

Biochemical and Molecular Screening of Varieties of Chili Plants that are Resistant against Fusarium Wilt Infection

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Pakistan holds the position of top chilies producers. So *Capsicum annuum* L. production in Pakistan should be promoted by combating against diseases. The only solution is to cultivate resistant varieties. Presently six chili varieties were treated with *Fusarium oxysporum* Schlecht. and screened for the most resistant and the most susceptible varieties. Representative varieties were evaluated for their biochemical and transcriptional profiles to discover the bases of antifungal-resistance. Results concluded that the most resistant variety was "Dandicut" and the most susceptible was "Ghotki". Tannins, coumarins, flavonoids, phenolics, Riboflavins and saponins were observed in higher quantities in Dandicut as compared to Ghotki. Defense related enzymes i.e. polyphenol oxidase, phenyl ammonia lyase and peroxidase were found in elevated amounts in Dandicut than in Ghotki. Transcriptional results showed that defense related genes i.e. PR2a, acidic glucanase; Chitinase 3, acidic; Osmotin-like PR5 and Metallothionein 2b-like had higher expression rates in Dandicut. Pearson's correlation coefficient revealed stronger direct interaction in signal transduction and salicylic acid pathway. Resistance of chili varieties is salicylic acid based. Results obtained from this study not only help to improve chili production in Pakistan but also facilitate variety development operations. Moreover, it also constructed a scale to evaluate innate resistance among varieties.

Keywords: biochemical resistance, *Capsicum annuum*, defense related enzymes, *Fusarium oxysporum*, transcriptional analysis

Introduction

The chili is actually a fruit pod from the plant belonging to the nightshade family of *Solanaceae*. Chili pepper has a very hot and pungent flavor. The medicinal effects of chilies were known to pre-Hispanic people, and the modern science has confirmed the medicinal as well as nutritional value of the crop. Large amounts of vitamin A, vitamin C, vitamin E, and vitamin B1-3 have been detected in chilies [1]. Indian subcontinent is the largest producer of chilies in the whole world with its annual production of millions tons [2]. The important exporter countries of chilies are India, South Africa, China, Pakistan, Mexico, Malawi, and Zimbabwe [3].

There are numerous reasons affecting directly or indirectly the chili yield in Pakistan. Mal-cultural practice is one of those major reasons of yield loss [4]. Phytophthora root rot is another fungal infection of chilies which is very common in Pakistan. It also affects the chili price in international market or even chili approach to market. Wilt of chili is caused by the *Fusarium oxysporum* also known as Fusarium wilt. In Pakistan, Fusarium wilt of chili has caused 15% to 20% yield losses in dry areas during the last few years [5]. In current agricultural practices, fungus is controlled with synthetic chemical fungicides which effectively do so but are also disturbing our environment [6]. Plants are rich in several phytochemicals which are known to play important role in plant metabolism and defense against several diseases [7-9]. Phytochemicals also have countless benefits to humans as they are exploited as natural pesticides, flavorings, fragrances, medicinal compounds, fibers, beverages, and food products. These compounds protect host against bacteria, fungi, herbivores, insects, and viruses that plague the plant [10].

Plant defensive compounds are actually the secondary metabolites of plant's vegetative part and produce [11, 12]. They are among the most potent and therapeutically beneficial bioactive compounds, but their main function is to defend plants against invading pathogens including bacteria, fungi, and viruses [13]. They are also essential to plant life by making food unpalatable to herbivorous predators [14]. Most of the defense biochemicals are antioxidants due to their redox properties, which allow them to act as reducing agents, hydrogen donors, and singlet oxygen quenchers.

Due to enormous antimicrobial activities of defense biochemicals, during recent years, researches have been diverted towards the disease control by using plant metabolites as they are not serious hazard for environment and also not harmful for human health. In the present study, natural plant defenses have been evaluated against a fungal disease and may be potentially used against fungal pathogen *F. oxysporum* in case of chilies.

Materials and Methods

Procurement of Chili Varieties and Fungal Culture. Six chili varieties (i.e., Mexi, Nageena, Talhari, Ghotki, Sanam, and Dandicut) were procured from agriculture seed market, Lahore, Pakistan. The pathogenic isolate of *F. oxysporum* was obtained from Fungal Biotechnology Lab, University of the Punjab, Lahore, Pakistan. The culture was stored at 4 °C and subcultured monthly.

Screening of Representative Varieties. A screening test was performed to evaluate the most resistant and the most susceptible variety of chilies against *F. oxysporum*. The pathogenicity test was performed by using the protocol of Shafique *et al.* [15]. Disease rating scale was made on the basis of disease incidence and disease severity. Disease incidence was

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observed as the symptoms appeared on the plant, and disease index was calculated with the help of following formula:

$$\text{Disease Severity} = \frac{\text{Area of Plant Part Affected}}{\text{Total Area}} \times 100$$

$$\text{Disease Index} = \frac{\text{Number of Plants in Particular Category}}{\text{Total Number of Plants}} \times 100$$

Quantification of Biochemicals. The representative varieties obtained through primary screening were further subjected to biochemical analysis to verify their quantitative potential.

Quantification of Tannins. Five hundred milligrams of plant material was extracted for 30 min with 50 mL of methanol (80%). Extracts were filtered twice to remove plant debris, and then, 50 mL of the end volume was achieved by adding the same solvent as previous one. To quantify the tannin contents, 500 μL of extract was transferred in a test tube. Then, the prepared chemical solutions, i.e., folin–ciocalteu reagent (500 μL), sodium carbonate (1 mL), and distilled water (8 mL), were added. Reaction mixture was incubated at room temperature for 30 min. The first light absorbance was recorded at 760 nm through spectrophotometer (model: UT 2100 UV, Utech Products Inc., USA). Then, 0.5 g of casein was added into glass flask prior to addition of equal volumes of extract and distilled water (5 mL of each). After 2 h of incubation at room temperature, the mixture was filtered and the weight of residue was recorded.

Quantification of Coumarins. Prewighed plant material (0.5 g) was extracted with 50 mL of methanol (80%), and all the plant debris were removed by filtration. After that, the extract concentration was adjusted to 1 mg/ml by adding 80% methanol in it.

For quantification of coumarins, 0.5 mL of extract was taken into a test tube. Then, equal volume of lead acetate solution was added to it prior to dilution with 9 mL of distilled water. After rigorous shaking, 2 mL of this mixture was transferred to a new tube. In that tube, 8 mL of HCl solution was also added and incubation period of half an hour was provided at room temperature. Then absorbance was recorded at 320 nm and compared with the calibration curve to determine total coumarin contents.

Quantification of Flavonoids. Plant material (0.5 g) was extracted with 80% methanol. Extraction process continued up to half an hour at hot plate. Then, plant debris was removed by filtering the mixture and final volume of extract was increased by adding the same solvent again to get the final concentration of 1 mg/ml.

Quantity of flavonoids was determined by taking 0.5 mL of plant extract into a test tube containing equal volume of acetic acid solution. Then, other reagents were added to that test tube in order of pyridine solution (2 mL), aluminum chloride solution (1 mL), and 80% methanol solution (6 mL). The mixture was incubated for 30 min at room temperature, and then, light absorbance was recorded at 420 nm and compared with calibration curve, drawn by rutin to quantify flavonoids in given samples.

Quantification of Total Phenolics. Plant sample (1 g) was extracted with 80% methanol at hot plate at 70 °C for 15 min. Then, plant debris was removed through filtration, and 1 mL of extract was added up with 5 mL of distilled water accompanied with 0.25 mL of folin–ciocalteu reagent. Reaction mixture was incubated at 25 °C, and absorbance was taken at 725 nm in a spectrophotometer and compared with calibration curve.

Determination of Riboflavin. Weighed 5 g of plant material was taken, and extraction was done with ethanol (50%) for 1 h with continuous shaking. After removal of plant debris, 10 mL of plant extract was added up with 10 mL of 5% potassium permanganate and 10 mL of 30% hydrogen peroxide. Mixture was heated for 30 min, and then, 2 mL of sodium sulfate (40%) was added into mixture. Afterwards, water was added to the mixture to make the final volume 50 mL and absorbance was taken at 510 nm.

Determination of Alkaloids. Five grams of plant material was taken and soaked in 20% acetic acid solution prepared in ethanol for 4 h and then filtered and evaporated in rotary evaporator to one fourth of original volume. Then, concentrated ammonium solution was added to precipitate alkaloids. Precipitant was collected through filtration and weighed [16].

Determination of β -glucans. The method of Gruppen *et al.* [17] was used to quantify β -glucans of plant sample. Five grams of plant sample was extracted with dis. water and filtered. Absolute ethanol was added into extracts to precipitate polysaccharides which were filtered. Then, arabinoxylans were removed by the addition of $\text{Ba}(\text{OH})_2$ into filtrate and water was added in filtrate in equal volume. Aqueous layer was taken and evaporated to obtain β -glucans.

Determination of Saponins. Plant material was taken and shaken well in 100 mL of 20% ethanol. Mixture was heated up to 55 °C for 4 h and then filtered and bi-extracted with same solvent. Both extracts were combined and evaporated to reduce the volume up to 40 mL. Then, half volume of diethyl ether was added into it, and after vigorous shaking the aqueous layer was recovered. Same procedure was repeated by using *n*-butanol, and this time, *n*-butanol layer was isolated. These extracts were given washing with 10 mL of 5% NaCl, and extracts were dried in rotary evaporator to get solid saponins [16].

Determination of Pectin. Water extracts of 5 g plant material were prepared at boiling temperature for 1 h and then filtered, and extracts were added with 1 mL of NaOH and 30 mL of water and kept overnight. Afterwards, extracts were neutralized with 5 mL of acetic acid solution. After 5 min of intact stay, extracts were added up with 2.5 mL of calcium chloride solution. Residues were allowed to dry in a preweighed beaker to get solid pectins.

Enzymatic Assays. To determine enzymatic activities, the crude enzyme extracts were obtained through crushing the 1 g plant material in 3 mL of sodium phosphate buffer (pH 6.8) and precipitated with acetone. Protein pallet was dissolved in 8 M urea solution and used as enzyme source in downstream enzymatic analyses. The method described by Mayer *et al.* [18] was used to determine polyphenol oxidase (PPO) activity in plant samples by taking the spectrophotometric absorbance at 495 nm. Likewise, the method of Burrell and Rees [19] was adopted to determine the activity of phenyl ammonia lyase (PAL) at 290 nm absorbance. Peroxidase activity was determined by using the method as described by Fu and Huang [20], and light absorbance was taken at 470 nm after 1 min interval.

Hydrocarbons Assays. One gram of plant material was crushed and extracted with *n*-heptane (10 mL). This extract was washed by equal volume of distilled deionized water, and upper organic layer was collected to record its absorbance for quantification of hydrocarbons [21]. Triterpenoids were quantified through the following equation:

$$(\epsilon_{190 \text{ nm}} = 90 \pm 5 \text{ mM}^{-1} \text{ cm}^{-1})$$

while carotenoids quantification was carried out by using following equation:

$$(\epsilon_{450 \text{ nm}} = 165 \pm 5 \text{ mM}^{-1} \text{ cm}^{-1})$$

Pathogenesis-related Gene Expression Analysis. Reverse transcriptase (RT) polymerase chain reaction (RT-PCR) was performed to analyze the expression of resistance specific genes of chilies varieties. RNA isolation from 1 g leaf tissues of both chili varieties was carried out separately by using kit of “biomol,” RiboEX (TM). The same kit was utilized to synthesize cDNA of both chili varieties.

Semi-quantitative RT-PCR. In this study, α -Tubulin was used as housekeeping gene due to its strong recommendation by Coker and Davies [22]. A primer set used for studying α -Tubulin expression was the same as used by Xu and Shi [23], at their recommended thermocyclic conditions in GeneAmp-2700 thermocycler (Applied Biosystems, 850 Lincoln Centre Drive, Foster City, CA, USA). Primer sequences targeting housekeeping gene were [F]: TGAACAACCTCATAAGTGGCAAAG and [R]: TCCAGCAGAAGTGACCCAAGAC. The PCR products were electrophorated on 1% agarose gel and compared to analyze the equal expression of housekeeping gene.

cDNA synthesized and constructed in previous reaction was used as template in separate reactions. The quantity of amplified product at the end of the reaction was assumed to be directly related with the initial transcriptome contents isolated from plants. It was considered as the extent of gene expression. Amplifications were recorded with six primer sets (Table 1). Temperature conditions were provided to each primer as recommended by Kavroulakis *et al.* [24]. PCR product was electrophorated on agarose gel (1%) to visualize the amplified bands.

Data Analysis. Standard errors of means of all the replicates of each treatment were computed using computer software Microsoft excel. All the data were analyzed by analysis of variance (ANOVA) followed by Duncan's new multiple range (DMR) test to separate the mean differences. Photographs of gel were analyzed through GELANALYZER (Lazar, Hungary) to determine the intensity of the band, molecular weight, and R_f value of each amplified gene. The intensity of gene expression was taken into account to determine interrelations of different plant pathways using DSAASTAT (Onofri, Italy). Moreover, it was also used to compare pathway values with each other according to Pearson's correlation coefficient (PCC).

Results

Pathogenicity Assay. Six chili varieties were taken and inoculated with *F. oxysporum* to evaluate the most resistant

and the most susceptible variety of chilies. Infection and visible characteristic symptoms were evident after 10 days of inoculation. Initially, the plants displayed yellowing on the leaves that was turned into chlorosis and necrosis; slowly, it was converted to wilting and, eventually, the death of the whole plant. Results obtained were portrayed in Figure 1 which depicted that the maximum disease was induced in variety Ghotki, so it was stated as the most susceptible variety. The following susceptible varieties were Mexi and Talhari, which were more or less equally attacked by the pathogen and exhibited nonsignificant difference in disease indexes after the attack of pathogen. Nageena ranked third in the susceptibility index of chili varieties as it displayed about 50% disease index. The most resistant variety of chilies against *Fusarium* wilt was Dandicut as it displayed only 16% disease index. Sanam was closely followed by the Dandicut among the resistant varieties and revealed 33% disease index (Figure 1).

Thus, Ghotki was the most susceptible and Dandicut was the most resistant variety among the representative varieties, respectively.

Biochemical Quantifications. The representative varieties were subjected to quantitative biochemical analysis to scrutinize their resistance potential, and the results obtained are summarized in Figure 2. Susceptible variety (Ghotki) exhibited 0.0358 g/kg tannin contents in comparison with 0.432 g/kg tannin contents of resistant variety (Dandicut) which were found to be 12 times more in resistant variety than susceptible variety.

In case of coumarins, the resistant variety exhibited about 11 times more coumarins content in comparison to susceptible

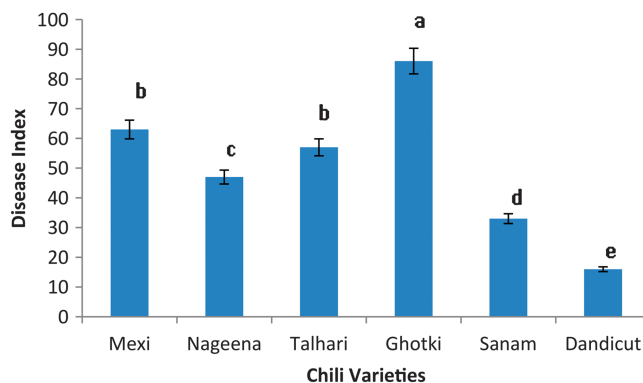


Figure 1. Disease index of selected chili varieties against *F. oxysporum*. Significance level of data was analyzed through Duncan's multiple range test at $P \leq 0.05$ and presented as values with different letters. Vertical bars indicate standard error of means of three replicates

Table 1. Pathogenesis related genes controlling innate antifungal resistance in plants

Gene family	Specific class	Accession numbers	Primer details	Tm (°C)	References
PR1	PR1b, basic PR1	AJ011520	F-CCAAGACTATCTTGCGGTTC	57.3	Van Kan <i>et al.</i> [25]
			R-GAACCTAAGCCACGATACCA	57.3	
PR2	PR2a, acidic glucanase	M80604	F-TATAGCCGTTGGAAACGAAG	55.3	Danhash <i>et al.</i> [26]
	PR2b, basic glucanase	M80608	R-TGATACTTTGGCCTCTGGTC	57.3	
F-CAACTTGCCATCACATTCTG			55.3		
R-CCAAAATGCTTCTCAAGCTC	55.3				
PR3	Chitinase 3, acidic	Z15141	F-CAACTTGCCATCACATTCTG	55.3	Danhash <i>et al.</i> [26]
	Chitinase 9, basic	Z15140	R-CCAAAATGCTTCTCAAGCTC	55.3	
PR5	Osmotin-like PR5	AY093593	F-AATTGTCAGAGCCAGTGTC	57.3	Rep <i>et al.</i> [27]
			R-TCCAAAAGACCTCTGATTGC	55.3	
PR7	P69A, subtilisin-like	Y17275	F-AATTGCAATTTAATGGTGC	49.1	Rep <i>et al.</i> [27]
			F-TAGCAGACCGTTAAGATGC	55.3	
MT2bL	Metallothionein 2b-like	EF584509	F-AACTGCAGAACAAGTGAAGG	55.3	Tornero <i>et al.</i> [28]
			R-AAC GTGATTGTAGCAACAGG	55.3	
			F-AGTACGCGGGGAGCAAC	57.3	
			R-TTTTTTTTTTTTTTTTTTTTTTTTTTTTGGAT	57.2	

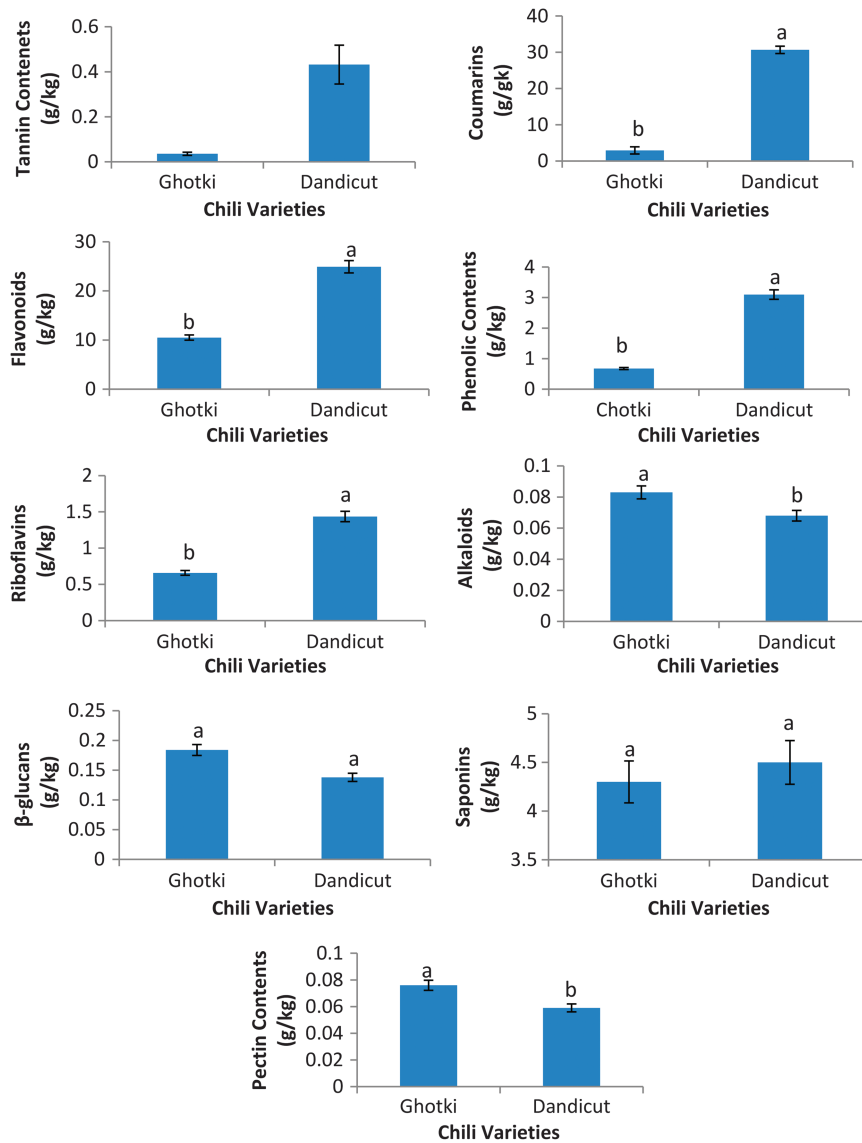


Figure 2. Comparison of different biochemical compounds of resistant and susceptible varieties. Significance level of data was analyzed through Duncan's multiple range test at $P \leq 0.05$ and presented as values with different letters. Vertical bars indicate standard error of means of three replicates

variety. It was evident from Figure 2 that resistant variety contained increased amounts of coumarins (30.67 g/kg) in their leaf tissues with respect to susceptible variety (2.918 g/kg).

Enhanced quantities of phenolics were presented in leaves of resistant chili variety than susceptible variety. Numerical value of phenolics in Dandicut was 3.1 g/kg, which was 4.5 times more than 0.675 g/kg phenolics of Ghotki.

Results revealed that about twice the amounts of riboflavin contents were detected in resistant variety with numeric values of 1.435 and 0.658 g/kg in leaf tissues of Dandicut and Ghotki, respectively.

Lesser quantities of alkaloids were recorded in chili variety exhibiting more antifungal resistance than the susceptible variety, as Ghotki exhibited 0.083 g/kg of alkaloids in their leaf tissues in comparison with 0.068 g/kg alkaloids of Dandicut.

The results demonstrated that the quantity of β -glucans was more in susceptible chili variety with lesser antifungal resistance as Ghotki revealed 0.184 g/kg of β -glucans, while 0.138 g/kg β -glucan contents were found in leaves of Dandicut.

The quantitative analysis of saponin contents of the representative varieties revealed that the resistant variety possessed about 5% more saponin contents than the susceptible chili variety as Figure 2 showed that the saponin content of Ghotki was 4.3 g/kg and Dandicut was 4.5 g/kg, respectively.

From the data analysis, it was found that 0.076 g/kg pectins were present in leaves of Ghotki in comparison to 0.059 g/kg pectin contents of Dandicut. Thus, more quantities of pectins were recorded in chili variety (Ghotki) with reduced antifungal resistance in comparison to Dandicut.

Analysis of Enzymatic Assays. The analysis of defense related enzymes of representative varieties revealed that the resistant variety possessed enhanced enzymatic activity as compared to susceptible one (Figure 3). It was clarified from the results that polyphenol oxidase activity had a direct interconnection with antifungal resistance as Ghotki showed 0.108 PPO activity in comparison with 0.16 activity of PPO in Dandicut leaves. About 20% increased PAL activity was detected in resistant variety. Similarly, Ghotki variety with reduced resistance was found to express 39.77 per oxidase (PO) activity; in contrast to it, Dandicut, with more antifungal resistance, exhibited 43.028 activity of PO (Figure 3).

Analysis of Hydrocarbons Assays. The representative varieties were further subjected to analysis of hydrocarbons to verify their quantitative potential. The results evidenced that the quantities of triterpenoids were found to be negatively associated with antifungal resistance of chili varieties, as Ghotki exhibited 25.2 g/kg triterpenoids, whereas Dandicut possessed 19.8 g/kg triterpenoid contents with more antifungal potential.

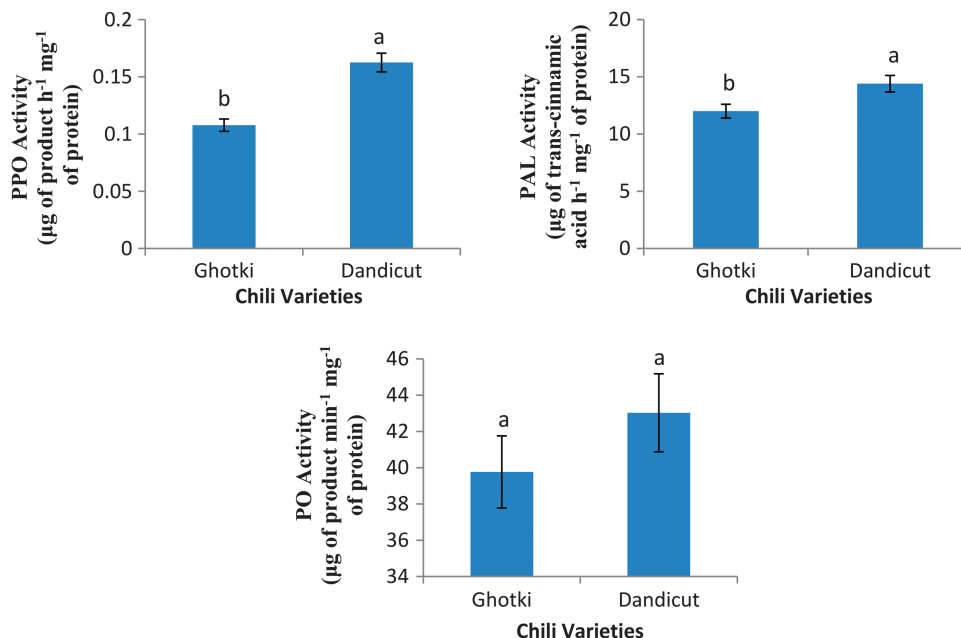


Figure 3. Comparison of defense related enzyme activity of resistant and susceptible varieties. Significance level of data was analyzed through Duncan's multiple range test at $P \leq 0.05$ and presented as values with different letters. Vertical bars indicate standard error of means of three replicates

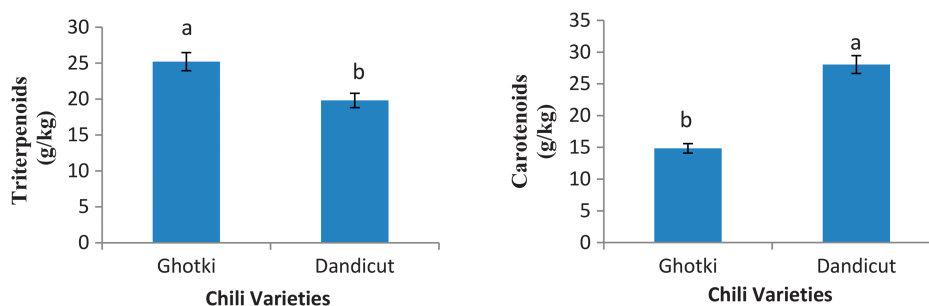


Figure 4. Comparison of triterpenoids and carotenoids of resistant and susceptible varieties. Significance level of data was analyzed through Duncan's multiple range test at $P \leq 0.05$ and presented as values with different letters. Vertical bars indicate standard error of means of three replicates

Conversely, carotenoid contents were found to be directly correlated with chili antifungal resistance, as Chili variety with lesser resistance had 14.85 g/kg carotenoids in comparison with 28.05 g/kg carotenoids of chili variety (Dandicut) with enhanced antifungal resistance (Figure 4).

Molecular Analysis. Molecular analysis revealed that the genes PR2a, acidic glucanase; chitinase 3, acidic; osmotin-like PR5; and metallothionein 2b-like had significantly greater expression in resistant variety while PR1b, basic PR1 and chitinase 9, basic genes did not express any transcriptional difference among varieties. Variability in plant innate

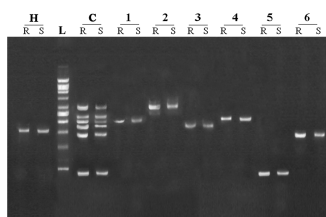


Figure 5. RT-PCR gel electrophoresis image of chili varieties for expression of defense related genes. H = housekeeping gene or reference gene (α -Tubulin, used as expression control). C = collective run of 6 defense related genes (5 μL of PCR products of lane 1, 2, 3...6 were loaded in a single well). 1 = PR2a, acidic glucanase; 2 = PR1b, basic PR1; 3 = chitinase 3, acidic; 4 = chitinase 9, basic; 5 = osmotin-like PR5; 6 = metallothionein 2b-like; R = resistant variety; S = susceptible variety

resistance did not affect the expression of these genes. Bands of two genes (PR2a, acidic glucanase and chitinase 3, acidic) of resistant variety were more than two times stronger than respective bands of susceptible variety (Figure 5) revealing strong association of gene expression with basal antifungal resistance of chilies. Osmotin-like PR5 and metallothionein 2b-like had slightly elevated expression in resistant variety, but elevation in their expression was not as remarkable as PR2a, acidic glucanase and chitinase 3, acidic.

Transcriptional increase of 33.25% and 28.52% was recorded in case of PR2a, acidic glucanase and chitinase 3, acidic, respectively. Transcript abundance of Osmotin-like PR5 was 39.31 ng/ μL in susceptible variety which was raised up to 44.17 ng/ μL in resistant variety. Similarly, band intensity of metallothionein 2b-like was 40.52 and 44.36 ng/ μL in susceptible and resistant variety, respectively. Transcript quantity of chitinase 9, basic was also elevated up to 25.69% in resistant variety (Figure 6).

The strongest direct interaction in resistant variety was found between signal transduction (ST) pathway and salicylic acid (SA) pathway (0.68). Jasmonic acid (JA) pathway had a direct but the poorest association with ST (0.25). SA and JA had a moderate direct interaction in resistant variety with PCC value of 0.34. Susceptible variety exhibited a significantly higher PCC value between SA and JA, indicating that both pathways had more pronounced interrelations in susceptible as compared to resistant chili varieties. SA was not found as strongly associated with ST in susceptible variety as was found in resistant. Thus, basal antifungal resistance of chilies

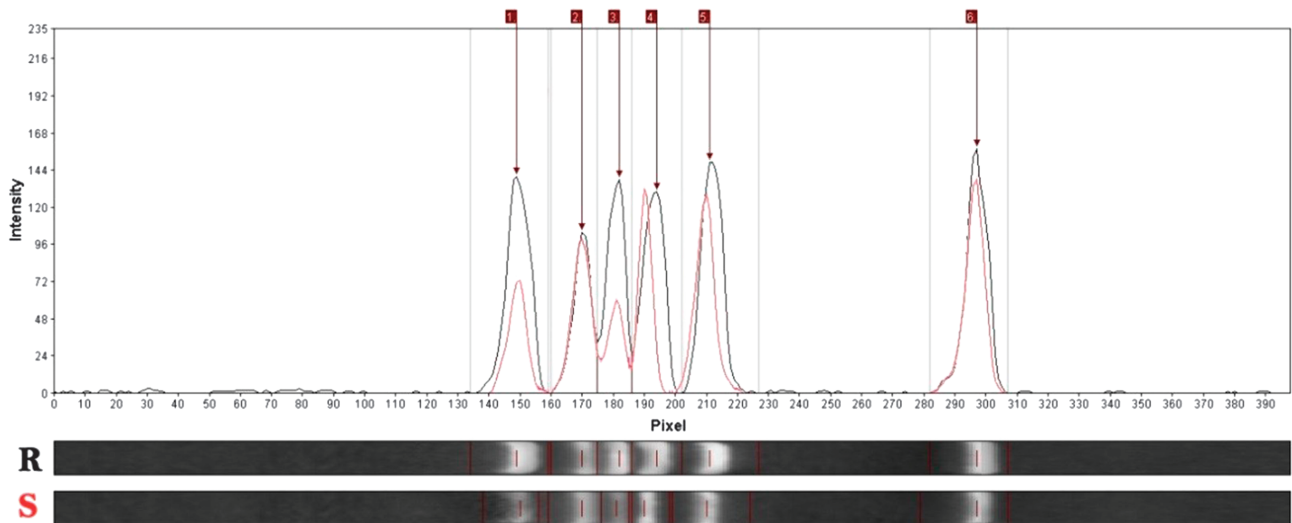


Figure 6. Band intensity graph of R and S lines under C category of “figure V.” Black line indicates genetic expression of resistant variety, whereas red line shows gene expression profile of susceptible variety. Bands of different genes have been tagged with different numbers, i.e., 1 = PR2a, acidic glucanase; 2 = PR1b, basic PR1; 3 = chitinase 3, acidic; 4 = chitinase 9, basic; 5 = osmotin-like PR5; and 6 = metallothionein 2b-like

was mainly dependent upon SA. A stronger correlation between JA and ST was recorded in susceptible chilies than resistant (Figure 7).

Discussion

Different varieties of same crop may have large differences in their innate resistance against pathogens. This variation in resistance depends upon the differences in defense weapons of plants, which are ultimately encoded by plant genome. Plant innate resistance may vary with the variety [29]. Different crop varieties may possess different levels of characters or even different characters. The present and future focus is on continuing improvement of agronomic traits such as yield and abiotic stress resistance in addition to the biotic stress tolerance of the present generation.

Tannin is a specialized group of plant originated phenolic compounds. The antimicrobial activity of tannins is basically dependent upon their ability to prevent microbial adhesion with host surface. Many researchers have reported that tannins are toxic to yeasts, filamentous fungi, and bacteria [30]. The present study is also the reminiscent of all these previous researches in which tannins have been reported as antimicrobial agents. It can be suggested that antifungal resistance in Dandicut is dependent upon increased quantities of tannins in them.

Coumarins are phenolic substances [31]. Higher concentration of coumarins is a sign of antipathogenic activity of plants. Similar results have been obtained in this study where resistant varieties of chilies exhibited greater amounts of coumarins

in their leaf tissues. The common herbs tarragon and thyme both contain caffeic acid which is effective against viruses [32], bacteria [33], and fungi [34]. Several studies had been conducted to evaluate the correlation between phenolic compounds and antioxidant activity. The antioxidant activity of Du-Zhong (*Eucommia ulmoides*) [35], ear mushrooms [36], and anise (*Pimpinella anisum* L.) seed [37] was found to correlate with the phenolic compounds.

The role of riboflavin as a plant defense activator in chili and bean against pathogens has been rarely investigated. The present study concludes that riboflavin production plays an essential role in the defense response towards the pathogen; thus, it supports the study of Sardooei [38] and confirms the positive role of riboflavin in defense response of plants. Likewise, alkaloids have their significant share in plant defenses. From the results of our present study, it may be concluded that resistance of chilies plant has no participation of alkaloids contents because resistant plant exhibited lesser quantities of alkaloids.

In controlling fungal plant pathogens, a variety of mechanisms contributes to the biocontrol activity of microbes. Cell-wall-degrading enzymes such as β -1, 3-glucanases, cellulases, proteases, and chitinases are involved in antagonistic activity of some biological control agents against phytopathogenic fungi [39]. In parallel to this, present investigation concludes that chitinase is not always sufficient for plant antifungal resistance; thus, glucans play their role in plant defenses. Saponins have also been found in elevated amounts in the resistant variety of chilies, which confirmed their antifungal activity inside plant

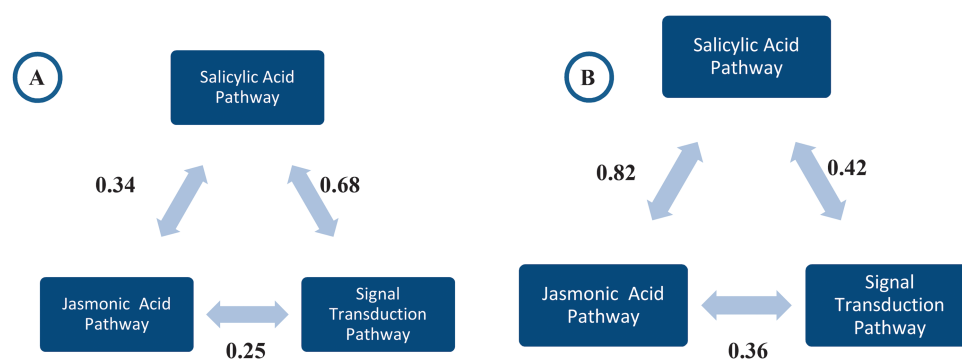


Figure 7. Correlation of plant pathways existing in different chili varieties. PCC values indicate the type and extent of interaction in two different pathways, while “A” and “B” have been used to tag resistant and susceptible chili variety, respectively

tissues [40]. These compounds, called phytoanticipins [41], are present constitutively in plants and seem to be involved in plant disease resistance because of their well-known antimicrobial activity [42, 43, 44]. However, many researches support the antifungal activity of pectin, but resistance of chilies is not dependent on pectin concentration because the current study has revealed that resistant varieties of chilies had lesser quantities of pectins.

In the present study, a change of defense related enzymes including PO, PPO, and PAL was detected in both chili varieties. Elevated amounts of defense related enzymes were found in resistant variety of chilies, which proved the active participation of defense related enzymes in constitutive antifungal resistance of chilies. Typically, this inducible resistance system as systemic acquired resistance (SAR) is effective against diverse pathogens including viruses, bacteria, and fungi [45]. The defense proteins make the plant resistant to pathogen invasion [46], and have been correlated with defense against pathogen invasion in cucumber [47].

Terpenes or terpenoids are active against bacteria [10], fungi [12, 48], and viruses. Presently, it can be concluded that these compounds do not play a significant role in plant antifungal defense. It has been proved that carotenoids are effective against various food-borne pathogens, such as *Staphylococcus aureus*, *Escherichia coli*, and *Salmonella enteritidis* [49–51]. Despite the broad antimicrobial spectrum, carotenoids were ineffective in certain plant materials [50, 51]. Thus, we suspect the involvement of carotenoids in the enhanced antimicrobial activity of carotenoids. Current research also proves that the carotenoids had antimicrobial activities that are responsible for constitutive antifungal resistance in chilies.

Plants have a variety of potential mechanisms at cellular level that might be involved in the tolerance to stress. These are involved primarily in avoiding the build-up of toxic concentrations at sensitive sites within the cell. It has been demonstrated that the physiological mechanism of resistance is not based on an enhanced synthesis of phytochemicals [52, 53] and that in resistant plants only a small proportion of stress factors seems to be coordinated by sulfur donor atoms [54, 55]. Some plants have been shown to have resistance stresses and resistance traits seem to be constitutive, as revealed by a study on a large number of European populations [56]. To evaluate the potential of resistance-mediated remediation, the genetics and physiology of resistance have to be investigated first. The current study is parallel to these researches in which resistance physiology has been studied. This would be very helpful in understanding the resistance mechanisms prevailing in plants.

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Author's Contributions

Sobiya S. and Shazia S. conceived the experiment. A.A. performed it. States and write up were completed by the efforts of all the authors.

Conflict of Interest

There is no conflict of interest between the authors.

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