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Influence of titanium foliar application on antioxidant enzyme activity and some biochemical attributes of corn

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Abstract

This study was conducted to evaluate the effect of solution titanium dioxide and titanium oxide on antioxidant enzyme activity and some biochemical attributes of corn during 2010 growing season in the research farm of Islamic Azad University of Shahryar –Shahr-e Qhods. The experimental design was randomized complete blocks arranged in factorial with four replications. The factors were included two growing stages i.e. four leafy stage and stem elongation for titanium application and five titanium concentration and sources including control (water), titanium oxide (Bulk) and three concentrations of 0.01%, 0.02%, and 0.03% of titanium dioxide nanoparticles. Antioxidant enzymes activity, malondialdehyde and 8-hydroxyguanosine, dityrosine, protein and membrane stability were assayed. The results showed that there was significant difference between growing stages regarding catalase, glutathione peroxidase, malondialdehyde, dityrosine and protein content. In addition, titanium caused an increase in antioxidant enzyme activity and decrease in malondialdehyde accumulation. In general titanium dioxide nanoparticle (0.03%) application improved antioxidant enzymatic system and prevent lipid peroxidation in corn plants. So it can be recommended to use this material on stressed plants.

Keywords: antioxidant enzyme, lipid peroxidation, protein, titanium, corn

Introduction

The ninth most abundant element and the second most abundant transition metal in the earth's crust is titanium. Titanium dioxide, also known as titanium (IV) oxide or titania, is the naturally occurring oxide of titanium, chemical formula TiO_2. When used as a pigment, it is called titanium white. Generally it comes in two different forms, rutile and anatase. Generally it is sourced from ilmenite, rutile and anatase. It has a wide range of applications, from paint to sunscreen to food colouring. Titanium has been considered as an inert element for a long time. However, since the 1930s its promotive effect on plant metabolism has begun to be appreciated (Carvajal and Alcaraz, 1998). Titanium has significant biological effects on plants, being beneficial at low and toxic at higher concentrations. Titanium is a very interesting chemical element, especially physiologically. It shows beneficial effects on various physiological parameters at low doses. It has been found that TiO_2 nanoparticles encourage spinach seed germination and plant growth (Zheng et al, 2005). Application of titanium dioxide on crops has been reported to promote plant growth, increase the photosynthetic rate, reduce disease severity and enhance yield by 30% (Chao et al, 2005). Previously physiological effect of titanium was studied on alfalfa and plant dry weight, plant height; chlorophyll and Fe, Mn, Mg, and Zn content were measured, titanium showed significant effect on above mentioned parameters (Kuzel et al, 2002). Many investigators (Dumon and Ernst, 1988) demonstrated the promotion of growth by titanium, whether applied as a fertilizer to the soil, or as a spray to the leaves. Reverte and coworkers (2000) reported the application of titanium significantly improved red paprika yield and fruit quality as well as photostability of ground peppers during storage. The positive effects of titanium were found on rape plant development (an increase of chlorophyll content and photosynthesis intensity), the yield and mass of a 1000 seeds of wheat, and the yield and sugar content in sugar beets (Grenda, 2003). The application of titanium has been found to show excellent efficacy in rice and maize by reducing the effect of Curvularia leaf spot and bacteria leaf blight disease incidence and severity (Chao et al, 2005).

To our knowledge, in the literature there is a lack of data referring to influence of titanium on antioxidant enzymes activity and biochemical changes in treated plants. So this experiment was conducted to evaluate the effect of different titanium concentrations and sources on antioxidant enzymes activity and some biochemical changes in corn. The objective of the study was to estimate the influence of titanium treatment on wheat and understanding which titanium source and concentration is better to use.

Materials and Methods

Study site and climate

Field experiment was conducted at the agricultural research farm in Islamic Azad University, Shahryar – Shahr-e Qhos branches, Tehran, Iran during the 2010 growing season. The highest and lowest temperature

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in study site were registered at -20°C and 38°C, respectively. Also mean annual precipitation was recorded 235 mm.

Experimental design and treatments

The experimental design was randomized complete blocks arranged factorial with four replications. The first factor was growth stages at two levels (four leafy stage and stem elongation stage) in addition, five different dose of titanium (control, 0.01%, 0.02%, 0.03% titanium dioxide nanoparticle and titanium oxide (bulk) were allocated to second factor.

Filed preparation and seed sowing

Prior to experiment soil samples were collected to determine the soil characteristics. The soil had clay loam texture containing 28.6% sand, 25.2% silt, and 46.2% clay. The soil pH and EC was measured as 7.7 and 3.4 mmhos cm^{-1}, respectively. When plots were prepared there were 40 plots with 3 m width and 4 m length. There was 2 m alley between plots and blocks to avoid lateral water movement. Before seed sowing 150 kg ha^{-1} super phosphate triple was mixed into the soil. Corn seeds (*Zea mays* L single cross 704) were sown by hand on 15th June at depth of 3 cm. The plant density was 10 plants per square meter. Irrigation was performed as furrow irrigation, immediately. Second irrigation was done three days after first one to get the best seed germination and seedling establishment. Weeds were controlled manually during growing season. It is worth mentioning that nitrogen fertilizer (300 kg ha^{-1} urea) was applied at eight leafy stage and tasseling.

Preparation of titanium dioxide nanoparticles and bulk titanium dioxide

In order to preparation of titanium dioxide nanoparticles 20 g titanium dioxide was dissolved in to the water and then 0.01 ml of solution was filled up to 1,000 ml. Thus different concentrations of titanium dioxide (0.01, 0.02, and 0.03%) were prepared. Ultrasound instrument was used to homogeneity of the solution. Bulk titanium dioxide was purchased from Advanced Material Company (US). To make bulk solution, 6 g titanium was dissolved in to 100 ml distilled water then 1 ml of solution was filled up to 1,000 ml.

Titanium foliar application

Titanium dioxide nanoparticles and titanium oxide (bulk) were sprayed on plants by a calibrated pressurized backpack sprayer (20 l capacity) at four leafy stage and stem elongation. Plants were treated by 500 ml titanium solution per square meter. Control plants were treated by distilled water.

Figure 1 - Titanium dioxide nanoparticles tracing on leaf surface.

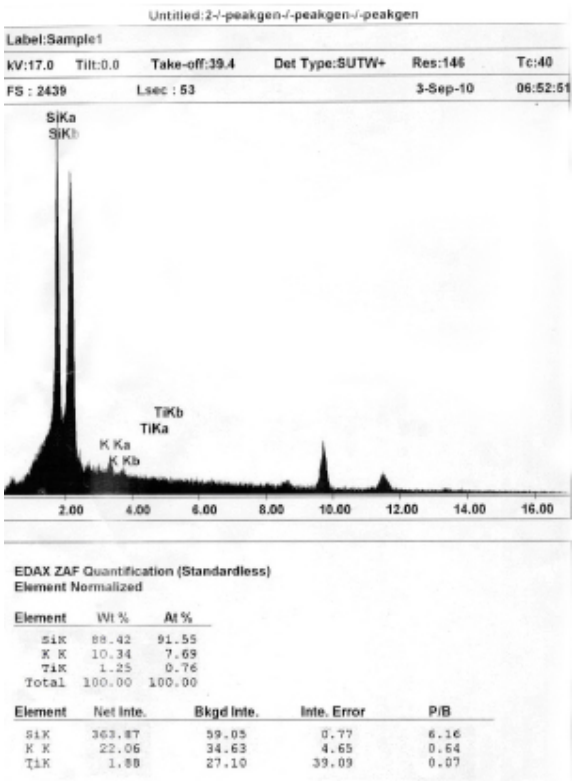

Figure 2 - Titanium nanoparticles were traced by scanning electron microscope (SEM). Titanium dioxide nanoparticles uptake percentage was estimated 1.25%.

Nanoparticle tracing

Titanium nanoparticles were traced by scanning electron microscope (SEM) on leaf surface (Figure 1). Titanium dioxide nanoparticles uptake percentage was estimated 1.25% (Figure 2).

Sampling and biochemical assays

Leaf samples were collected after five days after titanium foliar application. Samples were frozen in liquid nitrogen and kept in deep freezer under -80°C until biochemical analysis.

Antioxidant enzyme activity

Catalase activity was estimated by the method of Cakmak and Horst (1991). The reaction mixture contained 100 µl crude extract, 500 µl 10 mM H_2O_2, and 1,400 µl 25 mM sodium phosphate buffer. The decrease in the absorbance recorded at 240 nm for 1 min by a spectrophotometer.

Superoxide dismutase activity was determined by measuring the ability of the enzyme extract to inhibit the photochemical reduction of nitroblue tetrazolium according to the method of Giannopolitis and Ries (1977). The reaction mixture contained 100 µl of 1 µM riboflavin, 100 µl 12 mM L-methionine, 100 µl 0.1 mM EDTA (pH 7.8), 100 µl 50 mM Na_2CO_3 (pH 10.2), 100 µl 75 µM nitroblue tetrazolium in 25 mM sodium

phosphate buffer (pH 6.8) and 200 µl crude enzyme extract, in a final volume of 3 ml. Glass test tubes that contained the reaction mixture were illuminated with a fluorescent lamp (120 W), and identical tubes that were not illuminated served as blanks. After illumination for 15 min, absorbance was measured at 560 nm. One unit of superoxide dismutase activity was defined as the amount of enzyme which caused 50 % inhibition of photochemical reduction of nitroblue tetrazolium.

Glutathione peroxidase activity was measured according to the method of Paglia and Valentine (1997) in which 0.56 M (pH = 7) phosphate buffer, 0.5 M EDTA, 1 mM NaN_3, 0.2 mM NADPH were added to the extracted solution. Glutathion peroxidase catalyses the oxidation of glutathion by cumene hydroperoxide in the presence of glutathion reductase and NADPH, the oxidized glutathion is immediately converted to the reduced form with the concomitant oxidation of NADPH to NADP. The decrease in absorbance at 340 nm was measured with a spectrophotometer.

Determination of malondialdehyde in crude extract

The level of membrane damage was determined by measuring the amount of malondialdehyde which is the end product of lipid peroxidation according to De Vos et al (1991) method. In brief, samples were homogenized in 10 % trichloroacetic acid (w/v) and aliquots of the filtrates were heated (100°C for 30 min) in 0.25% thiobarbituric acid. The amount of malondialdehyde in the samples was determined from the absorbance at 532 nm, followed by correction for non-specific absorbance at 600 nm in a spectrophotometer. Concentration of malondialdehyde determined by extinction coefficient MDA (e = 155 lm cm^{-1}).

Dityrosine

Leaf samples were homogenized with 5 ml of 0.16 M Tris-phosphate, pH 7.5. The plant tissue homogenate was centrifuged at 5,000 g for 60 min to remove debris. o,o-dityrosine was recovered by gradient elution from the C-18 column (Econosil C18, 250mm × 10 mm) and was analyzed by reversed-phase HPLC with simultaneous UV-detection (280 nm). A gradient was formed from 10 mM ammonium acetate, adjusted to pH 4.5 with acetic acid, and methanol, starting with 1% methanol and increasing to 10% over 30 min. A standard dityrosine sample was prepared according to Amado et al (1984). Dityrosine was quantified by assuming that it's generation from the reaction of tyrosine with horseradish peroxidase in the presence of H_2O_2 was quantitative (using the extinction coefficient e_{315} = 4.5 mM^{-1} cm^{-1} at pH 7.5).

Determination 8-hydroxyguanosine in crude extract

Hydroxyguanosine was measured in the leaves essentially as described previously (Bogdanov et al, 1999). Briefly, an automated column switching method for 8OH-2'dG is based on the unique selectivity of the integral porous carbon column for purines. Samples were injected onto a C8 column and the band containing 8OH-2'dG was then quantitatively trapped on a carbon column. The selectivity of the carbon column for 8OH-2'dG allows elimination of interfering peaks by washing the column with a second mobile phase and then eluting 8OH-2'dG to an analytical C18 column with an identical mobile phase containing adenosine to displace 8OH-2'dG. Detection with series colorimetric electrodes provides qualitative certainty for 8OH-2'dG peak by response ratios.

Protein

The protein content of the crude extract was determined using bovine serum albumin (BSA) as a standard, according to the method of Bradford (1976). One millilitre of Bradford solution was added to 100 µl crude extract and absorbance recorded at 595 nm for estimate of total protein content. The protein concentration was calculated from a BSA standard curve.

Membrane stability

Leaf samples (0.5 g) were immersed into 10 ml of -2 bar mannitol solution (14.7 g mannitol per liter) and after 24 h electrical conductivity of the solution was measured.

Statistical analysis

All data were subjected to SAS software and Duncan's Multiple Range Tests was used to measure statistical differences between treatments.

Results

Analysis of variance showed that the effect of growth stage was significant on catalase, glutathione peroxidase, malondialdehyde, dityrosine, and protein (Table 1). In addition, titanium application has significant effect on activity of antioxidant enzymes, malondialdehyde content and protein in corn plants. Moreover, interaction effects between growth stage

Table 1 - Analysis of variance on antioxidant enzyme activity and some biochemical attributes affected by growth stages and titanium concentrations.

Sources of variation	d.f	Superoxide dismutase	Catalase	Glutathione peroxidase	Malondialdehyde	Dityrosine	8-OH-DG	Protein	EC
Replication	3	11932.0250 ns	45.225000 ns	17.350667 ns	16.526250 ns	1.60156250 ns	0.289950ns	17.9626 ns	0.89091ns
Growth stage	1	28037.0250 ns	2325.625000**	4840.000**	7136.912250**	47.98290250**	0.299290 ns	683.9290**	0.2402ns
Titanium concentration	4	151912.4125**	1742.337500**	1319.567125**	1648.269625**	0.96392750 ns	0.411253 ns	1699.905**	0.0996 ns
Growth stage × Titanium concentration	4	175772.3375**	1592.937500**	718.613125**	1697.585375**	0.78990250 ns	0.601958 ns	1204.435**	0.2708 ns
Error	27	7300.321	112.05833	36.00233	20.97255	0.45799954	0.468644	31.31156	0.1909
C.V		6.79	6.34	5.61	6.56	6.11	6.91	6.33	1.42

*, **, and ns: significant at 0.05 and 0.01 probability level and not significant, respectively.

Table 2 - Main effects of growth stages and titanium concentrations on antioxidant enzyme activity and some biochemical attributes.

Treatments	Superoxide dismutase	Catalase	Glutathione peroxidase	Malondialdehyde	Dityrosine	8-OH-DG	Protein	EC
Growth stages								
Four leafy	1231.20a	174.350a	117.940a	83.135a	12.1550a	9.9860a	84.125b	30.6200a
Stem elongation	1284.15a	159.100b	95.940b	54.420b	9.9645b	9.8130a	92.395a	30.7750a
Titanium								
Titanium dioxide 0.01	1367.63 ab	178.125 a	101.500 c	57.313 c	10.4525 b	9.6475 a	80.050 c	30.7375 a
Titanium dioxide 0.02	1282.88 b	171.250 a	116.588 b	63.538 b	11.2538 a	10.0375 a	112.650 a	30.5375 a
Titanium dioxide 0.03	1407.75 a	181.625 a	123.263 a	57.575 c	11.1125 ab	9.7650 a	90.625 b	30.8375 a
Bulk titanium	1111.00 c	154.750 b	102.150 c	86.513 a	11.1775 ab	9.8313 a	76.738 c	30.6500 a
Control	1119.13 c	147.875 b	91.200 d	83.950 a	11.3025 a	10.2163 a	81.238 c	30.7250 a

Values within the each column and followed by the same letter are not different at $P < 0.05$ by an ANOVA protected Duncan's Multiple Range Test.

and titanium concentration was significant on all assayed traits except for dityrosine, 8-hydroxyguanosine and membrane stability (Table 1). It is necessary to remark that, 8-hydroxyguanosine and membrane stability were not affected neither by growth stage nor by titanium application. Comparison of main effects is given in Table 2. There were significant differences between growing stages on catalase and glutathione peroxidase activity, malondialdehyde content, dityrosine and protein (Table 2). The results indicated that titanium dioxide nanoparticle application increased antioxidant enzyme activity and decreased malondialdehyde content compared with titanium oxide (bulk) or control treatment (Table 2). As can be seen from Table 3, the highest superoxide dismutase activity was obtained when titanium dioxide nanoparticle (0.03%) was applied at four leafy stage. On the contrary the lowest activity was observed in those plants which were treated with titanium oxide (bulk) at four leafy stage and control treatment (Table 3). Similarly, the highest catalase activity was related to titanium dioxide nanoparticle (0.03%) treatment that was sprayed on plants at four leafy stage (Table 3). By contrast, the lowest activity was found from treated plants with titanium oxide (bulk) and control treatment. Glutathione peroxidase activity was strongly affected by titanium dioxide nanoparticle (0.02 and 0.03%) application at four leaf stage. It is interesting to remark that application of titanium dioxide nanoparticle at stem elongation stage had not significant difference with control treatment (Table 3). Malondialdehyde as final product of lipid peroxidation significantly decreased when titanium dioxide nanoparticle was used at four leafy or stem elongation stages. The highest malondialdehyde content was achieved from titanium oxide (bulk) treatment and control plots. Obtained results from protein assay were almost erratic. In brief the highest protein was found when titanium dioxide nanoparticle (0.02%) was applied at four leafy stage while the lowest was observed from titanium dioxide nanoparticle (0.01%), titanium oxide (bulk) and control treatments.

Discussion

In our study, the activity of superoxide dismutase, catalase and glutathione peroxidase increased on account of titanium dioxide nanoparticles (0.03%) at four leafy stage of corn. There is lack of data referring to influence of titanium application on antioxidant enzymes activity in the literature. Nonetheless, Lu et al (2002) have shown that nano sized TiO_2 could in-

Table 3 - Interaction effects of growth stages and titanium concentrations on antioxidant enzyme activity and some biochemical attributes.

Growth stages	Titanium	Superoxide dismutase	Catalase	Glutathione peroxidase	Malondialdehyde	Dityrosine	8-OH-DG	Protein	EC
Four leafy stage									
	Titanium dioxide 0.01	1314.25 bc	187.000 b	109.675 b	66.800 bc	12.0500 a	9.9275 a	67.225 d	30.6750 a
	Titanium dioxide 0.02	1344.75 bc	189.250 b	140.175 a	67.900 b	12.3000 a	10.3950 a	127.875 a	30.2250 a
	Titanium dioxide 0.03	1588.25 a	206.000 a	140.175 a	53.775 ef	12.2750 a	9.9725 a	91.425 bc	30.8750 a
	Bulk titanium	966.00 d	146.750 ed	109.500 b	112.900 a	12.0250 a	9.5800 a	66.500 d	30.4500 a
	Control	942.75 d	142.750 e	90.175 c	114.300 a	12.1250 a	10.0550 a	67.600 d	30.8750 a
Stem elongation stage									
	Titanium dioxide 0.01	1421.00 b	169.250 c	93.325 c	47.825 f	8.8550 c	9.3675 a	92.875 bc	30.8000 a
	Titanium dioxide 0.02	1221.00 c	162.750 cd	93.000 c	53.600 ef	10.2075 b	9.6800 a	97.425 b	30.8500 a
	Titanium dioxide 0.03	1227.25 c	157.250 cde	106.350 b	61.375 bcd	9.9500 b	9.5575 a	89.825 bcc	30.8000 a
	Bulk titanium	1256.00 c	153.250 cde	98.800 c	60.125 cde	10.3300 b	10.0825 a	86.975 c	30.8500 a
	Control	1259.50 bc	153.000 cde	92.225 c	59.175 ed	10.4800 b	10.3775 a	94.875 bc	30.5750 a

Values within the each column and followed by the same letter are not different at $P < 0.05$ by an ANOVA protected Duncan's Multiple Range Test.

crease the soybean ability to absorb and utilize water and fertilizer, promote its antioxidant system, and in fact accelerate its growth. In addition, the effect of two titanium compounds, titanium ascorbate and titanium chloride, on some enzymatic activities, such as catalase, peroxidase, lipoxygenase and nitrate reductase in seeds, embryos, and seedlings and adult plants of red pepper (*Capsicum annuum* L.), have been studied by Carvajal et al (1994). A stimulatory effect of titanium they have reported for every iron-depending enzyme studied at all developing stages as well as for nitrate reductase but only for whole plants. In current study, it seems that titanium nanoparticles are able to promote antioxidant enzymatic system and conserve of plants against generated reactive oxygen species.

Decrease in malondialdehyde content may be due to positive effect of titanium to help antioxidant system for scavenging or neutralizing of free radicals. This finding proves that titanium is involving in lipid peroxidation and membrane stability process.

Protein content increased because of titanium application. This improvement in the protein is explained as a consequence of the beneficial effect of titanium on the absorption, translocation and assimilation processes. It has been reported that titanium application significantly increased Ca, iron (Fe), copper (Cu), and zinc (Zn) concentrations (Alcaraz-Lopeza et al, 2004) and improve plant growth (Zheng et al, 2005).

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