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Influence of technogenic pollution on the activity of catalase and ascorbate peroxidase in plant leaves

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Abstract
This article comparatively studied the activity of catalase (CAT, H_2O_2:H_2O_2-oxidoreductase, EC 1.11.1.6) and ascorbate peroxidase (APO, ascorbate peroxidase, EC 1.11.1.11) enzymes in the leaves of Amaranthus albus L., Chenopodium album L. and Atriplex tatarica L. species belonging to the families Amaranthaceae and Chenopodiaceae, widespread in some technogenic polluted areas (Ramana Iodine Plant, Sumgait Superphosphate Plant) in the Absheron Peninsula of Azerbaijan. The research was conducted during 2017-2019. A number of methods (Arinushkina, Hodges, Bates, Sedmak and Grossberg, Kumar and Knovles, Nakano and Asada,etc.) have been used to study the soil-climatic, floristic, and botanical-geographical composition of territories. It was found that among the species taken, A. tatarica was more resistant to stress than A. Albus and A. album. The study of CAT and APO activity in the leaves of the species, which are typical representatives of antioxidant enzymes, showed that APO activity is low in varieties (A. tatarica) that are resistant under normal natural conditions and high in other varieties (A. albus, A. album) that have lower natural resistance, which indicates that the level of natural resistance of the species plays an important role in the formation of adaptive responses to stress factors in C_4 plants.

Keywords: *A. albus, A. tatarica,* adaptation, catalase, *Ch. album,* pollution, stress

Introduction

In recent years global climate change, wars, scientific and technological progress, natural population growth and other factors have worsened the ecological situation on Earth, reduced arable land and caused serious damage to biodiversity. Therefore, one of the most important tasks facing modern science is to identify all types of stress - resistant plant varieties that grow in unfavorable conditions, and using these varieties as parent forms to create stress-resistant and high-yielding varieties [18, 23, 25].

It is known that the development of scientific and technological progress, on the one hand, leads to an increase in material well-being, on the other - to soil pollution, salinization and destruction of natural flora. It disturbs the natural soil structure, a large number of toxic chemical elements and their compounds absorbed into the soil, which leads to disruption of water-salt metabolism and root mineral nutrition of plants. In addition, the relative amount of water stored by plants in the leaves decreases, and the leaves become heavier as the amount of salt increases. Plant species that retain more water in their leaves during stress are more resistant to drought [31]. The fact that ~ 48-50% of favorable land plots in our Republic are not used for various reasons suggests that these problems are also relevant for our country [24].

The enzymes of the antioxidant system play an important role in the utilization of active oxygen radicals (AOR), such as O^{2-}, H_2O_2, $\dot{O}H^-$, and hydroxonium, formed in the tissues of living organisms, and in connection with the protection of organisms [11]. AOR is involved in processes such as signal transmission, gene expression, protein synthesis and decomposition, and so on, which control redox reactions in plants. High AOR generation in plants leads to disruption of many processes, including physiological and biochemical ones [19]. Many low-molecular compounds, such as ascorbic acid, glutathione, Proline, carotenoids, flavonoids, and so on, and large-molecular compounds, such as catalase (CAT), ascorbate peroxidase (APO), superoxide dismutase, and so on, play an active role in the formation of resistance in higher plants under extreme conditions [2].

The destructive effects of stress occur directly or indirectly through AOR formation [27]. Therefore, there is a growing interest in studying the activity of enzymes of the antioxidant system in plants growing under the influence of extreme factors [10]. From this point of view, the results of studying the activity of antioxidant enzymes, such as CAT and APO, in the organs of higher plants can provide valuable information when studying the biochemical mechanisms of adaptation to stress.

CAT breaks down H_2O_2, which is formed in living organisms during respiration, into water and molecular oxygen. CAT is one of the most active enzymes known to date and plays an important role in tissue respiration.

APO has both peroxidase and oxygenase activity, and also uses aromatic amines, benzidine, guaiacol, ascorbate, aromatic acids, some phenols, and other substances as substrates to catalyze a number of oxidation reactions [17]. The APO isoform in apoplasts has been found to be involved in auxin catabolism [16], wound healing [8], salt resistance [3], protection from pathogens [12] and so on. It also protects unsaturated fatty acids in plant plasma membranes from oxidative stress by changing the permeability of their membranes through peroxidation. CAT and APO activity and gene expression are regulated by abiotic and biotic factors [14].

With 8 out of 11 existing climate zones, Azerbaijan has rich natural resources and vegetation cover. To effectively use the land Fund of the Republic, it is necessary to protect it from all types of pollution. Large industrial facilities created in connection with the development of technological advances pollute adjacent territories with industrial waste, destroying the fauna and flora of these territories. Therefore, the restoration of soils and natural flora in polluted areas is one of the urgent tasks facing science.

Against this background, the main goal of this study was to compare the role of the antioxidant defense system enzymes CAT and APO in adaptive mechanisms in the leaves of the species *A. albus*, *Ch. album* and *A. tatarica* belonging to the families *Amaranthaceae* and *Chenopodiaceae.*

Materials and methods

The object of the study was the leaves of *Amaranthus albus* L. belonging to the *Amaranthaceae* family, as well as *Chenopodium album* L. and *Artiplex tatarica* L. species belonging to the *Chenopodiaceae* family, planted in polluted territories around Baku and Sumgayit (Ramana Iodine Plant (RIP) and Sumgait Superphosphate Plant (SSP)) as well as on soils taken from these territories (Fig.).

Fig 1: Leaves of plants used for research: a) *Amaranthus albus,* b) *Atriplex tatarica,* c) *Chenopodium album*

A number of methods have also been used to study the soil-climatic, floristic, and botanical-geographical composition of territories. The chemical composition of the soil was analyzed by Arinushkina [4], natural soil moisture by Alexandrova and Naydenova [1], pigment content by the Sims and Gamon spectrophotometric method [29], malondialdehyde (MDA) content by Hodges *et al*. [13], proline content by Bates *et al*. [6], and the amount of proteins was determined according to Sedmak and Grossberg [28]. The rate of photosynthesis (P_n) was determined by the LI-COR 6400 XT infrared gas analyzer (LI 6400 XT Postable Photosynthesis System; LI-COR 6400 Biosciences, US), the activity of the CAT enzyme by Kumar and Knovles [22], the activity of the APO enzyme by Nakano and Asada [26], and the amount of H_2O_2 by Velikova *et al* [30]. Statistical processing of the obtained results was carried out according to Dospekhov [9].

Results and discussion

At the beginning of the work, the soil-climatic and botanical-geographical features of the research territories were studied. It was found that the soils on the territories of the RIP and SSP are close to each other in terms of humus content, but differ in chemical composition. As can be seen from table 1, sulfates predominate in the soils around the SSP, and chlorides predominate in the soils around the RIP. Bicarbonates are practically absent in the soils around RIP. As for alkaline and alkaline earth metals, the total number of elements Ca + Mg and Na + K in the soils around the SSP is 25-30% higher than in the soils around the RIP (Table 1).

Table 1: Chemical composition of technogenically polluted soils and dynamics of meteorological factors in the studied territories

Content, (μequiv %)	Chemical composition of soil		Meteorological factors	SSP		RIP	
	SSP	RIP		july	september	july	september
HCO_3	4.1±0.01	0.1±0.01	LI, klx	103-110	80-90	105-110	80-90
Cl	3.5±0.02	10.1±0.35					
SO_4	7.4±0.11	1.8±0.65	RWC (%)	40-50	55-70	50-55	55-70
Ca+Mg	16.7±0.22	12,3±0.19					
Ca	9.7±0.02	9.1±0.21	AT, °C	35-40	30-35	35-40	30-35
Mg	6.8±0.01	3.8±0.08					
Na+K	9.6±3.02	6.9±3.45	SH (%)	40,0	45,0	35-40	40-45
Salt (%)	0.09	1.42					

This table also shows the dynamics of changes in the meteorological indicators of the experimental sites during the experiments. As can be seen from the table, light intensity (LI), relative water content (RWC), air temperature (AT), and soil humidity (SH) vary widely in the study areas. These indicators, presented in the table, provide the necessary information about the current soil and climatic conditions of the territory.

As with other enzymes, the activity of antioxidant enzymes depends on the amount of salt and relative water content accumulated in the leaves, as well as on the amount of harmful and toxic compounds of radioactive ions and heavy metal ions accumulated as a result of anthropogenic pollution. In addition, when analyzing adaptive signs of stress in plants, it is important to study the amount of MDA, the final product of peroxidation of the amino acid proline and lipids.

From this point of view, if we pay attention to Table 2, we will see that the RWC in the leaves of three plant species, which is typical for the studied territories, decreases with increasing vegetation period. Among the species taken, RWC was lowest in *A. tatarica* leaves, average for *A. albus* leaves, and highest in *Ch. album* leaves in the adjacent SSP and RIP territories. The table shows that the amount of proline and MDA in the leaves of all three species decreases significantly over time. If we compare plant species taken with a variable amount of MDA, we see that *A. tatarica* has the highest value in SSP, and *A. albus* in RIP. Two months later, the measurements were similar. As for the amount of the amino acid proline, *A. albus* has always had higher values than other species (Table 2).

Table 2: Dynamics of changes in the water content, the content of amino acid proline and MDA in the leaves during the experiment

Species	Territory	RWC in lieves, %		^{a}MDA·10^5		^{b}Proline	
		july	september	july	september	july	september
A.albus	SSP	84.92±8.37	73.41±8.99	0.31	0.31	46.3	39.4
	RIP	76.84±6.24	70.32±6.79	0.45	0.44	42.7	35.3
A.tataruca	SSP	74.3 ± 7.37	61.7±7.37	0.34	0.32	24.1	15.5
	RIP	70.1 ± 7.37	58.9±6.88	0.28	0.26	19.8	11.2
Ch.album	SSP	88.41±7.79	77.41±5,70	0.23	0.19	38.3	21.6
	RIP	80.36±7.96	71.44±4.36	0.27	0.16	33.9	15.6

Note: ^{a}MDA-mmole/g FW; ^{b}Proline-µmole/g FW.

As can be seen from Table 3, P_n in the leaves of the *A. tatarica* species has a lower value in both polluted areas compared to the *Ch. album* and *A. albus* species. Due to the high content of chlorophyll and carotenoids (Car) in the leaves of albus species, the leaves of these species had a high P_n content. The high content of P_n, chlorophyll and Car in the taken species makes them resistant to various types of stress.

If we compare Tables 3 and 4, we can see that an increase in the activity of the CAT and APO enzymes in the case of an increase in P_n in *A. albus* and *Ch. album* varieties is associated with an increase in energy demand in these species under stress. In this case, the studied enzymes help plants first preserve themselves and then their offspring, using more H_2O_2, which is released when the metabolism accelerates. The data obtained here can be used as a biochemical criterion for restoring the natural flora of anthropogenic polluted territories.

Table 3: Influence of technogenic pollution on the dynamics of changes in the rate of photosynthesis and the amount of pigments in plant leaves

Species	Territory	Rate of photosynthesis, P_n, µmol $CO_2 \cdot m^{-2} . s^{-1}$		The amount of pigments, mmole/ml					
				Xl (a/b)		Car		Car/Xl (a+b)	
		july	september	july	september	july	september	july	september
A.albus	SSP	18.3±3.21	9.9±1.21	2.28	2.13	29.1±3.21	34.1±3.68	2.11	3.01
	RIP	14.9±2.21	7.8±2.35	2.26	2.09	29.6±3.19	33.8±4.21	2.11	2.96
A.tatarica	SSP	5.1±3.43	4.8±1.26	1.95	1.86	25.4±2.37	32.7±3.76	2.31	2.55
	RIP	7.4±2.34	5.6±1.74	1.41	1.25	18.6±2.43	33.0±3.42	2.66	2.79
Ch.album	SSP	18.0±5.21	8.9±2.18	2.18	1.99	28.3±2.12	30.1±3.78	1.39	1.67
	RIP	12.3±4.18	7.8±2.23	1.64	1.52	27.9±2.13	29.9±3.91	2.04	2.0

It is known that the physiological and biochemical mechanisms of plant metabolism are difficult to imagine without enzymes. It can be noted that due to the activity of the CAT and APO enzymes, the main energy exchange enzymes in the leaves of plant species living in extreme conditions, H_2O_2, which is formed in plant metabolism and is the substrate of both enzymes, is toxic to cells. The CAT enzyme, which cleaves H_2O_2, together with the APO enzyme, are antioxidant enzymes that play a key role in protecting plants from oxidative stress. The activity of CAT and APO enzymes in the leaves of the studied *A. albus, A. tatarica* and *Ch. album* species gradually increases with time.

Table 4: Dynamics of changes in water parameters, amino acid proline and MDA in leaves during the experiment days

Species	Territory	H_2O_2, %		[a]CAT activity		[b]APO activity	
		july	september	july	september	july	september
A.albus	SSP	143.5	121.1	22.6±4.21	25.3±4.20	0,30±0,02	0,42±0,02
	RIP	126.2	109.8	20.5±4.94	24.5±4.33	0,29±0,01	0,32±0,01
A.tataruca	SSP	142,0	105.6	17.9±2.26	19.5±3.27	0,19±0,01	0,23±0,01
	RIP	119.4	99.8	17.7±4.19	18.5±4.45	0,15±0,01	0,19±0,01
Ch.album	SSP	154.7	149.8	20.4±3.93	24.5±4.37	0,27±0,01	0,34±0,02
	RIP	148.8	139.7	19.8±3.28	24.5±5.81	0,24±0,01	0,28±0,01

Note: [a]CAT activity-μmol H_2O_2/mg protein·min; [b]APO activity-μmol askorbat/mg protein·min.

In the leaves of these plants, an inverse correlation was observed between an increase in the activity of the CAT and APO enzymes and a change in the amount of H_2O_2 under both experimental conditions. It was found that with increasing enzyme activity in the leaves of these species, the amount of H_2O_2 decreased correspondingly over time. The increase in CAT and APO activity over time in anthropogenic contaminated areas seems to depend on the duration of stress, the level of contamination, and the systematic significance of species. Similar claims have been made in the literature that CAT and APO are involved in protecting plants from stress by neutralizing H_2O_2 in the leaves [5, 20].

It is known that antioxidant enzymes, in coordination with each other under conditions of control and stress in plant organisms, first convert superoxide anion radicals formed in plant tissues into H_2O_2 molecules. In the next step of the process, CAT breaks down H_2O_2 into water and oxygen. The physiological and biochemical function of APO is a continuation of the catalytic action of the CAT enzyme. APO removes metabolic peroxides from the organisms, preventing peroxidation of unsaturated fatty acids contained in membrane lipids [21].

According to the results of research, an alternative idea can be expressed that the low activity of APO in some species (*A. tatarica*) is a sign that the metabolism in the tissues of this variety is normal, and the variety is stable. It can be noted that, despite the "enzymes are activated under stress" pattern, the weak activation of APO under stress is explained by the resistance of the species and, as a result, the low formation of AOR. There is no need to increase the activity of the enzyme. The relevant literature indicates that CAT activity first increases and then decreases as salinity increases, but never falls below the control. There is no weakening of the activity of APO and superoxide dismutase enzymes. The authors explain this by adapting the variety to salinity [15]. Increased activity of the APO enzyme under stress in some species is associated with the synthesis of new Bavei APO isoforms [7].

Conclusions

1. It was found that the relative water content (RWC) in the leaves of plants growing on chloride soils (RIP) is always lower than the RWC in the leaves of plants growing in sulfate soils (SSP). In all three experimental plants, the RWC decreases depending on the duration of vegetation.
2. In contrast to sulphate soils, in chloride soils, the amount of MDA and the amino acid proline decreases over time, which plays an important role in cellular osmotic processes, which in turn indicates the natural adaptation of the taken plants to extreme factors.
3. Among the species taken, *A. tatarica* was more resistant to stress than *A. Albus* and *A. album*. Increasing the rate of photosynthesis (P_n) by increasing the amount of chlorophyll and carotenoid pigments also plays an important role in the observed adaptation processes.
4. The study of CAT and APO activity in the leaves of the obtained species, which are typical representatives of antioxidant enzymes, showed that APO activity is low in varieties (*A. tatarica*) that are resistant under normal natural conditions and high in other varieties (*A. albus*, *A. album*) that have lower natural resistance, which indicates that the level of natural resistance of the species plays an important role in the formation of adaptive responses to stress factors in C_4 plants.
5. The results of studying the physiological and biochemical mechanisms of plant adaptive responses to abiotic stress factors can be used as a marker for understanding the molecular mechanisms of plant adaptation to stress, help in studying problems such as the photosynthesis stability and plant ecology, while obtaining plant genotypes resistant to salt stress.

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