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Influence of Bulk and Nanoparticles Titanium Foliar Application on some Agronomic Traits, Seed Gluten and Starch Contents of Wheat Subjected to Water Deficit Stress

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Abstract

Titanium (Ti) is a very interesting chemical element, especially physiologically. Although Ti is not toxic for animals and humans, its effects on plants show remarkable concentration dependence. Whereas for plants, it shows beneficial effects on various physiological parameters at low doses. This study was conducted to evaluate the effect of bulk and nanoparticles titanium foliar application on some agronomic traits, seed gluten and starch contents of wheat under water deficit stress conditions during 2010-2011 growing seasons. The experimental design was randomized in complete blocks arranged in split-split plots with four replications. The factors included normal irrigation, water deficit stress (irrigation withholding at two growing stages of stem elongation and flowering), two growing stages for water deficit stress induction and titanium applications, five titanium concentrations, sources including control of titanium oxide (bulk), and three concentrations of 0.01%, 0.02%, and 0.03% of titanium dioxide nanoparticles. Plant height, ear weight, ear number, seed number, 1000-seed weight, final yield, biomass, harvest index, gluten, and starch contents were assayed. The results showed that water deficit stress caused significant decrease in plant growth, yield and yield components. In addition, among the different titanium treatments, titanium dioxide nanoparticles at 0.02% increased almost all agronomic traits including gluten and starch content. Thus, the application of titanium dioxide nanoparticles under conditions of water deficit stress is recommended.

Keywords: gluten, starch, titanium dioxide, wheat, yield and yield components

Introduction

Titanium is a chemical element with the symbol Ti and the atomic number 22. It has a low density and is a strong, lustrous, corrosion-resistant transition metal with a silver colour. Titanium has significant biological effects on plants, being beneficial at low levels but toxic at higher concentrations. Although titanium is not toxic for animals and humans, its effects on plants and bacteria show noteworthy concentration dependence. Whereas for bacteria it acts as an antibiotic (Yaghoubi *et al.*, 2000), for plants, it shows beneficial effects on various physiological parameters at low doses [e.g. biomass yield (Pais, 1993), essential element contents (Giménez *et al.*, 1990), chlorophyll contents (Carvajal *et al.*, 1994)] and is toxic at higher levels [chlorosis, retardation of growth (Hruby *et al.*, 2002)]. Although titanium is beneficial, it is not essential; titanium deficiency does not exist. Beneficial effects are often exploited by adding titanium to various complex micronutrient fertilizers. 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. Pais (1983) carried out numerous field experiments and found that titanium-chelate-treated apple trees yielded the fruits with higher soluble solids, sugar and acid content. The positive effects of titanium treatment were found on rape plant development (an increase of chlorophyll content and photosynthesis intensity), the yield and mass of a thousand seeds of winter wheat, and the yield and sugar content in sugar beets (Grenda, 2003). The most important effects of titanium on plants are enhancement of the yield, an improvement of some essential element contents in plant tissues, and an enhancement of the chlorophyll content in paprika (*Capsicum anuum* L.) (Hruby *et al.*, 2002). Plants interact with their atmospheric and edaphic environments strongly and are expected to be affected by exposure to engineered nanoparticles (Ruffini Castiglione and Cremonini, 2009). Lu *et al.* (2002) have shown that a combination of nano-sized SiO_2 and TiO_2 could increase the nitrate reductase enzyme in soybean (*Glycine max* L.), increase its abilities of absorbing and utilizing water and fertilizer, promote its antioxidant system, and, in fact, accelerate its germination and growth. Also, it has been found that TiO_2 nanoparticles encourage spinach (*Spinacia oleracea)* seed germination and plant growth (Zheng *et al.*, 2005). Navarro *et al.* (2008) stated that engineered nanoparticles could sequester nutrients on their surfaces, and thus, serve as a nutrient stock to the organisms, particularly those engineered nanoparticles having high specific surface area.

Whereas, in the literature there is little evidence on the effect of titanium on crop plants, we have studied the influence of titanium dioxide nanoparticles and common titanium oxide (bulk) on growth, yield and yield components, and also on gluten and starch of wheat under conditions of water deficit stress. The objective of this study, therefore, is to evaluate the response of wheat plant to titanium and water deficit stress.

Materials and methods

The field experiment was conducted at Shahriar, Iran (35°39′52″N, 51°03′33″E) during the 2010-2011 growing season. The yearly average precipitation (30 years long-term period) was 244 mm, mostly concentrated during the autumn and winter months (November to February). The experimental design was randomized complete blocks arranged in spilt-split plot with four replications. Irrigation was the main factor at two levels, normal irrigation, water deficit stress (irrigation withholding at two growing stages of stem elongation and flowering). Plant growth stages were considered as sub-factors and, finally, different doses of titanium (Control, 0.01%, 0.02%, 0.03% titanium nanoparticle and bulk titanium) were allocated to sub-sub plots. After plowing and disks in autumn, 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 were measured as 7.7 and 3.4 mm hos cm^{-1}, respectively. When plots were prepared there were 80 plots with 2 m width and 4 m length. There was a 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. Wheat seeds (*Triticum aestivum* L. c.v 'Pishtaz') were sown by hand on 15^{th} November at a depth of 3 cm. The planting density was 350 individuals per square meter. Irrigation was performed as furrow irrigation immediately. Second irrigation was done 3 days after the first one to get the best seed germination and seedling establishment. Weeds were controlled manually during the growing season. Water deficit stress treatment was induced by irrigation withholding in spring at two different stages, stem elongation and flowering. It is worth mentioning that nitrogen fertilizer (300 kg ha^{-1} urea) was applied at the tillering and booting stages. In order to prepare titanium dioxide nanoparticles, 20 g titanium dioxide was dissolved into water and then 0.01 ml of solution was filled up to 1000 ml. Thus, different concentrations of titanium dioxide (0.01%, 0.02%, and 0.03%) were prepared. An ultrasound instrument was used to homogenize the solution. Bulk titanium dioxide was purchased from Advanced Material Company (United States). To make bulk solution, 6 g solid of titanium was dissolved in 100 ml distilled water, then 1 ml of solution was filled up to 1000 ml. Titanium dioxide nanoparticles were sprayed on plants using a calibrated pressurized backpack sprayer (capacity 20 l) at the stem elongation and flowering stages at the end of water deficit stress induction. Plants were treated with 240 ml titanium solution per square meter. Control plants were treated with distilled water. At the end of the growing season, crop was harvested and the following traits were investigated: Plant height, ear weight per square meter, ear number per square meter, seed number per square meter, 1000 seed weight, final yield, biomass, and harvest index. Gluten and starch were measured according to the methods of Graveland and Henderson (2000) and Fulai *et al.* (2004), respectively. In order to purify gluten, 50 g of wheat flour was washed by chloroform to de-lipidation and then dissolved in 600 ml distilled water. The mixture was centrifuged at 1500x*g* for 10 min; sediment was dissolved in 0.5 M acetic acid. After adding 600 ml distilled water, pH was adjusted on 3.9. After 1 h, the samples were centrifuged at 500x*g* again for 10 min. Sediment was dissolved in acetic acid and used for electrophoresis procedures. In brief, after preparing SDS-PAGE, the extracted gluten was dissolved in Tris buffer, then loaded and run onto the gel. After about 100 min, the bands were fixed by trichloroacetic acid solution. After dying the gluten and standard bands, gluten concentration was estimated using a specific scanner. Starch content was estimated based on spectrophotometry. Thus, 50 g of wheat flour was washed by ethanol (80%) and, after boiling for 15 min, the samples were centrifuged at 20,000x*g* for 10 min. Sediment was dissolved in dichloromethane and centrifuged again at 4000x*g* for 10 min. Supernatant was removed gently and dried at 45°C. What remained was dissolved in distilled water and was passed from the hydroxyl column in order to separate the sugars. Remained starch was dissolved in sodium phosphate buffer and digested by digestive solution containing 100 ml of 15 mM $MgCl_2$ and 2.5 unit amyloglucosidase enzyme for 1 h at 55°C. Released glucose reacted with the amyloglucosidase enzyme and, after composing with dianisidine solution, the reaction was stopped using sulphuric acid. Absorbance was read at 540 nm and starch content was calculated according to the standard curve. Analysis of variance were carried out using the GLM procedure (SAS Institute, 2002), assuming that the residuals were random, homogenous and with a normal distribution about a mean of zero. Treatment means were compared using LSMEANS ($p \leq 0.05$).

Results and discussion

The results showed that the individual effect of irrigation, the combined effect of growth stages and titanium concentrations, and the combined effect of irrigation and growth stages had a significant impact on plant height. In addition, the triple combined effect was significant (Tab. 1). Comparison of means demonstrated that water deficit stress considerably decreased plant height (Tab. 2), although there was no significant difference between the two growing stages regarding plant height (Tab. 2). In the case of titanium application, the highest plants were harvested from the control plot, whereas the shortest plants were found in those plots which were treated with titanium dioxide 0.01% (Tab. 2). When it comes to the combined effects, results show that the highest plants were observed in non-stressed plots. By contrast, when titanium dioxide (0.01%) was applied on stressed plants during the flowering stage, the shortest plants were produced (Tab. 3). The results were strongly dependent on water deficit stress and growing stages, but we generalized some trends. The effect of water deficit stress was obvious on all the traits of wheat. Plant height was significantly affected by water stress. Plant height plays an important role in photosynthesis. Malik and Hassan (2002) and Khanzada *et al.* (2001) have earlier reported that shoot length was significantly reduced under water stress. Similarly, Inamullah *et al.* (1999) also observed that plant height in wheat varieties reduced significantly under water deficit stress when it was compared with irrigated plants.

The individual effect of titanium concentrations, the combined effect of irrigation and titanium concentrations, and the combined effect of growth stages and titanium concentrations were significant (Tab. 1). As can be seen from Tab. 2, there is no significant difference between complete irrigation and withholding irrigation regarding ear weight per square meter. Furthermore, the two growing stages were not statistically different. The maximum ear weight was observed when titanium dioxide nanoparticles (0.02%) were applied on plants; however, there was no significant difference between this treatment and titanium oxide (bulk) treatment (Tab. 2). The maximum and minimum ear weight per square meter were obtained when non-stressed plants were treated with titanium dioxide nanoparticles (0.02%) and titanium dioxide nanoparticles (0.01%) at the flowering stage, respectively (Tab. 3). Ear number per square meter was significantly affected by irrigation withholding and decreased due to water stress. There was no significant difference between two growing stages regarding ear number (Tab. 2). In addition, titanium dioxide nanoparticles at 0.02% concentration increased ear number (Tab. 2). The combined effects among irrigation, growing stages, and titanium concentrations indicate that the most ears were produced when titanium dioxide nanoparticles (0.02%) were applied on non-stressed plants at the stem elongation stage (Tab. 3), whereas the least ears were obtained from stressed plants and no titanium application at the flowering stage (Tab. 3). Qadir *et al.* (1999) also found that water deficit stress reduced the spikelets per spike in wheat. Tompkins *et al.* (1991) reported the significant suppressive effect of water deficit stress on the number of seeds per ear. Khanzada *et al.* (2001) and Qadir *et al.* (1999) have earlier reported that water deficit stress throughout the vegetative and reproductive development caused a significant reduction in the number of seeds per ear in wheat. The effects of irrigation and titanium concentrations were significant on seed number per square meter. In addition, all combined effects were significant as well (Tab. 1). Water deficit stress led to the decrease in seed number per square meter during both growing stages; however, there was no significant difference between the two growing stages (Tab. 2). Titanium application, whether titanium dioxide nanoparticles or titanium oxide (bulk), increased seed number per square meter compared with control, although titanium dioxide nanoparticles (0.02%) produced the highest number of seeds (Tab. 2). When the effects of three experimental factors were combined, the highest seed number was obtained when titanium dioxide nanoparticles (0.02%) and titanium oxide (bulk) were applied on non-stressed plants at the stem elongation and flowering stages, respectively (Tab. 3). Water deficit stress and titanium application had significant effects on 1000 seed weight but the effect of the growing stage was not significant (Tab. 1). An interaction among these factors showed a significant effect as well. Irrigation withholding decreased 1000 seed weight at both growing stages, whereas there was no significant difference between the stem elongation and flowering stages (Tab. 2). Application of titanium dioxide nanoparticles increased 1000 seed weight compared to titanium oxide (bulk) or control treatment (Tab. 2). The highest 1000 seed weight was observed when non-stressed plants were treated with titanium dioxide nanoparticles (0.02%) at the flowering stage; on the other hand, the lowest 1000 seed weight was obtained from those plants which were not treated with titanium but stressed at the flowering stage (Tab. 3). Analysis of variance showed that all individual and combined effects were significant except for the combined effect of irrigation and growth stage (Tab. 1). Water deficit stress drastically decreased final seed yield. In addition, water deficit stress induction at the flowering stage led to a significant decrease in seed yield compared to stress at the stem elongation stage (Tab. 2). Thus, the flowering stage is more sensitive to water stress. Generally, titanium application caused seed yield increment (Tab. 2), however, titanium dioxide nanoparticles (0.02%) application was more effective in improving yield (Tab. 2). The highest seed yield was obtained when non-stressed plants were treated with titanium dioxide nanoparticles (0.02%) during the stem elongation stage (Tab. 3).

According to the analysis of variance (Tab. 1), irrigation and titanium application had a significant effect on

Tab. 1. Analysis of variance on some agronomic traits, gluten and starch of wheat affected by irrigation, growth stages and titanium concentrations

Sources of variation	d.f	Plant height	Ear weight	Ear number	Seed number	1000-seed weight	Final yield	Biomass	Harvest index	Gluten	Starch
Replication	3	10.3744700^{ns}	387024.399**	113146.5458**	242843788**	13.3790000**	11762391.39**	260232957.0**	17.2812567^{ns}	0.034633^{ns}	1.553792^{ns}
Irrigation	1	188.6822450**	513.439^{ns}	79191.1125**	187099726**	459.8405000**	5087126.28**	36896861.3**	487.9732050**	244.7900450**	1485.226125**
Error (a)	3	18.9931550	46124.142	16416.8458	33201530	2.3968333	478053.33	3056771.5	8.6634083	0.1825350	0.583792
Growth stage	1	11.5520000^{ns}	12517.756^{ns}	437.1125^{ns}	3950583^{ns}	0.760500^{ns}	871593.88*	48285888.8^{ns}	69.0432800**	0.0540800^{ns}	0.210125^{ns}
Irrigation × growth stage	1	61.7058450*	2848.049^{ns}	49750.3125**	55244714**	0.000000^{ns}	413036.91^{ns}	131220.0^{ns}	73.8432450**	0.0014450^{ns}	0.351125^{ns}
Error (b)	6	34.7121225	12038.195	8331.9125	14375568	1.5722500	72452.27	8162096.3	6.1423958	0.1177192	1.087292
Concentration	4	30.3010456^{ns}	42413.617**	22763.9188^{ns}	70046909**	1.6265625**	1412461.84**	35401656.0**	108.815863**	0.9993081**	9.067813**
Irrigation × concentration	4	47.7314294*	108359.281**	69908.5813**	180688827**	18.6295625**	2143772.01**	31397328.5**	21.0887363**	2.9593919**	9.831438**
Growth stage × concentration	4	56.5422969**	221222.379**	172586.1438**	358371140**	11.4733125**	5192796.38**	143408542.1**	27.2678800**	0.3335081^{ns}	1.954188^{ns}
Irrigation × growth stage × concentration	4	193.8440606**	62698.164**	47961.0313**	113982329**	6.4478125**	3104268.13**	6225254.8^{ns}	68.0248325**	1.1266919**	5.862063**
Error	48	13.779679	6033.332	3643.335	5335882	0.4183125	156055.86	4067289	3.995112	0.1989550	1.392625
C.V		6.14	9.25	9.64	8.48	1.82	8.63	10.12	8.21	4.44	1.73

*, ** and ns: significant at 0.05, 0.01 probability level and no significant, respectively.

Tab. 2. Individual effects of irrigation, growth stages and titanium concentrations on some agronomic traits, gluten and starch of wheat

Treatments	Plant height(cm)	Ear weight ($g.m^{-2}$)	Ear number	Seed number	1000-seed weight(g)	Final yield ($kg.ha^{-1}$)	Biomass ($kg.ha^{-1}$)	Harvest index(%)	Gluten(%)	Starch(%)
Irrigation										
Normal irrigation	61.9093a	841.48a	657.08a	28796a	37.7725a	4826.2a	20602.3a	26.8138a	11.77825a	72.3400a
Water deficit stress	58.8378b	836.42a	594.15b	25711b	32.9775b	4321.8b	19244.0b	21.8743b	8.27975b	63.7225b
Growth stages										
Stem elongation	59.993a	851.46a	627.95a	27017.6a	35.4725a	4678.38a	19146.2a	25.2730a	10.00300a	68.0825a
Flowering	60.754a	826.44a	623.28a	27462.0a	35.2275a	4469.63b	20700.0a	23.4150b	10.05500a	67.9800a
Titanium										
Titanium dioxide 0.01	58.581b	831.18b	629.44b	27103.6b	35.6625a	4538.8b	19838.4bc	25.4119b	10.3406a	68.7313a
Titanium dioxide 0.02	59.973ab	899.93a	675.31a	30145.6a	35.7375a	4896.1a	21153.9ab	28.3394a	10.2350ab	68.5875a
Titanium dioxide 0.03	59.789ab	836.51b	625.75b	27460.7b	35.3438ab	4670.2ab	17782.4c	23.6744c	9.9438bc	68.3250ab
Bulk titanium	61.733a	866.14ab	628.50b	27249.5b	35.0750b	4670.0ab	21481.4a	22.1575d	9.8819c	67.5563bc
Control	61.792a	760.98c	569.06c	24239.7c	35.0563b	4094.8c	17782.4d	22.1369d	9.7438c	66.9563c

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

Tab. 3. Combined effects of irrigation, growth stages and titanium concentrations on some agronomic traits, gluten and starch of wheat

Irrigation	Growth stages	Titanium	Plant height (cm)	Ear weight $(g.m^{-2})$	Ear number	Seed number	1000-seed weight(g)	Final yield $(kg.ha^{-1})$	Biomass $(kg.ha^{-1})$	Harvest index(%)	Gluten(%)	Starch(%)
Normal irrigation	Stem elongation	Titanium dioxide 0.01	60.36 bcd	883.7 def	647.3 cdef	27820 bcdef	37.35 d	4986 cd	20100 de	26.34 bc	12.29 a	73.95 a
		Titanium dioxide 0.02	59.48 cde	1226.0 a	990.3a	43900a	38.65 bc	6576 a	20800 de	38.28 a	12.04 a	71.28 e
		Titanium dioxide 0.03	57.91 def	1037.4 bc	706.8 cd	30810 bc	38.05 bdc	5684 b	1886 g	27.10 b	11.92 ab	71.47 de
		Bulk titanium	65.80 ab	763.3 fghi	452.3 ij	18880 j	37.05 de	4193 fgh	16770 f	25.23 bcd	11.34 c	72.00 cde
		Control	59.71 cd	674.1 hi	718.3 c	18810 j	38.25 bcd	3760 hi	15980 f	26.57 bc	11.18 c	72.93 abc
	Flowering	Titanium dioxide 0.01	64.48 abc	909.6 cdef	535.3 ghi	22380 ghij	39.00 b	3895 ghi	16790 f	27.56 b	12.10 a	71.38 e
		Titanium dioxide 0.02	58.92 cde	727.3 ghi	477.3 hij	20380 ij	40.78 a	4246 fgh	16740 f	25.67 bc	12.38 a	72.25 bcde
		Titanium dioxide 0.03	63.07 abcd	718.0 ghi	570.0 fgh	24870 efgh	37.60 cd	4102 fghi	17060 f	27.14 b	11.94 ab	71.75 cde
		Bulk titanium	60.36 bcd	658.6 i	895.8 b	40710 a	35.05 gh	6384 a	28610 a	22.73 def	11.44 bc	73.58 ab
		Control	68.50 a	785.0 efghi	605.5 defg	25810defg	35.95 ef	4430efg	20690 de	21.53 ef	11.17 c	72.83 abcd
Water deficit stress	Stem elongation	Titanium dioxide 0.01	59.92 cd	931.5 cde	690.3 cde	29810 bcd	34.00 gh	4951cde	22110 cd	22.45 def	8.760 ef	64.58 g
		Titanium dioxide 0.02	60.13 cd	1105.5 ab	619.3cdefg	26980cdef	34.68 gh	5327 bc	25180 b	21.30 ef	9.375 d	66.30 f
		Titanium dioxide 0.03	64.08 abc	813.8 defghi	649.0 cdef	28480 bcde	32.23 j	3968 ghi	18500 ef	20.99 f	7.925 hij	61.68 i
		Bulk titanium	53.37 fg	688.8 hi	572.0 fgh	23830 fghi	33.50 hi	3969 ghi	16350 f	24.12 cde	7.123 j	62.78 hi
		Control	58.83 cde	675.2 hi	520.3 ghij	20870 hij	31.35 jk	3555 i	16790 f	20.36 f	7.665 ij	63.88 gh
	Flowering	Titanium dioxide 0.01	49.57 g	850.9 dfeg	645.0 cdef	28410 bcde	32.30 ij	4323 fgh	20360 de	25.13 bcd	8.975 de	65.03 g
		Titanium dioxide 0.02	61.01 bcd	825.7 defgh	614.5 cdefg	29330 bcd	32.55 ij	4322 fgh	21890 cd	20.63 f	8.470 efg	64.53 g
		Titanium dioxide 0.03	54.10 efg	776.9 fghi	577.3 fgh	25960 defg	33.50 hi	4225 fgh	16680 f	26.95 bc	8.400 fgh	62.93 hi
		Bulk titanium	66.90 a	786.4 efghi	594.0 efg	25580 defg	34.70 gh	3941 ghi	24200 bc	16.47 g	7.590 ij	61.88 i
		Control	60.13 cd	941.3 cd	432.3 j	31800 b	30.98 k	4634 def	23970 bc	20.18 f	8.075 ghi	63.68 gh

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

biomass. Water deficit stress decreased biomass regardless of the growing stage (Tab. 2). Moreover, titanium dioxide nanoparticles (0.02%) and titanium oxide (bulk) application produced the highest biomass (Tab. 2). The highest biomass was obtained when non-stressed plants were treated with titanium oxide (bulk) during the flowering stage (Tab. 3). This is in agreement with Zheng *et al.* (2005), who reported that the significant effect of titanium nanoparticles on spinach is probably attributed to the small particle size, which allows its penetration into the seed during the treatment period. It seems that bulk titanium could not penetrate into the plants; therefore, the results were not as marked as those of the treatment with nanoparticles. Increase of growth and yield may be due to the positive effects of titanium in different cellular mechanisms. For instance, improvement of photosynthesis and increase in chlorophyll content are two possible reasons for this. Owolade *et al.* (2008) reported that the seed yield of cowpea (*Vigna unguiculata* Walp) was increased when treated (as foliar application) with nano-sized titanium dioxide. They concluded that it may be due to the photocatalyst ability of the nano-sized titanium dioxide, which leads to an increased photosynthetic rate. Similar yield increases were reported in rice with a corresponding reduction in the incidence *Curvularia* leaf spot and bacteria leaf blight disease (Chao and Choi, 2005). Harvest index was affected by all individual and combined effects (Tab. 1). Water deficit stress decreased harvest index especially at the flowering stage (Tab. 2). Titanium dioxide nanoparticles (0.02%) caused the maximum harvest index, whereas titanium oxide (bulk) and control treatments were related to the minimum (Tab. 2). When the effects of the experimental factors were combined, the maximum harvest index was related to non-stressed plants treated with titanium dioxide nanoparticles (0.02%) at the flowering stage (Tab. 3).

Irrigation withholding and titanium application had a significant effect on gluten content but the growing stage had no significant effect on this trait (Tab. 1). It is worth mentioning that the combined effect of irrigation, growing stages, and titanium concentrations was significant as well (Tab. 1). A drastic decrease in 1000 seed weight was recorded. This result is in agreement with those of Khan *et al.* (2005) and Qadir *et al.* (1999), who observed that 1000 seed weight of wheat was reduced due to water stress. It might be concluded from the results of this study that water deficit stress caused a significant reduction in vegetative growth, yield, and yield components of wheat. We have suggested in our recent work that titanium increases wheat growth, yield and yield components and improves gluten and starch content of the seeds. Among the different titanium treatments, titanium dioxide nanoparticles at 0.02% concentration produced the highest yield and yield components and improved gluten and starch content, especially under conditions of complete irrigation. Zheng *et al.* (2005) demonstrated that titanium nanoparticles helped the water absorption by the spinach and improved growth. So, their results showed that the growth of spinach plants greatly improved at 250-4,000 ppm nano titanium dioxide concentrations, but there was no improvement at higher concentrations. Similarly, we have found that when titanium concentration was more than 0.02%, plant growth and production would be decreased. This finding shows that the positive effect of titanium depends on its concentration. In addition, particle size plays an important role in absorption by plants. Because titanium oxide (bulk) has greater size than titanium dioxide nanoparticles, it could not be absorbed by plants as easily as the nanoparticles

Gluten decreased due to water deficit stress and increased on account of titanium dioxide nanoparticles application (Tab. 2). The highest gluten content was observed when non-stressed plants were treated with titanium dioxide nanoparticles either during the stem elongation stage or during the flowering stage (Tab. 3). Starch content was affected and decreased because of water deficit stress (Tab. 1 and 2). There was no significant difference between the growing stages in terms of starch content (Tab. 2). Titanium application increased starch content in comparison with control treatment (Tab. 2). The highest starch content was obtained when non-stressed plants were treated with titanium dioxide nanoparticles (0.01%) during the stem elongation stage (Tab. 3). Gluten and starch content were affected by water deficit stress so that water deficit stress caused a significant decrease in these qualitative traits. Zhao *et al.* (2009) demonstrated that protein components are very sensitive to drought stress. An increase of gluten and starch in treated plants with titanium may be due to the positive correlation between titanium application and photosynthesis rate so that titanium application improved CO_2 metabolism in spinach. This increase in photosynthesis was attributed to an improvement of rubisco activity (Gao *et al.*, 2006).

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