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Ameliorative effects of TiO_2 nanoparticles and sodium nitroprusside on seed germination and seedling growth of wheat under PEG-stimulated drought stress[1]

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ABSTRACT – Seed germination and early seedling growth are sensitive to drought stress in wheat. A factorial experiment was arranged based on a completely randomized design with three replicates to study the impacts of TiO_2 nanoparticles (TiO_2 NPs: 0, 500, 1000 and 2000 mg.L) and sodium nitroprusside (SNP: 0 and 100 µM), as NO donor, on seed germination and seedling growth of wheat under polyethylene glycol (PEG)-induced drought stress (0, -0.4 and -0.8 MPa). Our results revealed that PEG-stimulated drought stress significantly decreased germination percentage (GP), germination energy (GE), germination rate (GR), root length (RL), shoot length (SL), root fresh weight (RFW), shoot fresh weight (SFW) and vigor index (VI) but increased mean germination time (MGT) in wheat seeds. However, application of TiO_2 NPs and SNP alone or in combination significantly enhanced GP, GE, GR, RL, SL, RFW, SFW and VI up to 23.72%, 50%, 33.74%, 85.38%, 93.28%, 73%, 91.91% and 91.04% respectively, but significantly reduced MGT up to 28.36% under severe drought stress. Our results showed that application of TiO_2 NPs and SNP alone or in combination can significantly alleviate the adverse effects of PEG-stimulated drought stress on seed germination and early seedling growth of wheat.

Index terms: Nitric oxide, polyethylene glycol, titanium nanoparticles, *Triticum aestivum*, water stress.

Efeitos benéficos de nanopartículas de TiO_2 e nitroprussiato de sódio na germinação de sementes e no crescimento de plântulas de trigo sob estresse hídrico estimulado por PEG

RESUMO – A germinação de sementes e o crescimento inicial de plântulas são sensíveis ao estresse hídrico no trigo. O delineamento experimental foi inteiramente casualizado, com três repetições, para estudar os impactos de nanopartículas de TiO_2 (NPs de TiO_2: 0, 500, 1000 e 2000 mg.L) e nitroprussiato de sódio (SNP: 0 e 100 µM), como NO doador, na germinação de sementes e no crescimento de plântulas de trigo sob estresse hídrico induzido por polietilenoglicol (PEG) (0, -0,4 e -0,8 MPa). Nossos resultados revelaram que o estresse hídrico estimulado por PEG reduziu significativamente a porcentagem de germinação (GP), energia de germinação (GE), taxa de germinação (GR), comprimento de raiz (RL), comprimento da parte aérea (SL), peso fresco da raiz (RFW) peso (SFW) e índice de vigor (VI), mas aumento do tempo médio de germinação (MGT) em sementes de trigo. No entanto, a aplicação de TiO_2 NPs e SNP isoladamente ou em combinação aumentou significativamente GP, GE, GR, RL, SL, RFW, SFW e VI até 23,72%, 50%, 33,74%, 85,38%, 93,28%, 73%, 91,91 % e 91,04% respectivamente, mas reduziu significativamente o TGM até 28,36% sob estresse hídrico severo. Nossos resultados mostraram que a aplicação de NPs de TiO_2 e SNP isoladamente ou em combinação pode aliviar significativamente os efeitos adversos da seca causada por PEG na germinação de sementes e no crescimento inicial de plântulas de trigo.

Termos para indexação: óxido nítrico, polietilenoglicol, nanopartículas de titânio, *Triticum aestivum*, estresse hídrico.

Introduction

Titanium is the ninth most abundant element in the earth's crust (Feizi et al., 2012). TiO_2 is the oxide form of titanium and occurs naturally in the environment (Cox et al., 2016). TiO_2 NPs are used in many products such as paints, papers, inks,

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coatings, catalysts, solar cells, plastics, soaps, antimicrobial and antifungal agents, alloys, textiles, food products, toothpaste, sunscreen and cosmetics, medicines and pharmaceuticals (Shi et al., 2013). TiO_2 NPs are the most produced nanoparticles worldwide and it is critical to track their fate in the environment and assess their toxic or beneficial effects on plant life (Moll et al., 2016). Seed germination and early seedling growth tests are sensitive, fast and effective methods to evaluate the effects of nanoparticles on plants (Ma et al., 2010). Recently TiO_2 NPs have been reported to enhance seed germination and plant growth. For instance, Feizi et al. (2013) proved that germination rate, germination percentage, germination value, mean daily germination, vigor index and shoot dry weight of fennel seeds were considerably improved upon exposure to TiO_2 NPs. In an earlier study they also demonstrated that application of TiO_2 NPs improved wheat seed germination indices but only mean germination time was significantly affected by TiO_2 NPs compared to untreated control (Feizi et al., 2012). Mahmoodzadeh and Aghili (2014) concluded that TiO_2 NPs even in high concentrations promoted seed germination and seedling growth of wheat compared to untreated control while low concentrations had inhibitory effects on wheat germination indices. Jaberzadeh et al. (2013) reported that foliar application of TiO_2 NPs improved yield, yield components as well as qualitative traits in wheat under drought stress condition. It was observed that exposure to TiO_2 NPs improved germination percentage, germination rate, root and shoot length, fresh weight, vigor index and the chlorophyll content of the parsley seedlings (Dehkourdi and Mosavi, 2013).

Nitric oxide (NO) is an endogenous signaling molecule that plays a key role in seed germination of different species under stress condition (Xiong et al., 2010). Nitric oxide occurs naturally in plants via four routes including (i) nitric oxide synthase, (ii) by plasma membrane-bound nitrate reductase, (iii) by mitochondrial electron transport chain, or (iv) by nonenzymatic reactions (Popova and Tuan, 2010). Sodium nitroprusside (SNP) and S-nitrosoglutathione (GSNO) are the most widely used NO donors in plant studies (Kováčik et al., 2014). Many studies suggested that the application of exogenous NO in the form of sodium nitroprusside (SNP), as a NO donor, is involved in enhancing plant tolerance to abiotic stress. For example, wheat seedlings treated by SNP showed increased activities of antioxidant enzymes, reduced oxidative damage, enhanced photosynthesis rate, and lowered water loss under drought stress (Tan et al., 2008). SNP treatment improved wheat seedling growth, regulated relative water content and ameliorated the oxidative damage under drought condition (Tian and Lei, 2006). Seed pre-treatment with SNP was also proved to improve salt stress tolerance in wheat. It seems that stress tolerance is related to increased antioxidant activity (Duan et al., 2007). Zheng et al. (2009) noted that exogenous SNP treatment significantly induced seed germination, germination rate, coleoptile and radicle weight under severe salt stress in wheat. Exogenous application of SNP alleviated the inhibition of rice seed germination and seedling growth induced by cadmium stress via counteracting the harmful effects of cadmium on germination index, vigor index, root and shoot length and fresh weight (He et al., 2014).

The reduction of wheat yield, depending on time and severity of drought and also other types of biotic and abiotic stresses, varies from 10 to 90% of its potential yield under non-stressed condition (Reynolds et al., 2005). As a major limiting factor affect crop production worldwide, water shortage during germination stage results in a reduction or even complete inhibition of seedling emergence and establishment (Kaya et al., 2006). Many germination-related processes such as gene transcription and translation, respiration and energy metabolism, early reserve mobilization and DNA repair could also occur during seed treatment (Varier et al., 2010), although often limited due to reduced water supply compared to normal germination (Chen and Bradford, 2000; Li et al., 2005). It is essential to ameliorate the negative effects of drought stress for obtaining optimum crop yields (Ashraf and Rauf, 2001).

Polyethylene glycol (PEG-6000) has been widely used to induce water shortage stress under laboratory condition because it is less possible to be absorbed by plants and is not phytotoxic (Michel and Kaufmann, 1973; Kaya et al., 2006). Seed germination and root elongation are two standard criteria of phytotoxicity suggested by U.S. Environmental Protection Agency – USEPA (1996). Wheat (*Triticum aestivum* L.) was selected as plant material for the current study as recommended for the testing of chemicals by USEPA (1996) and Organization for Economic Cooperation and Development – OECD (2003). This study was conducted to examine the effects of TiO_2 NPs and SNP on seed germination and early seedling growth of wheat under PEG-stimulated drought stress.

Material and Methods

Plant material

Wheat (var. Pishgam) seeds were supplied by the Seed and Plant Improvement Institute (SPII), Karaj, Iran. Seeds were kept in dry place at room temperature prior to use. The average germination rates of the tested seeds were greater than 90%, as indicated by our preliminary study.

TiO_2 NP characterization

TiO_2 NPs were purchased from Iranian Nanomaterials Pioneers Company (NANOSANY), Mashhad, Iran. As reported by the manufacturer, TiO_2 NPs used in current study had the following features: primary size: 10-25 nm, surface area: 200-240 $m^2.g^{-1}$, pH: 6-6.5, bulk density: 0.24 $g.cm^{-3}$, true density: 3.9 $g.cm^{-3}$, and 99% purity.

Preparation of TiO_2 NP suspensions

Stock solution of TiO_2 NPs (2000 mg.L) were prepared by dissolving TiO_2 NPs in deionized water and dispersed by ultrasonic vibration (100 W, 40 kHz) for 30 min. TiO_2 NP suspensions at concentrations of 0, 500, 1000, and 2000 mg.L were prepared by dilution of the stock suspension with deionized water. The suspensions were stirred for 1 min by small magnetic bars to prevent the aggregation of nanoparticles.

Drought stress treatments

To simulate moderate and severe drought stress conditions, osmotic stress was imposed by using PEG-6000 solutions at -0.4 and -0.8 MPa, as described by Michel and Kaufman (1973). The treatment with deionized water served as control. Then seeds were exposed to different concentrations of TiO_2 NPs (0, 500, 1000, and 2000 mg.L) and SNP (0 and 100 µM) alone or in combination.

Seed germination test

Wheat seeds were randomly selected and soaked in 5% sodium hypochlorite solution for 10 min and washed several times with distilled water to ensure surface sterility (USEPA, 1996). Sterilized seeds were evenly placed on 9 cm Petri dishes containing filter papers moistened with 9 mL of test solutions. Petri dishes were sealed with tape to prevent evaporation and stored at germinator under dark at 25 ± 1 °C for 7 days. Germination data were collected daily following International Rules for Seed Testing Association – ISTA (1976). Seeds were considered germinated once the radicle was 2 mm long (ISTA, 2009). At the end of experiment, length and fresh weight of roots and shoots were measured by ruler and digital balance respectively.

Germination percent was computed by following equation: $(GP) = (G_f/N) \times 100$.

Where, G_f is the total number of germinated seeds at the end of experiment and N is the total number of seeds used in the test.

Germination energy was recorded as describe by Amooaghaie et al. (2015): (GE) = number of germinated seeds after 3 days/number of total seeds.

Germination rate was determined based on Maguire (1982): $(GR) = (a/1) + (b-a/2) + (c-b/3) + ... + (n-n-1/N)$

Where, a, b, c and n are numbers of germinated seeds after 1, 2, 3 and N days from the start of imbibition.

Mean germination time was computed according to Ellis and Roberts (1981): $(MGT) = \Sigma N_i D_i / T$

Where, N_i is number of germinated seeds till i^{th} day and D_i is number of days from start of experiment till i^{th} counting and T is total germinated seeds.

Vigor index was calculated based on Vashisth and Nagarajan (2010): (VI) = germination % × seedling length (root + shoot).

Experimental design and statistical analysis

A factorial experiment was arranged based on a completely randomized design (CRD). All germination tests were performed in triplicate and data were reported as the mean of three replicates. To compare treatments, least significant difference (LSD) test performed using the *Statistical Analysis System* (SAS, version 9.3). In all cases, $p < 0.05$ was considered significant.

Results and Discussion

As it could be inferred from Figure 1A, TiO_2 NPs (500 and 2000 mg.L) in combination with SNP significantly promoted seed germination compared to untreated control under normal condition, whereas TiO_2 NPs or SNP treatments alone, had no significant impact on germination percentage of non-stressed seeds. PEG-induced moderate (-0.4 MPa) and severe (-0.8 MPa) drought stress resulted in significant reductions in germination percentage. However, application of different concentrations of TiO_2 NPs and SNP alone or in combination, significantly improved seed germination percentage up to 15% and 23.72% under moderate (-0.4 MPa) and severe (-0.8 MPa) drought stress stimulated by PEG solutions (Figure 1A). As shown in Figure 1B, germination energy significantly increased in non-stressed seeds by adding 500 mg.L TiO_2 NPs and SNP to the test medium, but application of TiO_2 NPs at 1000 and 2000 mg.L and SNP alone or in combination significantly reduced germination energy in wheat seeds under control condition. However, application of SNP alone or in combination with different concentrations of TiO_2 NPs enhanced germination energy up to 57.40% and 50% under moderate and severe drought stress induced by PEG solutions (Figure 1B). Application of TiO_2 NPs at 1000 mg.L or SNP alone or in combination significantly reduced germination rate in wheat seeds under normal condition. However, under moderate and severe drought stress application of TiO_2 NPs in combination

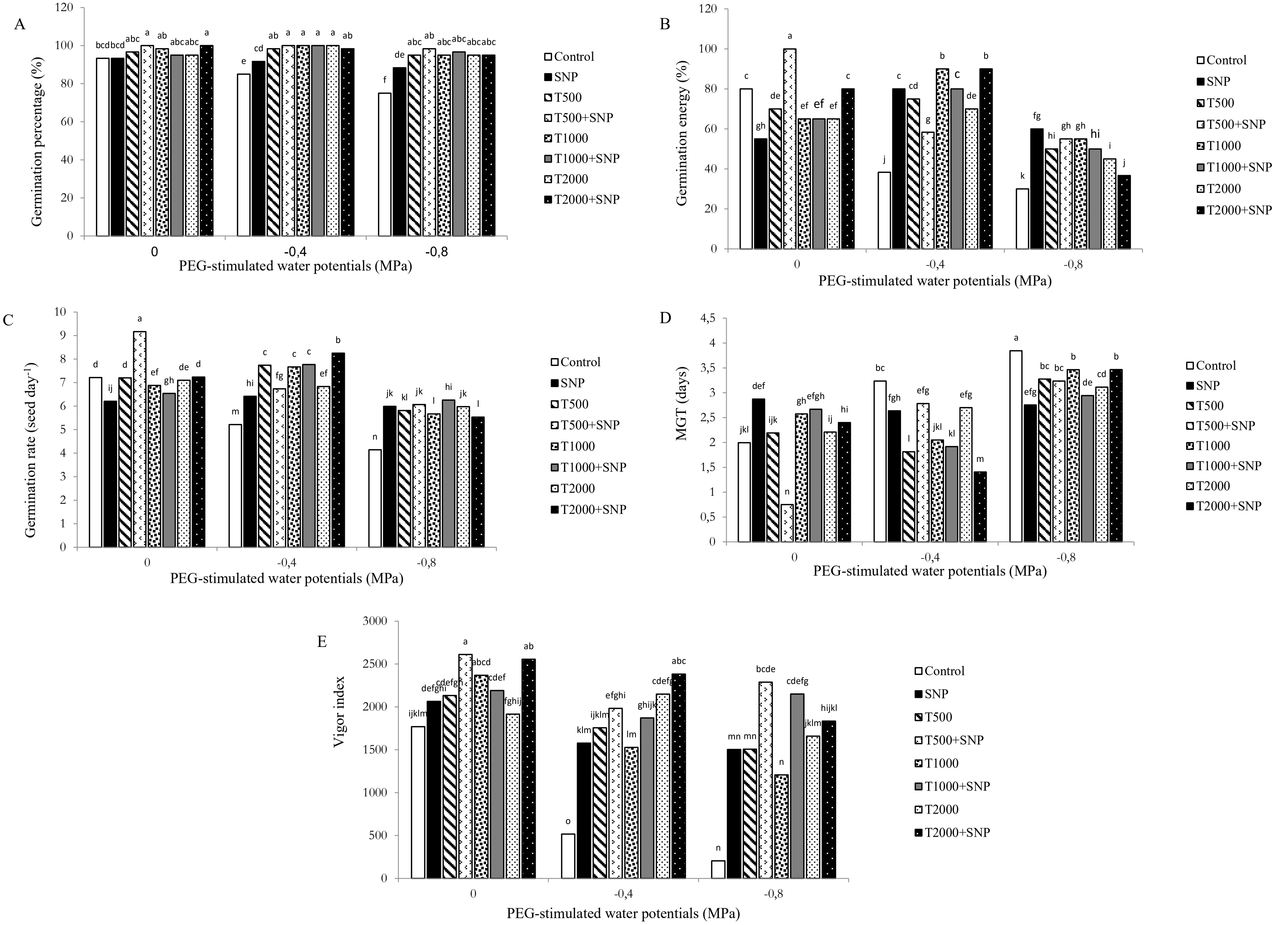

Figure 1. Impacts of TiO_2 NPs (T: 500, 1000 and 2000 mg.L) and SNP (100 µM) suspensions on A) Germination percentage, B) Germination energy, C) Germination rate, D) Mean germination time (MGT) and E) Vigor index of wheat under PEG-stimulated drought stress. Data are the mean of three replicates. Different letters represent significant differences between the treatment means at $p < 0.05$.

with SNP significantly enhanced germination rate up to 36.76% and 33.74% respectively (Figure 1C). Under non-stressed condition, only application of TiO_2 NPs at 500 mg.L in combination with SNP significantly shortened mean germination time. But under moderate and severe drought stress condition, application of different concentrations of TiO_2 NPs and SNP alone or in combination decreased mean germination time up to 56.56% and 28.36% in wheat seeds (Figure 1D). Drought stress induced by PEG solutions significantly decreased the length of roots and shoots. Under control condition, application of TiO_2 NPs at 2000 mg.L or SNP alone had no significant positive effect on root length, but application of TiO_2 NPs at 500 and 1000 mg.L alone or application of different concentrations of TiO_2 in combination with SNP significantly enhanced root length. In presence of moderate and severe drought stress created by PEG solutions, root length significantly increased up to 67.20% and 85.38% when seeds were treated by TiO_2 NPs and SNP alone or in combination (Figure 2A). Under non-stressed condition, no significant positive change observed in shoots length by application of TiO_2 NPs at 500 mg.L alone, but application of TiO_2 NPs at 1000 and 2000 mg.L or SNP alone or application of different concentrations of TiO_2 in combination with SNP significantly increased shoot length. Application of TiO_2 NPs in combination with SNP, significantly increased shoot length up to 82.55% and 93.28% under moderate and severe PEG-stimulated drought stress (Figure 2B). PEG-simulated water shortage triggered significant reductions in fresh weight of roots and shoots followed by a dose-dependent manner. In absence of water stress, root fresh weight showed no significant change in wheat seeds treated by TiO_2 NPs or SNP alone. However, application of TiO_2 NPs (500 and 2000 mg.L) in combination with SNP significantly increased root fresh weight under control condition. Under moderate and severe drought stress, root fresh weight significantly increased up to 65.41% and 73% respectively, by TiO_2 in combination with SNP treatments (Figure 2C). Under non-stressed condition, shoot fresh weight significantly enhanced by application of TiO_2 NPs and SNP alone or in combination. Shoot fresh weight significantly improved up to 85.76% and 91.91% respectively, under moderate and severe PEG-induced drought stress (Figure 2D). Under control condition, application of TiO_2 NPs at 2000 mg.L or SNP alone had no significant positive impact on vigor index but application of TiO_2 NPs at 500 and 1000 mg.L alone or in combination with SNP significantly enhanced vigor index in wheat seeds. However, application of TiO_2 NPs and SNP alone or in combination, significantly enhanced vigor index up to 78.29% and 91.04% under moderate and severe PEG-stimulated drought stress respectively (Figure 1E).

PEG-induced drought stress significantly reduced seed germination and early seedling growth of wheat which is fully consistent with the results of previous reports (Kaya et al., 2006). However, application of TiO_2 NPs alone or in combination with SNP treatments promoted seed germination and seedling growth in wheat under normal and stress conditions (Figures 1 and 2). Improved seed germination and seedling growth have been reported in wheat upon exposure to TiO_2 NPs (Feizi et al., 2012; Jaberzadeh et al., 2013; Mahmoodzadeh and Aghili, 2014). Khot et al., 2012 reported that TiO_2 NPs may induce oxidation-reduction reactions by the superoxide ion radical during germination, resulting in scavenging free radicals in the germinating seeds. On the other hand, oxygen produced in such process could also be consumed in respiration, which would further enhance germination. Nanoparticles may create large new pores in the seed coat, facilitate the process of water uptake inside the seed embryo and finally accelerate germination rate (Navarro et al., 2008). It was also shown that nanoparticles are capable to enlarge root pores or create new ones, leading to higher water and nutrient uptake by the roots and consequently improved root and shoot growth (Asli and Neumann, 2009). Increased growth of roots and shoots has been reported in TiO_2 NPs-treated wheat plants (Larue et al., 2012). It seems that TiO_2 NPs may regulate plant growth and play a role similar to plant hormones such as cytokinin and gibberellin as indicated by ability to induce plant cell division and cellular development (Sauret-Güeto et al., 2012). TiO_2 NPs may loosen cell wall and induce cell extension and growth of plant under drought stress (Mohammadi et al., 2016). TiO_2 NPs may also act as a fertilizer and enhance biomass accumulation by stimulating plant metabolic activities (Raliya et al., 2015). Increased water absorption by TiO_2 NPs causes enhanced nutrient uptake in plants leading to increased biomass production and plant height (Raliya et al., 2015). Marchiol et al. (2016) reported that TiO_2 NPs prolonged vegetative phase and increased dry matter accumulation in barley plants. The application of TiO_2 NPs significantly increased shoot and root fresh and dry weight and root area in *Zea mays* (Yaqoob et al., 2017). Protective effects of TiO_2 NPs may be due to antimicrobial properties of TiO_2 that improve plant tolerance against stress (Clément et al., 2013).

Our results showed that application of SNP alone or in combination with different concentrations of TiO_2 NPs promoted seed germination and early growth of wheat under control condition and drought stress created by PEG solutions (Figures 1 and 2). The growth-enhancing effect of SNP under drought stress has been reported in many crop species (Cechin et al., 2015). For example, improved wheat growth

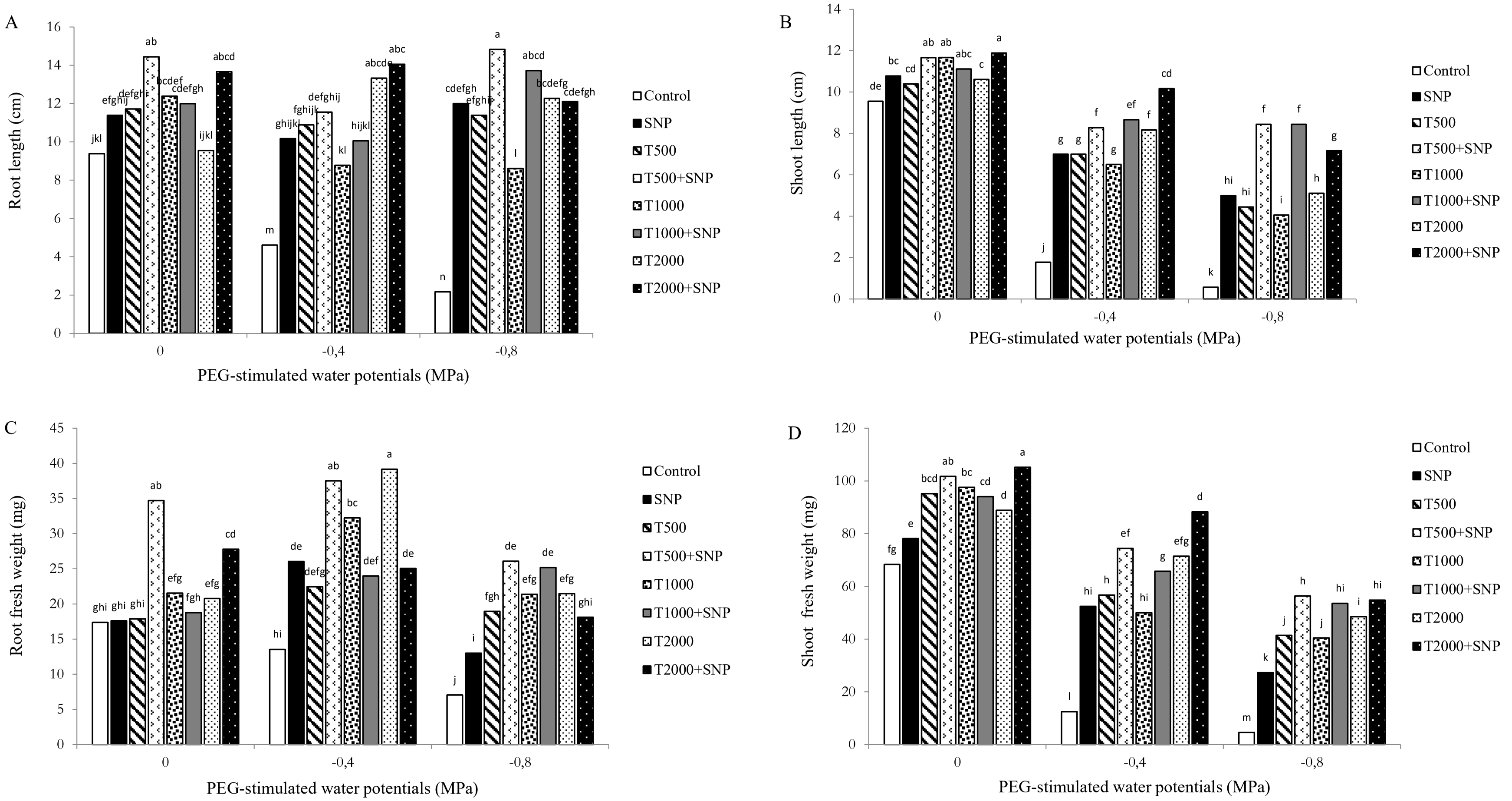

Figure 2. Impacts of TiO_2 NPs (T: 500, 1000 and 2000 mg.L) and SNP (100 μM) suspensions on A) Root length, B) Shoot length, C) Root fresh weight and D) Shoot fresh weight of wheat under PEG-stimulated drought stress. Data are the mean of three replicates. Different letters represent significant differences between the treatment means at $p < 0.05$.

parameters such as height and dry weight were observed under oxidative stress due to application of SNP (Tian et al., 2015). It is well known that SNP plays a key role in the regulation of phytohormones such as cytokinin, gibberellin and auxin which are required for cell elongation, cell division, and tissue differentiation, leading to optimal plant growth under stress condition (Mohamed et al., 2016). SNP promotes the uptake and transport of mineral elements during stress condition resulting in improved plant growth (Mohamed et al., 2016). It is known that SNP is responsible for embryo extension and reserve degradation under normal and osmotic stress conditions and plays a significant role in mobilization of α-amylase, β-amylase and protease in wheat seeds during early germination (Wu et al., 2013). Zheng et al. (2009) noted that exogenous SNP promoted seed germination and seedling growth in wheat under abiotic stress by increasing the metabolism of amylase and starch.

Conclusions

Our results showed that seed germination and early seedling growth of wheat were negatively affected by PEG-stimulated drought stress in a dose-dependent manner. However, application of TiO$_2$ NPs and SNP alone or in combination significantly improved wheat germination and growth under PEG-induced stress. Moreover, it was observed that protective impacts of TiO$_2$ NPs and SNP against drought stress enhanced when TiO$_2$ and SNP treatments were applied in combination. Nevertheless, growth-enhancing effects of TiO$_2$ and SNP treatments were more obvious under drought stress condition compared with non-stressed condition. In conclusion, it seems that application of TiO$_2$ NPs in combination with SNP can significantly alleviate the adverse effects of PEG-stimulated drought stress on seed germination and early seedling growth of wheat.

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