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The influence of anthropogenic environment on tannin content and activity of polyphenol oxidase in leaves of woody plants (with Naberezhnye Chelny as an example)

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Abstract

The dynamics of tannin content and activity of polyphenol oxidase in leaves of tree plant species growing in plantations of different ecological categories were studied. Their participation in adaptive reactions of woody plants to conditions of technogenic environment was shown. It was revealed that the tannin content in the leaves of plants increased during the whole period of active vegetation and reached the maximum value in August. An increase in the activity of polyphenol oxidase in the leaves of tree plants in the city planting has been noted, being the result of the intensive technogenic load. Reactions of different kinds of plants on conditions of growth depend on a degree of technogenic load and on the prevailing meteorological conditions during vegetation of plants. In our opinion, condensed tannins and polyphenol oxidase are the active participants of adaptation processes in woody plants in the context of technogenic stress.

Keywords: woody plants, technogenic environment, polyphenol oxidase activity, tannins.

1. INTRODUCTION

Plant organisms are the most important environment-forming component of the anthropogenic environment, which are affected by various stresses. The antioxidant system of plant protection acts as the most important stability mechanism under the influence of various anthropogenic stresses. Stress factors lead to a change in the functional activity of antioxidant systems. The papers of scientists from different countries note the important role of tannins and various enzymes, including polyphenol oxidase, in the protection of plants from various stresses of anthropogenic nature [1-8].

Many studies indicate the interconnection of the adaptive capabilities of the plant organism and the content of tannins, as well as the activity of polyphenol oxidase [1, 2, 9-17].

The scope of our research was to study the activity of polyphenol oxidase and the content of condensed tannins in the leaves of woody plants growing in the plantations of various ecological categories in a large industrial center of the Middle Volga region - the city of Naberezhnye Chelny.

2. METHODS

The objects of the study were the woody plants, such as resident species –Norway maple (*Acer platanoides* L.), small-leaved linden (*Tilia cordata* Mill.) and drooping birch (*Betula pendula* Roth.); the introduced species –box elder (*Acer negundo* L.) and rough-bark poplar (*Populus balsamifera* L.). The studied species grew in Naberezhnye Chelny as a part of the plantations of various ecological categories: main plantings (Avto-1 and Prospect Mira major highways) and sanitary protection zones (SPZ) of industrial enterprises of OJSC Kamaz: foundry and blacksmith plants being the main polluters of the city. The territories of the Chelninsky district forestry for the resident species and the territory of the “Grenada” city park for introduced species were selected as conditional control zones (CCZ). Trial areas (TA) of at least 0.25 ha had been formed in a regular way (5 TA per each plantation). The estimation of the degree of atmospheric air pollution in the places of growth of woody plants was carried out by us on the basis of materials of the Report on the ecological state of the Republic of Tatarstan for 2013-2016. The complex atmospheric pollution index (AP I= 15.3) characterized the state of atmospheric air pollution in the city as very high. The excess of the maximum permissible concentration for benz(a)pyrene, formaldehyde, phenols, carbon oxides and nitrogen was found. In the SPZ of industrial enterprises, the average annual excess of MPC was noted for the following substances: carbon monoxide - 2 times; oxides of nitrogen - 3 times; sulfur dioxide - 1.2 times; formaldehyde - 5 times; phenol - 1.7 times; and benz(a)pyrene - 1.9 times. In the zone of main plantings, the average annual excess of MPC was noted for the following substances: carbon monoxide - 3.4 times; formaldehyde - 3.8 times; phenol - 1.4 times; benz(a)pyrene - 1.5 times [18].

Within the trial plots, at least 10 species of each type were selected, numbered and evaluated for their physiological and biochemical indices of woody plants. The discount species had a good vital state and a middle-aged generative ontogenetic state (g_2). During the active vegetation period, i.e. in June, July and August, the median formation leaves on the annual vegetative shoot (from the lower third of the section of the crown of the southern exposition) were selected for the discount species. The median formation leaves are typical for the plant leaves, developing in the middle zone of a shoot and performing the photosynthesis function [19]. In the main plantations, part of the crown of the southern exposition was turned directly to the roadway of the avenue. The leaves were selected once and in one day in all types of plantings.

The activity of polyphenol oxidase was determined by a spectrophotometric method based on the measurement of the optical density of reaction products formed during the oxidation of pyrocatechol over a certain time interval [20]. The content of condensed tannins in leaves of woody plants was determined three times during vegetation (June, July, August) using the permanganatometric method (the Leventhal method in the

Kursanov modification). During 2017, the activity of polyphenol oxidase and the content of condensed tannins in plant leaves were determined.

Processing of materials was carried out with the use of the Statistica 5.5 statistical package. The methods of descriptive statistics and dispersion multivariate analysis (in the subsequent estimation of differences by the multiple comparison method - LSD-test) were used for interpretation of the materials obtained.

3. Results And Discussion

Dispersion multifactor analysis of the results revealed the reliability of the influence of species features (the level of importance $P<10^{-5}$), the complex conditions of the place of growth ($P<10^{-5}$), the vegetation period ($P<10^{-5}$), as well as the interaction of these factors ($P=5.3\cdot10^{-5}$) on the activity of polyphenol oxidase in leaves of woody plants (Table 1).

The activity of polyphenol oxidase in the leaves of small-leaved linden growing in the SPZ of industrial enterprises and main plantings (MP) in June, July and August exceeded that in species in the control plantations, except for the indices in the SPZ of industrial enterprises, where no significant differences were found.

Starting from July, in the small-leaved linden in the SPZ plantations, the activity of polyphenol oxidase was 0.56 act.u. higher as compared to that in CCZ, and in August the same indicator was 2.03 act.u. higher (LSD_{05}=0.08 act.u.). In MP in the small-leaved linden during the active vegetation period, polyphenol oxidase activity exceeded the control. Thus, in June, the activity of polyphenol oxidase was 0.80 act.u. higher for plants in the primary plantations, in comparison with the control ones. In July, the activity of the enzyme in all species in urban plantings was higher than that of the control ones. In August, under conditions of intensive technogenic load, the maximum activity of polyphenol oxidase in the leaves of all studied woody plants, except for small-leaved linden was noted. In July, in the small-leaved linden, the maximum activity of the enzyme in leaves was noted.

In the introduced species - rough-bark poplar and box elder - both in CCZ and in urban plantings, there was a similar dynamics of enzyme activity: an increase in activity from June to August. In resident species such as drooping birch, Norway maple and small-leaved linden in the CCZ, a significant increase was noted from June to July, and then a decrease - in August.

During the entire period of active vegetation, from June to August, the remaining resident species such as drooping birch and Norway maple and the introduced species such as rough-bark poplar and box elder, growing on the territory with technogenic load, showed a general tendency in increasing the activity of polyphenol oxidase. Thus, in June, in rough-bark poplar, an increase from 0.11 to 0.85 in the activity of the enzyme in SPZ and in the primary plantations was recorded; in July it was from 0.73 to 1.71; in August - from 1.05 to 2.41 act.u., as compared to the activity of polyphenol oxidase in the conditional control zone.

For the study period, some peculiarities in the dynamics of enzyme activity were noted in the drooping birch. In August, in the species of technogenic environment, the activity of the enzyme was higher by 2.22–2.70 act.u., as compared to the control. In June and August, in the SPZ plantations of the industrial enterprises and in the MP, the activity of the enzyme in drooping birch was higher than that in the CCZ, by 0.45 and 0.79, and by 2.22 and 2.70 act.u., respectively. In July, there were no significant differences between plantation types. Apparently, this was due to the species-specific reaction of the drooping birch to the complex of growth conditions and its active inclusion in the process of adaptation to technogenic stress.

In rough-bark poplar in MP and plantations of SPZ, the activity index of polyphenol oxidase exceeded that on the CCZ: in June - 0.11-0.85 act.u.; in July - 0.73-1.71; and in August - 1.05-2.41 act.u.

In representatives of the maple wood genus, the reaction was similar to that in a small-leaved linden and rough-bark balsamic, but some differences were observed. The enzyme activity in the Norway maple in the plantings of sanitary zones and MP was significantly higher than that in the CCZ in July (by 0.10 and 0.67 act.u.) and in August (by 1.58 and 2.91 act.u., respectively). Similar dynamics of enzyme activity was observed in box elder.

Dynamics of enzyme activity during the growing season under study showed that in all studied resident species in plantations of control zones the activity of polyphenol oxidase changed as follows: in June it was less than in July, and in July it was more than in August, while in the introduced species it increased significantly: in June it was less than in July, and in July it was less than in August.

Table 1 –The activity of polyphenol oxidase and the content of condensed tannins in the leaves of woody plants (Naberezhnye Chelny)

Kind of a woody plant	Functional zone	Activity of polyphenol oxidase, act.u.			Condensed tannins, mg/g dry matter		
		June	July	August	June	July	August
Drooping birch	CCZ	1.47	3.51	2.88	4.03	7.17	9.81
	SPZ	1.92	3.56	5.10	3.34	7.83	9.42
	MP	2.26	3.58	5.58	3.38	6.44	8.00
Small-leaved linden	CCZ	1.54	4.22	2.72	2.79	4.44	7.87
	SPZ	1.56	4.78	4.75	2.18	5.10	7.02
	MP	2.34	5.33	4.74	1.86	4.87	6.59
Norway maple	CCZ	1.28	2.96	2.03	4.50	6.57	8.13
	SPZ	1.36	3.06	3.61	4.66	5.79	7.20
	MP	1.34	3.63	4.94	4.20	5.43	7.25
Box elder	CCZ	0.97	3.35	4.50	3.95	5.24	7.19
	SPZ	1.21	3.78	5.65	3.84	4.55	6.82
	MP	1.41	4.30	6.37	3.68	5.02	6.58
Rough-bark poplar	CCZ	1.97	4.09	4.88	3.55	4.80	6.58
	SPZ	2.08	4.82	5.93	3.50	5.38	6.86
	MP	2.82	5.80	7.29	3.63	5.66	7.06
LSD_{05}		0.08			0.04		

In the SPZ plantations of industrial enterprises and in MP, the dynamics of enzyme activity in the introduced species did not change in comparison with control plantings, and in the resident species (Norway maple and drooping birch) it significantly changed and became similar to that of introducents: in June it was less than in July, and in July - less than in August. The exception was a small-leafed linden, for which in all types of plantations the dynamics of enzyme activity did not differ from the control plantings. The small-leafed linden differed from other resident species studied in later flowering periods associated with the underdevelopment of flower buds on the shoot in the previous vegetation year.

It should be noted that the introduced species were distinguished by the higher indices of enzyme activity in June and July in planting with the intensive technogenic load.

In our view, the increase in polyphenol oxidase activity seems to be a peculiar reaction of the cells to the increasing need for respiration caused by the high content of particulate matter and other pollutants that prevent normal gas exchange in the leaves of plants. Given that the high activity of the enzyme persists in the final stages of the vegetation of plants, it can be assumed that this may be caused by mechanical damage to tissues and cells of leaves.

Dispersion multifactor analysis of the results revealed the reliability of the influence of species features (the level of importance $P<10^{-5}$), the complex conditions of the place of growth ($P = 8.37 \cdot 10^{-5}$), the vegetation period ($P=1.16 \cdot 10^{-3}$), as well as the interaction of these factors ($P<10^{-5}$) on the activity of polyphenol oxidase in leaves of woody plants (see Table).

The results of the study have shown that in all studied kinds of plants and in all types of plantations the content of tannins in leaves increased during vegetation, reaching the highest values in August. The drooping birch and Norway maple grown in the conditional control zone, i.e. the resident species, had the highest values of the tannin index (9.81 and 8.13 mg/g dry mat., respectively).

In the maple genus representatives, similar changes in the content of tannins in the leaves of plants of urban plantations were observed: in June, July and August the content of tannins in plant leaves in plantations of industrial zones and in MP was lower than that in CCZ plantations. At the same time, the greatest decrease in comparison with CCZ was observed in the Norway maple in July (by 0.78 mg/g dry matter) and in August (by 0.93 mg/g of dry matter, with $LSD_{05} = 0.04$) in the industrial zones' primary plantations in July and August (1.14 and 0.88 mg/g of dry matter, respectively, with $LSD_{05} = 0.04$). This can be explained either as a decrease in the synthesis of this metabolite, or its intensive expenditure in the protective reactions of plants, as in these months, according to long-term data, the greatest level of atmospheric air pollution is observed in the city.

In June, the differences in the content of tannins in the leaves of small-leaved linden and drooping birch in urban plantations were similar. In MP and in the plantations of sanitary zones, the plants were characterized by lower content of tannins than in the case of CCZ. In July, the results were different from those of June. In the leaves of plants in SPZ, the content of tannins was significantly higher than that in CCZ. In addition, the content of tannins in the leaves of small-leaved linden was significantly higher in July and in the primary plantings (by 0.43 mg/g of dry matter) than in the plantations of the park and suburban zones.

Specificity of the plant reaction to growth conditions was observed in the rough-bark poplar: in June and July in the plantations of industrial zones, the content of tannins in the leaves of plants was lower as compared to that of CCZ, and then in August, on the contrary, it was higher in comparison with CCZ. In MP, the dynamics of the indicator was different for the entire study period, the content of tannins was higher than that in the CCZ.

Thus, in can be concluded that the reactions of different kinds of plants on conditions of growth depend on a degree of technogenic load and on the prevailing meteorological conditions during vegetation of plants.

4. Conclusion

The results of the studies conducted have shown that in SPZ plantations of industrial enterprises and in MP, in August the activity of polyphenol oxidase in all species of woody plants studied exceeds the indicators of park plantations. Most likely this is due to the accumulation of pollutants in the leaves of plants during vegetation. Activity of this enzyme increases most significantly in more resistant species such as drooping birch, rough-bark poplar and box elder, both in plantings of sanitary zones of industrial enterprises and in MP. This fact undoubtedly testifies to the stability of these two types both to industrial emissions of pollutants and to emissions of motor vehicles.

The tannin content in the leaves of plants increases during the whole period of active vegetation and reaches the maximum value in August. The dynamics of accumulation of tannins in leaves of woody plants has specific features. In our opinion, condensed tannins and polyphenol oxidase are the active participants of adaptation processes in woody plants in the context of technogenic stress.

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