Advances in Biology & Earth Sciences
Vol.7, No.3, 2022, pp.169-177

THE EFFECT OF SALICYLIC ACID ON THE CONTENT OF PHOTOSYNTHETIC PIGMENTS AND THE ACTIVITY OF ANTIOXIDANT ENZYMES IN COTTON SEEDLINGS

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Abstract. The effects of salicylic acid on the content of photosynthetic pigments and activity of antioxidant enzymes in *Gossypium barbadense* Pima 3-79 and *Gossypium hirsutum* TM1 cotton genotypes seedlings was studied. It was established that salicylic acid (SA) was involved in the regulation of the content of photosynthetic pigments in cotton seedlings, but genotypes of cotton differed significantly in sensitivity to this phytohormone. Maximum stimulation effect of SA in *G. barbadense* Pima 3-79 was observed at1mM and in *G. hirsutum* TM1 at 0.1 mM. At low concentration of SA (0.1 mM) catalase activity in both cotyledons was positively stimulated, but high of SA (1 mM) caused the activity inhibition. Peroxidase activity linearly increased depending on SA concentration in *G. barbadense* Pima 3-79, but the same effect was observed only at relatively high concentrations of SA (10 mM). SA did not induce SOD activity. At low concentrations of SA - 0.1 and 1 mM, SOD activity decreased in both genotypes, but at high concentration - 10 mM, it was practically at the same level as control. Impact of SA on polyphenol oxidase activity had a zigzag pattern depending on concentration. 0.1 and 10 mM of SA caused stimulation, but 1 mM caused stimulation of enzyme activity in both genotypes. So, SA participated in regulation of photosynthetic pigment and antioxidant enzyme activity, but showed both cotton genotype and antioxidant enzyme specify.

Keywords: *salicylic acid (SA), Gossypium barbadense, Gossypium hirsutum, photosynthetic pigments, antioxidant enzymes.*

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Received*: 16 September 2022;* ***Accepted****: 31 October 2022;* ***Published****: 8 December 2022.*

1. Introduction

Cotton production is a priority area of agriculture in the world. It is considered as a strategic and multifunctional product. More than 26 million tons cotton is produced annually in the world. However, the need for this product is growing from year to year. Main cotton producing countries are China, India, USA, Pakistan and Uzbekistan (Keerthi *et al.*, 2014; Voora *et al.*, 2020).

How to cite (APA):
Amrahov, N., Bayramova, S., Mammadova, R., Ojagverdiyeva, S., Aghazada, G., Alizada, Sh., Mammadov, Z. (2022). The effect of salicylic acid on the content of photosynthetic pigments and the activity of antioxidant enzymes in cotton seedlings. *Advances in Biology & Earth Sciences*, *7*(3), 169-177.

Even though the cotton plant by nature is relatively stable, its productivity significantly depends on the influence of various favourable and unfavourable abiotic and biotic environmental factors. İf these factors turn out to be unfavourable, their negative effect will be reflected in the growth and productivity of cotton. The presence of innate genetic factors of adaptation, which form the basis of the protective mechanisms of plants from negative environmental influences, includes the primary activation of the reactive oxygen species (ROS) formation, followed by their neutralization with the help of antioxidant enzymes. As it is known, the activation of ROS is a necessary condition for triggering the protective reactions of plants. Since ROS is harmful to cells, after performing its function, its excess should be immediately neutralized. Antioxidant enzymes take an active part in the neutralization process (Zhang *et al.*, 2014; Parida *et al.*, 2005; Munns *et al.*, 2008). Naturally, the progress of these processes in plants is regulated by phytohormones, among which salicylic acid (SA) also plays an important role. It was found that SA and some of its derivatives have special specificity and act differently on different types of plants. Plants also differ in sensitivity to different concentrations of SA (Ganesan *et al.*, 2001; Delaney *et al.*, 1994). Exogenous SA affects some physiological and biochemical processes in plants, including leaf cell ageing, seed germination (Morris *et al*., 2000; Rajou *et al.*, 2006), protein degradation (Guilfoyle *et al.*, 2015) and others. In addition, it acts as the immunostimulator, which gives rise to the assumption of its active participation in the activation of the immune response of plants (Vlot *et al.*, 2009).

Taking into account above mentioned, the influence of different concentrations of SA on the photosynthetic components and activity of antioxidant enzymes in seedlings resistant to fungal pathogens of cotton varieties, *Gossypium barbadense* Pima 3-79 and unstable to pathogenic fungi common cotton- *Gossypium hirsutum* TM1 were studied.

2. Materials and methods

The experiments were carried out on cotton genotypes: *Gossypium barbadense* Pima 3-79 and *Gossypium hirsutum* TM1. The seeds of these plants were pre-treated with 0.3% potassium permanganate for 5 minutes, then they were planted in 7 cm plastic cups containing perlite. The seedlings were grown in a phytotron (Taisite, GZX-300 E) at a temperature of 24–26°C, a humidity of 65–75%, and a light intensity of 4800 lux. The light length was 14/10 hours day/night.

Steiner's solution was used as a nutrient medium, with the addition of 0.1, 1 and 10 mM concentrations of SA during the entire growth period, starting from the first days of seedling formation. Two-week-old embryonic cotyledons were used for analyses.

***Determination of enzymatic activity**:*

Catalase activity was determined by the Mosheva gasometric method (Mosheva, 1982). First, 0.5 g of plant material was measured, which was homogenized with the gradual addition of 0.5 g of $CaCO_3$ and 20 ml of distilled water. Subsequently, the extract was transferred to a Landolt vessel (Erlenmeyer flask) with a neck on the side and catalase activity was measured. To start the reaction, 5 ml of 3 % H_2O_2 was added to the extract and during the entire reaction, it was stirred on a magnetic stirrer. The measurement was carried out after 3 minutes. After this time, the amount of oxygen

released could be read on the burette scale. The enzyme activity was expressed in ml/second*g (fresh sample).

The activity of guaiacol peroxidase (GPX, EC 1.11.1.7) was determined according to Chance and Maehly (Chance & Maehly, 1955). The reaction mixture contained 0.1 M Na-phosphate buffer, pH 7,2, 1mM EDTA, 30mM H_2O_2, 50mM guaiacol, and 50µl of enzyme extract in the final volume of 1 ml. The formation of tetraguaiacol was detected at 440 nm. The concentration of tetraguaiacol was calculated using the extinction coefficient of tetraguaiacol (26.6mM $^{-1}$ cm $^{-1}$). GPX activity was expressed as $_{\Delta}$A590• g^{-1}• min^{-1}.

Superoxide dismutase (SOD, EC 1.15.1.1) activity was determined spectrophotometrically by measuring its ability to inhibit photochemical reduction of nitroblue tetrazolium (NBT) as reported by Beaucham and Fridovic (Beauchamp, C., & Fridovich, I., 1971). The reaction mixture contained 50 mM K phosphate buffer, pH 7.8, with 0.1mM EDTA, 150µM NBT, 26mM methionine, 8 µM riboflavin, and 50 µl of enzyme extract in the final volume of 2,05 ml. Reaction mixtures were incubated for 8 min under light conditions. One unit of SOD activity was defined as the amount of enzyme that inhibits 50% of NBT photoreduction.

Polyphenol oxidase activity was determined by measuring the oxidation of 0.05 M catechol at 590 nm in 0.1 M potassium phosphate buffer with pH 7,2 (Ermakov, A. I *et al.,* 1987). The activity of polyphenol oxidase was expressed as U•min•g^{-1} FW.

Determination of the content of chlorophyll a, b and carotenoids

The analysis and calculation of the concentration of chlorophyll **a, b** and carotenoids were carried out using the Wellburn method (Wellburn, A. R. *et al.,* 1984). 1 gram of leaf tissue was taken, cut into small pieces and homogenized in a mortar in 20 ml of 80% acetone. Then the homogenate was passed through an F-grade filter paper and the optical density was measured in the resulting filtrate at wavelengths of 440, 644 and 662 nm. The results obtained were presented in mg/g of fresh sample.

Statistical analysis

The adsorption experiments were performed in triplicate and data was reported as mean ± SD. The regression coefficients (R2) values of isotherms and kinetics models were calculated using statistical functions of Microsoft Excel (version Office 7, Microsoft Corporation, USA).

3. Results

In both genotypes of cotton, under the influence of a low concentration of SA- 0.1 mM, catalase activity in cotyledons was positively stimulated (Fig. 1). In the genotype of *Gossypium barbadense* Pima 3-79, this increase compared to the control was 3.2-3.4 times, and in *Gossypium hirsutum* TM1 - 1.6-1.8 times. Catalase activity in the genotype of *Gossypium barbadense* Pima 3-79 decreased to the control level at subsequently tested concentrations of SA-1 and 10 mM. A tenfold increase in the concentration (up to 1 mM) of this phytohormone was accompanied by a noticeable decrease in the activity of the enzyme in the genotype *Gossypium hirsutum* TM1 and compared with the control, which remained at a fairly high level. However, even in this case, the high concentration of SA caused significant suppression of catalase activity, which was noticeably lower than in the control cotyledons.

The reaction of peroxidase to exogenous SA differed significantly from the activity of catalase. A significant difference was also manifested at the level of cotton genotypes. With an increase in the concentration of phytohormone in the medium, the induction of the activity of the cotyledons' enzyme *Gossypium barbadense* Pima 3-79 increased in a linear sequence, reaching its maximum value at a concentration of 10 mM, where the activity of the enzyme was 3.5-3.8 times higher compared to the control (Fig. 2). Positive stimulation of peroxidase activity in the genotype of *Gossypium hirsutum* TM1 was observed only at relatively high concentrations of SA (1 and 10 mM), whereas, at a low concentration of phytohormone, it was even noticeably lower than in the tissues of cotyledons of the control variant.

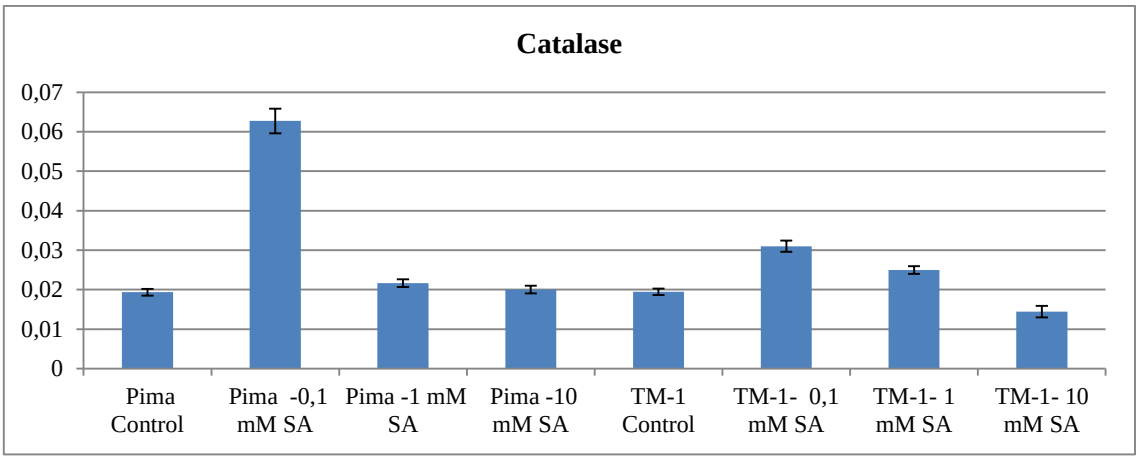

Fig. 1. Catalase activity in leaves of cotyledons of genotypes Gossypium barbadense Pima 3-79 and Gossypium hirsutum TM1 exposed to different concentrations of SA

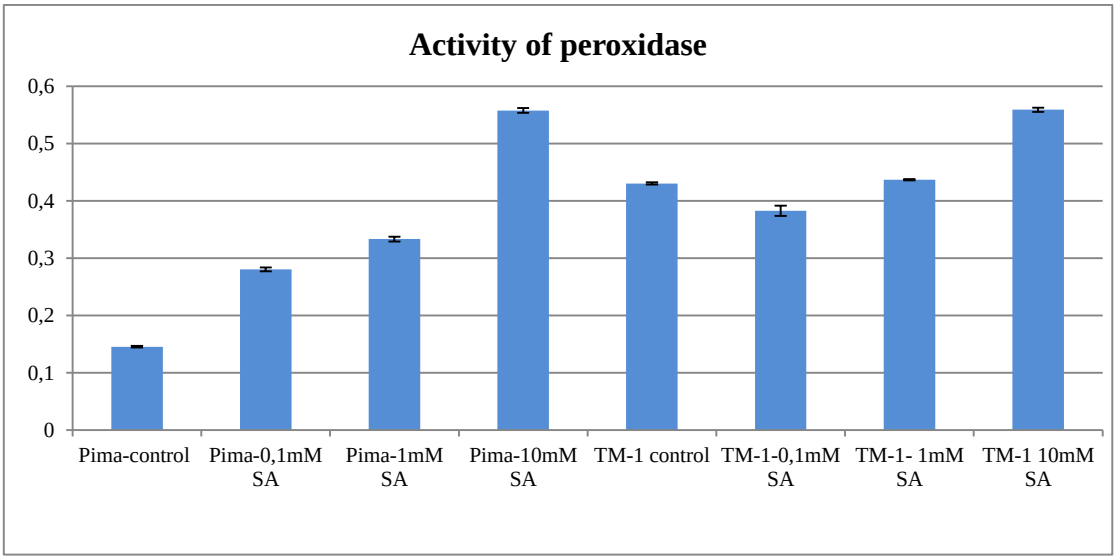

Fig. 2. Peroxidase activity (GPX) in cotyledons of genotypes: *Gossypium barbadense* Pima 3-79 and *Gossypium hirsutum* TM1 exposed to different concentrations of salicylic acid

The activity of SOD at concentrations of 0.1 and 1 mM of SA decreased in both genotypes (Fig.3). However, at its high concentration, it was practically at the level of the control variant.

The effect of SA on the activity of polyphenol oxidase in both genotypes of cotton was characterized by a similar zigzag pattern and significantly determined by its concentration (Fig.4). Low and high concentrations, i.e 0.1 and 10 mM, of SA caused stimulation of enzyme activity, and at a concentration of 1 mM in both genotypes, a noticeable suppression of activity in comparison with the control was observed.

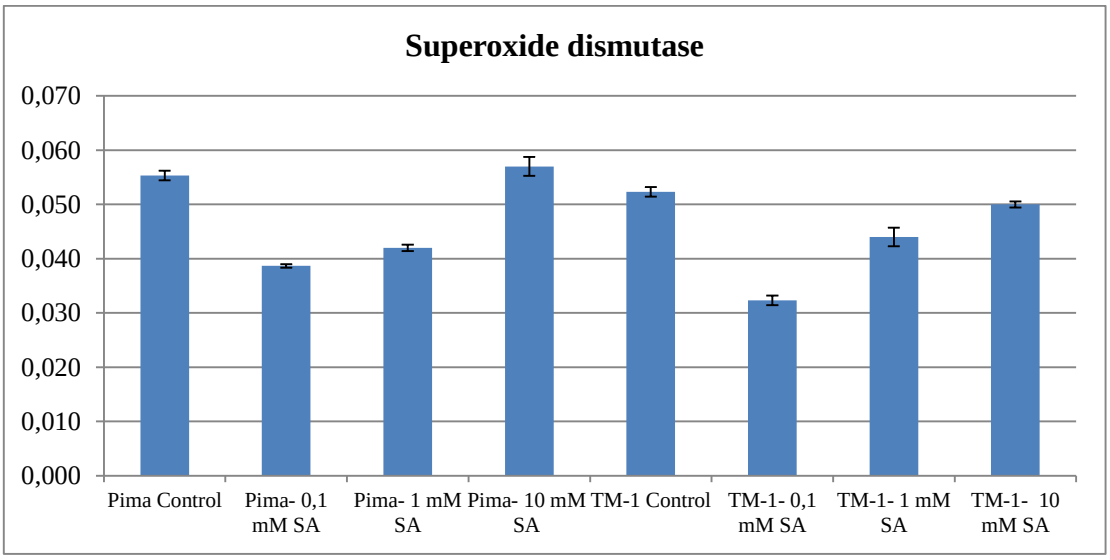

Fig. 3. SOD activity in the cotyledons of genotypes: *Gossypium barbadense* Pima 3-79 and *Gossypium hirsutum* TM1 exposed to different concentrations of salicylic acid

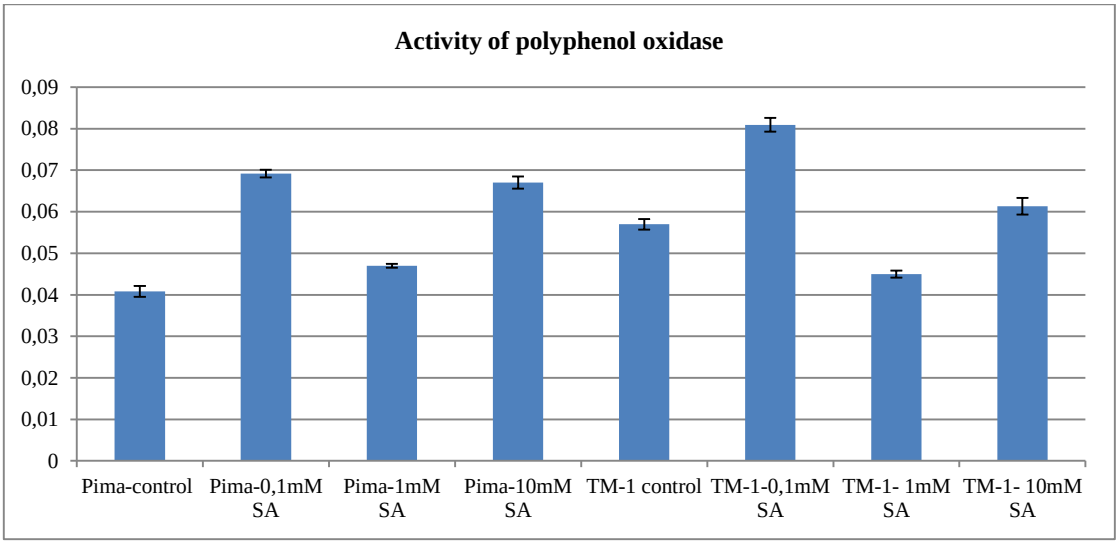

Fig. 4. The activity of polyphenol oxidase in the cotyledons of genotypes *Gossypium barbadense* Pima 3-79 and *Gossypium hirsutum* TM1 exposed to different concentrations of salicylic acid

The results of the analysis of the effect of SA on the content of chlorophyll a, b and carotenoids in the leaf tissues of seedlings *Gossypium barbadense* Pima 3-79 and *Gossypium hirsutum* TM1 are shown in Fig. 5. The most suitable SA concentration, at which the content of the studied pigments was the maximum for the genotype of

Gossypium barbadense Pima 3-79, was 1mM and for *Gossypium hirsutum* TM1 was 0.1 mM. From the obtained data it becomes obvious that SA is directly involved in the regulation of the content of photosynthetic pigments in cotton seedlings, and these genotypes differ significantly in sensitivity to SA.

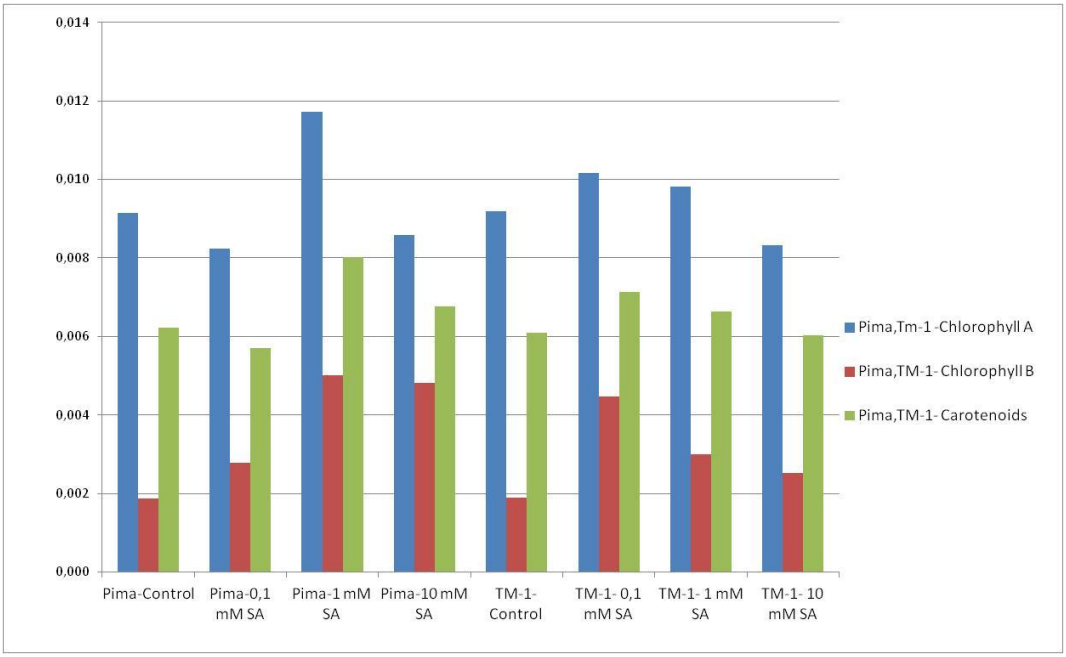

Fig. 5. The concentration of chlorophyll a, b and carotenoids in the cotyledons *Gossypium barbadense* Pima 3-79 and *Gossypium hirsutum* TM1 genotypes exposed to different concentrations of salicylic acid

4. Discussion

Salicylic acid is one of the important and generally recognized phytohormones that plays the role of an endogenous signalling molecule and, at so-called physiological concentrations, is involved in the regulation of many biochemical, and therefore physiological processes (Durrant *et al.,* 2004). Previous studies have shown that the negative effect of abiotic stress factors, which leads to a partial suppression of photosynthesis activity and reduces the concentration of carotenoids, can, to a certain extent, be restored by the stimulating effect of SA (Moustafa-Farag *et al.*, 2020). Our studies have shown that low concentrations (0.1 and 1 mM) of SA contribute to an increase in the content of the necessary components of the photosynthetic apparatus, viz chlorophyll a, b and carotenoids in the leaves of cotton seedlings. This effect was most pronounced in the *Gossypium hirsutum* TM1 genotype at SA concentration of 0.1 mM, and in the *Gossypium barbadense* Pima 3-79 genotype at its concentration of 1.0 mM. The obtained data indicate a difference in the level of susceptibility of the tested genotypes to SA, on the one hand, and the possibility of participation of SA in inducing the process of photosynthesis by increasing the concentration of the components of the photosynthetic apparatus, on the other hand. The influence of SA on the same processes at higher concentrations showed the opposite effect, which, is associated with the nonspecific behaviour of the phytohormone. Similar results were also obtained in experiments where SA was not introduced into a hydroponic solution but was used as a

spray at different concentrations against abiotic stress (Eraslan *et al.,* 2007; Tariq *et al.*, 2011).

One of the important properties of SA is its ability to induce the activity of antioxidant enzymes, which allow it to participate in the defence system of plants. Previous studies have identified a relationship between exogenous SA and antioxidant enzymes such as SOD, peroxidase, catalase, and polyphenol oxidase (Khalifa *et al.*, 2016). In the presence of salicylic acid, both genotypes showed a decrease in SOD activity at lower concentrations (0.1 and 1 mM) and a return to normal activity at a concentration of 10 mM of SA. The data are identical to previous studies, indicating a decrease in SOD activity at lower SA concentrations (Liu *et al.,* 2014). Peroxidase is also one of the important free radical scavenging enzymes (Sarvajeet *et al.,* 2010). It was found that maximum activity of peroxidase was manifested in both genotypes at a high SA concentration (10 mM). At the same time, the more sensitive Pima 3-79 genotype showed an increase in this activity starting from the minimum SA concentration, which continued with an increase in the concentration and reached its maximum value at 10 mM. Furthermore, peroxidase activity increased only at a concentration of 10 mM of SA as in the case of the TM-1 genotype. These results may indicate the participation of SA in the removal of free radical activity through the induction of peroxidase activity.

As shown in recent experiments, SA can reduce catalase activity under changing external abiotic factors (Min, Kyungwon *et al.,* 2021). As a result of our analysis, it was found that the activity of catalase increases as the concentration of SA increases. However, at its maximum concentration, the activity of the enzyme decreases, which may indicate a limitation of the role of catalase under such circumstances. A direct correlation between catalase activity and the components of the photosynthetic apparatus indicates a direct effect of SA on these systems, which was also demonstrated in previous studies (Dawood *et al.,* 2012). Moreover, an increase in catalase activity may indicate an increase in the concentration of hydrogen peroxide in the intercellular space, where various molecular forms of catalase responsible for ROS neutralization are localized (Patykowski *et al*., 2003). An increase in catalase activity in both genotypes at low SA concentrations, in this case, may also be associated with its activation in all parts of cells (both in the extracellular space and in the intracellular space). However, a decrease in catalase activity may be associated with an increased accumulation of ROS and partial blocking of the activity by these substances at high SA concentrations (Raskin, 1992; Taşgın *et al.,* 2006).

The involvement of PPO in the antioxidant defence of plants by minimizing ROS content has been demonstrated in several studies (Chandra *et al.,* 2007; Simaei *et al.,* 2012). A zigzag increase in the activity of polyphenol oxidase depending on different concentrations of SA may indicate the participation of this phytohormone in various genetic mechanisms for reducing the concentration of ROS inside cells, including polyphenol oxidase.

5. Conclusion

The effect of SA on both genotypes at low and moderate concentrations (0.1 mM and 1 mM) was stimulating, while the maximum concentration of 10 mM had a toxic effect. In all cases, there was a change in the parameters of antioxidant enzymes and components of photosynthetic pigments.

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