# Effects of rosehip fruit extract in two wheat varieties after herbicide treatment

**Eda Günay[1*], Gamze Baltacıer[1], Müge Teker Yildiz[1] & Okan Acar[2]**

[1] Department of Biology, Graduate School of Natural and Applied Sciences, Çanakkale Onsekiz Mart University, 17100 Çanakkale, Turkey, e-mail: gunayeg@gmail.com (*Corresponding author)

[2] Department of Biology, Faculty of Arts and Sciences, Çanakkale Onsekiz Mart University, 17100 Çanakkale, Turkey

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**Abstract** Extracts of medicinal plants can be used as biostimulants to overcome environmental stress conditions in cultivated plants. Herbicides are commonly used for weed control in wheat fields and accepted as abiotic stress factors for both weeds and cultivated plants. This study was aimed at determining the effects of rosehip (*Rosa canina* L.) fruit extract (20 %, 10 ml/pot, R) and tribenuron methyl (1 g/acre, H) on the antioxidant system in two wheat varieties (drought-tolerant *Triticum aestivum* cv. 'Tosunbey' and drought-sensitive cv. 'Sultan-95'). Wheat seedlings were divided into four groups: non-treated seedlings (C, Control), seedlings treated with rosehip fruit extract (R), seedlings treated with tribenuron methyl (H), and R seedlings treated with tribenuron methyl at the 28$^{th}$ day of the experiment (20 %, 10 ml/pot) (HR). R treatment increased root-shoot elongation in the 'Tosunbey' variety. All treatments decreased chlorophyll content in 'Tosunbey' but did not change it in 'Sultan-95'. R treatment did not alter superoxide dismutase (SOD) isoenzyme amount and peroxidase (POX) activities in 'Sultan-95' but increased lipid peroxidation (LP). However, 'Tosunbey' showed a low SOD isoenzyme amount and stable LP and cell membrane permeability (CMP). Thus, R treatment provided difference between these varieties. R treatment caused stable LP in the drought-resistant variety and increased it in drought-sensitive variety. LP did not decrease due to decrease in SOD isoenzyme amount and POX activity in 'Sultan-95' after HR treatment. However, HR treatment decreased LP and CMP and showed increased POX activity in 'Tosunbey'. Consequently, it was assumed that rosehip fruit extract treatment protects by inducing POX activity in the drought-resistant variety after herbicide treatment.

**Key words:** Antioxidant enzymes, oxidative stress, rosehip extract, tribenuron methyl, *Triticum aestivum*

## Introduction

Wheat (*Triticum aestivum* L.) is regarded as a strategic product for human nutrition worldwide. However, competition of weeds with wheat for water, light and nutrients decreases the yields (Olesen & al. 2004; Kaçan & Tursun 2019).

Chemical pesticides play an important and potential role in the control of pests and various plant-borne diseases (Sunar & Bulut 2019). Herbicides are xenobiotics used to control the growth and proliferation of weeds. They differ in their structure, but all show negative effects after penetrating the plant (Garkova & al. 2011). Herbicides are widely used in weed control in wheat production. For this purpose, herbicides containing tribenuron methyl (TM) are widely used in broadleaf weed control (Çakmak 2018; Pala & Mennan 2019). Furthermore, TM is very resistant to pho-

tolysis and is mobile in alkaline soils (Çakmak 2018). Herbicides cause stress in plants, when used continuously and in high doses (Sunar & Bulut 2019). Moreover that the enhanced herbicide doses increase retrotransposon polymorphism (15–53 %) in wheat seeds (Sunar & Bulut 2019). However, ineffectiveness and resistance in weeds may require the production of new types of herbicides.

Reactive oxygen species (ROS; $O_2^-$, $OH^-$, $H_2O_2$) are continuously produced during the metabolic processes in plant cells. Moreover, they can quickly react with other subcellular components and molecules resulting in oxidative damage (Demirbaş & Acar 2017). They are also involved in photosynthesis, signaling, pathogen defense, metal and xenobiotic detoxification, and regulation of growth (Bailey-Serres & Mittler 2006). Antioxidants produced under biotic and abiotic stress factors in plants protect against ROS. Increased ROS production in plant cells can be controlled by antioxidant systems, but excessive increase in ROS production may lead to degradation of the membrane lipids, proteins, pigments, and nucleic acids. Growth and yield reduction may result in plant death (Foyer & al. 1994).

Many studies have shown that herbicides cause ROS production and lipid peroxidation (LP) in both weeds and agricultural plants (Song & al. 2007; Jiang & Yang 2009). TM is an important herbicide used against weeds in wheat production (Garkova & al. 2011; El-Kholy & al. 2013; Enayati & al. 2016). This herbicide belongs to the sulfonylurea family. It kills weeds by inhibiting the synthesis of amino acids and stops cell division and growth.

Rosehip fruits are widely consumed as tea. While traditional uses of medicinal and aromatic plants such as rosehip (*Rosa canina* L.) are popular among the public (Haggag 2016; Mansori & al. 2016; Kaluzewicz & al. 2017), medicinal plant extracts can also be used as biostimulants to overcome stress conditions in cultivated plants.

This study was aimed at determining the effects of *Rosa canina* fruit extract (20 %, R) and TM (active herbicide substance, H) on the antioxidant system in two wheat varieties (drought-tolerant *T. aestivum* cv. 'Tosunbey' and drought-sensitive cv. 'Sultan-95'). The effects of TM treatment on 28-day-old R seedlings were also investigated (HR). In order to evaluate the effects, the focus was laid on physiological parameters, including root and shoot elongation, total chlorophyll content, superoxide dismutase (SOD) isoenzyme amounts, peroxidase activity (POX), lipid peroxidation (LP,) and cell membrane permeability (CMP).

## Material and methods

In this study, two wheat varieties were used as plant material – *Triticum aestivum* L. (*Poaceae*): drought-sensitive cv. 'Sultan-95', and drought-tolerant cv. 'Tosunbey'. The rosehip fruits (*Rosa canina* L., *Rosaceae*) were collected in the Çanakkale region of Turkey and extracted with dimethylsulphoxide (DMSO), according to Çabuk (2018). Accordingly, 5 g of rosehip fruit extract dissolved in DMSO were homogenized with 25 ml of distilled water. After filtration of the homogenate, a 20 % extract was prepared and used in the R treatments.

Wheat seeds were surface-sterilized with 5 % NaOCl for 5 min, thoroughly washed with distilled water and finally transferred to pots containing perlite. Wheat seedlings were grown in a growth chamber for three weeks (light/dark regime of 16/8 h at 25/20 C°, relative humidity 60–70 %, photosynthetic flux density (PAR of) 350 µmol $m^{-2}$ $s^{-1}$). They were grown in Hoagland nutrient solution (Steward 1983) during the experiment. Twenty-one-day-old seedlings were divided into four groups: the first group was of untreated plants (C, Control), the seedlings (R) in the second group were treated with rosehip fruit extract (20 %, 10 ml/pot) at the 21$^{st}$ day; in the third group, the seedlings (H) were treated with tribenuron methyl (1 g/acre); and the fourth group included TM treated R seedlings at the 28$^{th}$ day of the experiment (20 %, 10 ml/pot) (HR).

The active substance of the commercial herbicide is TM ( %75), an important herbicide against broad-leaved weeds in wheat fields. TM treatment was applied once at the 28$^{th}$ day in H and HR groups. Rosehip fruit extract was applied once at the 21$^{st}$ day in the R group. All treatments were applied by spraying (Fig. 1). TM and rosehip-extract treated seedlings were harvested with scissors at the 35$^{th}$ day and leaf samples were used for physiological (root and shoot length, total chlorophyll content) and biochemical analyses (SOD isoenzyme amounts, POX activity, LP, and CMP). Leaf samples were collected under cold chain (+4°C) for antioxidant enzyme analyzes and stored in deepfreeze (–18 °C).

## Physiological parameters

***Root and shoot length*:** Root and shoot length of plants was measured with a ruler.

***Total chlorophyll content*:** The total chlorophyll content of the samples was measured by Minolta chlorophyll meter (model SPAD-502) (Peryea & Kammereck 1997). The total chlorophyll content was measured on the leaves, before harvest, at the 35$^{th}$ day of the experiment.

## Biochemical parameters

***Peroxidase activity* (POX):** Tissue samples were homogenized with 2mL 0.05 M (pH 6.5) sodium acetate buffer. The homogenates were centrifuged at 4 °C and 13 000 rpm for 15 min. Peroxidase activity was measured by a spectrophotometer at 300 nm for 120 sec in 0.05 M (pH 6.5) sodium acetate buffer, 0.1 M pyrogallol, 0.09 M $H_2O_2$ reaction mixture of the samples. The enzyme unit was expressed as mg protein$^{-1}$ (Kanner & Kinsella 1983).

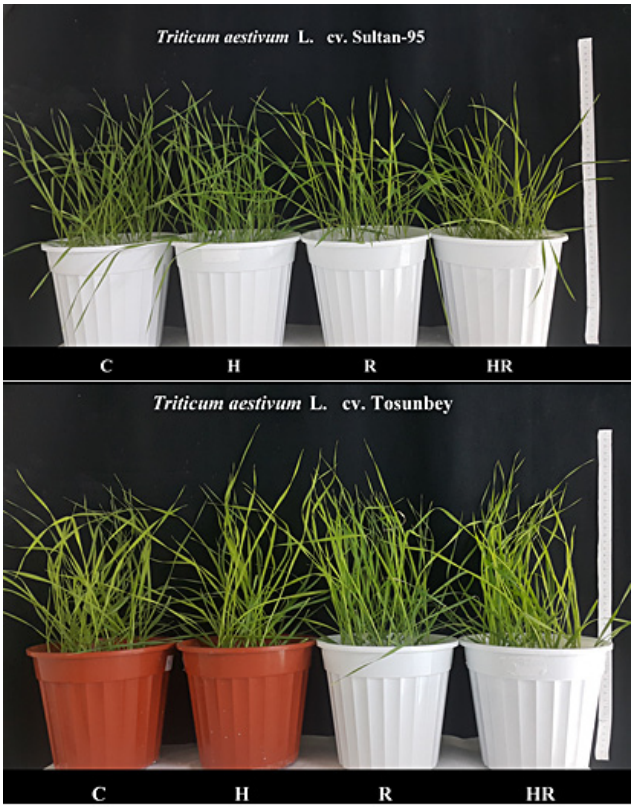



**Fig. 1.** Effects of herbicide treatment and rosehip extract on wheat seedlings. C – Control; R – Rosehip; H – Herbicide; HR – Rosehip, herbicide.

***Lipid peroxidation* (LP):** Lipid peroxidation was measured as TBARS amount. Leaf tissue was homogenized with 0.1 % trichloroacetic acid (TCA) solution. The reaction mixture, containing TCA and thiobarbituric acid (TBA), was added to the supernatant of the plant samples. Samples were then kept in a hot water bath for 30 min and transferred to an ice bath before centrifugation. Supernatants were measured within the range of calculated 532–660 nm (€=155 mM$^{-1}$ cm$^{-1}$) (nmol g FW$^{-1}$) (Madhava Rao & Stresty 2000).

***Cell membrane permeability* (CMP):** A hundred mg leaf sample was transferred to falcon tubes containing 10 mL of deionized water and incubated for two hours in a water bath (32 °C). Electrical conductivity of the medium was measured with an EC meter (EC1). The samples were then autoclaved at 121 °C for 20 min to remove the electrolytes of the tissues from the cell. Electrical conductivity (EC2) in this environment was measured by an EC meter by cooling down to 25 °C at room temperature. Electrolyte leakage (EL) was calculated by applying the following formula (Dionisio-Sese & Tobita 1998):

ES = EC1/EC2×100.

***Superoxide dismutase* (SOD) *isoenzyme amounts*:** Leaf tissue (0.1 g) was homogenized with 0.5 ml of homogenization buffer. The homogenates were then centrifuged at 14 000 rpm for 10 minutes at +4 °C. Samples homogenized with 50 mM Tris-HCl (pH 7.8) buffer containing 0.1 mM EDTA, 10 % Triton X, 1 mM PMSF and 2 % PVP were centrifuged. The protein amounts of the supernatants were then measured. Isoenzyme profiles were determined by the Native-Page Polyacrylamide Gel method (Laemmli 1970).

To prepare a vertical electrophoresis gel, the running gel was first placed at the bottom of the tank. One ml of n-butanol was added to solidify the gel and then washed with distilled water. After this, stacking gel was added in the upper part and combs were placed. Total protein content was calculated according to Bradford (1971).

SOD dye (5 µl) containing bromophenol blue and proteins of the samples were thoroughly mixed. Proteins mixed with the dye were loaded into the wells in the gel by pipette. Then, the gel running was started by applying electric current. For this purpose was used

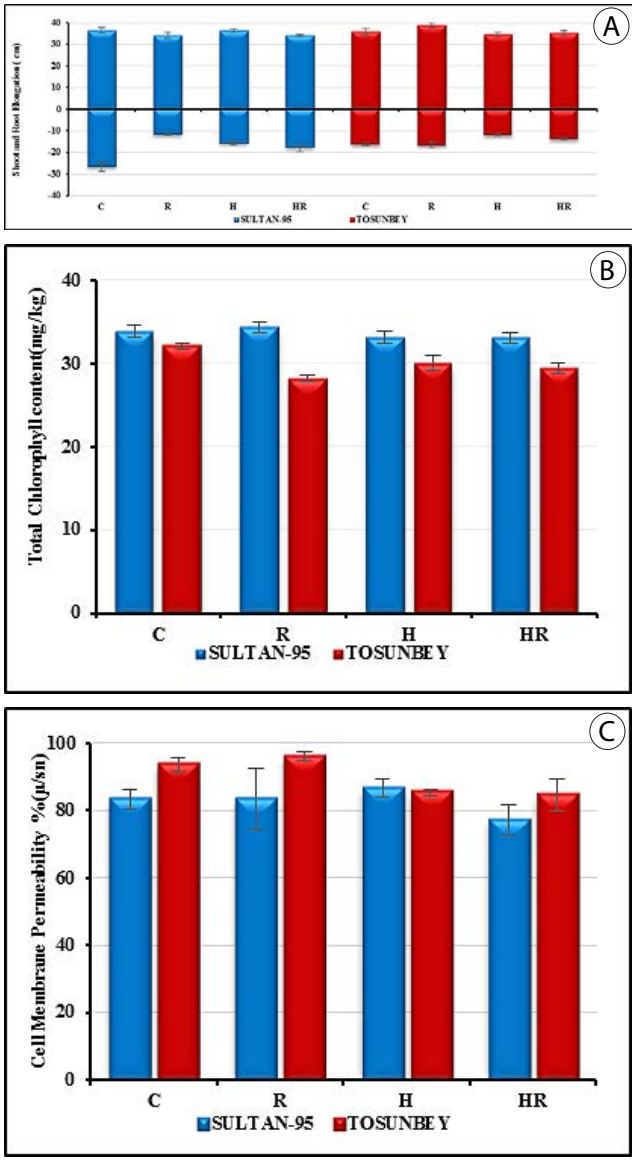
electric current for one-side gel running at 50 mA and for double-side gel running at 60 mA.

Samples were subjected to electrophoretic separation on separation (12.5 %) and stacking gels (4 %). SOD staining was performed with riboflavin and nitro blue tetrazolium (NBT) (Beauchamp & Fridovich 1971). Staining resulted in the formation of white bands by inhibition of NBT oxidation in the SOD localized regions on the gel. The gels were kept in the dark for 1 hour in the dye solution and the bands were shaken under light.

## Results

Root and shoot elongation increased with R treatment in 'Tosunbey' and decreased in 'Sultan-95' varities. Furthermore, H and HR treatments produced a decrease in both varieties (Fig. 2A). Total chlorophyll content in 'Tosunbey' decreased at all treatments but remained unchanged in 'Sultan 95' (Fig. 2B).

SOD isoenzyme analysis was performed with Native-PAGE. The amount of SOD isoenzyme in the leaf tissue of 'Sultan-95' did not change in the R treatment



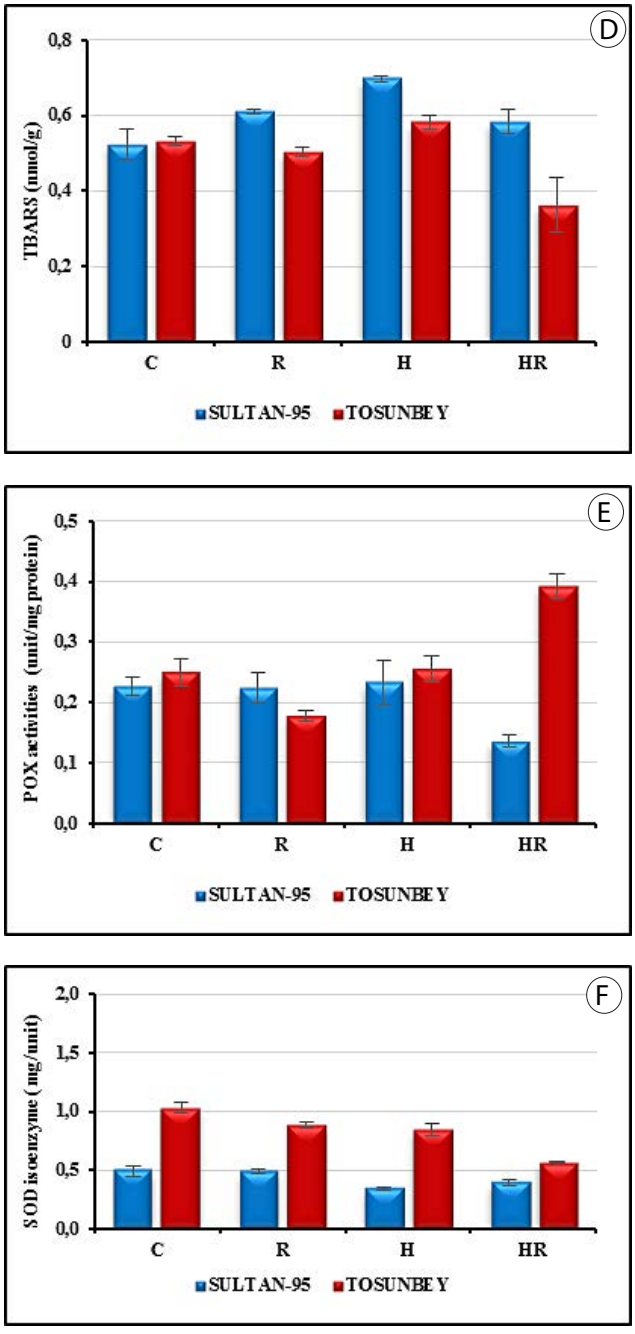




**Fig. 2.** Effects of herbicide treatment and rosehip extract on shoot and root elongation (A), total chlorophyll content (mg/kg) (B), cell membrane permeability (C), lipid peroxidation (LP) TBARS (nmol/g) (D), POX activities (unit/mg protein) (E), and SOD isoenzyme (mg/unit) (F). C – Control; R – Rosehip; H – Herbicide; HR – Rosehip, herbicide

group, as compared to the control one, but decreased with H treatment. Moreover, the resulting reduction in HR treatment was less than in H treatment. The amount of SOD isoenzyme decreased in 'Tosunbey' cultivar at all treatments (Fig. 2F, Fig. 3).

R treatments did not alter the SOD isoenzyme amount (Fig. 2F) and POX activities in 'Sultan-95' (Fig. 2E) but increased LP (Fig. 2D). On the contrary, LP and CMP did not change, although the amount of SOD isoenzyme decreased in 'Tosunbey' (Fig. 2C, D, F). Increase in LP was consistent with decrease in SOD isoenzyme amount and unchanged POX activities in both varieties. LP and CMP did not decrease owing to the decreased SOD isoenzyme amount and POX activity in 'Sultan-95' at HR treatment. However, HR treatment decreased LP and CMP (Fig. 2C, D), at characteristically increased POX activity in 'Tosunbey' variety (Fig. 2E).

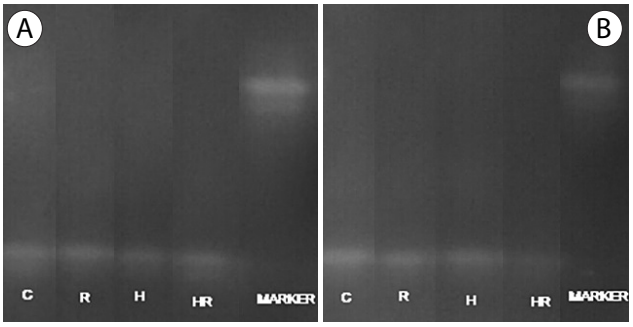



**Fig. 3.** SOD isoenzyme (mg/unit) of wheat variety. A. 'Sultan-95', B. 'Tosunbey'. C – Control; R – Rosehip; H – Herbicide; HR – Rosehip, herbicide

## Discussion

Many studies have shown that herbicides cause stress in plants (Ekmekci & Terzioglu 2005; Song & al. 2007; Miteva & al. 2010). Herbicides may induce herbicide phytotoxicity in non-target plants (Varshney & al. 2012; Caverzan & al. 2019). This can result in oxidative stress resulting from increased ROS concentrations due to herbicidal phytotoxicity (Wang & Zhou 2006; Yin & al. 2008; Jiang & Yang 2009; Garkova & al. 2011; Varshney & al. 2012; Cvetković & al. 2015; Ning & al. 2015; Caverzan & al. 2019). Oxidative stress may cause peroxidation of the cell membranes, which can damage the CMP. The superoxide radical is converted to $H_2O_2$ by the dismutation reaction catalyzed by SOD (Acar & al. 2001). Our results have shown that in all groups of the two varieties, the recommended dose of TM treatment reduced the SOD isoenzyme amounts. Similar results in wheat were reported by Wang & Zhou (2006) and Garkova & al. (2011). On the other hand, it is known that SOD activity increases or decreases depending on the type and dose of the herbicide treatment. Accordingly, SOD activities increase with high-dose paraquat-containing herbicide treatment, and decrease with low doses (Zhao & al. 2010). In contrast, it has been reported that low concentrations of herbicides containing isoproturon (Yin & al. 2008) and prometrine (Jiang & Yang 2009) increase SOD activity, while high concentrations decrease it. This suggests that high TM concentrations may increase SOD activity in wheat (Song & al. 2007; Yin & al. 2008; Jiang & Yang 2009; Guo & al. 2018).

Peroxidase plays an important role in detoxification of $H_2O_2$ in the result of SOD activity. Therefore, increased SOD activity with herbicide treatments also triggers out increase in the POX activities (Yin & al. 2008; Jiang & Yang 2009; Zhao & al. 2010; Cvetković & al. 2015). Although at some herbicide treatments SOD activities decrease, POX activities still increase (Wang & Zhou 2006). In our study, POX activities have increased in contrast to SOD isoenzyme amounts. This may be due to $H_2O_2$ produced by non-SOD sources (Wang & Zhou 2006). The POX activity of 'Tosunbey' variety has increased dramatically only with HR treatment, and decreased or did not change with R treatment. In 'Sultan-95', no treatment increased the POX activity.

The amount of TBARS in plants indicates oxidative damage, as well as cellular LP. Therefore, antioxidant enzymes such as SOD and POX are activated to reduce the herbicidal oxidative stress in wheat plants (Wang & Zhou 2006; Yin & al. 2008; Jiang & Yang 2009; Caverzan & al. 2019). Furthermore, it transpires that TM active herbicide causes higher LP in wheat (Faheed 2011). According to our results, R and H treatments decreased LP in 'Tosunbey' and increased it in all groups in 'Sultan-95'. This indicates that the drought-tolerant 'Tosunbey' reduces LP due to increased POX activities by HR. Similarly, high LP levels in 'Sultan-95' are associated with low POX activities. This supports the results obtained in similar studies on wheat (Song & al. 2007; Yin & al. 2008, Jiang & Yang 2009) (Fig. 2D).

Oxidative stress caused by herbicidal phytotoxicity is reportedly the major obstacle to high cere-

al yields (Varshney & al. 2012). Our results have shown that all herbicide-containing treatments inhibit growth in both varieties and that there is R-induced growth only in 'Tosunbey'. Similarly, it was observed that chlorophyll amounts did not increase in wheat seedlings. On the other hand, herbicides such as isopoturon (Yin & al. 2008), chlorimuron-ethyl (Wang & Zhou 2006) and prometrine (Jiang & Yang 2009) reportedly reduce the amount of chlorophyll in wheat. Our results have shown that TM application has reduced chlorophyll content in 'Tosunbey' and has not changed it in 'Sultan-95'. Accordingly, increased POX activity with HR seems to ensure protection only for CMP and LP in 'Tosunbey'. Furthermore, in 'Sultan-95', H and HR applications have partially induced chlorophyll content but did not increase it compared to control. This suggests that high TM concentrations in 'Tosunbey' can alter the amount of chlorophyll.

Our results indicated that the rosehip fruit extract treatment ensures protection against oxidative stress, and especially stimulates the POX activity in the drought-resistant wheat variety after herbicide treatment. Furthermore, reduction in the SOD activities for TM treatments appears to be acceptable as bioindicator for both wheat varieties.

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