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Physiological and biochemical effects of 2.4-D herbicide in wheat (*Triticum aestivum* L.) varieties

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ABSTRACT

In this work were investigated the physiological and biochemical responses depended on toxic effect caused by different concentrations of herbicides called as 2.4-Dichlorophenoxyacetic acid (2.4-D) herbicide in the seedlings that belong to three wheat varieties. The seeds which belong to wheat (*Triticum aestivum* L. Bayraktar cv., İkizce cv. and Tosunbey cv.) were used as plant material. 15-day old seedlings for each wheat variety were divided into 4 groups consisting of the same number of seedlings and four variety doses of herbicide (0, 100 µM, 300 µM ve 1000 µM) were applied for them. In these applications for seedlings were preferred hydroponic surrounding to root. Although three varieties were increased the growth of root and shoot elongation from the growth parameters of the seedlings, it leaded to a decrease in growth parameters in general in this herbicide. Although 2.4-D in the leaves were caused an increase in 100 µM dose for only the Bayraktar in amount of chlorophyll a+b. In ones except these, there was always been a decreased. The amount of carotenoid was resulted in the reduction of three varieties. Amount of MDA were increased in all three varieties. Glutathione (GSH) / oxidized glutathione (GSSG) ratios in leaves were increased in three varieties. The superoxide dismutase (SOD) activity in the leaves were increased at the Bayraktar and decreased in the İkizce and the Tosunbey. Catalase (CAT) activity showed to a decrease in three varieties. As a result, it was determined that the 2.4-D, which was toxic for wheat plants even in very low concentrations.

1. Introduction

It is known that herbicides can be absorbed by the cultivated plant seeds or weed seeds from soil during germination. However, herbicides move very slowly and hold onto clay and the organic substances in soil and this prevents them from being carried. If herbicides are applied after soil is made fit, their intake by plants increases. Also, a certain amount of herbicide is added in solutions to control the effects of herbicides and prevent them from harming the environment (Güngör 2005). As a result, in damaged plant cells, 2,4-D can bind to protein complexes during photosynthesis, stopping photosynthesis and causing a toxic effect that prevents the maturation of harmful plants (Mander and Liu 2010). Most of the herbicides evaporate from soil and disappear. These are the herbicides included in Thiocarbamate and Phenoxy groups. The herbicides such as 2.4-D, Clomazone, Triallate and Butylate have high vapor pressure. Solar rays cause herbicide molecules to become inactive. This is called chemical degradation. Light is quite important in the degradation of herbicides (Başaran and Serim 2010). Herbicides trigger the formation of the reactive oxygen species which cause oxidative stress. Increased activity of antioxidant enzymes such as SOD and CAT increase is a result of the detoxification mechanism which provides the decrease of lipid peroxidation (Santos and Silva 2015).

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In a study conducted by spraying 23 µM 2.4-D periodically to young and mature leaves of pea plant, oxidative damage in protein and membrane lipids was determined after 72 hours. It was observed that glutathione content increased. Leaves of the mature pea plant had a higher GSH/GSSG rate. In young plant leaves, SOD enzyme activity increased and CAT enzyme activity decreased (Pazmino et al. 2011). 9 macrophyte plants (aquatic) were kept in 2.4-D at concentration of 10-3000 µg/L $^{-1}$. It was observed that the root length of the plant grew more at low concentration and less at high concentration. A decrease was observed in leaf length in the concentration of 300-1000 µg/L $^{-1}$ (Belgers et al. 2007). In a previous study, it was determined that the amount of MDA increased and PRL amount decreased when maize, pea and wheat plants were treated with 2.4-D and atrazine (Alexieva et al. 2003). *Eleusine indica* and *Digitaria adscendens* plants were treated with 100 µM 2.4-D herbicide for 2 days and their root and leaf length decreased and leaf chlorophyll content decreased (Sunohara et al. 2010). In a study examining the effect of atrazine on *Acorus calamus* (sweet edge), *Lythrum salicaria* (Purple loosestrife) and *Scirpus tabernaemontani* (Softstem bulrush) seedlings, it was reported that there was a delay in the plant growth, a decrease in chlorophyll content, and an increase in MDA content (Wang et al. 2015). In a study investigating the effect of imazapic on the leaves of tobacco seedlings, it was reported that catalase activity, carotenoid content, GSH and MDA amounts increased, the plant growth delayed, and the total chlorophyll amounts decreased (Kaya and Doğanlar 2016). Fenoxaprop-p-ethyl herbicide was sprayed to leaves of wheat (*Triticum aestivum* L.) seedlings at the end of 1st, 5th, and 10th days and SOD activity, MDA and GSH amounts increased, CAT activity and chlorophyll amounts decreased and excepted increases and decreases were determined in carotenoids (Akbulut et al. 2018). In the study examining the topic effect on leaves of *Triticum aestivum* L., cv. Mironovskaya 808 (wheat), Secale cereale L. cv. Estafeta tatarstana (rye), and *Zea mays* L. cv. Kollektivnyi 172MV (maize) seedlings, it was reported that the lipid peroxidation, superoxide anion, total antioxidant activity, catalase and ascorbate peroxidase activities increased (Lukatkin et al. 2013).

In this study, the various growth parameters (root-shoot elongation growth, seedling mg dry weight (DW)/g $^{-1}$ fresh weight (FW)) at seedlings of three wheat varieties due to the toxic effect formed by the different concentrations of the 2.4-D herbicide were determined. Pigment analysis, malondialdehyde amount, reduced glutathione/oxidized glutathione rate, superoxide dismutase, and catalase activity were determined in the leaves. It was determined that the 2.4-D herbicide, which was toxic for wheat plants even in very low concentrations. 2,4-D herbicide is effective both in the control of a synthetic auxin hormone derivative and weeds in agricultural areas. Plants cause disorders in many plant functions, such as phloem transport, absorption and photosynthesis, when used or exposed in excess of 2,4-D herbicide. The fact that this herbicide, which is prohibited in Europe, is obtained very easily and with low cost in Turkey and unconsciously used threatens both our agriculture and health severely.

2. Materials and Methods

2.1. Applications to plant material

In order to grow wheat seedlings which are our experiment material, completely uniform seeds were selected (*Triticum aestivum* L., Bayraktar cv., İkizce cv. and Tosunbey cv.), soaked with tap water and kept in the dark at 23-25°C for 6 hours. At the end of this period, the seeds were placed in germination boxes with lids where they could breathe and left to germinate in the dark at 23-25oC for 3 days. Then germinated seeds with equal length of radicula were selected (homogenous) and planted in pots filled with a mixture of sand (3/1) and field soil (3/2) previously prepared in certain proportions. Seedlings were watered twice a week with an equal amount of tap water in the long day period (16/8) until 15 days in normal daylight. Of these seedlings, completely homogenous growing seedlings were selected and used as experimental material. 15 days of wheat seedlings were divided into 4 groups containing equal number of seedlings and pure water in 250 ml dark glass containers was used as hydroponic medium. Seedlings were exposed to 2.4-D (Alfa aeser) (0, 100 µM, 300 µM, and 1000 µM) at different concentrations for 1 day. All physiological and biochemical analyses were replicated three times for each treatment. For each analysis, 2 g of leaf tissue was used.

2.2. Determination of chlorophyll (a + b) and carotenoid content

0.5 g of fresh leaf tissue was used separately from all treated groups. The leaf tissue, which was cut into small pieces in a sterile mortar, was crushed with 40 ml of 80% acetone for 3-4 minutes and a green extract was obtained. Later, this extract was filtered by vacuuming through a buffer funnel. The remaining residue was crushed again with 30 ml of 80% acetone. This process was continued until the color of the residue completely discolored, and the filtrates were combined by filtration through the buhner funnel. The remaining colorless residue was washed with 80% acetone and the final volume of the total filtrate was completed to 100 ml. All these operations were done separately for each group. Then, the absorbances of the extracts at 440, 645, 652 and 663 nm wavelengths were read against the curve in the spectrophotometer (Shimadzu UVmini-1240). From the absorbance values obtained, mg.g^{-1} pigment was determined in fresh weight (Witham 1971).

2.3. Getting growth parameters

For this purpose, since homogenous seedlings were used initially in all groups, only final height measurements were taken as basis and height differences were calculated. After the 2,4-D herbicide application, the final weights of the seedlings

were determined. Dry weights fixed at intervals of 3-6 hours were determined in 105°C oven by making suitable packaging and markings. Thus, the amount of dry matter per fresh weight was determined ($mg^{-1}DW.G^{-1}$ FW) (Baltepe et al., 1982).

2.4. Malondialdehyde (MDA), reduced glutathione (GSH) \ oxidized glutathione (GSSG) ratio analysis

2 g of fresh leaves were taken, placed in falt tubes and homogenized for 4 minutes by adding 10 ml Tris (hydroxymethyl) aminomethane (TRIS Base, TRIS HCL, Ethylenediaminetetraacetic acid (EDTA)). The mixtures in falcon tubes were centrifuged (Hettich zentrifugen universal 32 R) at 6000-7000 rpm for 10 minutes. Phase separation occurred at the end of this process. 1 ml glass samples taken from the supernatant (upper phase) of each sample were put into test tubes. 1 ml of 10% perchloric acid (10 ml of perchloric acid, 90 ml of pure water soluble) was added to it and it is waited for two hours at +4˚C for 10 minutes at 5000 rpm. centrifuged. Then, 1 ml was taken and measured in HPLC (High performance liquid chromatography, Shimadzu prominence-İ LC-2030C) by taking into vials (Yilmaz et al. 2009, Karataş et al. 2002).

2.5. Superoxide dismutase (SOD; EC 1.15.1.1) analysis

The upper phase was taken to the epondorf. 2.6 ml sod buffer, 100 µl sample, 250 µl epinephrine, 50 µl xanthinoxidase (XO) were added to the glass tubes, respectively. This mixture was vortexed and kept in the dark for 30 minutes. Then, the absorbances of the extracts at 485 nm wavelengths were read against the curve in the spectrophotometer (Mourente et al. 1999).

2.6. Catalase (CAT; EC 1.11.3.6) analysis

The upper phase was taken to the epondorf. 1.9 ml phosphate buffer, 100 µl sample was taken into the glass tubes and absorbance was measured at 240 nm, 1 ml of H2O2 was added and a measurement of up to 60 seconds was made in 15 seconds (Aebi, 1984).

2.7. Statistical analyses

The results were analyzed using one-way ANOVA (SPSS 15.0 Evaluation Version Production Mode Facility). The difference between treatments was considered significant at $p<0.01$-0.05. Duncan's test was performed to compare means.

3. Results

3.1. Growth parameters

When the effects of the treatments performed on the seedlings on the growth parameters of the roots and leaves were examined, significant differences were determined in all groups.

Decreases of 7.69%, 26.92%, and 50% were determined in the root lengths of the seedlings treated with 2.4-D herbicide in the 100 µM, 300 µM and 1000 µM concentrations of Bayraktar wheat, respectively, compared to the control group. A decrease of 44%, 18.67%, and 9.33% were determined in 100 µM, 300 µM and 1000 µM concentrations, respectively, in İkizce wheat. Decreases of 19.78%, 20.88%, and 15,38% were determined in 100 µM, 300 µM and 1000 µM concentrations in Tosunbey, respectively ($P\leqslant0.05$) (Table 1).

A decrease of 54.17%, 16.67% and a decrease of 91.67% were determined in the offshoot lengths of the seedlings treated with 2.4-D herbicide in the 100 µM, 300 µM and 1000 µM concentrations, respectively, Bayraktar wheat, compared to the control group. A decrease of 45.71% and increases of 34.29%, and 5.71% were determined in 100 µM, 300 µM and 1000 µM concentrations in İkizce wheat, respectively. Decreases of 90.63%, 3.13%, and 56.25% were detected in 100 µM, 300 µM and 1000 µM concentrations, respectively, in Tosunbey wheat ($P\leqslant0.05$) (Table 1).

Decreases of 12.19%, 28.08% and 26.89% were determined in the dry weight amounts (mg KA. g^{-1} TA) per fresh weight in the seedlings treated with 2.4-D herbicide in the 100 µM, 300 µM, and 1000 µM concentrations in Bayraktar wheat, respectively, compared to the control group. Decreases of 11.60%, 13.63%, and 33.39% were determined in 100 µM, 300 µM, and 1000 µM concentrations, respectively in İkizce wheat. Decreases of 23.55%, 22.11%, and 48.55% were determined in 100 µM, 300 µM and 1000 µM concentrations, respectively in Tosunbey wheat ($P\leqslant0.05$) (Table 1).

3.2. Changes in the amounts of chlorophyll (a+b) and carotenoid

Decreases of 7.24%, 9.94%, and 1.67% were determined in the leaves of the 2.4-D-treated seedlings in terms of amount of photosynthetic pigment in 100 µM, 300 µM, and 1000 µM concentrations, respectively, in Bayraktar wheat, compared to the control group. Decreases of 2.35%, 9.52%, and 12.81% were determined in 100 µM, 300 µM, and 1000 µM concentrations, respectively, in İkizce wheat. Decreases of 5.39%, 10.77%, and 13.97% were determined in 100 µM, 300 µM, and 1000 µM concentrations, respectively, in Tosunbey wheat ($P\leqslant0.05$) (Table 1).

Decreases of 7.98%, 9.20%, and 10.89% were determined in the leaves of the seedlings to which 2.4-D herbicide was treated in terms of carotenoid amounts in 100 µM, 300 µM and 1000 µM concentrations, respectively, in Bayraktar wheat, compared to the control group. Decreases of 2.49%, 3.07%, and 4.02% were determined in 100 µM, 300 µM, and 1000 µM concentrations, respectively, in İkizce wheat. Decreases of 8.01%, 8.54%, and 9.52% were determined in 100 µM, 300 µM,

and 1000 µM concentrations, respectively in Tosunbey wheat (P≤0.05) (Table 1).

Table 1. Root shoot height, dry weight and amounts of photosynthetic pigments changes in the *Triticum aestivum* L. Bayraktar cv., İkizce cv. and Tosunbey cv. seedlings applied to 2.4-D

	Seedling Growth		**Dry Weight**	**Chlorophyll (a+b)**	**Carotenoid**
	(mm/cm^{-1})		(mg DW/g^{-1} FW)	(mg.g^{-1} FW)	(mg.g^{-1} FW)
GROUPS	**ROOT**	**SHOOT**			
B-Control	0,026±0,068	0,024±0,003	0,755±0,042	1.257±0.015	0.652±0.002
B-100 µM	0,024±0,012	0,011±0,014*	0,663±0,050	1.348±0.014*	0.600±0.002*
B-300 µM	0,019±0,021	0,020±0,006*	0,543±0,050*	1.132±0.014*	0.592±0.002*
B-1000 µM	0,013±0,012	0,002±0,007*	0,552±0,060*	1.236±0.014*	0.581±0.002*
İ-Control	0,075±0,017	0,035±0,005	0,638±0,039	1.491±0.015	0.522±0.001
İ-100 µM	0,042±0,012*	0,019±0,009*	0,564±0,040	1.456±0.013	0.509±0.002*
İ-300 µM	0,061±0,009*	0,047±0,013*	0,551±0,021	1.349±0.015*	0.506±0.002*
İ-1000 µM	0,068±0,020*	0,037±0,008*	0,425±0,029*	1.300±0.015*	0.501±0.002*
T-Control	0,091±0,032	0,032±0,007	0,692±0,039	1.467±0.014	0.562±0.002
T-100 µM	0,073±0,012*	0,003±0,003*	0,529±0,036*	1.388±0.018*	0.517±0.002*
T-300 µM	0,072±0,011*	0,031±0,008*	0,539±0,021*	1.309±0.011*	0.514±0.003*
T-1000 µM	0,077±0,007*	0,014±0,007*	0,356±0,032*	1.262±0.014*	0.510±0.003*

***:**Compared to the control group at p≤0.05 probability levels. Values are mean of three replicates, Bayraktar: B, İkizce: İ, Tosunbey: T

3.3. Changes in reduced glutathione (GSH)/oxidized glutathione (GSSG) ratios

Increases of 14.78% and 20.39% and a decrease of 33.41% were determined in the GSH/GSSG ratio in the leaves of the seedlings treated with 2.4-D herbicide in the 100 µM, 300 µM, and 1000 µM concentrations, respectively, in Bayraktar wheat compared to the control group. Increases of 5.10% and 24.47% and a decrease of 24.88% were determined in 100 µM, 300 µM and 1000 µM concentrations, respectively, in İkizce wheat. Increases of 36.63%, 113.19%, and 56.47% were determined in 100 µM, 300 µM and 1000 µM concentrations, respectively, in Tosunbey wheat (P≤0,05) (Table 2).

3.4. Changes in malondialdehyde (MDA) amount

Increases of 62.51%, 86.76% and 75.96% were determined in the MDA amounts of the leaves of the seedlings treated with 2.4-D herbicide in the 100 µM, 300 µM, and 1000 µM concentrations, respectively, in Bayraktar wheat, compared to the control group. A decrease of 7.74%, an increase of 16.01%, and a decrease of 18.95% were determined in 100 µM, 300 µM, and 1000 µM concentrations, respectively, in İkizce wheat. Increases of 18.98%, 25.43%, and 62.87% were determined in 100 µM, 300 µM, and 1000 µM concentrations, respectively in Tosunbey wheat (P≤0.05).

3.5. Changes in superoxide dismutase (SOD) activity

Increases of 24.43%, 111.36% and 89.20% were determined in the SOD activity in the leaves of the seedlings treated with 2.4-D herbicide in the 100 µM, 300 µM and 1000 µM concentrations, respectively, for Bayraktar wheat compared to the control group. Decreases of 10.02%, 23.31%, and 5.59% were determined in 100 µM, 300 µM and 1000 µM concentrations in İkizce wheat, respectively. Decreases of 10.26%, 19.33%, and 34.13% were determined in 100 µM, 300 µM and 1000 µM concentrations, respectively, in Tosunbey wheat (P≤0.05) (Table 2).

3.6. Changes in catalase (CAT) activity

Decreases of 49.62%, 42.83% and 5.51% were determined in the CAT activity in the leaves of the seedlings treated with 2.4-D herbicide in the 100 µM, 300 µM and 1000 µM concentrations, respectively, for Bayraktar wheat compared to the control group. Decreases of 50.39%, 27.17%, and 38.55% were determined in 100 µM, 300 µM and 1000 µM concentrations, respectively, in İkizce wheat. Decreases of 44.79%, 54.88%, and 50.65% were determined in 100 µM, 300

μM and 1000 μM concentrations of Tosunbey wheat, respectively (P≤0.05) (Table 2).

Table 2. GSH/GSSG, MDA amounts, SOD and CAT activities changes in the *Triticum aestivum* L. Bayraktar cv., İkizce cv. and Tosunbey cv. seedlings applied to 2.4-D

	GSH/GSSG	MDA	SOD	CAT
GROUPS		(nmol.g-1 FW)	unite/gr	(μg/g)
B-Control	10.557±0.272	296.375±17.804	0.176±0.037	339.159±16.336
B-100 μM	12.117±0.318	481.625±20.450*	0.219±0.050	170.874±11.210*
B-300 μM	12.710±1.126*	553.50±59.540*	0.372±0.044*	193.887±4.375*
B-1000 μM	7.030±0.495*	521.510±46.813*	0.333±0.062	320.460±3.135*
İ-Control	6.410±0.860	284.250±1.635	0.429±0.016	309.673±11.303
İ-100 μM	6.737±0.919	262.250±10.687	0.386±0.038	153.614±1.246*
İ-300 μM	8.171±1.180	329.750±14.126	0.329±0.022*	225.531±3.805*
İ-1000 μM	4.815±0.504	230.375±50.326	0.405±0.027	190.291±3.296*
T-Control	8.097±0.696	133.667±16.598	0.419±0.253	356.419±2.491
T-100 μM	11.063±0.949*	159.043±15.510	0.376±0.034	196.764±2.491*
T-300 μM	17.262±1.196*	167.660±8.747	0.338±0.026	160.806±7.610*
T-1000 μM	3.525±0.036*	217.710±5.135*	0.276±0.042*	175.908±2.593*

*:Compared to the control group at p≤0.05 probability levels. Values are mean of three replicates, Bayraktar: B, İkizce: İ, Tosunbey: T

4. Discussion

In the present study, the responses of wheat seedlings to different concentrations of 2.4-D herbicides were investigated. Bayraktar wheat increased in the root lengths of the seedlings treated with 2.4-D herbicide. In İkizce wheat increased in 300 μM and 1000 μM concentrations. At all concentrations of Tosunbey wheat and 100 μM concentration of İkizce decreased. The offshoot lengths of the seedlings treated with 2.4-D herbicide were determined an increase in 300 μM and a decrease in 100 μM and 1000 μM concentrations for Bayraktar wheat. In İkizce wheat were determined a decrease of 100 μM and increases of 300 μM and 1000 μM concentrations. Tosunbey wheat decreased in 100 μM 300 μM and 1000μM concentrations. At all concentrations of 2.4-D herbicide caused a significant decrease in terms of ratio of dry weight per fresh weight in wheat seedlings. It was found that 2.4-D treatment was most inhibitive on the root length among the growth parameters and this effect was the most apparent in Tosunbey wheat (Belgers et al. 2007, Sunohara et al. 2010). It was determined that 2.4-D (Belgers et al. 2007; Sunohara et al., 2010) application caused very effective growth delays on the offshoot lengths of wheat seedlings, especially in Tosunbey wheat. 2.4-D (Santos and Silva 2015) herbicide caused a significant decrease in terms of the dry weight amounts of wheat seedlings, especially in Tosunbey wheat.

Chlorophyll (a+b) contents in leaves of wheat seedlings decreased in all concentrations of 2.4-D herbicide. Carotenoid contents in leaves of wheat seedlings were influenced by 2.4-D herbicide in other words pigment breakdown increased (Santos and Silva 2015). The chlorophyll content of plants changes under stress. The reason for the decrease in chlorophyll content is the oxidative stress induced by herbicides. Because chlorophyllase enzyme and chlorophyll degradation increase under stress conditions (Kaya and Doğanlar 2016). The herbicide treatment causes important photo-dynamic damages in the photo-system by photo-inhibition and decreases photosynthesis production. Herbicides cause oxidative stress in chloroplasts and lead to the increase of the reactive oxygen species and, therefore, lead to the damage of the photosynthetic devices due to chlorophyll degradation (Santos and Silva 2015). In terms of chlorophyll (a+b) amounts, 2.4-D herbicide caused significant decreases mostly in Tosunbey wheat (Sunohara et al. 2010, Wang et al. 2015, Kaya and Doğanlar 2016). In terms of carotenoid amounts in wheat seedlings; the pigment destruction increased mostly in Bayraktar wheat (Santos and Silva 2015) in 2.4-D treatment. In macroscopic terms, necrosis and chlorosis were observed intensely in the leaves of the seedlings treated with the aforementioned herbicides and softening was reported, especially in roots, due to tissue destruction. Carotenoids play a shield-protection mechanism role against oxidative damage. Carotenoids included in chloroplast protect chlorophyll against free radicals in photo-oxidation risk and

they do this by reacting with free radicals themselves. Chlorophyll molecules which are stimulated by taking photon react with oxygen, singlet oxygen shape, superoxide anion, hydroxyl radicals, and hydrogen peroxide. This harms especially lipid derivative compounds included in cells and membrane systems. Thus, these pigments are exposed to oxidative damage due to stress (Santos and Silva 2015).

In the MDA amounts increased in the leaves of the Bayraktar wheat seedlings treated with 2.4-D herbicide (Alexieva et al. 2003, Wang et al. 2015, Kaya and Doğanlar 2016, Lukatkin et al. 2013). While MDA amounts in leaves of wheat seedlings caused an increase only 300 µM concentration, regarding 100 µM and 1000 µM concentrations determined a decrease in leaves of İkizce wheat. MDA amounts in leaves of Tosunbey wheat seedlings also increased in 100 µM, 300 µM, and 1000 µM concentrations. 2.4-D herbicide had an increasing effect mostly in Bayraktar wheat in the leaves of Bayraktar wheat seedlings in terms MDA amount (Alexieva et al. 2003, Wang et al. 2015, Kaya and Doğanlar 2016, Lukatkin et al. 2013). Akbulut and Yiğit (2010) reported in their study conducted on maize (*Zea mays* cv. Martha F1) seedlings that Atrazine herbicide caused an increase in MDA amount when it was treated at low dose and it caused a decrease in MDA amount when it was treated at high dose. Significant increases have been determined in the groups included in the studies in MDA amount, which is another important parameter demonstrating the oxidative stress intensity and is the typical end product of the lipid peroxidation reaction in the cell membrane. Also, it was determined that MDA amount decreased especially for the high herbicide doses (Akbulut and Yiğit 2010). The fatty acid profile (saturated and unsaturated fatty acid rates) in bio-membranes change under oxidative stress. The increase in MDA level showed that the oxidative stress-related lipid peroxidation increased in biomembranes of plants (Wang et al. 2015).

GSH/GSSG ratio of the seedlings treated with 2.4-D herbicide were determined an decrease in 1000 µM and a increase in 100 µM and 300 µM concentrations for Bayraktar and İkizce wheat (Pazmino et al. 2011, Kaya and Doğanlar 2016). The GSH/GSSG ratio of the Tosunbey wheat seedlings treated with 2.4-D herbicide increased in all concentrations (Pazmino et al. 2011, Kaya and Doğanlar 2016). The highest increase was observed in Tosunbey wheat for 2.4-D herbicide in terms of GSH/GSSG ratios in the leaves of wheat seedlings (Pazmino et al. 2011, Kaya and Doğanlar 2016) and a decrease was determined in 1000 µM concentration. Glutathione reductase enzyme (GR) converts oxidized glutathione (GSSG) to reduced glutathione (GSH) with a reaction based on NADPH+. Reduced glutathione is an important antioxidant that plays a role in the defense against oxidative stress and is not enzymatic. The increase in glutathione reductase activity is the indicator of the plant defense in oxidative stress. GSH and GR constitute the compounds of ascorbate-glutathione metabolism which plays a role in reacting to stress in plants (Kaya and Doğanlar 2016; Abou-Zeid et al., 2020).

An increase was determined in Bayraktar wheat in 2.4-D herbicide in the leaves of wheat seedlings in terms of SOD enzyme activity (Pazmino et al. 2011, Santos and Silva 2015) and a decrease was observed in İkizce and Tosunbey wheats (Romero-Puertas et al. 2004). The antioxidant system has an important role in protecting cell components from the damages of reactive oxygen species produced under stress. The production of reactive oxygen species in plant cells is low under optimal growth conditions. However, the increase in the production and accumulation of reactive oxygen species in most of the environmental stresses bring the deterioration of cell homeostasis (Wang et al. 2015). The SOD activity increasing under stress conditions has demonstrated that especially the superoxide radical reactive oxygen species are overproduced. Because it has a role in removing superoxide radical from SOD chloroplasts and superoxide radical is converted to H2O2. Herbicide toxicity is formed when SOD activation, which increases significantly in the antioxidant system, gets involved (Santos and Silva 2015; Sharma et al., 2012; Abou-Zeid et al., 2020; Linu and Girija 2020).

Decreases of CAT activity were determined in the leaves of all the wheat seedlings treated with 2.4-D herbicide in the all concentrations. In terms of CAT enzyme activity in wheat seedling leaves, Tosunbey wheat has the highest decrease in 2.4-D (Pazmino et al. 2011) herbicide (Akbulut et al. 2018). Catalase is found in the organelles named peroxidase in all the plant cells and it keeps H2O2 (H2O+½ O2) level at a certain level for cells and plays a protective role. The catalase enzyme detoxifies H2O2 at high concentration and provides that plant eliminates stress with the least loss. That the activity of antioxidant enzymes such as SOD and CAT increased is a result of the detoxification mechanism which provides a decrease of lipid peroxidation. The oxidative damage in the seedling leaves increases SOD and decreases the other enzymes, thus causing an imbalance in the enzyme activities and probably is associated with the H_2O_2 increase in the chloroplasts (Santos and Silva 2015; Sharma et al., 2012; Abou-Zeid et al., 2020; Linu and Girija 2020).

Consequently, it was determined that the 2.4-D herbicide, which was toxic for wheat plants even in very low concentrations. 2,4-D herbicide is effective both in the control of a synthetic auxin hormone derivative and weeds in agricultural areas. Plants cause disorders in many plant functions, such as phloem transport, absorption and photosynthesis, when used or exposed in excess of 2,4-D herbicide. The fact that this herbicide, which is prohibited in Europe, is obtained very easily and with low cost in Turkey and unconsciously used threatens both our agriculture and health severely. As our results revealing this situation are very limited and, we could not discuss our results in a very large platform and sometimes we had to give the indirect studies as references.

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