Environment Protection Engineering

Vol. 37 2011 No. 1

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PHYSIOLOGICAL AND BIOCHEMICAL ACTIVITY OF SPRING WHEAT (*TRITICUM VULGARE*) UNDER THE CONDITIONS OF STRESS CAUSED BY CADMIUM

The effect of cadmium on physiological and biochemical activity of spring wheat cv. Alba was studied within the concentrations of 0.025–5 mM. The content of chlorophyll a and b, carotenoids, the intensity of assimilation of CO_2 and transpiration as well as the activity of catalase, peroxydase, superoxide dismutase in the phase of two cotyledones and in the phase of shooting in wheat were determined. The applied doses of cadmium significantly decreased the content of determined assimilation dyes, reduced the intensity of assimilation of CO_2 and transpiration and caused an increase in the index of use of water in the photosynthesis in all the studied growth phases of spring wheat. A clear stimulating influence of the applied doses of cadmium on the activity of catalase, peroksydase and superoxide dismutase in spring wheat was noticed.

1. INTRODUCTION

As compared to other metals [1], cadmium is characterised by the largest concentration both in the soil and in the plant. Due to its high mobility, bioavailability and characteristic physicochemical properties it was regarded the metal of particularly high toxicity [2, 3]. Its presence causes blockades of key metabolic reactions by destabilization of functional groups and by structural changes of the enzymes engaged in the reactions [4–7]. One of the most dangerous effects of the activity of cadmium, due to its non-specific and reactive character, is generating reactive form of oxygen, including free radicals leading to considerable damages in the lipid composition of membranes [3, 8–10]. Typical reactions of neutralization of the excessive reactive forms of oxygen can be the activation of antioxidative enzymes, i.e. catalase, peroxidase and superoxide dismutase [11–13].

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The aim of the study was to determine the activity of oxidative stress enzymes (catalase, peroxidase and superoxide dismutase), the content of assimilation dyes and the intensity of CO_2 assimilation and transpiration in various growth stages of wheat growing in soil with the addition of cadmium and fertilized with NPK (nitrogen, phosphorous, and potash).

2. MATERIALS AND METHODS

Spring wheat cv. Alba was taken for the investigation. Two series of experiments were carried out in three replications in controlled conditions at 23 °C, light intensity 100 $\mu E\cdot m^{-2}\cdot s^{-1}$, photoperiodism 12/12 h. The soil used in the experiment belonged to the good wheat complex 2 soil of light silty loam composition and the 1.2% content of organic carbon. Humidity of the soil was kept at the level of 60% of maximum water capacity. Cadmium was introduced to the soil in the form of water solutions of cadmium nitrate ($Cd(NO_3)_2$), in the following concentrations: 0.025, 0.05, 0.5 and 5 mM, whereas fertilizers in the form of water solutions, according to the recommendations for the good wheat complex 2 soil – 115 kg $N\cdot ha^{-1}$, 50 kg $P_2O_5\cdot ha^{-1}$, 30 kg $K_2O\cdot ha^{-1}$. The following combinations were used in the experiment: 1 – the control, 2 – NPK, and samples containing NPK with cadmium in the following doses: 3 – NPK + 0.025 mM; 4 – NPK + 0.05 mM; NPK + 0.5 mM; 6 – NPK + 5 mM, 7 – 0.025 mM; 8 – 0.05 mM; 9 – 0.5 mM; 10 – 5 mM. The differentiated doses of cadmium and fertilizers were added to the soil according to the experimental combination. Germinated grains of wheat were sown to the soil prepared in this way. The determination of the studied parameters (the content of chlorophyll a and b, carotenoids, the intensity of CO_2 assimilation and transpiration, and the activity of catalase, peroxidase and superoxide dismutase) were carried out in two growth phases of the plant: two cotyledons (the 7th day of the studies) and shooting (14th, 21st, 28th day of the studies). The content of assimilation pigments in leaves was determined by means of the Lichtenthaler and Welburn method [14]. The intensity of photosynthesis and transpiration was measured (repeating the measurement four times) using the TPS-2 gas analyzer manufactured by PP Systems (UK), at constant lighting 2053 $\mu mol\cdot m^{-2}\cdot s^{-1}$.Based on the obtained results of the intensity of assimilation and transpiration photosynthetic effectiveness of the use of water (ω_F) was calculated. The activity of peroxidase was defined by the Chance and Machly method [15], the activity of catalase by Lück''s method [16] and that of superoxide dismutase, using the method of Abassi et al. [17].

The results were worked out by means of the method of two-factor variance using the Duncan test at a significance level $NIR_{0.05}$. Using the Pearson's linear correlation coefficient (r), correlation between the concentration of cadmium in leaves and the examined physiological features of wheat was shown.

3. RESULTS AND DISCUSSION

Spring wheat cv. alba was characterised by a differentiated content of photosynthetic pigments, the activity of catalase, peroxidase and superoxide dismutase and the intensity of CO_2 assimilation and transpiration depending on the level of soil contamination with cadmium (Tables 1–3, Fig. 1).

A significant influence of cadmium was observed on the content of photosynthetic pigments and the intensity of CO_2 assimilation and transpiration of spring wheat. A negative effect of interaction time of cadmium on the defined physiological parameters was also noticed. Mineral fertilization increased the amount of chlorophyll a and b and of carotenoids, and it slightly changed the intensity of CO_2 assimilation and transpiration in the examined plant. Under fertilization, the average content of chlorophyll a increased by 6%, chlorophyll b by 14%, and carotenoids by 13% as compared to the control plants (Table 1).

The applied doses of cadmium significantly decreased the content of the determined dyes in wheat (Table 1). The average content of chlorophyll a and b in the control plants amounted to about 2.37 $mg \cdot g^{-1}$ of fresh matter. After adding a 5 mM dose of cadmium, a decrease in the content of chlorophyll a by 34%, chlorophyll b by 37% and carotenoids by 29% with respect to the control plant was observed in wheat (Table 1). The amount of chlorophyll a, b and carotenoids in the examined plant, when the highest dose and mineral fertilization were applied, constituted respectively 73%, 68% and 78% with respect to the control plant (Table 1). Chen and Kreeb [18] recorded over a twofold decrease in chlorophyll in maize under the influence of heavy metals as compared to the control. Hou et al. obtained a decreased amount of chlorophyll a by 46%, chlorophyll b by 32% and carotenoids by 48% in *Lemna minor* after the application of a 20 $mg \cdot dm^{-3}$ dose [5]. Erdei et al. [12] recorded a 60% decrease in the content of chlorophyll in barley after the application of cadmium. In the carried out studies, a stimulating effect of the lowest dose of cadmium – 0.025 mM, on the content of the determined photosynthetic dyes in spring wheat, was also observed. A 5% increase was noticed in the content of chlorophyll b, and a 10% increase in the content of carotenoids in wheat at the shooting stage after the application of 0.025 mM of cadmium with respect to their concentration in the studied plant in the phase of two cotyledones (Table 1). An increased amount of chlorophyll b in stonecrop (Sedum) after the application of Cd in the dose of 10^{-4} M was obtained by Zhou and Qiu [19]. The studies carried out by Nikolić et al. [4] showed, in poplar growing in a 10^{-4} M Cd medium, an increase in the content of chlorophyll b by 49%, and carotenoids by 10% in relation to the control plant. Based on the value of the correlation coefficient, a significant negative correlation relationship was observed between the content of photosynthetic pigments (chlorophyll a and b and carotenoids) and the concentration of cadmium in the leaves of spring wheat (Fig. 1a, b).

Table 1

Toxic impact of cadmium on the content of photosynthetic pigments in leaves of spring wheat (*Triticum vulgare*)

Day	Dose of Cd [mM]									
	Control	NPK	NPK +0.025	NPK + 0.05	NPK + 0.5	NPK + 5	0.025	0.05	0.5	5
Chlorophyll a [mg·g^{-1} f.m.] (% of control)										
7	1.59 ±0.57	1.48 ±0.33	1.39 ±0.12	1.40 ±0.08	1.54 ±0.18	1.24 ±0.01	1.31 ±0.11	1.47 ±0.04	1.37 ±0.03	1.12 ±0.03
14	1.60 ±0.11	1.77 ±0.18	1.46 ±0.01	1.27 ±0.07	1.53 ±0.15	1.15 ±0.14	1.40 ±0.10	1.39 ±0.10	1.32 ±0.03	1.10 ±0.02
21	1.77 ±0.06	1.87 ±0.01	1.30 ±0.06	1.52 ±0.03	1.43 ±0.08	1.32 ±0.06	1.21 ±0.01	1.10 ±0.02	1.29 ±0.02	1.08 ±0.08
28	1.67 ±0.06	1.91 ±0.01	1.67 ±0.22	1.20 ±0.23	1.12 ±0.01	1.11 ±0.12	1.36 ±0.01	1.33 ±0.21	1.21 ±0.25	1.05 ±0.02
Average	1.66 ±0.20 (100)	1.76 ±0.13 (106)	1.46 ±0.10 (88)	1.38 ±0.10 (83)	1.40 ±0.11 (84)	1.21 ±0.08 (73)	1.32 ±0.06 (80)	1.32 ±0.09 (80)	1.26 ±0.08 (76)	1.09 ±0.04 (66)
$LSD_{0.05}$ day – 0.112; dose – 0.232;										
Chlorophyll b [mg·g^{-1} f.m.] (% of control)										
7	0.62 ±0.15	0.74 ±0.13	0.49 ±0.01	0.64 ±0.05	0.55 ±0.09	0.52 ±0.02	0.55 ±0.02	0.56 ±0.04	0.57 ±0.05	0.46 ±0.01
14	0.63 ±0.11	0.65 ±0.19	0.60 ±0.03	0.56 ±0.01	0.57 ±0.11	0.48 ±0.04	0.57 ±0.05	0.56 ±0.11	0.54 ±0.04	0.45 ±0.02
21	0.78 ±0.14	0.95 ±0.03	0.65 ±0.02	0.57 ±0.17	0.54 ±0.02	0.49 ±0.07	0.58 ±0.04	0.53 ±0.03	0.54 ±0.05	0.46 ±0.02
28	0.80 ±0.03	0.89 ±0.09	0.75 ±0.16	0.53 ±0.06	0.46 ±0.01	0.45 ±0.03	0.58 ±0.01	0.49 ±0.07	0.47 ±0.02	0.44 ±0.01
Average	0.71 ±0.11 (100)	0.81 ±0.11 (114)	0.62 ±0.06 (87)	0.59 ±0.07 (83)	0.53 ±0.06 (75)	0.48 ±0.04 (68)	0.57 ±0.03 (80)	0.54 ±0.06 (76)	0.53 ±0.04 (75)	0.45 ±0.02 (63)
$LSD_{0.05}$ day – 0.098, dose – 0.118										
Carotenoids [mg·g^{-1} f.m.] (% of control)										
7	0.59 ±0.17	0.61 ±0.07	0.54 ±0.05	0.51 ±0.06	0.58 ±0.06	0.46 ±0.01	0.49 ±0.04	0.57 ±0.01	0.54 ±0.03	0.44 ±0.03
14	0.60 ±0.04	0.66 ±0.07	0.57 ±0.01	0.50 ±0.01	0.56 ±0.05	0.48 ±0.03	0.56 ±0.04	0.55 ±0.02	0.58 ±0.02	0.46 ±0.01
21	0.72 ±0.01	0.74 ±0.01	0.55 ±0.01	0.61 ±0.01	0.59 ±0.03	0.54 ±0.02	0.50 ±0.01	0.43 ±0.02	0.54 ±0.02	0.46 ±0.01
28	0.62 ±0.02	0.80 ±0.07	0.74 ±0.11	0.52 ±0.10	0.50 ±0.01	0.46 ±0.04	0.57 ±0.01	0.54 ±0.08	0.50 ±0.06	0.42 ±0.04
Average	0.63 ±0.06 (100)	0.71 ±0.06 (113)	0.60 ±0.04 (95)	0.55 ±0.05 (87)	0.56 ±0.03 (89)	0.49 ±0.03 (78)	0.53 ±0.03 (84)	0.52 ±0.03 (82)	0.52 ±0.03 (82)	0.45 ±0.02 (71)
$LSD_{0.05}$ day – 0.073, dose – 0.103;										

Table 2

Intensities of CO_2 assimilation, transpiration and the photosyntetic efficiency of water use in wheat growing in soil with various doses of cadmium

Day	Combination in experiment; dose of Cd [mM]									
	Control	NPK	NPK +0.025	NPK + 0.05	NPK + 0.5	NPK + 5	0.025	0.05	0.5	5
CO_2 assimilation (μmol $\cdot m^{-2} \cdot 7s^{-1}$) (% of control)										
7	4.53 ±0.51	4.49 ±0.39	2.29 ±0.20	1.83 ±0.15	2.01 ±0.23	1.43 ±0.17	1.97 ±0.09	1.52 ±0.40	1.44 ±0.21	1.17 ±0.13
14	4.03 ±0.22	3.68 ±0.79	2.48 ±0.39	2.27 ±0.35	1.15 ±0.19	1.12 ±0.04	2.16 ±0.31	1.56 ±0.27	1.02 ±0.09	0.92 ±0.13
21	3.25 ±0.53	3.32 ±0.21	1.56 ±0.19	1.74 ±0.36	1.11 ±0.08	1.03 ±0.28	1.62 ±0.08	1.21 ±0.25	1.05 ±0.14	0.94 ±0.09
28	3.11 ±0.41	2.88 ±0.38	1.79 ±0.06	1.15 ±0.10	1.03 ±0.08	0.59 ±0.07	1.27 ±0.17	1.19 ±0.11	0.56 ±0.14	0.43 ±0.10
Average	3.73 (100)	3.59 (96)	2.04 (55)	1.75 (47)	1.33 (36)	1.04 (28)	1.76 (47)	1.37 (37)	1.02 (27)	0.87 (23)
$LSD_{0.05}$ day – 0.589, dose – 0.778										
Transpiration [mmol$\cdot m^{-2} \cdot s^{-1}$) (% of control)										
7	1.21 ±0.09	1.06 ±0.03	0.66 ±0.06	0.57 ±0.03	0.55 ±0.10	0.37 ±0.15	0.46 ±0.10	0.37 ±0.07	0.32 ±0.04	0.28 ±0.06
14	1.17 ±0.09	0.87 ±0.07	0.45 ±0.06	0.50 ±0.06	0.32 ±0.03	0.23 ±0.06	0.51 ±0.06	0.29 ±0.04	0.18 ±0.02	0.16 ±0.04
21	0.95 ±0.07	0.89 ±0.12	0.47 ±0.10	0.43 ±0.07	0.25 ±0.05	0.18 ±0.03	0.39 ±0.03	0.30 ±0.03	0.11 ±0.02	0.12 ±0.04
28	0.71 ±0.09	0.76 ±0.04	0.35 ±0.04	0.19 ±0.07	0.31 ±0.06	0.07 ±0.03	0.23 ±0.07	0.21 ±0.04	0.13 ±0.05	0.11 ±0.03
Average	1.01 (100)	0.90 (89)	0.48 (47)	0.42 (41)	0.36 (35)	0.21 (21)	0.40 (39)	0.29 (29)	0.18 (18)	0.17 (17)
$LSD_{0.05}$ day – 0.134, dose – 0.241										
Photosyntetic efficiencyof water use [μmol$\cdot$mmol^{-1}]										
7	3.74	4.23	3.47	3.21	3.65	3.86	4.28	4.11	4.50	4.18
14	3.44	4.23	5.51	4.54	3.59	4.87	4.23	5.37	5.66	5.75
21	3.42	3.73	3.32	4.05	4.44	5.72	4.15	4.03	9.54	7.83
28	4.38	3.78	5.11	6.05	3.32	8.42	5.52	5.67	4.31	3.91

Malinowska [20] and Łukasik et al. [21] also noticed a negative significant correlation between the content of cadmium and chlorophyll a and b in leaves. By cumulating in chloroplasts, cadmium destroys the structure of these organelles and thus inhibits the synthesis of photosynthetic pigments [3, 7, 8]. It can also cause damage to the membranes of chloroplasts and thylakoids by increased production of free radicals [22].

Table 3

Activities of antioxidant enzymes in wheat plants depending on the Cd^{2+} doses added to soil

Stages of wheat development	Term of analysis (day)	Combination in experiment; dose of Cd [mM]										$LSD_{0.05}$
		Control	NPK	NPK +0.025	NPK + 0.05	NPK + 0.5	NPK + 5	0.025	0.05	0.5	5	
Catalase activity (CAT) [μM $H_2O_2 \cdot g^{-1}$ f.m.$\cdot min^{-1}$]												
Two leaf stage	7	0.337	0.341	0.341	0.371	0.420	0.786	0.346	0.376	0.439	0.796	0.004
Shooting	14	0.464	0.459	0.468	0.498	0.561	1.201	0.488	0.468	0.551	1.274	0.014
	21	0.332	0.322	0.346	0.424	0.561	1.006	0.351	0.424	0.561	1.089	0.007
	28	0.283	0.302	0.327	0.337	0.434	0.854	0.327	0.341	0.429	0.849	0.003
Peroxidase activity (POX) [μM purpurogaline$\cdot g^{-1}$ f.m.$\cdot min^{-1}$]												
Two leaf stage	7	0.443	0.502	0.513	0.858	0.963	1.080	0.528	0.875	1.050	1.163	0.002
Shooting	14	0.237	0.267	0.299	0.492	0.547	0.585	0.278	0.501	0.580	0.614	0.002
	21	0.594	0.605	0.708	1.114	1.284	1.478	0.768	1.177	1.269	1.535	0.002
	28	0.682	0.697	0.803	1.237	1.576	1.606	0.859	1.254	1.587	1.634	0.004
Superoxide dismutase activity (SOD) [$U \cdot g^{-1}$ f.m.$\cdot min^{-1}$]												
Two leaf stage	7	106.5	104.6	112.4	135.6	166.6	179.2	116.2	131.7	166.6	183.5	4.472
Shooting	14	54.2	58.1	67.8	62.0	77.5	89.4	56.2	83.3	102.7	104.2	5.750
	21	112.4	118.2	122.1	141.4	166.6	172.5	125.3	135.6	176.3	188.4	6.846
	28	34.8	40.6	48.4	67.8	77.5	78.7	36.8	63.9	58.1	66.2	6.404

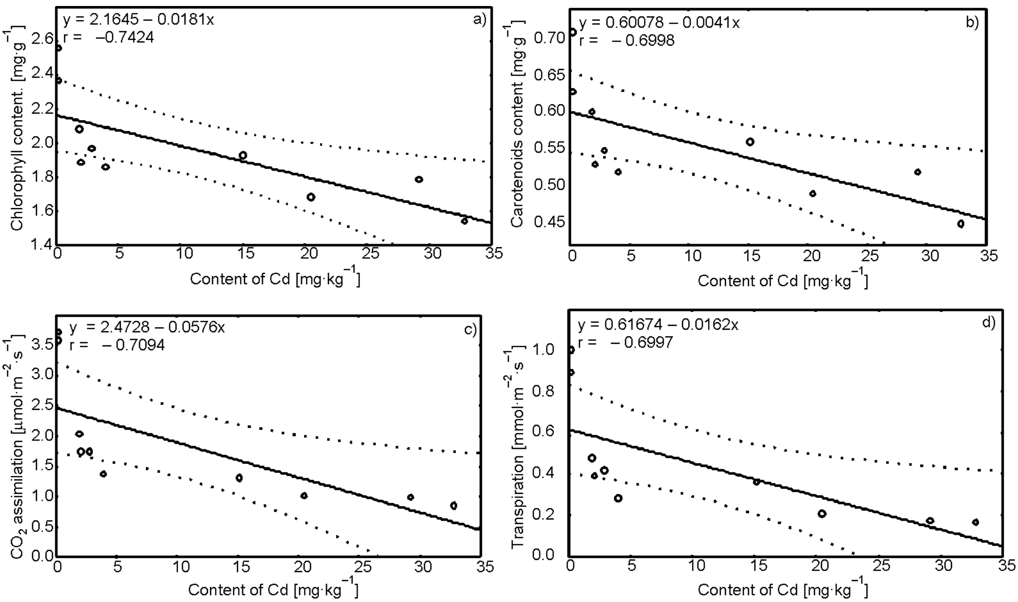

Fig. 1. Dependences of the contents of photosynthetic pigments ($mg \cdot g^{-1}$ of fresh matter, a, b), CO_2 assimilation (c) and transpiration (d) on the content of cadmium in leaves of spring wheat

A significant decrease was observed in the intensity of the examined physiological processes both at the stage of two cotyledones and in the phase of shooting in spring wheat growing in a soil with various doses of cadmium added. The highest decrease in the intensity of CO_2 assimilation and transpiration was recorded after a maximum dose of cadmium (5 mM) had been applied. The intensity of the photosynthesis process in such cultivation conditions decreased by 77%, whereas that of transpiration by 83% in relation to the control plant (Table 2). The decrease in the intensity of photosynthesis and transpiration can be related to the disturbances in functioning of the photosynthectic apparatus of the plants, the decrease in turgor, the inhibition of the transport of electrons in the process of photosynthesis and the decrease in the activity of carboxylase RuBP [4, 5, 7].

Photosynthetic effectiveness of the use of water is often a decisive index of productivity of plants under stress conditions [23]. The calculated index differed depending on the applied doses and of the growth stage of the plant. The increase in the photosynthetic effectiveness was observed in all stages of the experiment. Its highest 2.5-fold increase with respect to the control plants was obtained after the application of the dose of 0.5 and 5 mM at the shooting stage (21st day of studies) (Table 2). This results, first of all, from the low intensity of transpiration. Similar reactions of plants to the influence of heavy metals were recorded by Malinowska and Smolik [24]. As results from the value of the correlation coefficient, close relationships between the defined physiological processes and the concentration of cadmium in leaves of spring wheat were observed (Fig. 1c, d). The values of the correlation coefficients r for CO_2 assimilation and transpiration amounted to –0.7094 and –0.6697. Malinowska [20] reported a negative significant correlation between the intensity of photosynthesis and the content of cadmium in the leaves of plants.

The activity of catalase in wheat growing in the control soil in the phase of two leaves amounted to 0.337 mg $H_2O_2 \cdot g^{-1}$ of fresh matter$\cdot min^{-1}$. The enzyme reached the highest activity in all the combinations at the stage of shooting on the 14th day of the experiment (Table. 3).

The applied doses of cadmium (0.025–5 mM) caused a significant increase in the activity of catalase in all the examined growth phases of wheat. In the phase of shooting (21st day of measurement) the applied dose 5 mM + NPK caused an increase in the activity of catalase by 202%, whereas the highest dose of cadmium without fertilizers resulted in an increase by 228% with respect to the control plant (Table 3).

In both phases of wheat growth an increase in the activity of peroxydase was observed with an increase in the dose of cadmium in soil. A significant growth in the activity of this enzyme at an approximate level was observed during the whole experiment. The most distinct stimulation was recorded in the phase of two leaves, i.e. by 19%, 97%, 137% and 162% respectively for the doses of cadmium exceeding permissible amount of metal in the soil.

A significant stimulating effect of all the applied concentrations of NPK and cadmium salt on the activity of superoxide dismutase in the examined growth phases of wheat was also noticed. The enzyme reached the lowest activity in the phase of shooting during the last measurement (Table 3). The observed increase in the activity of the enzymes can be caused by an increase in the production of reactive forms of oxygen in plants. The activation of catalase in small radish under the influence of $CdCl_2$ applied in the concentrations from 0.25 to 1 mM is reported by Vitória et al. [25]. Hegedüs et al. [26] showed that the addition of of cadmium in concentrations ranging from 0.1 mM to 0.3 mM had a stimulating effect on the activity of peroxydase in wheat. A significant increase in the activity of peroxydase was observed in the leaves of the examined plants; whereas no significant changes in its activity were noticed in the roots with respect the control plants. Saffar et al. [27] found that cadmium at various concentrations o (50, 85 i 100 µM of $CdCl_2$) decreased the activity of catalase in seedlings of *Arabidopsis thaliana*, while an increase in the activity of peroxydase was observed. The experiment carried out by Ammar et al. [28] showed an increase in the activity of superoxide dismutase in tomato plants upon increasing concentration of cadmium salt (1–50 µM Cd^{2+}).

4. CONCLUSIONS

The applied doses of cadmium (0.05–5 mM) significantly reduced the content of defined photosynthetic pigments in all the studied phases of growth of spring wheat.

Upon increasing the doses of cadmium, the intensity of assimilation of CO_2 and transpiration in wheat significantly decreased while the index of use of water in photosynthesis increased.

The content of chlorophyll a and b and carotenoids significantly decreased upon increasing concentration of cadmium in the leaves of spring wheat.

Cadmium had a clear stimulating effect on the activity of catalase, peroxydase and superoxide dismutase in the studied growth phases of spring wheat.

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