RESEARCH ARTICLE

SABRAO Journal of Breeding and Genetics
56 (3) 1060-1071, 2024
http://doi.org/10.54910/sabrao2024.56.3.14
http://sabraojournal.org/
pISSN 1029-7073; eISSN 2224-8978

ALTERNARIA ALTERNATA FUNGUS EFFECTS ON PHYSIOLOGICAL AND BIOCHEMICAL PROCESSES OF SOYBEAN

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SUMMARY

This study assessed the leaf pigments and the activity of peroxidase, polyphenol oxidase, and phenylalanine ammonia-lyase enzymes in soybean local cultivars, i.e., Genetic-1, Tomaris, Baraka, Nafis, and Sochilmas, under the influence of the phytopathogenic fungus *Alternaria alternata*. In local soybean (*Glycine max* L.) cultivars during the budding and flowering periods, the *A. alternata* micromycetes influenced and decreased the chlorophyll a and b index compared with the control, validating the relationship of soybean leaf chlorophyll with the phytopathogenic micromycetes. Compared with the control variant, carotenoid content in the leaves of some soybean cultivars increased to varying degrees in variants with phytopathogenic micromycetes while decreasing in other genotypes to varying degrees. An outcome of the study revealed that the amount of leaf pigments in local soybean varieties infected with the phytopathogenic micromycete *A. alternata* is higher during the plant's flowering than during its budding period. Compared with other soybean cultivars, the Baraka and Nafis appeared with enhanced peroxidase enzyme activity by the artificial exposure to the *A. alternata* fungus. The results further revealed that polyphenol oxidase enzyme activity also increased by 117.7% and 152.0% in the cultivars Tomaris and Baraka, respectively, under the influence of *A. alternata* micromycetes compared with the control. The activity of phenylalanine ammonia-lyase enzyme was higher in the soybean cultivars Tomaris and Nafis under the influence of *A. alternata* than in other cultivars and the control. The activity of the peroxidase, polyphenol oxidase enzyme, and phenylalanine ammonia-lyase heightened in all the soybean cultivars under the influence of the phytopathogenic micromycete *A. alternata* compared with the control treatment.

Communicating Editor: Dr. Anita Restu Puji Raharjeng

Manuscript received: October 31, 2023; Accepted: February 9, 2024

Citation: Matniyazova H, Tillaboyeva D, Ergasheva G, Shaxmurova G, Yuldashov U, Sherimbetov A (2024). *Alternaria alternata* fungus effects on physiological and biochemical processes of soybean. *SABRAO J. Breed. Genet.* 56(3): 1060-1071. http://doi.org/10.54910/sabrao2024.56.3.14.

Keywords: Soybean (*G. max* L.), *Alternaria alternata*, budding, flowering, chlorophyll, carotenoid, peroxidase, polyphenol oxidase, phenylalanine ammonia-lyase, control, physiological and biochemical traits

Key findings: In the physio-biochemical defense system of the soybean (*G. max* L.), it was evident that a sharp increase of the protective enzymes occurred in resistant genotypes by showing considerable tolerance to the fungus *A. alternata*. Therefore, the local soybean cultivars Tomaris and Nafis were the choice resistant cultivars to *A. alternata* that can serve as base materials in future breeding programs to develop the soybean-resistant cultivars to alternariosis.

INTRODUCTION

In soybean (*G. max* L.) growing countries, phytopathogenic micromycetes infection is one of the primary factors affecting crop yield. Worldwide, around 26%–30% of the various profitable crops, such as cotton, wheat, and soybeans, are lost at production due to biotic and abiotic stress conditions (Oerke and Dehne*,* 2004). So far, more than 30 different diseases caused by various races of fungi, bacteria, and viruses have been recognized in soybeans (Ranjan *et al*., 2019).

Alternaria (*Alternaria spp.*) of chickpea, mung bean, phaseolus, and soybean crops have a remarkable negative impact on the agricultural economy of various countries. Among the *Alternaria* genus species, i.e., *Alternaria alternata (Fr.) Keissler*, *Alternaria tenuissima* (Need and T. Nees: Fr.) Wiltshire and several other species cause *Alternaria* diseases in crop plants, including legumes, as well as, in animals and humans (Khasanov *et al*., 2013). As reported, *A. Alternata,* as an individual species, caused disease in leaves, stems, and pods of soybeans and peas under natural conditions (Khasanov *et al.*, 2013). In the Republic of Uzbekistan, two types of *Alternaria* species, strains of *Alternaria alternata* and *Alternaria tenuissima,* are the causative agents on leguminous crops like soybean, chickpea, mung, and beans, detected black brown necrosis, black burn on the stem, and black sunken spot disease on the pods (Sherimbetov *et al*., 2020).

In *A. alternata,* the infection sources are widespread, mainly including mycelia in deciduous leaves and dead branches. *A. alternata* produces spores during May of the following year, spreading along winds and rains. With the growth of shoots, the disease peaks were evident in July and August (Xue *et al*., 2006; Song, 2013). *A. alternata* can also infect the fruits after taking off the outer cover in October, which causes red color spots on the peel and considerably affects the fruit quality (Dang *et al.*, 2018).

In photosynthesis, plants convert light energy into chemical energy to form organic products from inorganic materials, which serve as nutrients for growth and development in plant life (Simkin *et al*., 2020). Disruption of the ultrastructure of the chloroplasts by biotic and abiotic stress conditions leads to a decline in the chlorophyll content, reducing photosynthetic activities (Sidhu *et al*., 2017). In chlorophyll a and b and total chlorophyll, the reported decline attained influence from several stress conditions (Hamani *et al*., 2020; Matniyazova *et al*., 2022a, b, 2023). As antioxidants, carotenoids help to protect the chloroplasts and maintain the chlorophyll content (Kacharava *et al*., 2009).

Given the stress conditions, the dynamic balance among the formed and accumulated reactive oxygen species (ROS) and enzymes that neutralize them is upset. The enzymes polyphenol oxidase (PPO), peroxidase (POD), and phenylalanine ammonia-lyase (PAL) constantly try to stabilize the balance and detoxify ROS that contributes to plant resistance (Khatun and Chatterjee, 2011). Peroxidase is an enzyme of the oxidoreductase class, actively involved in the oxidation of phenols, suberization, and lignification of plant cell walls in response to phytopathogenic microorganisms. The said resistance mechanism links with the activity of

the peroxidase enzyme. Peroxidase is also vital in protecting crop plants from the detrimental effects of (ROS produced because of photosynthesis and respiration (Sharma *et al*., 2012).

Peroxidases are members of a large family of enzymes involved in hydrogen peroxide removal. Among them, the main difference depends upon the reducing substrate used, i.e., ascorbate peroxidase uses ascorbate, guaiacol peroxidase guaiacol, and pyrogallol peroxidase pyrogallol (Mourato *et al*., 2012). The PPO and its iso-enzymes provide a mechanism for defensive reactions in crop plants by obstructing phenol oxidation and cell damage. Therefore, it results in the inactivation of exo-enzymes and the activation of the synthesis of lignin in the damaged area of the plant cell walls to avoid the further spread of the pathogens (Tyuterev, 2002). In the activity of this enzyme, a sharp enhancement quickly prevents the spread of the phytopathogen in the crop plants.

Phenylalanine ammonia-lyase (PAL) is a chief phyto-immunity enzyme contributing to pathogen-host relationships (Khatun and Chatterjee, 2011). The PAL contributes to the biosynthesis of phytoalexins and phenolic compounds. Increased production of phenolic compounds, phytoalexins, lignin, and salicylic acid, associated with the plant's resistance to phytopathogens, often corresponds with the increased PAL activity in response to fungal infections (Raj *et al*., 2006). Defense-related enzymes elucidated the importance of peroxidase, polyphenol oxidase, and phenylalanine ammonia-lyase, and these enzymes have been comprehensively investigated as part of the plant defense system against pathogens (Sun *et al*., 2013; Ye *et al*., 2013; Qin *et al*., 2015; Han *et al*., 2016; Xie *et al*., 2017). The presented study sought to determine the number of chloroplast pigments and the activities of peroxidase, polyphenol oxidase, and phenylalanine ammonia-lyase enzymes in the leaf parts of the soybean local cultivars under the influence of phytopathogenic micromycetes that cause alternariosis disease.

MATERIALS AND METHODS

The latest study commenced at the Institute of Genetics and Plant Experimental Biology, Academy of Sciences, Republic of Uzbekistan. The effects of *A. alternata* phytopathogenic micromycetes attained scrutiny on five local soybean cultivars (*G. max* L.), i.e., Sochilmas, Genetik-1, Orzu, Tomaris, and Baraka. The phytopathogenic strains of the *A. alternata* came from the collection of "Phytopathogenic and other microorganisms unique scientific object" at the Institute of Genetics and Plant Experimental Biology, Academy of Sciences, Republic of Uzbekistan.

For the biomaterial preparation from *A. alternata* fungal strains, fungi growing in an artificial climate chamber (12 h of light, with a temperature of 25 °C–26 °C during the day and 21 °C–22 °C at night) took three to 15 days. Potatoes with sucrose (1000 ml of potato extract, 20 g of sucrose), grown in a nutrient medium, had a temperature of 25 °C–26 °C for 15 days to grow fungi. Then, fungi inoculation ensued 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 continued to be planted with soybeans in phytopathogenic soil mixed with 4 kg of oats per 100 m^2 at the specialized experimental field and, then, proceeded to transfer to soybean planting fields following the methodology of Solovyova (1951).

The presented study determined at the budding and flowering periods the number of chloroplast pigments and the activities of peroxidase (PO), polyphenol oxidase (PPO), and phenylalanine ammonia-lyase (PAL) enzymes in the leaf samples of soybeans (*G. max* L.).

Extraction and determination of pigment concentration

For determining the amount of pigments, the leaf samples from three to four soybean plants underwent calculation from the growth point. Each leaf's 50 mg placed in a test tube reached

homogenization in 5 ml of 95% ethyl alcohol solution (Lichtenthaler and Wellburn, 1983). The homogenate proceeded centrifugation at the speed of 5000 for 12 min. The amounts of chlorophyll a and b and carotenoids determination used an Agilent Cary 60 UV-Vis spectrophotometer at 664, 649, and 470 nm in the resulting extract. Based on these indicators, measuring amounts of chlorophyll a and b and carotenoids followed in the local soybean cultivar leaves using the following equations (Nayek *et al*., 2014):

$Ch\text{-}a = 13.36A_{664} – 5.19\ A_{649}$
$Ch\text{-}b = 27.43A_{649} – 8.12\ A_{664}$
$C\ x+c = (1000A_{470} –2.13C_a\text{-} – 97.63C_b)/209$

Extraction and assay of antioxidant enzyme

For enzyme extractions, the grinding of frozen leaf samples (0.5 g) into a fine powder using a mortar earlier placed in an ice bath and a pestle pre-cooled with liquid nitrogen and homogenized in 50 mM potassium phosphate buffer (pH 7.8) containing 1 mM ascorbate and 2% (w/v) polyvinylpolypyrrolidone. Homogenates proceeded centrifugation at 20,000x g for 30 min at 4 °C.

Peroxidase (POX; EC 1.11.1.7) activity measurement transpired by detecting the increase in absorbency at 460 nm upon oxidizing o-Dianisidine (Boyarkin, 1951). The 50 µl enzyme extraction added to the 2.85 ml of reaction mixture consisted of 1.85 ml 0.1M HAC-NaAC buffer (PH5.0), 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 incubation took 10 min at room temperature. Stopping the reaction ensued by adding 1 ml of 2.5 N H_2SO_4. The absorption of purpurogallin formed incurred scrutiny at 495 nm, with the blank prepared by adding 2.5 N H_2SO_4 at zero time for the same assay mixture. The PPO activity's expression was in U min^{-1} mg^{-1} protein (U = change in 0.1 absorbance min^{-1} mg^{-1} protein).

Verifying the phenylalanine ammonia-lyase (PAL, E.C. 4.3.1.5) activity in the leaf extract used the method of Ochoa and Salgado (1992), with slight modifications. Both control and infected leaves (0.2 g fresh weight) bore extraction 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 served as the PAL enzyme extract. The assay mixture contained 100 µl of extract, 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 incubated for 1 h at 37 °C had the reaction terminated by adding 0.5 ml of 6 M HCl; then, sample absorbance measuring was at 290 nm. The calibration curve construction used cinnamic acid. The blank had the same constituents except that adding the extract was after the HCl solution (Ochoa and Salgado, 1992).

Protein concentration

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

Statistical analysis

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

RESULTS

Studying the amount of chloroplast pigments in the leaves of local soybean cultivars (*G. max* L.) artificially infested with phytopathogenic

fungi occurred during the budding and flowering periods. The amount of chlorophyll a during the budding period of soybean showed a decline in the plants artificially infested with *A. Alternata* with varying degrees compared with the control plants. Among the soybean cultivars infected with *A. alternata*, the cultivars Genetik-1 and Tomaris had the highest amount of chlorophyll a (3.08 ± 0.11 mg/g and 3.04 ± 0.41 mg/g, respectively) versus other cultivars, and the cultivar Sochilmas (1.90 ± 0.39 mg/g) had a low index (Table 1).

The amount of chlorophyll b studied during the budding period in the leaves of soybean cultivars revealed a reduction in the plants artificially infested with *A. alternata* compared to the control plots. Among the soybean cultivars infected with *A. alternata*, the genotype Tomaris had a higher content of chlorophyll b (1.29 ± 0.27 mg/g) than other genotypes, and cultivar Sochilmas had the lowest value for the said physiological trait (0.75 ± 0.28 mg/g).

The sign of total chlorophyll content during the budding phase emerged varying, similar to the case of chlorophyll a and b content. In the leaves of soybean cultivars, the study of carotenoid content continued under laboratory conditions. A result of the action of phytopathogenic fungi indicated that the amount of carotenoids increased at different degrees compared with the control in the soybean genotype leaves (Table 2). Among the soybean cultivars artificially infested with *A. alternata*, the highest index of carotenoid content appeared in the Genetic-1 cultivar (1.62 ± 0.34 mg/g) compared with other soybean genotypes, with the lowest index found in cultivar Baraka (1.04 ± 0.14 mg/g).

The amount of chlorophyll a also assessed during the flowering period of soybeans provided a decline in chlorophyll a in the plants artificially infested with *A. alternata* at varying degrees compared with the control plants (Table 3). Among the soybean cultivars infected with *A. alternata*, as compared with other soybean cultivars, the genotypes Tomaris and Nafis had a higher amount of chlorophyll a (1.88 ± 0.05 and 1.62 ± 0.35 mg/g, respectively), while the cultivar Genetic-1 showed the lowest value for the said variable (0.96 ± 0.05 mg/g).

The chlorophyll b in the leaves of soybean cultivars assessed during the flowering period indicated a decrease in the plants artificially infested with *A. alternata*, with varying ratios compared with the control treatment. Among the soybean cultivars affected by *A. alternata*, the cultivar Tomaris owned the highest chlorophyll b content (0.83 ± 0.02 mg/g) as compared to other soybean cultivars, with the cultivar Baraka giving the lowest content (0.44 ± 0.42 mg/g).

The results further enunciated that the sign of total chlorophyll content during the flowering period also varied in the soybean genotypes similar to the chlorophyll a and b. Exposure to the phytopathogenic fungi resulted in an increased amount of carotenoids in different degrees compared with the control in the soybean leaves (Table 4). Among the soybean cultivars affected by *A. alternata*, carotenoids were high in the cultivar Tomaris (0.71 ± 0.05 mg/g) compared with the other four cultivars, and the cultivar Baraka gave a low value (0.43 ± 0.20 mg/g). Thus, it was apparent that due to the effects of phytopathogenic micromycetes on local soybean cultivars, the amount of chlorophyll a and b, total chlorophyll, and carotenoids in soybean cultivars was superior during the flowering period compared with the budding period.

In the plant leaves of one-month-old sprouts, detecting the activities of peroxidase, polyphenol oxidase, and phenylalanine ammonia-lyase enzymes ensued in laboratory conditions at the budding stage of soybean cultivars grown in a specialized experimental field. In the presented experiments, assessing the activity of the peroxidase enzyme in plant leaves of local soybean cultivars continued under the control variant (without phytopathogenic micromycetes) and in the variants with phytopathogenic micromycetes *A. alternata*.

In the group of local soybean cultivars studied in the control option, the highest indicator of peroxidase enzyme activity was in the cultivar Genetic-1 (78.62 ± 1.14 E/mg protein) compared with the other cultivars,

with the lowest indicator recorded in the soybean cultivar Nafis (61.00 ± 1.94 E/mg protein) (Figure 1). Under the influence of the phytopathogenic micromycete *A. alternata*, the activity of the peroxidase enzyme in local soybean cultivars Genetik-1 and Sochilmas decreased by 25.2% and 19.8%, respectively, while in cultivars Tomaris and Baraki, the said activity showed an enhancement of 10.4%, 35.9%, and 62.4%. It was also noticeable that the activity of the peroxidase enzyme was higher in the cultivars Baraka and Nafis, artificially infested with the fungus *A. alternata*, compared with the other cultivars.

Table 1. Amount of pigments in the plant leaves of the local soybean cultivars during the budding period.

No.	Cultivars	Chlorophyll a		Chlorophyll b	
		Control	*A. alternata*	Control	*A. alternata*
1	Genetic-1	3.83±0.26	3.08±0.11	1.74±0.06	1.09±0.61
2	Baraka	3.28±0.23	2.95±1.00	1.96±0.40	1.14±0.16
3	Tomaris	4.01±0.18	3.04±0.41	1.77±0.27	1.29±0.27
4	Nafis	3.15±0.09	1.88±0.18	1.93±0.09	0.83±0.23
5	Sochilmas	2.75±0.28	1.90±0.39	1.17±0.18	0.75±0.28

Table 2. Amount of pigments in the plant leaves of the local soybean cultivars during the budding period.

No.	Cultivars	Total chlorophyll		Total carotenoids	
		Control	*A. alternata*	Control	*A. alternata*
1	Genetic-1	5.57±0.10	4.17±0.66	1.32±0.10	1.62±0.34
2	Baraka	5.24±1.28	4.09±0.12	0.91±0.70	1.04±0.14
3	Tomaris	5.78±0.33	4.33±0.49	0.64±0.25	1.48±0.25
4	Nafis	5.08±0.15	2.71±0.33	0.90±0.23	1.19±0.16
5	Sochilmas	3.92±0.45	2.65±0.33	0.71±0.27	1.09±0.04

Table 3. Amount of pigments in the plant leaves of the local soybean cultivars during the flowering period.

No.	Cultivars	Chlorophyll a		Chlorophyll b	
		Control	*A. alternata*	Control	*A. alternata*
1	Genetic-1	1.79±0.19	0.96±0.05	0.68±0.09	0.55±0.07
2	Baraka	3.08±0.26	1.54±0.37	1.90±0.33	0.44±0.42
3	Tomaris	1.96±0.22	1.88±0.05	0.86±0.19	0.83±0.02
4	Nafis	2.17±0.11	1.62±0.35	0.97±0.12	0.71±0.22
5	Sochilmas	1.67±0.12	1.46±0.01	0.76±0.11	0.68±0.03

Table 4. Amount of pigments in plant leaves of local soybean cultivars during the flowering period.

No.	Cultivars	Total chlorophyll		Carotenoids	
		Control	*A. alternata*	Control	*A. alternata*
1	Genetic-1	2.47±0.22	1.51±0.05	0.39±0.04	0.53±0.14
2	Baraka	4.98±0.42	1.99±0.18	0.58±0.20	0.43±0.20
3	Tomaris	2.83±0.24	2.72±0.05	0.68±0.15	0.71±0.05
4	Nafis	3.14±0.16	2.33±0.03	0.65±0.05	0.59±0.20
5	Sochilmas	2.43±0.07	2.14±0.41	0.59±0.07	0.50±0.05

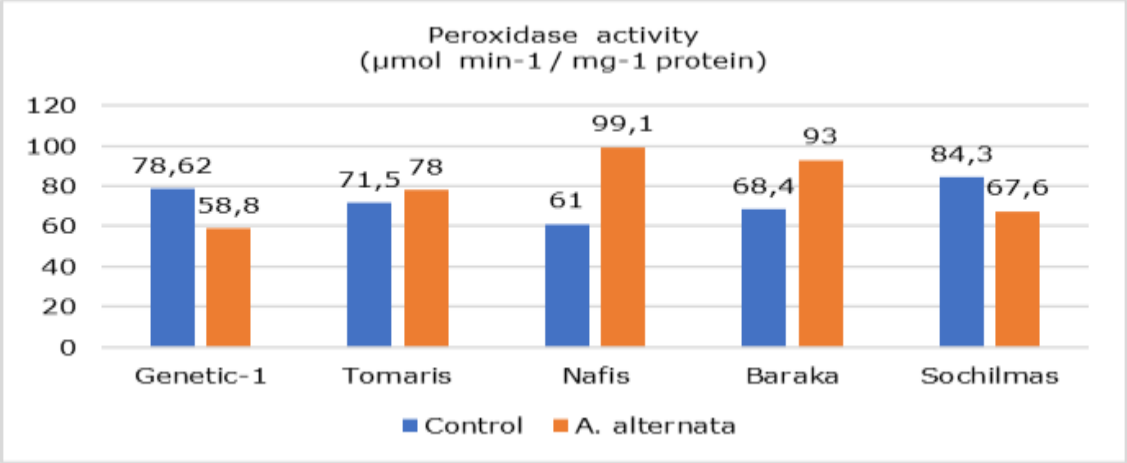

Figure 1. Activity of the peroxidase enzyme in the plant leaves of soybean local cultivars during the flowering period under the influence of *A. alternata*.

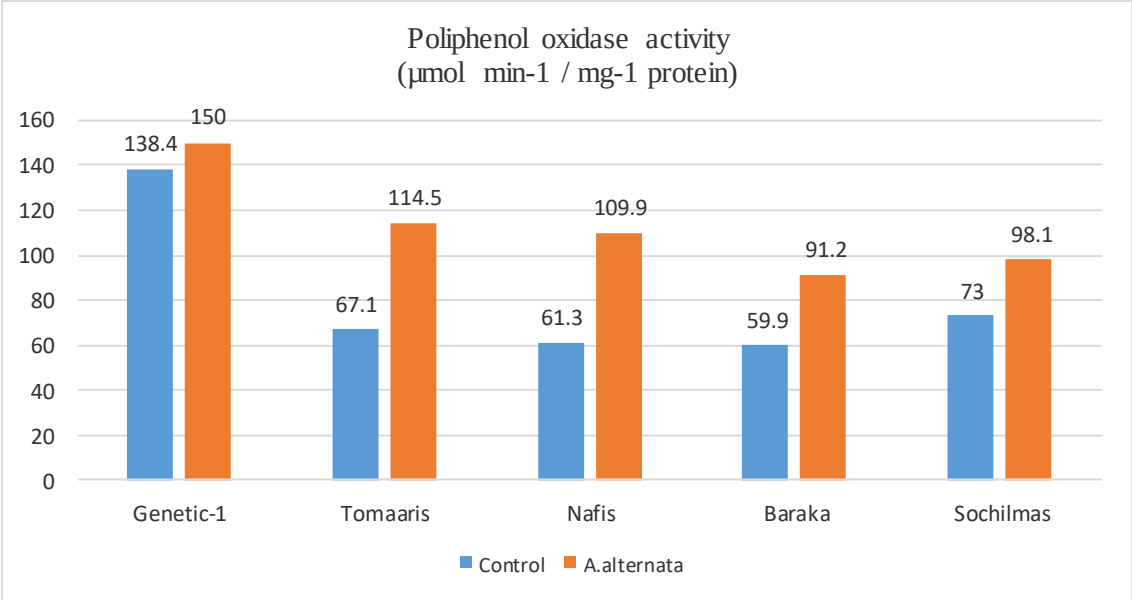

Figure 2. Activity of the polyphenol oxidase enzyme in the plant leaves of soybean local cultivars during the flowering period under the influence of *A. alternata*.

Compared with the control variant, the activity of the peroxidase enzyme in plants of local soybean cultivars differed under the influence of phytopathogenic micromycetes in varying degrees. The enzyme polyphenol oxidase is one of the essential enzymes of plant resistance to stress factors, and, therefore, probing its activity. The results revealed that the activity of this enzyme was higher under the influence of phytopathogenic micromycetes compared with the control variant (Figure 2). The effect of phytopathogenic plant micromycetes on the activity of the polyphenol oxidase enzyme in the leaves of all studied domestic soybean cultivars during the budding period was also prominent.

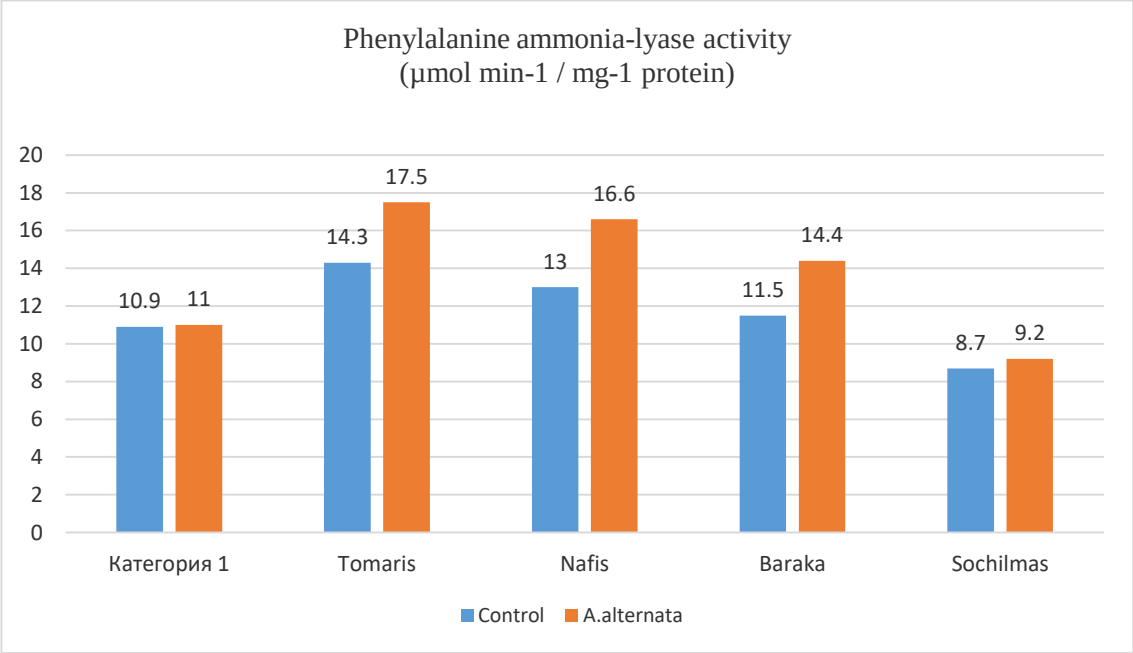

Figure 3. Activity of the enzyme phenylalanine ammonia lyase in the plant leaves of soybean local cultivars during the flowering period under the influence of *A. alternata*.

In the local soybean cultivars with the control variant, the highest indicator of polyphenol oxidase enzyme activity appeared in the cultivar Genetic-1 (138.44 ± 1.88 E/mg protein) compared with the other soybean cultivars, with the lowest indicator observed in the genotype Baraka (59.94 ± 3.20 E/mg protein). Analysis of the results revealed that the activity of the polyphenol oxidase enzyme increased in all the soybean cultivars under the influence of the phytopathogenic micromycete *A. alternata* compared with the control treatment. The results enunciated that in the soybean cultivars Genetik-1, Sochilmas, Nafis, Tomaris, and Baraka, the activity of the polyphenol oxidase enzyme attained a rise of 8.4%, 34.4%, 79.4%, 70.6%, and 52.2%, respectively (Figure 2). In the presence of the phytopathogenic micromycete *A. alternata*, it was noteworthy that the activity of the polyphenol oxidase enzyme was higher in the local soybean cultivars Tomaris, Nafis, and Baraka, compared with the other cultivars.

In these experiments, the activity of the enzyme phenylalanine ammonia-lyase also gained evaluation in the plant leaves of the local soybean cultivars. Phenylalanine ammonia-lyase is an indicator enzyme of high sensitivity to stress, a biochemical marker of structural and protective compounds (Figure 3). In the control variant, the highest activity of the phenylalanine ammonia-lyase enzyme in the local soybean cultivars was evident in the genotype Tomaris (14.3 ± 0.64 E/mg protein) compared with the other cultivars, and the lowest values emerged in the cultivar Sochilmas (8.7 ± 0.47 E/mg protein). Based on the results obtained, it was clear that under the influence of the phytopathogenic micromycete *A. alternata*, the activity of the phenylalanine ammonia-lyase enzyme increased in all local soybean cultivars to varying degrees compared with the control. The soybean cultivars Genetic-1, Sochilmas, Baraka, Nafis, and Tomaris increased by 0.9%, 5.7%, 25.2%, 27.6%, and 22.3%, respectively.

The activity of the enzyme phenylalanine ammonia-lyase was higher in the soybean cultivars Baraka and Nafis under the influence of the phytopathogenic micromycete *A. alternata* compared with the

other cultivars. The increased activity of these enzymes in soybean cultivars may be an effective defense response against pathogen infection and a reaction to induced resistance in the soybean plants. Based on the obtained results, phenylalanine ammonia-lyase, polyphenol oxidase, and peroxidase enzymes can serve as physiological and biochemical markers of resistance in the local soybean cultivars to biotic stresses, especially to phytopathogenic microorganisms.

DISCUSSION

This study presented the chloroplast pigment content and the activity of peroxidase, polyphenol oxidase, and phenylalanine ammonia-lyase enzymes in the leaves of five local cultivars of soybean (*G. max* L.) under the influence of phytopathogenic micromycetes. Phytopathogenic fungi develop the oxidative stress that causes plant cell membrane damage and chlorophyll degradation. The fungus *Alternaria* adversely affected photosynthetic activity and caused leaf necrosis and a decrease in the content of chlorophyll and carotenoids (Dehgahi *et al*., 2015).

This research also determined the leaf pigment content in the leaves of infected and control plants during budding and flowering periods. The results indicated a decrease in the chlorophyll a, b, and total chlorophyll contents. A significant decrease (53.3%) in the total chlorophyll content during the budding period was evident in the soybean cultivar Nafis with the infection background at 2.71 mg/g) compared with the control variant and without fungal infection (5.08 mg/g). During the flowering period, a considerable decrease was remarkable in the infectious background (1.99 mg/g) compared with the control treatment (4.98 mg/g), detecting the same in the soybean cultivar Baraka. Saleem *et al.* (2012) observed similar findings on the diseased leaves of broad bean plants infected by *A. alternata.* Pati *et al.* (2008) also reported a decline in leaf chlorophyll content while working on the disease profile of *Withania somnifera* suffering from *A. alternata.*

Based on the reduction percentage in chlorophyll in infected samples, it is presumable that the pathogen *A. alternata* affects the chlorophyll to a maximum extent. However, the carotenoid content rose by 56.7% in the disease-infected leaves compared with healthy ones. These results also explained the antioxidant properties of carotenoids. However, in the research work carried out by Zarger *et al.* (2014), they reported that the carotenoid content decreased up to 14% in leaf samples affected by *A. alternata*.

In crop plants, the pathogen infections provoke the production of reactive oxygen species (ROS) as one of the earliest responses in pathogen-plant interaction (Collin*,* 2019). Plants evolve specific protective mechanisms involving antioxidant molecules and enzymes to defend themselves against oxidants (Jiang and Zhang, 2002; Núñez *et al.,* 2003). Therefore, antioxidants and antioxidative enzymes, such as superoxide dismutase (SOD), catalase (CAT), peroxidase (POD), and ascorbate peroxidase (APOX), function to interrupt the cascades of uncontrolled oxidants in some organelles (Jiang and Zhang, 2001; Harinasut *et al.,* 2003). In tolerant plants, POD activity emerged higher to protect plants against oxidative stress conditions (Sreenivasulu *et al.,* 1999; Yuldashov *et al., 2023*).

By studying the activity of peroxidase enzyme in plant leaves of local soybean cultivars, it was apparent that cultivars Baraka and Nafis had higher activity compared with the control version under the influence of *A. alternata* fungus than other cultivars. Past studies authenticated that resistant cultivars have a higher polyphenol oxidase activity than susceptible genotypes (Raj *et al*., 2006). The activity of the enzyme polyphenol oxidase was superior in the tissues of millet plants infected with mold than in healthy plants (Shetty *et al*., 2001). A similar situation appeared in pear fruits infected with the pathogen *Erwinia amylovora* (Honty *et al.,* 2005).

Inducing damage on the polyphenol oxidase enzyme has proven to increase in *Sclerospora graminicola* of millet and *Alternaria triticina* of wheat, which enhances the oxidation of anti-pathogenic phenolic

compounds to more toxic forms, i.e., quinones (Tyagi *et al.,* 2000). In the presented study, the activity of polyphenol oxidase enzyme in local soybean cultivars, Nafis and Tomaris infected with *A. alternata,* was prevalent in the genotypes Tomaris and Nafis (70.6% and 79.4 %, respectively).

Phenylalanine ammonia-lyase (PAL) is an indicator enzyme of high sensitivity to stress conditions, which is a biochemical marker for structural and protective compounds. PAL is vital in the resistance mechanism to microorganisms (Jayaraj *et al.*, 2010; Tahsili *et al.*, 2014). Therefore, the activity of phenylalanine ammonia-lyase enzyme also attained probing in plants infected with *A. alternata*. As a result, it revealed that the activity of phenylalanine ammonia-lyase enzyme increased in plants infected with *A. alternata* compared with healthy plants. As shown in Figure 3, a significant increase in the activity of the phenylalanine ammonia-lyase enzyme appeared in the soybean local cultivars Tomaris, Nafis, and Baraka.

CONCLUSIONS

The soybean plant's physiological (chlorophyll a and b and carotenoids) and biochemical (PO, PPO, and PAL) properties proved to be related to the effects of *A. alternata* fungus. It was also evident that the activity of PO, PPO, and PAL attained maximum increases in the samples with minimal decrease in the content of chloroplast pigments in soybean plants infected with *A. alternata* phytopathogenic micromycetes. Local soybean cultivars Tomaris, Nafis, and Genetic-1 were selections for resistant genotypes to *A. alternata* fungus. These cultivars can also serve as a base material in future genetic selection studies to develop the soybean resistant cultivars to Alternaria disease and are options to grow in areas with a high risk of damage by *A. alternata*.

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