genotype in comparison to moderately resistant and susceptible genotypes. Total soluble sugar was high in resistant in comparison to moderately resistant and susceptible genotype.

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6. Conflict of interest

None.

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