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PHYSIOLOGICAL AND BIOCHEMICAL PROPERTIES OF SOYBEAN CULTIVARS INFECTED WITH PHYTOPATHOGENIC FUNGI

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SUMMARY

An investigation on soybean (*Glycine max* L.) commenced at the experimental field of the Institute of Genetics and Plant Experimental Biology, Academy of Sciences, Republic of Uzbekistan. The purposive study aimed to determine the effects of *Fusarium solani* phytopathogenic micromycetes on the physiological and biochemical composition of five soybean cultivars, viz., Sochilmas, Genetic-1, Nafis, Tomaris, and Baraka. The results revealed in the budding and flowering stages, the soybean cultivar leaves infected with *F. solani* showed decreased amounts of chlorophyll a and b compared with the healthy plants (control). In the control comparison against the soybean variants with phytopathogenic micromycetes, some soybean cultivars showed enhanced contents of carotenoids in the leaves, and others revealed a decline in carotenoids to varying degrees. The peroxidase enzyme activity was higher in soybean cultivars Tomaris and Nafis artificially infected with *F. solani* than the other cultivars. It was evident that the peroxidase enzyme activity under the influence of *F. solani* in the leaves of studied soybean cultivars increased by 20.76%, 43.6%, and 35.4%, respectively, in Baraka, Tomaris and Nafis cultivars. Results further indicated that under the influence and stressful conditions of *F. solani,* the activity of the polyphenol oxidase enzyme enhanced by 84.1% and 117.1%, respectively, in soybean cultivars Tomaris and Nafis. The phenylalanine-ammonia-lyase enzyme activity was also higher in the said cultivars with *F. solani* infection compared with the control. The earlier situation confirmed that the soybean plant leaves' physiological and biochemical parameters are closely associated with the phytopathogenic micromycetes.

Keywords: Soybean (*Glycine max* L. Merr.), genotypes, chlorophyll, carotenoids, budding, flowering, pigment, enzyme, peroxidase, catalase

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Key findings: The physio-biochemical defense system of the soybean exhibited the protective enzymes' sharp increase in resistant genotypes, showing a significant resistance to the fungus *F. solani*. Therefore, the local soybean cultivars Tomaris and Nafis became the choices as resistant cultivars to *F. solani* that can serve as a base material to develop the soybean-resistant cultivars to fusariosis in future breeding programs.

INTRODUCTION

Various phytopathogenic microorganisms have a broader negative impact on the world's food crop production, especially soybean, as one of the most important oilseed crops. Among oilseed crops, soybean occupies a prominent position, as its grains contain fats (18%–24%), proteins (36%–40%), carbohydrates (26%–34%), and minerals (5%–8%) (Arioglu, 2014).

Nowadays, in soybean-growing countries, one of the main problems is the infection of soybeans with phytopathogenic micromycetes, causing significant losses to the crop. Worldwide, around 26%–30% of produced crops, particularly profitable ones, such as, cotton, wheat, and soybeans, fail due to diverse diseases and abiotic stress conditions. So far, more than 30 diseases caused by various races of fungi, bacteria, and viruses have been identified in soybeans (Ranjan *et al*., 2019).

Fungi-causing diseases are one of the critical biotic factors for productivity losses in leguminous crops. Fungal infections cause a decrease of 15%–80% in legume yields (Horoszkiewicz-Janka *et al*., 2013). The spread of some fungal pathogens turns into an epiphytotic (epidemic) process, which leads to the failure of plantations (Deneke, 2018). One of the diseases causing the utmost economic damage to legumes is fusariosis (*Fusarium spp.*), mainly triggered by *F. solani*, *F. oxysporum*, *F. gibbosum,* and *F. culmorum* (Maui, 2015; Rejapova *et al*., 2020; Sampaio *et al*., 2020; Matniyazova *et al*., 2022, a, b; Matniyazova *et al.*, 2023).

Fusarium occurs in all soybean-growing areas and can damage soybeans throughout the vegetation period. In particular, various symptoms, such as, the death of seedlings before reaching the soil level, necrosis of the seed coat, drying of the growing point of seedlings, rotting of roots and root necks, wilting, slowing of growth, thinning of pods, rotting and falling of leaves, flowers, pods, and grains, and reduction of seed fertility are visible. However, the observed highest disease rate manifests during the flowering period. In addition, the plants infected with *fusarium* cause flowers and seed nodes in the center to fall off. *Fusarium* wilt appears in the pods at the end of the growing season as spots and wounds. On average, a determined 25%–30% of mass lesions came from *fusarium* injuries (Maui *et al*., 2016).

In photosynthesis, plants convert light energy into chemical energy and form organic products from inorganic substances, which serve as nutrients for plant growth and development (Simkin *et al*., 2020). Biotic and abiotic stress conditions disrupt the ultrastructure of chloroplasts, leading to a decline in chlorophyll content, which reduces photosynthetic activities (Sidhu *et al*., 2017). A decrease in chlorophyll a and b and total chlorophyll was distinct under several stress conditions' influences (Hamani *et al*., 2020; Matniyazova *et al*., 2022, a, b). As antioxidants, carotenoids help to protect the chloroplast and maintain chlorophyll content (Kacharava *et al*., 2009).

In addition, phytopathogenic fungi damage leads to a cell wall protein collapse and excessive production of reactive oxygen species (ROS) (Meena *et al*., 2016). The ROS primarily contribute to various mechanisms, ranging from developmental to defense processes in plants often linked with disease tolerance. However, overproduction of ROS affects developmental and physiological processes by damaging cell membranes, proteins, and the photosynthetic machinery, i.e., carotenoids and chloroplast in the host plants (Das and Roychoudhury, 2014). The antioxidant guard mechanism is the most protruding response toward enhanced ROS molecules by acting as foragers. In ROS

scavenging, the failure of these antioxidants results in oxidative stress (Shereefa and Kumaraswamy, 2016).

Stress disturbs the dynamic balance between the formed and accumulated ROS and enzymes that neutralize them. Enzymes polyphenol oxidase (PPO), peroxidase (POD), and phenylalanine ammonia-lyase (PAL) constantly stabilize this balance, detoxifying ROS that contributes to plant resistance (Khatun and Chatterjee, 2011). Peroxidase is an enzyme belonging to the oxidoreductase class, actively involved in the oxidation of phenols, suberization, and lignification of plant cell walls in response to phytopathogenic microorganisms. This resistance mechanism relates to the induction of peroxidase enzyme activity. Another essential function of peroxidase is protecting crop plants from the harmful effects of ROS produced from photosynthesis and respiration (Sharma *et al*., 2012).

The PPO and its isoenzymes provide mechanisms for defense reactions in crop plants by obstructing phenol oxidation and cell damage. As a result, the inactivation of exoenzymes and the activation of synthesis of lignin in the damaged area of the plant cell wall become vital in avoiding further pathogen spread (Tyuterev, 2002). A sharp and significant increase in the activity of this enzyme quickly prevents a widespread of the phytopathogen in the plant, which averts its diffusion to other parts of the plant.

Phenylalanine ammonia-lyase (PAL) is a key phytoimmunity enzyme directly involved in pathogen-host relationships (Khatun and Chatterjee, 2011). PAL involved in the biosynthesis of phytoalexins and phenolic compounds. Increased production of phenolic compounds, phytoalexins, lignin, and salicylic acid, associated with plant resistance to phytopathogens, often has links with increased PAL activity in response to fungal infections (Niranjanraj *et al*., 2006). The main goal of this study was to determine the amount of chloroplast pigments and the activities of peroxidase, polyphenol oxidase, and phenylalanine ammonia-lyase enzymes in the leaf parts of soybean local cultivars under the influence of phytopathogenic micromycetes that cause fusariosis and alternariosis diseases.

MATERIALS AND METHODS

The soybean (*Glycine max* L.) experiment commenced at the Institute of Genetics and Plant Experimental Biology, Academy of Sciences, Republic of Uzbekistan. The infection effects of *F. solani* phytopathogenic micromycetes on five soybean local cultivars, i.e., Sochilmas, Genetic-1, Nafis, Tomaris, and Baraka attained investigation. These phytopathogenic strains came from the unique object collection of phytopathogens and other microorganisms at the Institute of Genetics and Plant Experimental Biology, Academy of Sciences, Republic of Uzbekistan.

Biomaterial preparation of *F. solani* strains

Fungi growing progressed in an artificial climate chamber (12 h of light, with temperatures of 25 °C–26 °C during the day and 21 °C–22 °C at night) for three to 15 days. Potatoes with sucrose (1000 ml of potato extract, 20 g of sucrose) also grown in a nutrient medium at 25 °C–26 °C continued for 15 days to grow fungi. The fungi inoculation proceeded on sterile oat grain to produce infective biomass under laboratory conditions and grown for 15 days in an artificial climate chamber. Biomass prepared from micromycetes advanced to planting with soybeans in phytopathogenic soil mixed with 4 kg of oats per 100 m^2 of a unique experimental field and later transferred to a soybean-planted area, according to the method of Solovyova (1951).

In the presented study, determining the amount of chloroplast pigments at the budding and flowering stages and the activities of peroxidase (PO), polyphenol oxidase (PPO), and phenylalanine ammonia-lyase (PAL) enzymes continued in the leaf samples of five soybean cultivars.

Extraction and determination of pigment concentration

This experiment determined the amount of pigments in the leaves from the soybean plant samples' 3–4 leaves, calculating from the growth point. Fifty milligrams of each leaf sample, placed in a test tube, gained homogenization in 5 ml of 95% ethyl alcohol solution (Lichtenthaler and Wellburn, 1983). The homogenate underwent centrifugation at a speed of 5000 rpm for 12 min. The amounts of chlorophyll a and b and carotenoids in the resulting extract attained determination by an Agilent Cary 60 UV-Vis spectrophotometer at 664, 649, and 470 nm. Based on these indicators, calculating the amounts of chlorophyll a and b and carotenoids in soybean leaves used the following equations (Nayek *et al*., 2014):

$$\text{Chl a} = 13.36A_{664} - 5.19\ A_{649}$$

$$\text{Chl b} = 27.43A_{649} - 8.12\ A_{664}$$

$$\text{C x+c} = (1000A_{470} - 2.13C_a - 97.63C_b)/209$$

Extraction and assay of antioxidant enzymes

For enzyme extractions, frozen leaf samples (0.5 g) were ground into a fine powder, used a mortar placed in an ice bath and a pre-cooled pestle with liquid nitrogen, and then homogenized in 50 mM potassium phosphate buffer (pH 7.8) containing 1 mM ascorbate and 2% (w/v)polyvinylpolypyrrolidone. The resulting homogenates received centrifugation at 20,000 × g for 30 min at 4 °C.

Measuring peroxidase (POX; EC 1.11.1.7) activity continued by detecting the increase in absorbency at 460 nm as o-Dianisidine oxidization ensues, according to the method of Boyarkin (1951). The 50 µl enzyme extraction's addition to the 2.85 ml reaction mixture contained 1.85 ml 0.1M HAC-NaAC buffer (PH 5.0), 0.25% o-Dianisidine, and 0.1 ml 0.3% H_2O_2.

Polyphenol oxidase (PPO, EC 1.14.18.1) activity estimation used the method of Kumar and Khan (1982). The reaction mixture preparation comprised 2 ml of 0.1 M sodium phosphate buffer (pH 6.5), 0.5 ml of crude enzyme extract, and 1 ml of 0.1 M catechol. The assay mixture sustained incubation for 10 min at room temperature. The reaction stopped by adding 1 ml of 2.5 N H_2SO_4. The absorption of purpurogallin formed received examination at 495 nm. The blank's preparation continued by adding 2.5 N H_2SO_4 at zero time for the same assay mixture. The PPO activity was expressed in U min^{-1} mg^{-1} protein (U = change in 0.1 absorbance min^{-1} mg^{-1} protein).

Phenylalanine ammonia-lyase (PAL, E.C. 4.3.1.5**)** activity determination in the leaf extract employed the method of Ochoa and Salgado (1992), with slight modifications. Both control and infected leaves (0.2 g fresh weight) gained extracting in 600 µl 50 mM Tris-HCl buffer (pH 8.8) containing 1 mM EDTA, 15 mM mercaptoethanol, and 50 mM ascorbic acid at 4 °C. The collected supernatant became the PAL enzyme extract. The assay mixture contained 100 µl of isolates, 100 mM Tris-HCl buffer (pH 8.8), 0.5 ml of 10 mM L phenylalanine, and 0.4 ml of deionized water. The mixture continued to incubation for one hour at 37 °C, with the reaction terminated by adding 0.5 ml of 6 M HCl and sample absorbance measured at 290 nm. The calibration curve's construction used cinnamic acid. The blank had the same constituents except that adding the extract was after the HCl solution.

Protein concentration

Estimation of the total soluble protein content of the samples followed the technique of Lowry *et al.* (1951) by adding Folin–Ciocalteu's (1 N) reagent to plant extract and reading absorbance at 720 nm after a reaction time of 2 min. The sample protein content, expressed as equivalent microgram bovine serum albumin (BSA) per 0.1 ml sample (µg protein 0.1 ml^{-1}), attained verification from a standard curve of BSA versus absorbance.

Statistical analysis

Data analysis continued using StatView (SAS Institute Inc., Cary, NC, USA) with one-way ANOVA, followed by a Fisher PLSD post hoc test ($P < 0.05$ and $P < 0.01$).

RESULTS

In the leaves of five soybean cultivars (artificially infested with phytopathogenic fungi), the magnitude of chloroplast pigments diminished during budding and flowering period. The amount of chlorophyll a studied during the budding period of soybean cultivars, indicated that in soybean plants artificially infested with *F. solani*, shrank in varying degrees compared with the control variant (healthy plants). The soybean cultivars, i.e., Baraka, Tomaris, and Genetic-1 infected with *F. solani*, gave the highest content of chlorophyll a (3.19 ± 0.11, 3.28 ± 0.41, and 3.28 ± 0.41 mg/g, respectively), and cultivars Nafis and Sochilmas had the low values (1.66 ± 0.34 and 1.61 ± 0.27 mg/g, respectively) (Table 1).

The studied amount of chlorophyll b in the leaves of soybean cultivars during the budding period also provided a reduction in chlorophyll b contents in the soybean plants artificially infested with *F. solani* in different degrees compared with the control plants. Among the soybean cultivars (infected with *F. solani*), chlorophyll b was high in cultivars Tomaris and Genetic-1 (1.18 ± 0.25 and 1.32 ± 0.32 mg/g, respectively). A low index (0.69 ± 0.23mg/g) appeared in the cultivar Sochilmas. The sign of total chlorophyll content during budding also indicated a similar change to that with chlorophyll a and b contents (Table 2).

Studying the amount of carotenoids in the leaves of soybean cultivars also ensued under laboratory conditions. As a result of the action of phytopathogenic fungi, the amount of carotenoids in the soybean leaves increased in different degrees compared with the control. Among soybean cultivars (artificially infected with *F. solani*), the highest index of carotenoid content emerged in the soybean cultivar Tomaris (1.50 ± 0.18 mg/g), with the lowest index observed in the cultivar Sochilmas (0.99 ± 0.09 mg/g).

The chlorophyll a content also attained scrutiny during the flowering period of the soybean cultivars. Results revealed that in the soybean plants (artificially infested with *F. solani*), a reduction in the chlorophyll a amount appeared in varying degrees compared with the control plants. Among the soybean cultivars (infected with *F. solani*), the Tomaris and Nafis cultivars had the highest content of chlorophyll a (1.89 ± 0.28 and 1.78 ± 0.15 mg/g, respectively), and cultivar Sochilmas had a low index (0.47 ± 0.04 mg/g) (Table 3).

As for the amount of chlorophyll b in the leaves of soybean cultivars during the flowering period, a decrease occurred in chlorophyll b contents in plants artificially infested with *F. solani* with varied levels. Among the soybean cultivars (infected with *F. oxysporum*), the cultivars Tomaris and Nafis had the highest amount of chlorophyll b (0.87 ± 0.09 and 0.75 ± 0.05 mg/g, respectively), and soybean cultivars Sochilmas and Baraka showed the low index (0.33 ± 0.10 and 0.55 ± 0.09 mg/g, respectively). The total chlorophyll content during the flowering period also varies similarly with the content of chlorophyll a and b.

As a result of the action of phytopathogenic fungi, it was apparent that the amount of carotenoids in the soybean cultivar leaves also enhanced in different degrees versus the control. Among soybean cultivars (artificially infected with *F. solani*), the highest carotenoid content index emanated in the soybean cultivar Tomaris (0.69 ± 0.13 mg/g), and the lowest index of the said pigment was in the cultivar Sochilmas (0.18 ± 0.03 mg/g) (Table 4).

Thus, evidence proved the effect of phytopathogenic micromycetes on the amount of chlorophyll a and b, total chlorophyll, and carotenoids in the soybean cultivars was superior during the flowering period than during the budding period. *Fusarium* disease can damage the soybean plant throughout the growing season. Past studies also reported that the highest manifestation of the disease was usually during the flowering period (Arias, 2012).

Table 1. Amount of physiological pigments in the soybean cultivars at the budding stage.

No.	Cultivars	Chlorophyll a		Chlorophyll b	
		Control	*F. solani*	Control	*F. solani*
1	Genetic-1	3.83±0.26	3.01±0.24	1.74±0.06	1.32±0.32
2	Baraka	3.28±0.23	3.19±0.11	1.96±0.40	1.00±0.57
3	Tomaris	4.01±0.18	3.28±0.41	1.77±0.27	1.18±0.25
4	Nafis	3.15±0.09	1.66±0.34	1.93±0.09	1.10±0.14
5	Sochilmas	2.75±0.28	1.61±0.27	1.17±0.18	0.69±0.23

Table 2. Amount of physiological pigments in the soybean cultivars at the budding stage.

No.	Cultivars	Total chlorophyll		Carotenoids	
		Control	*F. solani*	Control	*F. solani*
1	Genetic-1	5.57±0.10	4.34±0.40	1.32±0.10	1.30±0.25
2	Baraka	5.24±1.28	4.19±0.74	0.91±0.70	1.39±0.16
3	Tomaris	5.78±0.33	4.46±0.48	0.64±0.25	1.50±0.18
4	Nafis	5.08±0.15	2.76±041	0.90±0.23	1.11±0.18
5	Sochilmas	3.92±0.45	2.30±0.30	0.71±0.27	0.99±0.09

Table 3. Amount of physiological pigments in the soybean cultivars at the flowering stage.

No.	Cultivars	Chlorophyll a		Chlorophyll b	
		Control	*F. solani*	Control	*F. solani*
1	Genetic-1	1.79±0.19	1.19±0.29	0.68±0.09	0.61±0.23
2	Baraka	3.08±0.26	1.09±0.14	1.90±0.33	0.55±0.09
3	Tomaris	1.96±0.22	1.89±0.28	0.86±0.19	0.87±0.09
4	Nafis	2.17±0.11	1.78±0.15	0.97±0.12	0.75±0.05
5	Sochilmas	1.67±0.12	0.47±0.04	0.76±0.11	0.33±0.10

Table 4. Amount of physiological pigments in the soybean cultivars at the flowering stage.

No.	Cultivars	Total chlorophyll		Carotenoids	
		Control	*F. solani*	Control	*F. solani*
1	Genetic-1	2.47±0.22	1.80±0.37	0.39±0.04	0.46±0.18
2	Baraka	4.98±0.42	1.64±0.17	0.58±0.20	0.38±0.06
3	Tomaris	2.83±0.24	2.76±0.34	0.68±0.15	0.69±0.13
4	Nafis	3.14±0.16	2.53±0.09	0.65±0.05	0.48±0.11
5	Sochilmas	2.43±0.07	0.80±0.09	0.59±0.07	0.18±0.03

The activities of peroxidase (POD), polyphenol oxidase (PPO), and phenylalanine ammonia-lyase (PAL) enzymes proceeded verification in laboratory conditions at soybean cultivars' budding stage grown in a specialized experimental area. In the studied experiment, the activity of the peroxidase enzyme in the leaves of soybean cultivars (with phytopathogenic micromycetes *F. solani*) compared with the control treatment (without phytopathogenic micromycetes) revealed that the control's soybean cultivars gave the highest index of peroxidase enzyme activity (in the soybean cultivar Genetic-1 at 78.62 ± 1.14 μmin^{-1} / mg^{-1} protein). On the other hand, the lowest index of the said enzyme was distinct in the cultivar Nafis (61.00 ± 1.94 μmin^{-1} / mg^{-1} protein) (Figure 1).

The activity of the peroxidase enzyme decreased by 32.9% and 5.9% in the soybean cultivars Genetic-1 and Sochilmas, respectively, by 20.76% and 43.6% in the cultivars Baraka, Tomaris, and Nafis under the influence of the phytopathogenic micromycete *F. solani* compared with the control variant, which increased by 35.4% (Figure 1). Under

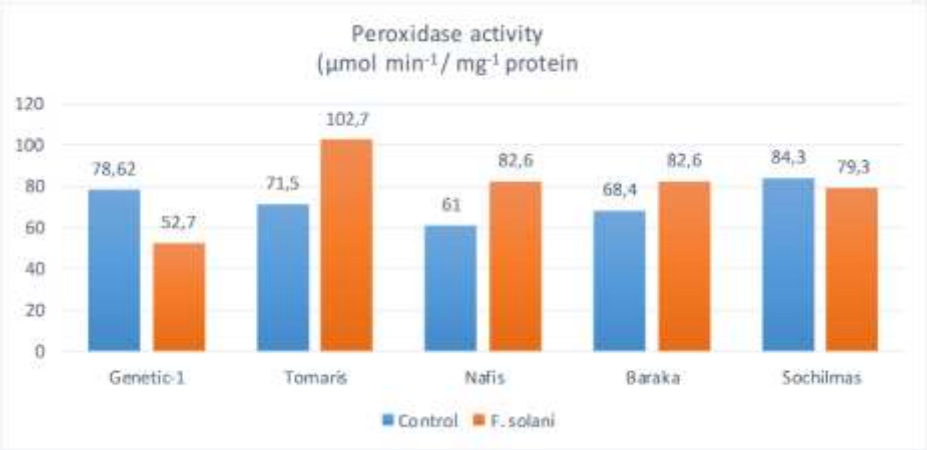

Figure 1. Activity of peroxidase enzyme in the leaves of soybean cultivars during the flowering period under the influence of phytopathogenic micromycete.

these biotic stress conditions, the peroxidase enzyme activity was higher in soybean cultivars Tomaris and Nafis versus other cultivars. Compared with the control option, the peroxidase enzyme activity in soybean cultivars under the influence of phytopathogenic micromycetes altered to a different extent (Figure 1).

During these experiments, the polyphenol oxidase enzyme movement, one of the significant enzymes in the plant's resistance to stress factors, also gained monitoring. Results revealed that the activity of this enzyme was higher under the influence of phytopathogenic micromycetes compared with the control variant without phytopathogenic micromycetes (Figure 2). Phytopathogenic micromycetes in plants proved to affect the polyphenol oxidase enzyme activity in the leaves of all domestic soybean cultivars studied during the budding period. The soybean cultivars studied in the control option revealed the highest index of the polyphenol oxidase enzyme activity was in cultivar Genetic-1 (138.44 ± 1.88 µmin^{-1} / mg^{-1} protein), and the lowest index resulted in the soybean cultivar Baraka (59.94 ± 3.20 µmin^{-1} / mg^{-1} protein).

An analysis of the obtained results further revealed that the activity of the polyphenol oxidase enzyme enhanced in differing levels in all the soybean cultivars under the influence of *F. solani* compared with the control. It showed an increase of 17.9%, 42.4%, 51.2%, 104.2%, and 87.1% in soybean cultivars Genetic-1, Baraka, Sochilmas, Tomaris, and Nafis, respectively (Figure 2). Under biotic stress conditions, the activity of polyphenol oxidase enzyme occurred higher in soybean cultivars Nafis and Tomaris than in other cultivars.

The study of the phenylalanine ammonia-lyase enzyme activity in soybean cultivar leaves also persisted. Phenylalanine ammonia-lyase is an indicator enzyme of high sensitivity to stresses, and it is a biochemical marker for structural and protective compounds. In the control option (without phytopathogenic micromycetes), the highest indicator of phenylalanine ammonia-lyase enzyme activity was apparent in the soybean cultivar Tomaris (14.3 ± 0.64 µmin^{-1} / mg^{-1} protein), and the lowest came from the cultivar Sochilmas (8.7 ± 0.47 µmin^{-1} / mg^{-1} protein) (Figure 3). The outcomes indicated that the phenylalanine ammonia-lyase enzyme activity increased in all the soybean cultivars under the influence of phytopathogenic micromycete *F. solani* compared with the control. These increases were 12.8%, 16.1%, 23.4%, 36.9%, and 53.1% in the cultivars Genetic-1, Sochilmas, Baraka, Nafis, and Tomaris, respectively (Figure 3).

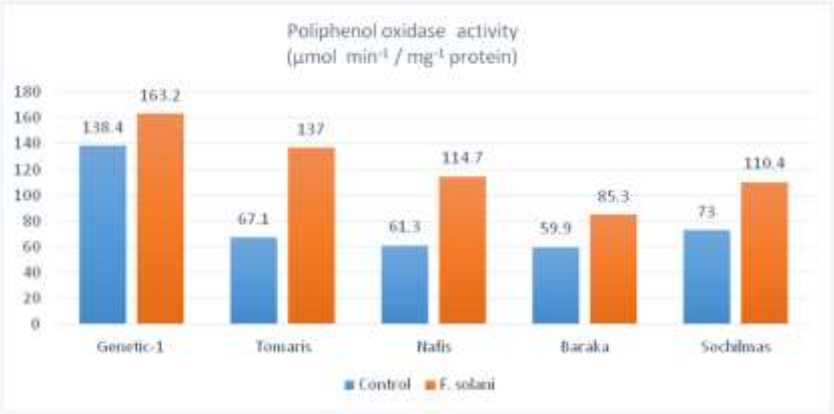

Figure 2. Activity of polyphenol oxidase enzyme in the leaves of soybean cultivars during the flowering period under the influence of phytopathogenic micromycete.

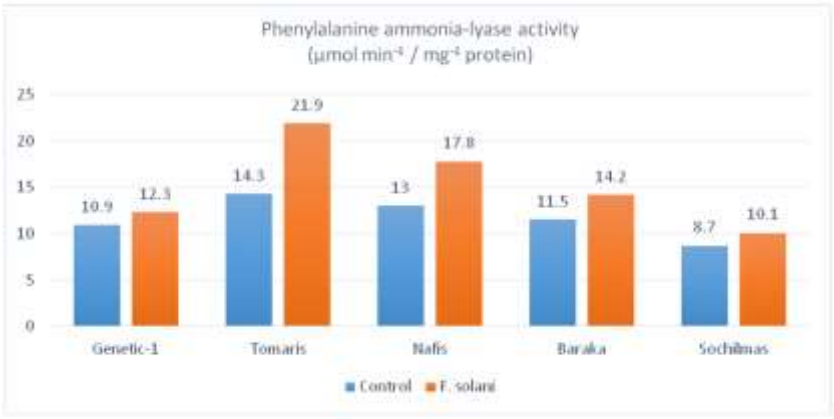

Figure 3. Activity of phenylalanine ammonia-lyase enzyme in the leaves of soybean cultivars during the flowering period under the influence of phytopathogenic micromycete.

Under the phytopathogenic stress conditions, the activity of the phenylalanine ammonia-lyase enzyme was higher in the cultivars Nafis and Tomaris than in other soybean cultivars. The increased activity of enzymes in these soybean cultivars may be an influential defense response against pathogen infection and a reaction ` to induced resistance in plants. From the results, it is possible to show phenylalanine ammonia-lyase, polyphenol oxidase, and peroxidase enzymes as physiological and biochemical markers of resistance in local soybean cultivars to biotic stresses, especially to phytopathogenic microorganisms.

DISCUSSION

In the prevailing study, the amount of chloroplast pigments (chlorophyll a and b, total chlorophyll, and carotenoids) and the activity of peroxidase, polyphenol oxidase, and phenylalanine ammonia-lyase enzymes attained measuring in the leaves of five local cultivars of soybean (*Glycine max* L.) under the influence of phytopathogenic micromycetes. Under the influence of *Fusarium solani*, plant cell membrane damage, and chlorophyll degradation are also effects of oxidative stress. The chlorophyll and carotenoid pigments are contributors to plant photosynthesis, found in

photosystems I and II, imparting their vital role in light harvesting. Carotenoids inhibit oxidative stress by quenching the singlet oxygen ($^1O_2$) and triplet chlorophyll (^{3}Chl) and protect photosynthetic machinery. As antioxidants, carotenoids help protect the chloroplast and maintain chlorophyll content (Kacharava *et al*., 2009; Hanafiah *et al*., 2021).

Structural variations of chloroplasts revealed that chloroplasts showed more sensitivity to stress conditions than other cell organelles. Reducing chlorophyll content on pathogen invasion indicates cell damage in canola plant tissues (Khallal, 2007). In the presented study, photosynthetic pigment contents decreased during budding and flowering after inoculation. Thus, it implies that the effect of phytopathogenic micromycetes on the amount of chlorophyll a and b, total chlorophyll, and carotenoids in the soybean cultivars was superior during the flowering period than the budding stage. The fusariosis disease can damage the soybean plant throughout the growing season; however, the highest manifestation of the disease was during the flowering period (Arias, 2012).

In the past study of Martinez *et al*. (2018), the least amount of chlorophyll (a and b and total chlorophyll) and carotenoids were visible in infected leaves of resistant genotypes, indicating that tomato-resistant genotypes can maintain their photosynthetic pigment under stress conditions. However, such variations in the photosynthetic attributes are general signs of stress conditions. Therefore, sustaining chlorophyll content in plants upon pathogen invasion is vital, as it will permit plant cells to continue photosynthesis.

The oxidative overproduction of ROS belongs to the earliest defense responses against pathogen invasion in crop plants (Lubaina and Murugan, 2013). Balancing the effects of oxidative stress, plants have developed an arsenal of defense mechanisms against pathogen outbreaks (Kholova *et al*., 2013). In the timely study, the peroxidase activity was different in the influence of the micromycete *F. solani* on soybean cultivars because the peroxidase enzymes decompose the hydrogen peroxide (H_2O_2) involved in the oxidation of phenolic and non-phenolic substrates (Passardi *et al*., 2007). However, in this study, the POD activity was higher in soybean-resistant genotypes.

The activity of peroxidase enzyme, studied in the leaves of soybean cultivars, indicated a higher action emerged in soybean cultivars Tomaris, Nafis, and Baraka than in two other cultivars, Genetic-1 and Sochilmas with micromycete *F. solani*. Higher levels of peroxidase activity resulted exclusively from fungal infection, in reports of Rosta *et al*. (2002) in *Alternaria brassicae*-infected Chinese cabbage. Higher peroxidase activity appeared in diseased leaves than in healthy leaves of *Capsicum annuum* L. (Meena *et al*., 2008). Paranidharan *et al.* (2009) observed higher peroxidase activity in rice leaf sheaths infected with *Rhizoctonia solani.* Increases in peroxidase activity could correlate with infection in crop plants and the polymerization of cinnamyl alcohols to lignin as catabolized by peroxidase lignification, leading to disease resistance. Infection with plant pathogens led to induction in POX activity in plant tissues, and a higher increase showed in resistant plants compared with the susceptible ones (Mydlarz and Harvell, 2006).

By studying the polyphenol oxidase activity in the soybean plant leaves, the higher polyphenol oxidase mobility surfaced in cultivars Genetic-1, Tomaris, and Nafis with micromycete *F. solani* compared with the control variant. In the existing study, the PPO activity emerged to be higher in *fusarium*-infected leaves than non-infected leaves, with the PPO activity enhanced in soybean cultivars Genetic-1, Tomaris, and Nafis compared with the cultivars Baraka and Sochilmas relatively to fusariosis. Niranjanraj *et al.* (2006) observed similar results in seedlings of resistant cultivars with better PPO activity than susceptible ones. Similarly, the higher PPO activity manifested in pearl millet tissues infected with the DM fungus than in healthy tissues (Shetty *et al*., 2001). A similar phenomenon has also appeared in pear fruits' resistant cultivars infected with the *Erwinia amylovora* pathogen (Honty *et al*., 2005).

Phenylalanine ammonia-lyase (PAL) activity in soybean leaves exhibited enhancements with *F. solani* in all genotypes compared with the control. In this experiment, PAL activity proved to be higher in leaves infected with *fusarium* than in non-infected leaves, and PAL activity increases resulted in soybean cultivars Tomaris and Nafis versus three other cultivars, Baraka, Genetic-1, and Sochilmas. The promising results were consistent with those of Li *et al*. (2011), who observed a significant increase in PAL activity after injection of resistant and non-resistant cotton species (*Gossypium barbadense*) with the *V. dahliae* strain V991. The increased activities of peroxidase, polyphenol oxidase, and phenylalanine ammonia-lyase due to the influence of phytopathogenic fungi in soybean cultivars indicate that these enzymes can play a specific role in the activation of plant defense mechanisms.

CONCLUSIONS

The influences of *Fusarium solani* on local soybean cultivars revealed that the amount of chloroplast pigments decreased, with the activities of peroxidase, polyphenol oxidase, and phenylalanine ammonia-lyase enzymes enhanced. The study also determined that the physiological (chlorophyll a and b and carotenoids) and biochemical (POD, PPO, and PAL) properties of the soybean cultivars proved connected to the fungus *F. Solani* effects. Likewise, POD, PPO, and PAL activities attained maximum increases in soybean cultivars with a minimal decrease in chloroplast pigment contents infected with the phytopathogenic micromycete *F. solani*. In the physio-biochemical defense reaction, it was evident that the number of protective enzymes in resistant genotypes increased sharply, and their role was significant in the resistance of the fungus *Fusarium solani* in the soybean-resistant cultivars. Soybean cultivars Tomaris and Nafis showed distinction as resistant ones to the *Fusarium solani,* hence, could serve as a base material in future breeding programs to develop soybean-resistant cultivars to fusariosis.

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