**Physiological and biochemical basis of resistance against *Colletotrichum capsici* (Sydow) in chilli germplasm grown under field conditions**

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**Novelty statement:** Chilli is an important spice/vegetable of the world and used in almost all vegetable cooking. Its production is hampered by *Colletotrichum capsici* which cause a great loss to farmers in the form of production and profit. Sustainable way to cope with this is to develop tolerance against the disease which cause no additional cost to the farmers and is also environment friendly relevant to pesticide use. Therefore, in this study we investigated the physiological and biochemical basis of tolerance against *Colletotrichum capsici* and identified best resistant genotypes. This study will create basis of development of a breeding program against *Colletotrichum capsici*.

**Abstract**

Screening of chili cultivars having natural resistance against anthracnose can be helpful in inducing resistance in susceptible cultivars through hybridization. Also, little is known about the biochemical basis of resistance in chili cultivars against anthracnose. In this regard, a field experiments were conducted during 2011 and was repeated in 2012 to investigate the resistant and susceptible cultivars in available chilli germplasm (total 30 cultivars) against anthracnose. As per disease rating, results revealed 18 cultivars to be resistant, 7 moderately resistant, 2 susceptible and 3 highly susceptible. The three resistant and three susceptible cultivars were selected and re-grown as pot experiment in 2013 to observe the comparative accumulation of biochemical compounds e.g. total soluble phenolics, reducing sugars, non-reducing sugars, total soluble sugars, total proteins, chlorophyll a & b and total chlorophyll contents. There was significant variation (*p ≤ 0.05*) in the concentration of these compounds in reaction group (inoculated and un-inoculated), type (resistant and susceptible) and in cultivars of the host plants as clear from nested random effect analysis of variance. More concentration of above stated biochemical compounds concentration in resistant cultivars comparative to susceptible ones might be the reason for their resistance against anthracnose.

**Keywords:** Climate change; Biochemical basis; disease resistance; *Capsicum annuum* L.; anthracnose

**INTRODUCTION**

*Capsicum* species originated in the tropical Americas are believed to have been consumed by humans since about 7500 BC. Christopher Columbus brought peppers to Europe on his return trip from the New World, after reportedly naming the fruit ‘red pepper’, due to its similarity in texture and taste to the unrelated black pepper, *Piper nigrum* (Bosland & Votava, 2003). *Capsicum* introduced quickly throughout Europe and into Asia, becoming an increasingly important and highly prized spice for many civilizations.

*Capsicum* commonly known as chili or pepper is amongst the most popular vegetables of the world following potato and tomato. Chili fruit is the source of a wide range of phenols, antioxidants, carotenoids, capsaicinoids, vitamins (A, C and E) and minerals like phosphorous, potassium, calcium, magnesium, iron and sulfur (Bosland & Votava, 2003; Marin et al., 2004). Annually, it is cultivated on an area of 1.5 mha around the world and Asia contributes about 46% of the total area. During 2017-18, an area of 65.1 ‘000’ ha was under the chili cultivation with 148.1 ‘000’ tons production in Pakistan (Pakistan Bureau of Statistics, 2018). Production of chilies not only fulfills 88% of the country’s requirement, but also helps in earning foreign exchange. Its production for spice, vegetable and other uses is increasing continuously.

The anthracnose disease in chilies was reported for the first time in Indo-Pak by Sydow in 1913 from Coimbatore of Madras Presidency. It affects the yield directly by infecting fruits and indirectly by infecting stems and leaves and yield loss up to 50-95% has been reported around the world (Bosland & Votava, 2003; Pakdeevaraporn et al., 2005; Poonpolgul & Kumphai, 2007; Saxena et al., 2016). Earlier, about 17% crop infection caused by anthracnose disease (*Colletotrichum* spp.) was reported in Punjab, Pakistan during 2015-16 (Haq et al., 2013; Bashair et al., 2016).

Plenty of measures against anthracnose have been suggested such as the use of systematic and contact fungicides as seed dressings and foliar sprays. However, these chemicals have not provided satisfactory results in controlling anthracnose of chilies due to development of tolerance in casual organism against these fungicides (Staub, 1991; Peres et al., 2004). Therefore, it is need of the hour to find out an economical, environment friendly and sustainable solution. Until now, no variety has been reported as resistant to anthracnose of chilies (Haq et al., 2013; Bashair et al., 2016).

One possible solution might be the exploration and exploitation of the full potential of resistant sources available. Thus, screening of available chili germplasm is a pre-requisite to identify the source of resistance against *Colletotrichum capsici* (Ridzuan et al., 2018). Moreover, screening of varieties/cultivars with natural resistance against a certain disease and exploring possible mechanisms is the first step in finding out the resistant genes (Pakdeevaraporn et al., 2005; Haq et al., 2013) which then can be transgressed to other high yielding genotypes. Generally, resistance in plants against various diseases have been associated with phenolic compounds. Carbohydrates and mineral elements also play an important role in inducing resistance (Ghosh et al., 2003; Chanchaichaovivat et al., 2008). Further, plants can also resist against pathogenic infection by increasing chlorophyll concentration in their leaves, thus enhancing the photosynthesis rate (Dietrich et al., 2005).

Based on the above discussion, it would imperative to screen available chili germplasm against anthracnose and determination of various biochemical changes in the form of phenolic, protein and sugar contents. The obtained results would be helpful in the identification of resistant sources which could be used for further tolerance in susceptible cultivars through breeding and genetic engineering/hybridization techniques. Based on above assumptions, the present was conducted to screen out the available germplasm of chili and to find out comparative biochemical changes between a resistant and susceptible cultivar. Up to our knowledge, no study has reported the screening of chili cultivars against anthracnose disease and biochemical basis of its resistance.

**MATERIALS AND METHODS**

**Establishment of disease screening nursery during year 2011-12**

Seeds of 26 cultivars (C-19, C-72, C-33, C-68, C-302, American dwarf, Arunalu, BSS-269, Ghotki, Hot Queen, Harmal, Kurni, Wonder King, Anaheim, P-6, Sabazpari, Skyline-1, Skyline-2, Loungi, Talhar, Tatapuri, FSD-1, FSD-2, KA-2, Neelum and Maha) were taken from Ayub Agricultural Research Institute (AARI) Faisalabad, Pakistan while that of four cultivars (namely Sanam, Golapeshawari, NARC-4 and Burewala) were purchased from the local market of Faisalabad. Seeds of all the cultivars were sown during 1st week of January 2011 and 2012 to raise nursery in the earthen pots (17 × 13 cm) at the experimental area of Department of Plant Pathology, University of Agriculture, Faisalabad. The earthen pots were filled with one kg of sterilized soil per pot. After 50 days, chili seedlings were transplanted in field with a plot size of 6 m × 3 m on ridges (0.6 m apart), following randomized complete block design (RCBD) in triplicate. All the recommended agronomic practices such as weeding, hoeing, etc. were applied to keep the crop in healthy condition. The plots were exposed to natural epidemics during the complete span of the growing season. The highly susceptible cultivar “Loungi” was planted as spreader on the boarders of field area. At maturity, (125 days after sowing), three plants from each row were randomly selected and ten fruits (comprising of immature, mature and fully ripened) from each plant were picked and observed for the assessment of anthracnose disease incidence during each picking. In total, seven pickings were taken during full reproductive period of the crop during 2011 and 2012. Data regarding disease were recorded by following modified scale in Gopinath et al. (2006) i.e. immune = 0% disease incidence, resistant = 1-25%; moderately resistant = 26-50%; moderately susceptible = 51-75% and susceptible = 76-100%. The disease incidence (D.I.) was recorded by using the following formula:

$$Disease incidence=\frac{Number of infected fruits}{Total number of fruits}×100$$

(Assessment regarding D.I was based upon examining the diameter (mm) of lesions produced on chilli fruits. As this study focused mainly on determination of biochemical factors which are thought to be the basis of resistance against anthracnose, so D.I (%) has been calculated). (Aklilu et al., 2018).

**Establishment of chili pepper nursery for determination of biochemical changes**

Seeds of 3 resistant (Sanam, C-72 and Talhar) and 3 susceptible cultivars (Golapeshawari, Loungi and Tatapuri) were selected and sown in the earthen pots at the experimental area of Department of Plant Pathology, University of Agriculture, Faisalabad during 2013. For nursery preparation, six earthen pots (17 × 13 cm) were used with 1 kg of sterilized soil per pot. Resistance in cultivars against anthracnose was based on the percentage of disease incidence like the cultivars with disease incidence ranging from 12-23% were called as resistant and with 77-82% as susceptible. Remaining protocols were followed as described earlier during establishment of disease screening nursery. On early flowering stage, inoculation was done to whole plants with the spore suspension of *Colletotrichum capsici* at 1 × 106 spores per liter (measured by haemocytometer) with the help of a sprayer in the evening. Koch postulates were followed for the proof of pathogenicity. Recommended cultural practices like weeding, hoeing etc. were performed. Percentage of disease incidence and its ranking according to the scale given in Table 1 was calculated.

**Data collection and determination of biochemical compounds**

The plant populations were comprised of two reaction groups i.e. inoculated and un-inoculated. Each group consisted of two reaction types, namely resistant and susceptible. The resistant reaction type had three chili cultivars viz: C-72, Talhari, Sanam; while susceptible reaction type composed of three cultivars viz: Golapeshawari, Tatapuri and Loungi. The plant samples (leaves for chlorophyll types and fruits for remaining biochemical attributes) from both resistant and susceptible cultivars were collected, stored in refrigerator at 4 °C and standard analytical methods were employed for the estimation of different physiological and biochemical attributes following nested design (Gomez & Gomez, 1984). All the physiological and biochemical analysis were performed in triplicate. There were 18 samples to be tested for each biochemical attributes and in total, 144 samples were assessed. Nested design can be referred as multifactor assessment as levels of cultivars are similar in both reaction types but not identical for different levels of another factor. In the present study, number of cultivars in each reaction types were three i.e. susceptible and resistant but these were not same in both the reaction types. Therefore, nested design was followed for statistical analysis of physiological and biochemical parameters.

Total soluble phenolic (TSPh) contents were determined by following the method of Julkenen-Titto (1985). Briefly, 0.1 g fresh ground plant sample (fruits) was refluxed with 1 mL acetone (80%) at 50 °C in a water bath, centrifuged for 5 minutes at 12000 rpm and the extract/supernatant was stored at -20 °C in a microfuge tube until it was used for further analysis. An aliquot (100 µL), taken from the extract was diluted with Milli Q water to 1 mL in a test tube and Folin-ciocalteu reagent (0.5 mL) was added and mixed. Then, 2.5 mL of Na2CO3 (7.5%) was added immediately and volume was made to 5 mL with Milli Q water, then vortexed for 5-10 seconds and placed in the dark for 20 minutes. The absorbance of samples was measured at 750 nm using a spectrophotometer (UV–vis model 1601, Shimadzu, Kyoto, Japan). The standard curves were prepared from 20, 40, 60, 80 and 100 µg of Gallic acid (prepared from 100 µg mL-1 stock).

Total soluble sugars (TSS) were quantified from fruit samples by the method of Yemm & Willis (1954), reducing sugars (RS) by the procedure of Somogyii (1952). The concentration of non-reducing sugars (NRS) was calculated by subtracting reducing sugars from total soluble sugars.

Total proteins (TP) in the plant sample were calculated after nitrogen determination through Kjeldahl apparatus. A known amount of oven dried sample (WI) was taken in a long neck Kjeldahl flask. Five grams of digestion mixture containing K2SO4 and CuSO4 and 25 mL of concentrated sulfuric acid were added. The sample was boiled in a digestion hood, initially at low temperature and then at vigorous boiling till the contents become clear. After cooling, the contents of the flask were diluted with distilled water in a 250 mL volumetric flask. A 10 mL of this solution was transferred to the micro Kjeldahl distillation apparatus and it was distilled in the presence of 10 mL of 40 % NaOH solution. The ammonia so produced was collected in a beaker containing 10 mL of 2 % of boric acid solution having 2 drops of methyl red as an indicator. The distillate was titrated against standard 0.1 N sulfuric acid to light pink point. The percentage of nitrogen was calculated according to the following formula (Kjeldahl, 1983).

$$Nitrogen (\%)=\frac{0.1 N H2SO4 × 0.0014× 250 × 100}{WI ×100}$$

The total protein percentage of the sample was calculated using the following formula:

$$Total Protein =Nitrogen (\%)×6.25$$

Total chlorophyll contents (TC) were quantified from inoculated and un-inoculated plant samples by following the method as described by Arnon (1949). Fresh leaves (0.25 g) were taken and extracted overnight with 5 mL acetone (80%) at -10 °C. The extract was centrifuged at 14000 rpm for 5 minutes and the absorbance of the supernatant was measured at 645 nm and 663 nm by spectrophotometer. The chlorophyll (Chl) a, b, and total were calculated by using the formulae of Inskeep & Bloom (1985).

**Statistical analysis**

The statistical tests were performed using the PROC MIXED procedure of the Statistical Analysis System (SAS®, 1990) using the software Mini Tab. Data were analyzed statistically and means were separated by Duncan’s multiple range test (DMRT) at the 5 % level of probability.

**RESULTS**

**Screening of chili cultivars against anthracnose during 2011-12**

During 2011-12, none of the cultivars exhibited immune response (0% disease incidence) against anthracnose disease among the thirty chili cultivars tested (Table 2). During 2011, eighteen cultivars (Sanam, C-19, C-72, C-33, American dwarf, Arunalu, BSS-269, Burewala, Ghotki, Hot Queen, Harmal, Kurni, Wonder King, Anaheim, P-6, Sabazpari, Skyline-1 and Skyline-2) expressed resistant response with disease rating scale of 2 (1-25% disease incidence), while seven cultivars (Talhar, FSD-1, FSD-2, KA-2, NARC-4, Neelum and Maha) showed moderately resistant response with disease rating scale of 3 (26-50% disease incidence). Two cultivars (C-68 and C-302) displayed moderately susceptible response with disease rating scale of 4 (51-75% disease incidence) while high susceptibility was shown by three cultivars (Tatapuri, Golapeshawari and Loungi) with highest disease rating scale of 5 (76-100% disease incidence) during the year 2011.

During 2012, highest disease incidence of 82.5, 79.6, 81.5, 60.1 and 61.7% was observed in Tatapuri, Golapeshawari, Loungi, C-68 and C-302, respectively. Moderate resistance was displayed by seven cultivars (Talhar, FSD-1, FSD-2, KA-2, NARC-4, Neelum and Maha with disease rating scale of 3 (26-50% disease incidence). The lowest disease incidence was recorded in case of Sanam, C-19, C-72, C-33, American dwarf, Arunalu, BSS-269, Burewala, Ghotki, Sabazpari, Skyline-2, Kurni, Anaheim, Wonder King, P-6, Harmal, Skyline-1 and Hot Queen with disease rating scale of 2 (1-25% disease incidence).

**Phenolic and sugar contents**

Non-significant difference was observed between inoculated and un-inoculated plants (255.0 and 350.4 µg mL-1) regarding total soluble phenolic (TSPh) contents of the plants (Table 3 and 4). Resistant and susceptible plants showed 328.8 and 276.7 µg mL-1 TSPh contents at *p* ≤ 0.05. The maximum and minimum contents of TSPh was observed in C-72 (371.8 µg mL-1) and Golapeshawari (232.1 µg mL-1). The component termed as “type” expressed total variance of 65.8% as shown in Table 3.

Regarding reducing sugars (RS), significant variation was observed in types and cultivars (Table 3). The average concentration of RS in inoculated and un-inoculated plants were 0.06 and 0.07 mg g-1 FW, respectively with no variation expressed. A variation of 53.7 and 46.3% of the total variance was noticed in type and cultivars, respectively during anthracnose attack. Resistant and susceptible cultivars contained 0.08 and 0.05 mg g-1 FW average concentration of RS. The maximum and minimum concentration of RS was recorded in Sanam (0.095 mg g-1 FW) and Loungi (0.042 mg g-1 FW), respectively (Table 4).

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Types and cultivars varied significantly in case of non-reducing sugars (NRS) with 67 and 33% of total variance expressed while non-significant variation was observed between inoculated and un-inoculated plants with an average NRS contents of 0.048 and 0.051 mg g-1 FW. The maximum (0.073 mg g-1 FW) and minimum (0.026 mg g-1 FW) NRS were observed in Sanam and Golapeshawari, respectively (Table 4).

Total soluble sugar (TSS) concentration had no significant variation in groups with an average TSS concentration of 0.092, 0.117 mg g-1 FW in inoculated and un-inoculated plants, respectively as shown in Table 3 and 4. Significant variation (63.6%) was expressed by resistant and susceptible cultivars with an average TSS concentration of 0.133 and 0.078 mg g-1 FW, respectively. The cultivars expressed significant variation as 36.4 % of the total variance. Sanam and Golapeshawari showed the maximum (0.165 mg g-1 FW) and minimum (0.063 mg g-1 FW) TSS concentration, respectively (Table 4).

**Protein and chlorophyll contents**

Groups and types showed no variance while 100% variability was noticed in cultivars regarding total protein (TP) contents (Table 5). The resistant and susceptible type cultivars showed an average protein contents of 258.3, 208.7 µg g-1 FW at *p*≤ 0.05. The maximum and minimum TP concentration un-inoculated (248.0 µg g-1 FW) and inoculated plants (219.0 µg g-1 FW), respectively.

A total variance of 95.6, 4.2 and 0.1% of Chlorophyll a (Chl a) was shown by group, type and cultivars, respectively significant at *p* ≤ 0.05 as shown in Table 5. Un-inoculated plants expressed the maximum Chl a concentration (0.9 mg g-1 FW) while that of the minimum (0.5 mg g-1 FW) was observed in inoculated plants. The maximum and minimum concentration of Chl a was recorded in C-72 (0.8 mg g-1 FW) and Loungi (0.7 mg g-1 FW), respectively (Table 6).

Significant variation was expressed in groups (96.4%) and cultivars (0.7%) of the total variance in Chlorophyll b (Chl b) contents (Table 5). Inoculated and un-inoculated plants contained 0.2 and 0.7 mg g-1 FW average concentration of Chl b, respectively. Similarly, resistant and susceptible cultivars had 0.5 and 0.4 mg g-1 FW concentration of Chl b, respectively. The maximum (0.5 mg g-1 FW) and minimum (0.4 mg g-1 FW) concentration of Chl b was observed in Talhar and Tatapuri, respectively (Table 6).

Regarding total chlorophyll (TC) contents, groups, types and cultivars expressed significant results (*p* ≤ 0.05) with 98.0, 1.1 and 0.2% of the total variance (Table 5). An average TC contents of 1.2 and 1.1 mg g-1 FW were observed in un-inoculated and inoculated plants while that of the resistant and susceptible plants contained 1.2 and 1.1 mg g-1 FW, respectively. The maximum (1.2 mg g-1 FW) and minimum (1.1 mg g-1 FW) contents of TC was recorded in case of Sanam and Tatapuri cultivars, respectively (Table 6).

**Discussion**

Use of varieties/cultivars with resistant traits has been the most economical, safe and an effective strategy for the management of plant diseases (Mohammed, 2013). In this regard, transfer of genes related to pathogen resistance is the only possible solution to protect the susceptible varieties/cultivars from disease incidence. For this purpose, screening of varieties/cultivars with natural resistance against a certain disease and exploring possible mechanisms is the first step in finding out the resistant genes sources that could be used and transferred to susceptible varieties in order to develop resistance in them (Pakdeevaraporn et al., 2005; Haq et al., 2013). The present study was based on screening available germplasm of chili for relative resistance/susceptibility against anthracnose of chilies. During this study, thirty (30) cultivars were tested for resistance against anthracnose of chilies and screened, based on disease incidence and disease rating scale. Moreover, the concentration of biochemical compounds such as phenols, proteins and sugars were also determined in order to explain the basis for their resistance/susceptibility against anthracnose.

From the results, it was clear that none of the cultivars tested were immune against anthracnose of chilies. Several cultivars (Sanam, C-19, C-72, C-33, American dwarf, Arunalu, BSS-269, Burewala, Ghotki, Hot queen, Harmal, Kurni, Wonder King, Anaheim, P-6, Sabazpari, Skyline-1 and Skyline-2) expressed natural resistance against anthracnose. Earlier, Haq et al. (2013) conducted screening of a few chili cultivars (11) against anthracnose disease and none had resistance against anthracnose of chilies. No further analysis was done to order to find out the reasons behind the resistance or susceptibility. Similar results have also been reported in chilies against anthracnose (Taylor, 2007; Jaihan et al., 2018). Moreover, disease resistance either horizontal (broad spectrum), vertical (race specific) or both (based upon R-gene) is thought to be broken by the continuous, frequent and over exploitation of same race specific resistant cultivars in the same geographic vicinity. In addition, development of new races and more aggressive strains of the pathogen with more virulence due to gene shuffling, mutation and horizontal gene transfer might disturb the host prevailing resistance (Agrios, 2005). Therefore, continuous testing of resistance in a variety or cultivars against a certain disease needs to be renewed.

A slight rise in disease incidence was observed during the year 2012 as compared to the previous year. This may be due to little inoculum available during the previous study year 2011. The sclerotia of the *Colletotrichum capsici* had maximum survivability in soil up to the depth of 5 cm. Another factor may be rainfall that had occurred during the end of March and in the start of April, 2012, which lowered the air temperature, enhanced relative humidity (80%) and thus all these environmental factors favored the progression of anthracnose (Garg et al., 2009).

Plant phenolics are secondary metabolites which are required for proper pigmentation, development, reproduction, tolerance to pathogens and for many other activities in plants. These compounds exist in different parts of the plant, such as root, shoot, leaf and flower. Phenolic intermediates play an important role in enhanced expression of resistance and are ubiquitous in plants (Leucuta et al., 2005; Lattanzio et al., 2006). In the present study, the production of phenolic contents was related to resistance as more total phenolic contents were noted in resistant cultivars (328.80 µg mL-1) as compared to the susceptible ones (276.7 µg mL-1). Earlier, a similar increase in total phenolic contents of chilies and onion was also observed and suggested to be due be a source of resistance against anthracnose caused by *Colletotrichum* spp. (Prathibha et al., 2013; Srivastava & Kumar, 2013). Decrease in phenolic contents in susceptible cultivars might be due to the oxidative polymerization of phenolics into melanin in necrotic tissues or the incorporation of phenols into lignin (Anand et al., 2009). However, contrary to current findings, Bharathi et al. (2004) observed that with the pathogenesis progression, there was a gradual increase of phenols in infected plants. This increase might be due to the production of insoluble material by the causal agent which may contain phenols or might be due to increased activity of certain enzymes which are very crucial for pathogen metabolism.

In resistant cultivars, production of total protein (TP), reducing- (RS), non-reducing- (NRS) and total soluble sugar (TSS) contents were more as compared to the susceptible ones which might be a reason for their increased resistance against anthracnose disease. Similar results have been noted in guava fruits infected by *Colletotrichum* spp. where a decrease in carbohydrates (RS, NRS, TSS) and protein was recorded possibly due to accumulation of insoluble solid materials in the diseased area (Saud et al., 2000). Khodke & Wankhede (2000) reported that TSS were decreased in chili pepper after infection by *Colletotrichum capsici.* Decrease in carbohydrate contents in susceptible cultivars may also be due to the deficiency of photosynthetic pigments, the magnitude of which has been reported to be directly proportional to the rate of photosynthesis (Ghosh et al., 2003). During pathogenesis, the enzymatic activities of pathogen may cause hydrolysis of sugars, thereby decreasing TSS contents rapidly in susceptible cultivars as compared to the resistant ones (Ghosh et al., 2003). Moreover, fungus uses the sugars and starch as a carbon source during respiration, and also as a source of energy for survival (Chanchaichaovivat et al., 2008).

In the resistant cultivars of both un-inoculated and inoculated chili plants, chlorophyll a (Chl a), chlorophyll b (Chl b) and total chlorophyll (TC) contents were found slightly higher i.e. 0.8, 0.45 & 1.2 mg g-1 as compared to the susceptible cultivars i.e. 0.7, 0.43 and 1.1 mg g-1, respectively. Similar results were reported by Mistry et al. (2008), where the decrease in TC contents was noted in susceptible chili cultivars infected by *Colletotrichum capsici.* It may also be due to lesion growth and stimulation of chlorosis and necrosis due to fungal attack which reduced the green leaf area; however, photosynthetic activity of the remaining green leaf tissues was apparently not impaired (Dietrich et al., 2005). Due to fungal attack, the production of different types of toxic substances in the host plant cells may also be the cause of reduced chlorophyll contents in chili plants as these are known to disturb chlorophyll synthesis and functionality (Perez-Grajales et al., 2018).

**Conclusions**

From the observed results, it was found that resistance to anthracnose disease of chilies was dependent on the cultivar under investigation, therefore selection of the genotypes should be made very carefully. Regarding, biochemical parameter, the highest phenolic, total protein and sugar contents were observed in resistant chili cultivars while that of the lowest were observed in highly susceptible cultivars which suggests role of these biochemicals in anthracnose tolerance and these biochemical may be used as biomarkers for screening of chili germplasm against anthracnose disease.

**Declarations**

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**Conflict of interest** the authors declare that they have no known competing financial interests or personal relationships that could appear to influence the work reported in this paper.

**Ethics approval** Not applicable

**Consent to participate** Not applicable**Consent for publication** All authors have read and approved this manuscript and have no competing interest

**Availability of data and material** Not applicable**Code availability** Not applicable

**Authors' contributions** MB designed the research and conducted the experiment and wrote initial draft, NJ supervised the work, and SU, MH, AS, MI performed statistical analyses, discussion and review and editing,

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**Table 1: Disease rating scale of infected fruits plant-1**

|  |  |  |  |
| --- | --- | --- | --- |
| Rating scale  | Infected fruits per plant (%) | Response  | Symbol  |
| 1 | 0 | Immune | I |
| 2 | 1-25 | Resistant | R |
| 3 | 26-50 | Moderately resistant | MR |
| 4 | 51-75 | Moderately susceptible | MS |
| 5 | 76-100 | Susceptible  | S |

**Table 2: Reaction of chili Germplasm against anthracnose during year 2011 and 2012**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Sr. No.** | **Cultivars** | **Rating** | **Disease incidence (%)** | **Response** |
| **2011** | **2012** | **2011** | **2012** | **2011** | **2012** |
| 1 | Sanam | 2 | 2 | 11.1±0.51 st | 15.08±1.02 r | R | R |
| 2 | C-19 | 2 | 2 | 10.6 ±0.57 t | 16.5±0.52 u | R | R |
| 3 | C-72 | 2 | 2 | 13.3±0.54 tq | 17.5±1.02 t | R | R |
| 4 | C-33 | 2 | 2 | 12.6±0.52 qr | 19.8±0.52 q | R | R |
| 5 | American Dwarf | 2 | 2 | 12.40±0.57 qr | 16.7±0.50 u | R | R |
| 6 | Arunalu | 2 | 2 | 14.7±0.05 o | 20.4±0.51 p | R | R |
| 7 | BSS-269 | 2 | 2 | 11.8 ±0.51 rs | 19.3±0.51 r | R | R |
| 8 | Burewala | 2 | 2 | 13.6±0.52 p | 18.5±1.03 s | R | R |
| 9 | Ghotki | 2 | 2 | 21.1±0.51 l | 23.4±1.03 m | R | R |
| 10 | Hot Queen | 2 | 2 | 24.1 ±0.51 ij | 17.5±1.03 t | R | R |
| 11 | Harmal | 2 | 2 | 23.3±0.52 jk | 24.4±0.52 kl | R | R |
| 12 | Kurni | 2 | 2 | 11.9±0.52 rs | 13.9±0.52 w | R | R |
| 13 | Wonder King | 2 | 2 | 14.7±0.12 o | 21.9±0.51 o | R | R |
| 14 | Anaheim | 2 | 2 | 16.0±0.52 n | 20.5±0.57 p | R | R |
| 15 | P-6 | 2 | 2 | 24.0 ±1.01 j | 23.1±0.54 m | R | R |
| 16 | Sabazpari | 2 | 2 | 12.8±0.52 pq | 22.5±0.52 n | R | R |
| 17 | Sky line-1 | 2 | 2 | 23.1±0.50 k | 24.2±0.57 kl | R | R |
| 18 | Sky line-2 | 2 | 2 | 19.2±0.51 m | 24.1±0.05 l | R | R |
| 19 | Talhari | 3 | 3 | 30.3±0.51 l | 33.8±0.51 fg | MR | MR |
| 20 | FSD-1 | 3 | 3 | 28.1 ±0.52 g | 32.5±0.13 h | MR | MR |
| 21 | FSD-2 | 3 | 3 | 27.2±0.52 g | 33.5±0.523 g | MR | MR |
| 22 | KA-2 | 3 | 3 | 26.1±0.52 h | 31.4±1.02 j | MR | MR |
| 23 | NARC-4 | 3 | 3 | 29.1±0.52 f | 34.2±0.52 f | MR | MR |
| 24 | Neelum | 3 | 3 | 28.0±0.52 g | 32.0±0.50 i | MR | MR |
| 25 | Maha | 3 | 3 | 25.0±0.51 i | 25.6±0.51 k | MR | MR |
| 26 | C-68 | 4 | 4 | 54.5±1.03 d | 60.1±0.51 e | MS | MS |
| 27 | C-302 | 4 | 4 | 55.6±1.02 c | 61.7±0.52 d | MS | MS |
| 28 | Tatapuri | 5 | 5 | 78.4±1.03 a | 82.5±0.52 a | S | S |
| 29 | GolaPeshawari | 5 | 5 | 76.5±0.52 b | 79.6±1.08 c | S | S |
| 30 | Loungi | 5 | 5 | 77.6±0.52 a | 81.5±1.03 b | S | S |

\*Mean values in a column sharing similar letters do not differ significantly as determined by the DMR test (*p* ≤ 0.05); R=Resistant, MR=moderately resistant, MS=moderately susceptible, S=susceptible

**Table 3: Nested ANOVA for biochemical factors (total soluble phenols, reducing sugars, non-reducing sugars and total soluble sugars) of inoculated and un-inoculated chili plants**

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **SOV** | **DF** | **SS** | **F value** | **P>F** | **MS** | **Variance component** | **% of total** |
| **Total soluble phenols (µg mL-1)** |
| Group (A) | 1 | 78537.72 | 0.28 | 0.648NS | 78537.72 | -3694.71 | 0.0 |
| Type (B) | 2 | 556103.90 | 61.47 | 0.000\* | 278051.9 | 10130.68 | 65.8 |
| Cultivar (C) | 8 | 36189.90 | 0.857 | 0.555NS | 4523.74 | -83.58 | 0.0 |
| Error | 96 | 506495.58 |  |  | 5275.99 | 5275.99 | 34.2 |
| Total | 107 | 1.18E+06 |  |  |  | 15406.67 |  |
| **Reducing sugar (mg g-1 FW)** |
| Group (A) | 1 | 0.0038  | 0.394  | 0.594NS | 0.0038  | -0.000 | 0.0 |
| Type (B) | 2 | 0.0192 | 4.490 | 0.049\* | 0.0096 | 0.000 | 53.7 |
| Cultivar (C) | 8 | 0.0171 | 7.13  | 0.000\* | 0.0021 | 0.000 | 46.3 |
| Error | 96 | 0.0000 |  |  | 0.0000 | 0.000 | 0.0 |
| Total | 107 | 0.0401 |  |  |  | 0.000 |  |
| **Non-reducing sugar (mg g-1 FW)** |
| Group (A) | 1 | 0.0046 | 0.410  | 0.587NS | 0.0046 | -0.000 | 0.0 |
| Type (B) | 2 | 0.0224  | 7.060 | 0.017\* | 0.0112 | 0.00 | 67.0 |
| Cultivar (C) | 8 | 0.0127 | 1.89  | 0.000\* | 0.0160 | 0.000 | 33.0 |
| Error | 96 | 0.0000 |  |  | 0.0000 | 0.000 | 0.0 |
| Total | 107 | 0.0396 |  |  |  | 0.001 |  |
| **Total soluble sugar (mg g-1 FW)** |
| Group (A) | 1 | 0.0167  | 0.401 | 0.591NS | 0.0167 | -0.0000 | 0.0 |
| Type (B) | 2 | 0.0830  | 6.256  | 0.023\* | 0.0415  | 0.001 | 63.6 |
| Cultivar (C) | 8 | 0.0531  | 0.85  | 0.000\* | 0.00667 | 0.001 | 36.4 |
| Error | 96 | 0.0000  |  |  | 0.0000 | 0.000 | 0.0 |
| Total | 107 | 0.1527 |  |  |  | 0.002 |  |

\* = significant at *p*≤ 0.05 while NS = non-significant

**Table 4: Concentration of total soluble phenols (TSPh), reducing sugars (RS), non-reducing sugars (NRS) and total soluble sugars (TSS) in reaction groups (inoculated versus un-inoculated), types (resistant versus susceptible) and cultivars of chili plants**

|  |
| --- |
| **Total soluble phenols (µg mL-1)** |
| Cultivars (C) | Sanam | C-72 | Talhar | Golapeshawari | Loungi | Tatapuri |
| Type (B) | Resistant | Susceptible |
| Group (A) | Inoc\* | Unin | Inoc | Unin | Inoc | Unin | Inoc | Unin | Inoc | Unin | Inoc | Unin |
| Amount of TSPh in (C) | 222.6 | 272.6 | 281.5 | 462.0 | 311.6 | 422.1 | 195.5 | 268.7 | 230.4 | 360.3 | 288.6 | 316.3 |
| Av. amount of TSPh in (C) | 247.6 | 371.8 | 366.9 | 232.1 | 295.3 | 302.5 |
| Av. amount of TSPh in (B) | 328.8 | 276.7 |
| Av. amount of TSPh in (A) |  Inoculated = 255.0 Un-inoculated = 350.4 |
| **Reducing sugar (mg g-1 FW)** |
| Amount of RS in (C) | 0.09 | 0.10 | 0.05 | 0.08 | 0.06 | 0.07 | 0.04 | 0.05 | 0.042 | 0.041 | 0.051 | 0.072 |
| Av. amount of RS in (C) | 0.095 | 0.065 | 0.065 | 0.045 | 0.042 | 0.061 |
| Av. amount of RS in (B) |  Resistant = 0.08 Susceptible = 0.05 |
| Av. amount of RS in (A) |  Inoculated = 0.06 Un-inoculated = 0.07 |
| **Non-reducing sugar (mg g-1 FW)** |
| Amount of NRS in (C) | 0.071 | 0.074 | 0.036 | 0.061 | 0.049 | 0.061 | 0.025 | 0.026 | 0.082 | 0.034 | 0.035 | 0.051 |
| Av. amount of NRS in (C) | 0.073 | 0.049 | 0.055 | 0.026 | 0.058 | 0.043 |
| Av. amount of NRS in (B) |  Resistant = 0.059 Susceptible = 0.042 |
| Av. amount of NRS in (A) |  Inoculated = 0.048 Un-inoculated = 0.051 |
| **Total soluble sugar (mg g-1 FW)** |
| Amount of TSS in (C) | 0.161 | 0.169 | 0.085 | 0.135 | 0.111 | 0.132 | 0.066 | 0.069 | 0.051 | 0.075 | 0.080 | 0.123 |
| Av. amount of TSS in (C) | 0.165 | 0.111 | 0.122 | 0.068 | 0.063 | 0.106 |
| Av. amount of TSS in (B) |  Resistant = 0.133 Susceptible = 0.078 |
| Av. amount of TSS in (A) |  Inoculated = 0.092 Un-inoculated = 0.117 |

\*Inoc=inoculated, Unin=un-inoculated

**Table 5: Nested ANOVA for biochemical parameters (Total Protein, Chlorophyll a, Chlorophyll b and Total Chlorophyll) of inoculated and un-inoculated chili plants**

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **SOV** | **DF** | **SS** | **F value** | **P>F** | **MS** | **Variance component** | **% of total** |
| **Total protein (µg g-1 FW)** |
| Group (A) | 1 | 22265.18 | 0.546 | 0.537NS | 22265.18 | -343.34 | 0.0 |
| Type (B) | 2 | 81611.07 | 0.634 | 0.555NS | 40805.53 | -871.24 | 0.0 |
| Cultivar (C) | 8 | 514631.71 | 950485.7 | 0.000\* | 64328.96 | 7147.66 | 100.0 |
| Error | 96 | 6.50 |  |  | 0.07 | 0.068 | 0.0 |
| Total | 107 | 618514.45 |  |  |  | 7147.72 |  |
| **Chlorophyll a (mg g-1 FW)** |
| Group (A) | 1 | 3.82 | 45.83 | 0.021\* | 3.82 | 0.069  | 95.6 |
| Type (B) | 2 | 0.17 | 141.01 | 0.000\* | 0.08 | 0.003 | 4.2 |
| Cultivar (C) | 8 | 0.005 | 13.20 | 0.000\* | 0.001 | 0.000 | 0.1 |
| Error | 96 | 0.004 |  |  | 0.00 | 0.000 | 0.1 |
| Total | 107 | 3.99 |  |  |  | 0.072 |  |
| **Chlorophyll b (mg g-1 FW)** |
| Group (A) | 1 | 4.74 | 1960.40 | 0.001\* | 4.74 | 0.088  | 96.4 |
| Type (B) | 2 | 0.005 | 0.30 | 0.749NS | 0.002 | -0.000 | 0.0 |
| Cultivar (C) | 8 | 0.064 | 3.06 | 0.004\* | 0.008 | 0.001 | 0.7 |
| Error | 96 | 0.25 |  |  | 0.003 | 0.003 | 2.9 |
| Total | 107 | 5.06 |  |  |  | 0.091 |  |
| **Total chlorophyll (mg g-1 FW)** |
| Group (A) | 1 | 17.24 | 172.16 | 0.006\* | 17.24 | 0.317  | 98.0 |
| Type (B) | 2 | 0.20 | 12.49 | 0.003\* | 0.10 | 0.003 | 1.1 |
| Cultivar (C) | 8 | 0.06 | 3.034 | 0.004\* | 0.008 | 0.001 | 0.2 |
| Error | 96 | 0.26 |  |  | 0.004 | 0.003 | 0.8 |
| Total | 107 | 17.76 |  |  |  | 0.324 |  |

\* = significant at *p*≤ 0.05 while NS = non-significant

**Table 6: Concentration of total proteins (TP), chlorophyll a (Chl a), chlorophyll b (Chl b) and total chlorophyll (TC) in reaction groups (inoculated versus un-inoculated), types (resistant versus susceptible) and cultivars of chili plants**

|  |
| --- |
| **Total protein (µg g-1 FW)** |
| Cultivars (C) | **Sanam** | **C-72** | **Talhar** | **Golapeshawari** | **Loungi** | **Tatapuri** |
| Type (B) | **Resistant** | **Susceptible** |
| Group (A) | **Inoc\*** | **Unin** | **Inoc** | **Unin** | **Inoc** | **Unin** | **Inoc** | **Unin** | **Inoc** | **Unin** | **Inoc** | **Unin** |
| Amount of TP in (C) | 161.5 | 210.8 | 280.7 | 381 | 323.9 | 192.0 | 150.9 | 190.2 | 256.0 | 328.4 | 140.8 | 185.8 |
| Av. amount of TP in (C) | 186.1 | 330.9 | 258.0 | 170.6 | 292.2 | 163.3 |
| Av. amount of TP in (B) | Resistant = 258.3 Susceptible = 208.7 |
| Av. amount of TP in (A) | Inoculated = 219.0 Un-inoculated = 248.0 |
| **Chlorophyll a (mg g-1 FW)** |
| Amount of Chl a in (C) | 0.6 | 1.0 | 0.6 | 1.0 | 0.6 | 0.9 | 0.5 | 0.9 | 0.5 | 0.9 | 0.5 | 0.9 |
| Av. amount of Chl a in (C) | 0.8 | 0.8 | 0.8 | 0.7 | 0.7 | 0.7 |
| Av. amount of Chl a in (B) | Resistant = 0.8 Susceptible = 0.7 |
| Av. amount of Chl a in (A) | Inoculated = 0.5 Un-inoculated = 0.9 |
| **Chlorophyll b (mg g-1 FW)** |
| Amount of Chl b in (C) | 0.2 | 0.7 | 0.2 | 0.7 | 0.3 | 0.7 | 0.2 | 0.7 | 0.2 | 0.7 | 0.2 | 0.7 |
| Av. amount of Chl b in (C) | 0.4 | 0.5 | 0.5 | 0.4 | 0.4 | 0.4 |
| Av. amount of Chl b in (B) | Resistant = 0.45 Susceptible = 0.43 |
| Av. amount of Chl b in (A) | Inoculated = 0.23 Un-inoculated = 0.66 |
| **Total chlorophyll (mg g-1 FW)** |
| Amount of TC in (C) | 0.8 | 1.6 | 0.8 | 1.6 | 0.8 | 1.6 | 0.7 | 1.6 | 0.7 | 1.5 | 0.7 | 1.5 |
| Av. amount of TC in (C) | 1.2 | 1.2 | 1.2 | 1.1 | 1.1 | 1.1 |
| Av. amount of TC in (B) | Resistant = 1.2 Susceptible = 1.1 |
| Av. amount of TC in (A) | Inoculated = 0.8 Un-inoculated = 1.6 |

\*Inoc=inoculated, Unin=un-inoculated