RESEARCH ON THE ACTIVITY OF SOME OXIDATIVE ENZYMES FROM POTATO LEAVES

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Abstract

Oxidative enzymes catalyze the decomposition of reactive oxygen species: hydrogen peroxide, superoxide, hydroxyl and singlet oxygen, which are formed during the normal plant physiological processes**.** The hydrogen peroxide is decomposed by peroxidase and catalase along with superoxide dismutase. This research studies the activity of those enzymes in potato leaves from plants subjected to thermo-hydric stress. The activity of oxidative enzymes studied had a similar dynamic in the potato leaves. The highest activity was determined during budding phase, in which the leaves are most sensitive to the action of thermo-hydric stress. All three enzymes analyzed had increased activity in the leaves of plants grown at a 50% AHI hydric regime compared with those from the variants grown at 80% AHI regime. Under thermo-hydric stress conditions the highest activity of catalase, peroxidase and superoxide dismutase was determined in Robusta variety, which proved to be more sensitive to the action of these factors, and the lowest activity of those enzymes was determined for Sante and Tresor varieties.

Key words: *Solanum tuberosum L.,* thermo hydric stress, catalase, guaiacol peroxidase, superoxid dismutase.

Enzymes are specialized proteins that catalyze biological reactions, regulate the metabolic activity of plants and the biochemical processes in cells (Gheorghe et al, 2001).

Intracellular enzymes are localized in different cellular compartments, where they regulate certain metabolic processes. Histochemical methods and the study of cellular components separated by gradient centrifugation where used to reveal the distribution of intracellular enzymes (Frennema 1976).

Among the existing enzymes in plant tissues the oxidative ones catalyze the decomposition of reactive oxygen species: hydrogen peroxide, superoxide, hydroxyl and singlet oxygen, which are formed during the normal plant physiological processes: photosynthesis, photorespiration, breathing, β-oxidation of fatty acids. Synthesis of reactive oxygen species intensifies under thermo hydric stress.

Oxidative free radicals through their action can break lipids, proteins, pigments and nucleic acids. Their action on lipid membranes, can produce alcohols, aldehydes, alkanes or malondialdehyde, the last substance being considered as a marker for assessment the action of free radicals.

The hydrogen peroxide is decomposed by one of the two Fe metaloenzimes: peroxidase and catalase along with superoxide dismutase. These enzymes are indeed "scavenger" of oxygen free radicals.

MATERIAL AND METHOD

The research was conducted with biological material taken from the Research Station for Plant Development on Sand Dăbuleni, Dolj County.

Following varieties of *Solanum tuberosum L.* were studied: "Astral", "Magic", "Robusta", "Sante" and "Tresor".

Potato plants grown on sandy soil in two active humidity intervals (A.H.I.) 50% and 80% were analyzed.

The plant material previously frozen at -20 ° C was grounded in a mortar in the presence of quartz sand at 4 ° C. 0.1M potassium phosphate buffer, pH 7.4 was added over the powder obtained in a ratio of 1:5 weight: volume and transferred in to Corning tubes. After vortex the tubes were left at 4 ° C for 3 hours. The homogenate was centrifuged at 15,000 rpm at 4 ° C for 10 minutes. The supernatant was used for enzymatic activity determination.

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Catalase activity was spectophotometric determined at 240nm, adapting the method proposed by Beers and Sizer (1952). Reaction mixture contained 960µl 0.1M K phosphate buffer, pH 7.2, 30µl H_2O_2 5% and 10µl protein extract.

Guaiacol peroxidase activity was spectophotometric determined at 436 nm using the method described by Bergmeyer (1974). The reaction mixture contained 660 µl, 0.1M K phosphate buffer, pH 7.2; 166 µl guaiacol solution 18mM; 166 µl H_2O_2 8mM solution and 330 µl protein extract.

All the enzymatic activities were calculated for a period of 1 minute and reported to the protein concentration determined with Bradford (1976) method.

RESULTS AND DISCUSSIONS

Catalase acts on a decomposition of hydrogen peroxide into water and molecular oxygen, a process that takes place in the peroxisome and glycosome. The most intense activity of catalase was determined during budding (*table 1*).

The average activity of the catalase from the leaves of all the variants analyzed in this period was 0.0184 U/mg protein. During the period of blossoming the activity decreased to 0.0053 U/mg protein and during tuberization reached 0.0031 U/mg protein. Among the three growing seasons analyzed the flowering period was the most sensitive to thermo hydric stress favoring increased catalase activity due to the accumulation of free radicals. The maturation process of the leaves decreased the activity of this enzyme, probably due to their adaptation to thermo hydric stress.

Under water stress, when potato plants were maintained in at 50% AHI catalase activity intensified. Thus, the mean activity of this enzyme, determined in leaves of five varieties of potato was 0.0499 U/mg protein, compared to the amount of 0.0079 U/mg protein, as in leaves from plants cultivated in a system of 80%AHI. Water stress caused stimulation of catalase activity.

Analysis of catalase activity in leaves of five potato varieties revealed that the highest values were determined for Astral and Robusta varieties that were more sensitive to water stress, correlated with the accumulation of large quantities of hydrogen peroxide. The lowest catalase activity correlated with the accumulation of a lower content of hydrogen peroxide was found in varieties Magic and Tresor.

Table 1

Catalase activity in leaves of potato (U/mg protein)

Variety	Irrigation degree	Budding	Blooming	Tuberization	Average
Astral	50 % AHI	0.0328	0.0052	0.0027	0.0135
	80 % AHI	0.0121	0.0049	0.0042	0.0071
	Average	0.0224	0.0050	0.0034	0.0103
Magic	50 % AHI	0.0103	0.0075	0.0020	0.0066
	80 % AHI	0.0126	0.0028	0.0029	0.0061
	Average	0.0114	0.0051	0.0024	0.0063
Robusta	50 % AHI	0.0472	0.0034	0.0017	0.0174
	80 % AHI	0.0165	0.0057	0.0023	0.0082
	Average	0.0318	0.0045	0.0020	0.0128
Sante	50 % AHI	0.0113	0.0033	0.0017	0.0054
	80 % AHI	0.0236	0.0080	0.0048	0.0121
	Average	0.0174	0.0056	0.0032	0.0087
Tresor	50 % AHI	0.0121	0.0052	0.0036	0.0070
	80 % AHI	0.0058	0.0074	0.0053	0.0062
	Average	0.0089	0.0063	0.0044	0.0066
Average		0.0184	0.0053	0.0031	0.0089

Guaiacol peroxidase activity in potato leaves. Peroxidase is structurally and functionally similar to catalase, but it catalyzes the reaction between oxygen peroxide and oxygen peroxide acceptor or hydrogen donor substrate: ascorbic acid, glutathione, or phenols, which are oxidized.

Results show that just like in the case of catalase activity, guaiacol peroxidase activity was more intense during the budding (*table 2*). Thus the average activity of peroxidase, in all the variants analyzed in this period was 0.6639 U / mg protein. During the blooming period fell to 0.2085 U / mg protein and reached 0.1393 U / mg protein during tuberization. Resulting that just as with catalase activity the budding period was the most sensitive to thermo hydric stress, favoring increased peroxidase activity correlated with the accumulation of free radicals.

In water stress conditions (50% AHI) average activity of peroxidase in the leaves from the five potato varieties was 0.3775 U / mg protein, more than 1.27 times as compared with the average value determined in variants grown on a 80% AHI scheme (0.2962 IU / mg protein).

Table 2

Guaiacol peroxidase activity in potato leaves (U/mg protein)

Variety	Irrigation degree	Budding	Blooming	Tuberization	Average
Astral	50 % AHI	0.8670	0.1787	0.1063	0.3840
	80 % AHI	0.4939	0.1410	0.1426	0.2588
	Average	0.6804	0.1598	0.1245	0.3214
Magic	50 % AHI	0.6635	0.2536	0.1732	0.3634
	80 % AHI	0.5476	0.2537	0.2066	0.3359
	Average	0.6056	0.2536	0.1899	0.3496
Robusta	50 % AHI	1.9067	0.2051	0.1026	0.7381
	80 % AHI	0.7137	0.5864	0.1081	0.4694
	Average	1.3102	0.3957	0.1053	0.6037
Sante	50 % AHI	0.3209	0.1889	0.2265	0.2415
	80 % AHI	0.6865	0.1176	0.0993	0.3011
	Average	0.5037	0.1532	0.1629	0.2713
Tresor	50 % AHI	0.2467	0.0592	0.1750	0.1603
	80 % AHI	0.1924	0.1016	0.0530	0.1157
	Average	0.2195	0.0804	0.1140	0.1380
Average		0.6639	0.2085	0.1393	0.3368

Although peroxidase activity (mean value of 0.3368 U / mg protein) was higher than that of catalase (0.0089 U / mg protein), during the periods analyzed it fell by 4.76 times compared with that of catalase activity which decreased by 5.96 times.

Analysis of guaiacol peroxidase activity in leaves of five potato varieties revealed that the highest values were determined as in catalase activity in Robusta variety (0.7381 U / mg protein), which was more sensitive to water stress, and lowest guaiacol peroxidase activity was determined in variety Tresor.

The activity of superoxide dismutase

Superoxide dismutase (SOD) catalyzes the conversion of superoxide radical (O_2^-) to hydrogen peroxide and water, which serve to reduce the oxidation processes in the tissues.

The analytical data presented (*table 3*) reveals the trend of reducing the enzyme activity during the growing season. Thus, the average activity of superoxide dismutase was maximum during the budding: 22.9850 U / mg protein. The activity of this enzyme decreased 8.29 times during the blooming period reaching a value of 2.6626 U / mg protein.

This enzyme presents the most intense activity in early phenophases, especially in the budding period which seems to be the most sensitive to thermo hydric stress.

Water stress in the 50% AHI regime determined increased activity of the superoxide dismutase enzyme in the potato leaves which achieved an average of 9.6713 U / mg protein.

In the case of leaves from plants grown in a regime of 80% of the AHI, the activity of superoxide dismutase was lower by a factor of 1.21, reaching 7,9818 U / mg protein.

Under thermo hydric stress, the highest activity for superoxide dismutase was determined in Robusta variety (18.9422 U / mg protein), which proved to be more sensitive to the action of these factors, and the lowest activity of this enzyme was determined in Sante and Tresor varieties.

Table 3

The activity of superoxide dismutase from potato leaves (U/mg protein)

Variety	Irrigation degree	Budding	Blooming	Tuberization	Average
Astral	50 % AHI	27.0522	3.1141	1.3494	10.5052
	80 % AHI	23.7448	2.0537	1.9522	9.2502
	Average	25.3985	2.5839	1.6508	9.8777
Magic	50 % AHI	16.7586	2.7976	0.6105	6.7222
	80 % AHI	22.0564	2.3908	3.2300	9.2307

	Average	19.4075	2.5942	1.9202	7.9764
Robusta	50 % AHI	52.0808	3.5387	1.2070	18.9422
	80 % AHI	19.3337	7.6514	4.3050	10.4300
	Average	35.7072	5.5950	2.7560	14.6861
Sante	50 % AHI	15.8246	1.1254	0.0624	5.6708
	80 % AHI	22.2734	0.6279	0.8250	7.9088
	Average	19.0490	0.8766	0.4437	6.7898
Tresor	50 % AHI	14.5940	2.1562	2.7987	6.5163
	80 % AHI	7.1320	1.1699	0.9660	3.0893
	Average	10.8630	1.6631	1.8823	4.8028
Average		22.0850	2.6626	1.7306	8.8265

CONCLUSIONS

The activity of oxidative enzymes studied: catalase, peroxidase and superoxide dismutase had a similar dynamic in the potato leaves.

The highest activity for these three oxidative enzymes was determined in budding phase, in which the leaves are most sensitive to the action of thermo-hydric stress.

All three enzymes analyzed had increased activity in the leaves of plants grown at a 50% AHI hydric regime compared with those from the variants grown at 80% AHI regime.

Under thermo-hydric stress conditions the highest activity of catalase, peroxidase and superoxide dismutase was determined in Robusta variety, which proved to be more sensitive to the action of these factors, and the lowest activity of those enzymes was determined for Sante and Tresor varieties.

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