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FORTIFYING THE FUTURE: BREEDING FUSARIUM WILT-RESISTANT CHICKPEA THROUGH BIOCHEMICAL UNDERSTANDING IN WESTERN HIMALAYAN KASHMIR

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ABSTRACT

Fusarium wilt, caused by *Fusarium oxysporum* f. sp. *ciceri,* poses a significant threat to chickpea production worldwide. The disease causes extensive yield losses, necessitating an in-depth understanding of resistance mechanisms to develop resistant cultivars. This study aims to investigate the biochemical basis of resistance in chickpea genotypes against Fusarium wilt to facilitate sustainable disease management and breeding strategies. A total of 117 chickpea genotypes were screened for resistance to Fusarium wilt under controlled conditions. The genotypes were categorized into resistant and susceptible groups based on their disease response. Biochemical profiles, including phenolic compounds, flavonoids, tannins, glycine betaine, proline, chlorophyll content, and DPPH antioxidant activity, were analyzed and compared between resistant and susceptible genotypes. Correlation analysis was also performed to explore relationships between biochemical traits associated with resistance. The study revealed varying degrees of resistance among chickpea genotypes, ranging from highly resistant to highly susceptible. Resistant genotypes exhibited significantly higher levels of phenolic compounds, flavonoids, tannins, and glycine betaine compared to susceptible genotypes, suggesting their roles in disease resistance. Correlation analysis revealed a strong positive correlation between chlorophyll content and DPPH antioxidant capacity in resistant genotypes, suggesting a potential synergistic role in enhancing resistance through improved antioxidant activity. These findings offer critical insights into the biochemical traits associated with Fusarium wilt resistance, contributing to the development of strategies for breeding resistant chickpea cultivars.

Keywords **:** *Chickpea, Fusarium, Secondary metabolites, Biotic stress*

Introduction

Chickpea (*Cicer arietinum* L., 2n = 16) commonly known as Bengal gram or garbanzo bean is one of the oldest (earlier than 9,500 BC) and widely cultivated pulse crop. It is a highly self-pollinating annual grain legume and is the third major legume in the world widely accounting for 11.67 million tons annually (FAO, 2019). Chickpeas are a widely grown crop found in dry regions and areas with moderate rainfall. Over 50 countries cultivate chickpeas, including those around the Mediterranean Sea, Central Asia, East Africa, Europe, Australia, and the Americas with India being the largest producer (Dadon *et al.,* 2017). India, while being the largest producer and importer of chickpea is also a major exporter ranking second only to Australia (FAO, 2019). India grows chickpea in

about 96.26 lakh ha producing 93.78 lakh tonnes which represents 35 per cent and 46 per cent of the national pulse acreage and production, respectively (Annual Report of DPD 2017-18). India cultivates both Kabuli (white seeded) and desi (brown seeded) types representing two diverse gene pools of chickpea but the production of later is more dominant. However, rarely pea-shaped chickpea types are available which may be a result of a cross between desi and kabuli types that has resulted in a sort of mixed populations (Muehlbauer and Tullu, 1997).

A significant portion of the global population struggles with food insecurity and malnutrition due to the high cost of nutritious food. With the rising global population, food growers around the world will have to increase food production significantly despite challenges like climate change, limited land and water resources, and a growing desire for varied, protein-rich diets among populations. Chickpea has multifarious role in human health and nutrition. The seeds of chickpea are rich in protein (24.6 per cent), carbohydrate (64.6 per cent) and vitamins (Abu-Salem and Abou, 2011). The carbohydrates and proteins, together constitutes 80 per cent of the total dry seed weight. Since chickpea plays the pivotal role of supplying protein source in the vegetarian diet, it is also called as the 'poor man's meat'. The mineral component is rich in phosphorous (343mg/100g), calcium (186mg/100g), magnesium (141mg/100g), iron (7mg/100g) and zinc (3mg/100g). Important carotenoids including β-carotene, lutein, zeaxanthin, β-cryptoxanthin, lycopene and α-carotene are also present in chickpea. (Abbo *et al*., 2005). On a dry seed weight basis, it has more amount of β-carotene than "golden rice" endosperm or red coloured wheat and it could potentially be utilized as a source of dietary carotenoids (Jukanti *et al.,* 2012). In spite being an economically important crop, chickpea productivity is low because of yield losses due to abiotic (drought, cold and salinity) and biotic stresses (*Helicoverpa armigera*, *Ascochyta* blight, *Fusarium* wilt and Botrytis grey mold). The yield losses due to abiotic stresses overreach 6.4 million tons than those caused by biotic stresses 4.8 million tons (Ryan, 1997).

Fusarium wilt, a disease caused by the fungus *Fusarium oxysporum* f.sp. *cicer*, can devastate chickpea crops, reducing yields by up to 90 percent (Varshney *et al*., 2013). *Fusarium* wilt is caused by about eight races of *Fusarium oxysporum* f. sp. *cicer* (Foc) affecting all major chickpea growing areas and at least three Foc races are known to exist in India (Gurjar *et al.,* 2009). *Fusarium* wilt invades plant roots and blocks the xylem, resulting in obstruction of nutrients to the plant, eventually leading to plant death. It can survive many years in soil even without its host and hence, poses a serious challenge for disease management (Haware *et al.,* 1996). Development of Foc resistant chickpea cultivars through breeding programs is the most effective way to counter fungal attack. However, pathogen variability and mutability result in loss of host resistance and remain as main hurdles for plant breeders. *Fusarium* wilt is one of the widely distributed diseases of chickpea depending on varietal susceptibility and climatic conditions (Jimenez-Diaz *et al.,* 1989; Patil *et al.,* 2015). The disease is prevailing mostly in the Indian subcontinent, Spain, Ethiopia, Mexico, Tunisia, Turkey and the United States (Ghosh *et al.,* 2013). Since the disease is soil borne, chemical control is not effective and practical to implement (Sharma *et al.,* 2017).

Disease interactions are complex in nature and factors leading to resistance or susceptibility in plant remain largely obscure. In response to stresses, plants exhibit a diverse range of responses at the cellular and molecular levels when confronted with biotic stresses like fungal infections. The resistance of plants to various diseases has been linked to alterations in biochemical parameters. These biochemical changes may be constitutively present in the plant or induced in response to the stress. The modulation of these biochemical factors, either by increasing their levels or activities, under stress conditions is indicative of the severity of the stress and the plant's capacity to resist it. This study investigated the biochemical responses of chickpea cultivars, both susceptible and resistant to wilt disease, by quantifying key biochemical compounds including glycine betaine, total phenols, tannins, flavonoids, chlorophyll, proline, alkaloids, 2,2-diphenyl-1-picrylhydrazyl (DPPH) and total soluble sugars. Analysis of infected plants from both cultivar types aimed to elucidate the biochemical mechanisms underlying disease resistance.

Materials and Methods

Plant material, isolation of the pathogen and inoculum preparation

The experimental materials comprised of 117 chickpea germplasm lines procured from Indian Institute of Pulses Research (IIPR) in Kanpur, Rajmata Vijayaraje Scindia Krishi Vishwavidyalaya (RVSKVV) in Gwalior and Dry Land Agricultural Research Station (DARS) in Budgam. Isolation of wilt fungi from affected plant samples was carried out using tissue isolation method described by (Rangaswamy and Mahadevan, 1999) under aseptic condition. The infected chickpea plant roots were

washed under running tap water to remove excess soil adhered to the root zone and dried on blotter paper before isolation to avoid contamination. These roots were then cut into small pieces of size 2–3 mm with sterilized blade. These bits were then surface sterilized with 2% sodium hypochlorite solution for one minute and rinsed with sterilized water at three intervals to remove traces of sodium hypochlorite on the root. Then each bit was dried on a blotter paper and four bits of each were placed on the Potato Dextrose Agar (PDA) medium poured plates and were incubated at 28±2°C for seven days in an incubator (Aneja, 2003). The obtained pure cultures were preserved and maintained on Potato Dextrose Agar (PDA) medium throughout the investigation.

Preparation of sick pots and sowing of genotypes

Soil was collected in autoclavable bags and sterilized in autoclave for one hour. Sand was added to the soil to facilitate proper drainage and aeration in pots. Finally the mass multiplied fungus inoculum was added in 1: 10 proportion to soil and thoroughly mixed, thus the soil was made sick. Plastic pots of size 20 cm diameter were taken and surface sterilized with 0.1% $HgCl_2$. The sick soil was filled in sterilized pots $1/4^{th}$ of its capacity. The pots were watered lightly and incubated for 4 days. Five seeds of each chickpea genotype was surface disinfected with 4% sodium hypochlorite solution for 30 seconds and pregerminated in moist paper towels in petri dishes for 3 days, and then planted in the pots. The seedlings maintained in sterilized soil without inoculums were served as control. Disease screening was done by recording incidence of the disease. The disease incidence was recorded using 1-5 disease rating proposed by Nene *et al.* (1981). Plants were observed periodically up to 55 days after sowing (DAS) for wilt symptoms and the per cent disease incidence was calculated (Jamil and Ashraf, 2020).

$$PDI = \frac{\text{Number of wilted plants pot - 1}}{\text{Total number of plants pot - 1}} \times 100$$

Chickpea samples of both resistant and susceptible plants were collected after 10 days post inoculation and all the biochemical estimations were done in triplicate and the results on fresh weight basis are statistically analyzed and reported.

Biochemical characterisation

To elucidate the biochemical mechanisms underlying Fusarium wilt resistance, ten contrasting genotypes (five resistant and five susceptible) were selected based on greenhouse screening. These genotypes have undergone comprehensive biochemical characterization.

Leaf chlorophyll

Arnon's (1949) acetone method was used to determine the amount of chlorophyll. In brief, 1g of fresh leaf tissue was crushed in a mortar and pestle with 80% acetone, then filtered and centrifuged. A UV-Vis spectrophotometer was used to detect the absorbance between 645 and 662 nm. The formula developed by Lichtentaler and Wellburn (1983) was used to determine the amounts of chlorophyll a and chlorophyll b.

Chlorophyll a (µg/g) = (11.75 x Abs 662 nm) – (2.350 x Abs 645 nm)

Chlorophyll b (µg/g) = (18.61 x Abs 645 nm) – (3.960 x Abs 662 nm)

Proline content

The proline content of leaves was calculated by applying the Bates et al. (1973) method. The procedure involves homogenizing a leaf sample (0.05 g) in 5 ml of 3% aqueous sulfosalicylic acid, centrifuging the homogenate at 15000 rpm for a duration of 10 min, and adding 2 ml of the supernatant to 2 ml of glacial acetic acid and 2 ml of acidic ninhydrin (heat 1.25 g ninhydrin in 30 ml of glacial acetic acid and 20 ml 6M H3PO4 with agitation until dissolved). Boil the mixture for 60 minutes in a water bath at 100°C. Putting up an ice route stopped the reaction. After adding 4ml of toluene to the mixture and thoroughly mixing it, absorbance at 520 nm was measured using toluene as a blank. L-Proline concentrations in series were used to create the concentration standard curve.

Phenols

The Folin–Ciocalteu method was used to measure total phenolic compounds spectrophotometrically, according to Singleton's (1999) procedure. The reference sample was distilled water, and the standard was gallic acid (GA). In brief, 4 mL of distilled water, 0.4 mL of methanolic leaf extract, and 0.4 mL of Folin-Ciocalteu reagent were combined in a 10 mL volumetric flask, and the mixture was left to react for five minutes. The total phenolic content (TPC), which is expressed as milligrams of gallic acid equivalents per gram of dry matter (mg GAE/g), was measured at 730 nm using a UV-VIS spectrophotometer after this incubation, which was followed by the introduction of 4 mL of 7% Na_2CO_3 solution and a 90-minute incubation in the dark at room temperature.

Flavonoids

The aluminum chloride technique was used to determine the flavonoid concentration. In brief, 1.9 mL of distilled water, 0.1 mL of 10% aluminum chloride hexahydrate, 0.1 mL of 1M potassium acetate, and 2.8 mL of distilled water were combined with 0.1 mL of leaf extract. For forty minutes, the reaction mixture that was produced was incubated at room temperature. At 415 nm, the solution's absorbance was then measured. The total amount of flavonoids in the extract was measured and reported as micrograms of rutin equivalents (µg RE) per milligram.

Alkaloids

Alkaloids were calculated using the Dragondroffs technique (Ajanal, 2012) including bromocresol green (BCG) solution (made by boiling 69.8 mg of bromocresol green with 3 ml of 2N NaOH and 5 ml of distilled water until completely dissolved and the solution was diluted to 1000 ml). By using 0.2 M citric acid to bring the pH of 2M sodium phosphate (71.6 gm Na_2HPO_4 in 1 L distilled water) down to 4.7, phosphate buffer solution (pH 4.7) was prepared. After transferring around 1 ml of the leaf extract solution to a separatory funnel, the pH was neutralized and three times washed with 10 ml of chloroform. This was mixed with 5 ml of phosphate buffer and 5 ml of BCG solution. Using a UV Spectrophotometer, the complex's absorbance in chloroform was measured at 470 nm in the spectrum. Graded concentrations of atropine were created by dissolving 1 ml of pure atropine in 10 ml of distilled water.

Tannins

The Vanillin-hydrochloride method was utilized to assess the amount of tannin. 2.5 ml of Vanillin-HCl reagent (made by mixing equal volumes of 8% HCl and 4% vanillin right before usage) was added to a 0.5 ml leaf extract. For 20 minutes, the mixture was incubated at room temperature. A UV/Visible spectrophotometer was used to detect the absorbance at 500 nm, and the tannin content was expressed in milligrams of tannic acid equivalents (mgTAE) per gram of dried sample.

DPPH assay (2,2-diphenyl-1-picrylhydrazyl)

The radical scavenging activity of different extracts was determined by using DPPH assay according to Chang (2001). 0.1mM DPPH solution was prepared by dissolving 4mg of DPPH in 100ml of ethanol. Different volumes (2 - 20µl) of plant extracts were made up to 40µl with DMSO and 2.96ml DPPH (0.1mM) solution was added. The reaction mixture was incubated in dark condition at room temperature for 20 min. After 20 min, the absorbance of the mixture was read at 517 nm. 3ml of DPPH was taken as control. Ascorbic acid (10mg/ml DMSO) was used as reference.

Glycine betaine

For glycine betaine, leaves were taken from the plants grown under normal and disease stressed conditions and analysis was carried out according to the method of Grieve and Grattan (1983). Leaf extract was prepared in 20 ml test tubes by chopping 0.5 g leaves in 5 ml of toluene-water mixture (0.05% toluene). All the tubes were mechanically shaken for 24 h at 25°C. After filtration 0.5 ml of extract was mixed with 1 ml of 2 N HCl solution and 0.1 ml of potassium tri-iodide solution (containing 7.5 g iodine and 10 g potassium iodide in 100 ml of 1 N HCl) was added and shaken in an ice cold water bath for 90 min. 2 ml of ice-cooled water was added after gentle shaking. 10 ml of 1, 2 dichloroethane (chilled at -10°C) was poured in it. By passing continuous stream of air for 1-2 minutes, two layers were separated, upper aqueous layer should be discarded and optical density of organic layer was recorded at 365 nm.

Total soluble sugars

Tissues of shoot (0.1 g) was crushed and then homogenized with 2 ml of 80% ethanol in shaker at 200 rpm in 5-ml tube for 1 h. Centrifuged at 6000 × g for 10 min and then equal volumes of chloroform was added to the supernatant, completely mixed and further centrifuged at 12,000 × g for 10 min. 0.2 ml of aqueous part was transferred to a new tube and mixed with 4.8 ml anthrone reagent and then boiled for 15 min. Optical density of samples was measured at 620 nm by spectrophotometer. The content of soluble sugar was calculated according to the standard curve (Mandre, 2002; Jin, 2007).

Data analysis: Excel 2010 was used to process the experimental data and generate graphs while R studio 4.3.0 was used for correlation analysis.

Results

Disease reaction

Based on a pooled analysis of greenhouse screening data from 2022 and 2023, a total of 117 chickpea genotypes were evaluated for resistance to Fusarium wilt. The results indicated a distribution of resistance levels, with three genotypes exhibiting complete resistance, 12 showing resistance, 15 demonstrating moderate resistance, 32 displaying moderate susceptibility, 38 classified as susceptible, and 17 exhibiting complete susceptibility (Figure 1). The AUDPC (Area Under Disease Progress Curve)

graph provides a visual representation of disease progression over time for 117 chickpea genotypes (Figure 2). The AUDPC is calculated by summing the area under the disease progress curve for a specific time period, in this case, from 0 to 35, 35 to 45, and 45 to 55 days after inoculation. Higher the AUDPC value, the more severe the disease progression. The graph shows a clear increase in AUDPC over time, indicating that the disease progresses as days go by. The steepest increase in AUDPC occurs between 45 and 55 days, suggesting that the disease intensifies significantly during this period.

Biochemical characterization of resistant and susceptible genotypes

Resistant chickpea genotypes exhibited significantly higher mean levels of phenolic compounds (78.51) and flavonoids (51.93) compared to susceptible genotypes (66.90 and 50.16, respectively). Although both resistant and susceptible genotypes displayed similar mean chlorophyll content (45.07 vs. 44.23), resistant genotypes had significantly higher mean tannin content (1.47) than susceptible genotypes (1.17). Interestingly, resistant genotypes showed slightly lower mean proline content (22.77) compared to susceptible genotypes (24.93). However, resistant genotypes exhibited significantly higher mean glycine betaine content (117.62) than susceptible genotypes (16.22). Both resistant and susceptible genotypes displayed similar mean alkaloid content (4.93 vs. 4.70). Furthermore, resistant genotypes had higher mean total soluble sugar content (60.00) compared to susceptible genotypes (44.63). Both resistant and susceptible genotypes exhibited similar mean DPPH values (1.63 vs. 1.62), indicating comparable antioxidant activity (Table 1).

Mean performance of biochemical profile of resistant and susceptible genotypes:

Mean performance of resistant and susceptible genotypes for biochemical parameters is presented in Table 2.

Phenols

Phenols are secondary metabolites known for their antioxidant and antimicrobial properties. Genotypes K9 and Y39 exhibited the highest phenolic content (92.02 and 91.03, respectively), suggesting a robust defence mechanism against Fusarium wilt. Genotypes K16 and K17 had lower phenolic levels (69.78 and 64.72, respectively), indicating a weaker defence response.

Flavonoids

Flavonoids are another class of secondary metabolites with antioxidant and antifungal properties. Genotypes K9 and D35 showed the highest flavonoid content (66.14 and 60.39, respectively), suggesting enhanced resistance to Fusarium wilt. Genotypes Y39 and K17 had lower flavonoid levels (42.95 and 49.4, respectively), indicating a weaker defence response.

Chlorophyll

Chlorophyll is essential for photosynthesis and plant growth. All genotypes had relatively similar chlorophyll levels, suggesting that Fusarium wilt infection did not significantly affect photosynthetic activity. However, genotype K9 had the highest chlorophyll content (51.24), indicating a potentially stronger photosynthetic capacity.

Tannins

Tannins are polyphenolic compounds with antimicrobial properties. Genotypes K9 and K16 had the highest tannin content (1.78 and 1.7, respectively), suggesting a stronger defence mechanism against Fusarium wilt. Genotypes D2 and D30 had lower tannin levels (0.89 and 1.17, respectively), indicating a weaker defence response.

Proline

Proline is an amino acid that accumulates under stress conditions, acting as an osmoprotectant and antioxidant. Genotypes D35 and K14 had the highest proline content (29.71), suggesting a stronger stress tolerance mechanism. Genotypes Y39 and D2 had lower proline levels (18.14 and 16.6, respectively), indicating a weaker stress tolerance response.

Glycine Betaine (GB)

Glycine betaine is another osmo-protectant that helps maintain cell turgor under stress. Genotype K16 had the highest GB content (131.01), indicating a strong osmotic adjustment capacity. Genotypes K6 and D2 had the lowest GB levels (8.43 and 3.71, respectively), suggesting a weaker osmotic adjustment capacity.

Alkaloids

Alkaloids are nitrogen-containing compounds with various biological activities, including antimicrobial properties. All genotypes had relatively similar alkaloid levels, suggesting that alkaloids may not play a major role in Fusarium wilt resistance in these genotypes. But genotypes Y39 and K16 had slightly higher alkaloid content (5.05 and 5.03, respectively) compared to other chickpea genotypes.

Total Soluble Sugars (TSS)

Total soluble sugars can act as energy sources and osmoprotectants. Genotype K17 had the highest TSS content (81.76), suggesting a strong energy storage and osmotic adjustment capacity. Genotypes K6 and D2 had the lowest TSS levels (37.6 and 40.04, respectively), indicating a weaker energy storage and osmotic adjustment capacity.

DPPH Radical Scavenging Activity

DPPH radical scavenging activity is a measure of antioxidant capacity. All genotypes had similar DPPH radical scavenging activity, suggesting that antioxidant capacity may not be a major factor in Fusarium wilt resistance in these genotypes.

Trait association for biochemical responses in resistant genotypes

The correlation analysis was carried out in case of resistance genotypes (Figure 3). A strong positive correlation (0.9) was observed between chlorophyll and DPPH, indicating a potential synergistic effect of these compounds in enhancing antioxidant capacity. Similarly, a positive correlation was found between proline - TSS (0.8) and tannin - DPPH (0.8) , suggesting a coordinated response to stress. Interestingly, a strong negative correlation (-1.0) was observed between proline and alkaloids. Additionally, a negative correlation (-0.7) was found between both TSS and flavonoids with alkaloids, suggesting a potential inverse relationship in their regulation or function. These findings provide valuable insights into the complex interplay of biochemical factors contributing to Fusarium wilt resistance in chickpea.

Trait association for biochemical responses in susceptible genotypes

The correlation analysis was carried out in case of susceptible genotypes (Figure 4). A strong positive correlation (0.9) was observed between proline and DPPH, suggesting a potential synergistic effect of these compounds in defense mechanisms. Similarly, a positive correlation was found between phenol and Glycine betaine (0.8), phenol and tannin (0.8). Interestingly, a strong negative correlation was observed between DPPH and chlorophyll (-1.0), proline and chlorophyll (-1.0) suggesting a potential trade-off between defense mechanisms and photosynthetic activity.

Discussion

In this study, we evaluated the resistance of diverse chickpea genotypes to Fusarium wilt and characterized the biochemical responses associated with resistance. The results of our greenhouse screening experiments revealed a wide range of resistance levels among the tested genotypes, from complete resistance to complete susceptibility. These findings highlight the genetic diversity for Fusarium wilt resistance within the chickpea germplasm. The AUDPC analysis provided a quantitative measure of disease progression over time, confirming the differential responses of genotypes to the pathogen.

Biochemical analysis revealed significant differences in the levels of various secondary metabolites between resistant and susceptible genotypes. Resistant genotypes had significantly higher levels of phenolic compounds, flavonoids, and tannins, which are known to possess antimicrobial properties and can contribute to disease resistance. These compounds may act as physical barriers or toxic substances to the pathogen. These results align with previous research by Rathod and Vakharia (2011), Belkar (2018), and Jyothi (2018) on phenol activity changes in response to pathogen infection. Recently Parmar and Gohel (2024) also reported that both resistant and susceptible germplasm exhibited increased phenol levels in response to disease. However, resistant germplasm showed a significantly higher accumulation of phenols compared to susceptible germplasm. Earlier research has indicated that pathogen elicitation can trigger the upregulation of isoflavone biosynthesis and accumulation pathways in legumes (Dixon and Sumner, 2003). Kumar (2015) reported that flavonoids were among the primary metabolites differentially expressed in chickpea root tissue in response to *Fusarium oxysporum* infection. Yang (2024) also highlighted the significant upregulation of various phenolic acids and flavonoids, particularly those derived from the flavonoid biosynthesis pathway, as differentially accumulated metabolites (DAMs) in response to Fusarium infection.

Glycine betaine, an osmo-protectant, was also significantly higher in resistant genotypes, suggesting its role in maintaining cellular homeostasis under stress conditions. Glycine betaine, as reported by Nagaraju in 2017, is a potent compound capable of enhancing pearl millet's defense against downy mildew. By stimulating the plant's defense mechanisms, it effectively hinders the pathogen's infection process.

Interestingly, resistant genotypes had slightly lower proline levels compared to susceptible genotypes. While proline is often associated with stress tolerance, its role in Fusarium wilt resistance may be complex and genotype-specific. It is possible that other mechanisms, such as the activation of defence-related enzymes or the induction of systemic acquired resistance, may contribute to the observed differences in resistance.

Correlation analysis revealed interesting relationships among biochemical parameters in both resistant and susceptible genotypes. In resistant genotypes, a strong positive correlation was observed between chlorophyll and DPPH, suggesting a potential synergistic effect of these compounds in enhancing antioxidant capacity. This finding is supported by previous studies, which have shown that chlorophyll can act as an antioxidant and protect plant cells from oxidative damage (Pérez-Gálvez, 2020 and Lanfer-Marquez, 2005). Additionally, a positive correlation was found between proline and TSS, indicating a coordinated response to stress. This suggests that proline accumulation may be linked to increased sugar synthesis, which can provide energy for defense responses.

In susceptible genotypes, a strong positive correlation was observed between DPPH and proline, suggesting a potential synergistic effect of these compounds in defense mechanisms. However, a negative correlation was observed between chlorophyll-proline and chlorophyll and DPPH, indicating a potential trade-off between defense mechanisms and photosynthetic activity. This suggests that susceptible genotypes may allocate resources to defense at the expense of growth and development.

Conclusion

Overall, our results suggest that a combination of biochemical factors, including phenolic compounds, flavonoids, tannins, glycine betaine, and proline play a substantial role in the plant defense mechanism against biotic stresses like Fusarium wilt in chickpea. By understanding the complex interplay of these factors, we can develop effective breeding strategies to improve the resistance of chickpea cultivars to this devastating disease. Further research is needed to elucidate the specific mechanisms underlying these biochemical interactions and their contribution to Fusarium wilt resistance in chickpea.

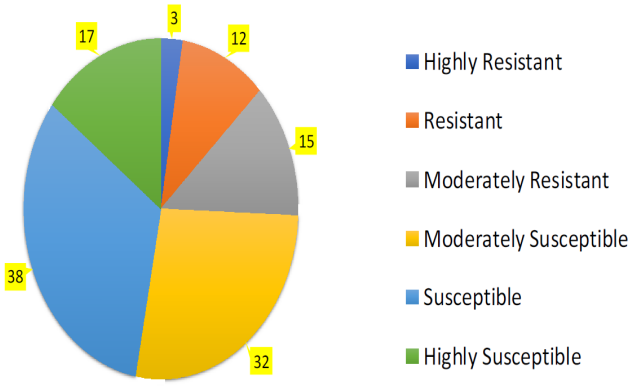

Fig. 1 : Number of chickpea Genotypes in Each Disease Category

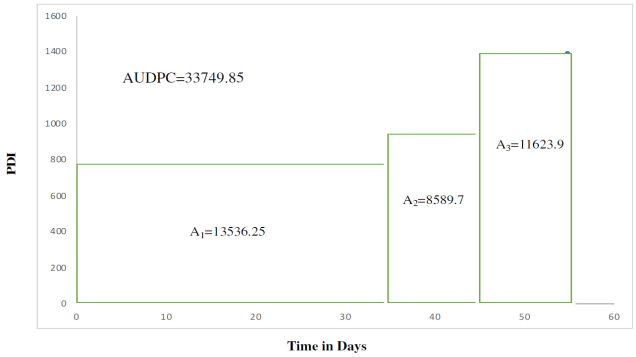

Fig. 2 : Area calculated under the disease progress curve in chickpea genotypes

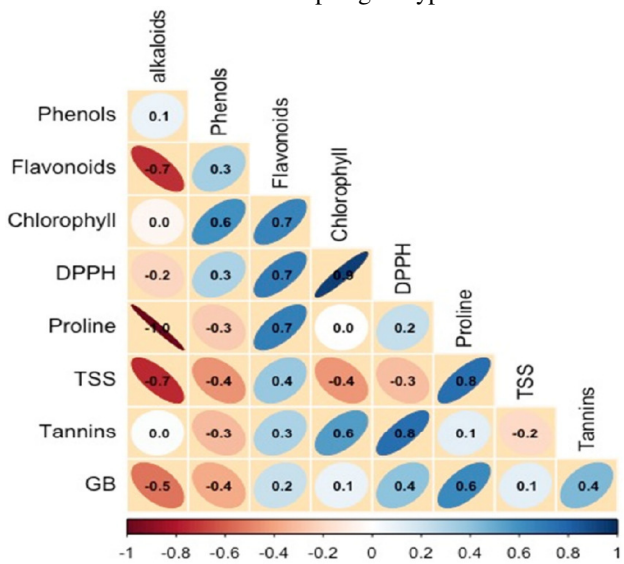

Fig. 3 : Correlation heat map showing association between biochemical parameters in resistant genotypes

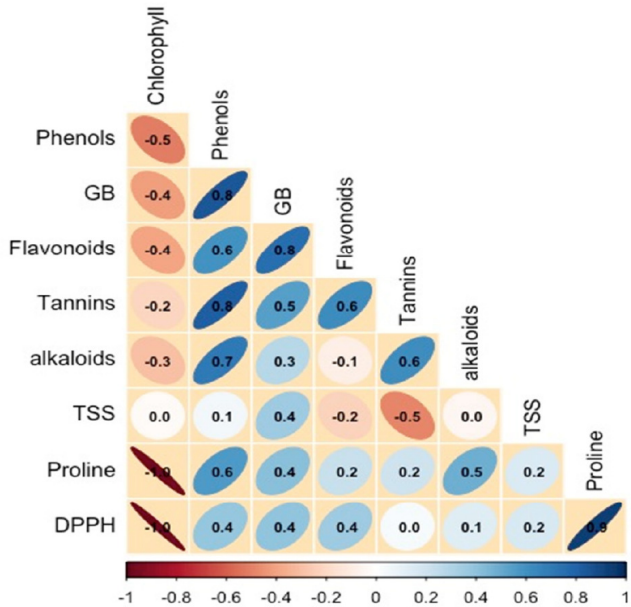

Fig. 4: Correlation heat map showing association between biochemical parameters in susceptible genotypes

Table 1: Fold increase of biochemical parameters in resistant genotypes under *Fusarium oxysporum* inoculation

Parameter	Mean Resistant	Mean Susceptible	Fold increase
Phenol	78.51	66.90	0.17
Flavonoids	51.93	50.16	0.04
Chlorophyll	45.07	44.23	0.02
Tannins	1.47	1.17	0.25
Proline	22.77	24.93	-0.09
Glycine betaine	117.62	16.22	6.25
Alkaloids	4.93	4.70	0.05
Total sugars	60.00	44.63	0.34
DPPH	1.63	1.62	0.00

Table 2: Mean performance for biochemical parameters in resistant and susceptible genotypes in response to fusarium wilt in chickpea genotypes

Genotype	Phenols	Flavonoids	Chlorophyll	Tannins	Proline	GB	Alkaloids	TSS	DPPH
K 16	69.78	40.75	44.42	1.70	20.71	131.01	5.03	33.94	1.63
K 17	64.72	49.40	42.83	1.59	22.26	108.21	4.97	81.76	1.63
D 35	75.01	60.39	43.43	1.30	29.71	130.96	4.70	94.70	1.63
K 9	92.02	66.14	51.24	1.78	23.03	117.26	4.91	44.19	1.63
Y 39	91.03	42.95	43.45	0.97	18.14	100.68	5.05	45.41	1.63
K 6	74.41	46.72	44.79	1.61	23.80	8.43	4.83	37.60	1.62
K 14	52.06	41.56	43.33	0.57	29.71	5.17	4.69	49.80	1.62
D 2	40.39	50.80	45.71	0.89	16.60	3.71	4.53	40.04	1.62
D 30	76.98	50.56	45.35	1.17	21.74	30.01	4.71	53.22	1.62
D 41	90.63	61.14	41.96	1.60	32.80	33.80	4.72	42.48	1.62

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