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Distribution and effects of ionic titanium application on energy partitioning and quantum yield of soybean under different light conditions

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Abstract

Soybean growth and development response to ionic titanium application have never been investigated. Therefore, such study is needed to better explain the titanium (Ti) application for soybean crop. For the first time, we studied the effects of application of two Ti concentrations (12.5 and 25 mg L^{-1}) on photosynthetic and chlorophyll (Chl) fluorescence parameters of soybean under normal light (NL) and shade conditions (SC). Compared to NL, SC significantly decreased Chl contents, leaf area (LA), leaf thickness (LT), plant dry mater (PDM), photosynthetic and Chl fluorescence parameters, total soluble sugar, and Ti uptake. Overall, Ti application (12.5 mg L^{-1}) had more distinct effects on LA, LT, PDM, Chl content and Chl fluorescence parameters. In conclusion, these results implied that an appropriate Ti content can improve plant morphological and anatomical features by enhancing the photosynthetic characteristics, especially under shade conditions.

Additional key words: anatomical structure; biomass; foliar application; *Glycine max*; photosynthesis; titanium uptake.

Introduction

Titanium (Ti) is the ninth most abundant element in the Earth's crust and makes up about 0.25% by moles and 0.57% by mass of the crust (Buettner *et al*. 2012). Its content in the soil is approximately 1–20 g kg^{-1} and the average is 6.8 g kg^{-1} (Zhang *et al.* 2012). Ti is the second most abundant transition metal, after Fe, and the elemental abundance of Ti is about five times lower than that of Fe and 100 times greater than that of Cu. Some studies have shown that Ti is a beneficial element for plant growth (Lyu *et al.* 2017) and in recent years, Ti is used as micronutrient in the agriculture. It can enhance the growth and yield of crops and seedlings about 10–20% (Feizi *et al*. 2013) and can increase the quality of fruits by enhancing their contents of protein, soluble sugar, and vitamin C. It increases crop physical strength and resistance to pesticide residue, drought, freezing, heat, and disease, which ultimately improves plant's ability to acquire fertilizers (Choi *et al.* 2015). However, its existence in an insoluble stable state (TiO_2) makes the use of Ti difficult and only small amount is absorbed by plants (Lyu *et al.* 2017). Ti salt solution is highly unstable and can only exist at high acidity. In order to increase its absorption by plants, it is necessary to provide soluble, non-sedimentary, and long-term stable Ti. Zhang *et al.* (2012) invented a continuous method and production device for producing hydrolysis-resistant stable ionic Ti (Ti^{4+}) which makes Ti^{4+} existing in a salts solution soluble, long-term stable, and non-sedimentary, so it could be easily absorbed by plants. However, to the best of our knowledge, there is no report about its physiological activities in plants. In addition, by

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Abbreviations: Chl – chlorophyll; C_i – intercellular CO_2 concentration; E – transpiration rate; g_s – stomatal conductance; LA – leaf area; LDM – leaf dry matter; LT – leaf thickness; NL – normal light; PDM – plant dry matter; P_N – net photosynthetic rate; q_p – coefficient of photochemical quenching; SC – shade condition; TiL – titanium in leaves; TiR – titanium in roots; TiS – titanium in stem; TSS – total soluble sugar; TTi – total titanium accumulation; $\Phi_{(f,D)}$ – quantum yield of constitutive nonregulatory nonphotochemical quenching; $\Phi_{(NPQ)}$ – quantum yield of light-induced regulatory nonphotochemical quenching; $\Phi_{(P)}$ – effective quantum yield of PSII; $\Phi_{(PSII)}$ – maximum quantum yield of PSII photochemistry.
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selecting the appropriate genotypes and nutrient contents, crop yield and quality could be increased under stress conditions (Raza *et al*. 2018).

Shade stress is one of common abiotic stresses, and plant suffers from it when grown in high density, greenhouse, intercropping, and agroforestry systems. Furthermore, air pollution, haze, and decreasing solar radiation lead to lesser PAR (Fu *et al.* 2015). Plants decrease net photosynthetic rate (P_N) and optimize the agronomic traits in order to cope with poor quality and quantity of light under shade stress. Shade influences plant life in multi-faceted ways, creating new and variable environmental settings. Shade has detrimental impacts on plant growth, such as a decrease in biomass, leaf area and thickness, stem diameter (Wu *et al.* 2017), and ultimately yield. In maize soybean intercropping, shade of maize limits photosynthesis and is considered as a major threat to soybean growth (Su *et al.* 2014). Recent studies have shown that shade blocks the energy transport from PSII to PSI, reduces leaf thickness, palisade and spongy tissues, and results in lower photosynthetic capacity (Wu *et al.* 2017, Yao *et al*. 2017). In case of tropical pastures, shade reduces the root growth and net assimilation rate (Ludlow *et al.* 2017). Furthermore, high planting density disrupts the photosynthesis by destroying chloroplasts and thylakoid structure of plant leaf (Ren *et al.* 2017).

To overcome shade stress, several practices have been suggested to optimize the plant growth and photosynthesis, such as the use of shade-tolerant cultivars (Menglu *et al.* 2016), plant growth regulators (Yan *et al.* 2015), and an appropriate NH^{4+}:NO_3^- ratios (Hu *et al.* 2017). However, the role of ionic Ti in plant biology, specifically, under shade stress, has not been investigated. Therefore, the current study was aimed to gain more understanding of the role of Ti in soybean. The objectives of this study were: *1*) to investigate morphological and anatomical changes in soybean leaves; *2*) to explore the changes in chloroplast structure, P_N, and Chl fluorescence parameters by Ti under light and shade conditions.

Materials and methods

Titanium ion solution characterization: Titanium, which has an atomic number 22 and atomic mass 47.88, is a transition element belonging to Group 4 (IVB) in the middle of the periodical table. Ti occurs at oxidation states of Ti^{2+}, Ti^{3+} (titanous), and Ti^{4+} (titanic), of which Ti^{2+} and Ti^{3+} are unstable, while Ti^{4+} is the most stable ion. In this experiment, we used ionic titanium (Ti^{4+}), which was provided by *Taigu Science and Technology Ltd.* (Tianjing, China). The concentration was 3.5 g(Ti) L^{-1}.

Plant growth, exposure, and experimental conditions: Soybean (*Glycine max* L.), cv. Nandou 032-4, was used in this study. The experiment was conducted in a greenhouse of Sichuan Agricultural University (29°59'N, 103°00'E). Seeds were first sterilized with 0.5% sodium hypochlorite solution for 15 min, and then germinated in the dark for 4 d in two plastic boxes. Seedlings were cultured hydroponically in full strength Hoagland nutrient solution in a growth chamber characterized by a 12-h dark/12-h light photoperiod, 28°C day/25°C night temperature, and approximately 60% relative humidity. When the first trifoliate leaf developed, the seedlings were transferred to shade and light conditions and different Ti treatments were applied. Following concentrations of Ti solution were used: 0, 12.5, and 25 mg L^{-1}, named as T_0, T_1, and T_2, respectively. The Ti was sprayed two times with the 7-d interval after emergence. Each treatment was replicated three times. Seedlings were allowed to grow for 30 d.

Light environment: Shade condition (SC) was provided by one layer of green filters (*Q-MAX 122*, Hampshire, UK) as 27% of full sunlight. The R:FR ratio under shade was (0.5–0.6). Normal light (NL) condition was provided by simply placing the pots in the growth chamber without the shade net.

Leaf area (LA) and leaf thickness (LT): Leaf area was measured by using (*CI-203 CID*, *Bio-Science Portable Instruments for Precision Plant Measurement Inc.*, WA, USA). Leaf thickness was measured by using *Image J2x 1.42q*.

Plant dry matter (PDM): Plants were sampled from each replicate for morphological analysis; stem and aboveground biomass were measured. All aboveground parts of soybean plants were exposed to 105°C for 1 h and dried to constant mass at 75°C to determine the biomass of the stems and leaves.

Photosynthetic characteristics and Chl content: The net photosynthetic rate (P_N), stomatal conductance (g_s), intercellular CO_2 (C_i), and transpiration (E) were measured with a portable photosynthesis system (*Model LI-6400*, *LI-COR Inc.*, Lincoln, NE). *SPAD-502* (*Minolta*, Japan) apparatus was used for the measurement of leaf Chl content. Latest fully expanded leaves were selected to measure photosynthetic parameters on plants between 08:00 and 11:00 h using following settings: PAR_i = 1,000 μmol(photon) m^{-2} s^{-1}, stomatal ratio = 0.5, flow = 500 μmol mol^{-1}, and reference CO_2 concentration was 400 μmol mol^{-1}. Physiological measurements were taken when P_N, g_s, and fluorescence were stable (P_N: slope < 1 and g_s: slope < 0.05 for 45 s).

Chl fluorescence parameters: Chl fluorescence technology (*FluorImager software*, *Technologia LTD version 2.2.2.2*) was used to investigate soybean response to ionic Ti both under light and shade conditions. We calculated: *1*) effective quantum yield of PSII, $\Phi_{(P)}$; *2*) quantum yield of light-induced regulatory nonphotochemical quenching, $\Phi_{(NPQ)}$; *3*) quantum yield of constitutive nonregulatory nonphotochemical quenching, $\Phi_{(f,D)}$; *4*) coefficient of photochemical quenching, q_P; and *5*) quantum yield of PSII photochemistry, $\Phi_{(PSII)}$, according to Humplík *et al.* (2015) and Lazár (2015).

Total soluble sugar (TSS) content was measured with anthrone colorimetric reagents method (Wu *et al.* 2017). Powder [0.1 g (DM)] of well-ground leaves was put into a 10-mL centrifuge tube with 10 mL of 80% ethanol. The tube was incubated in a water bath of 80°C for 30 min, and then centrifuged (3,000 × *g*, 15 min). The residue was extracted again twice using 80% ethanol. The three supernatants were combined and mixed. One mL of supernatant and one mL of distilled water were added into 10-mL test tube. Anthrone reagent (0.2%) of 4 mL was mixed with concentrated H_2SO_4 in a test tube and placed in boiling water bath for 15 min. After cooling, the absorbance was read at 620 nm with spectrophotometer (*MAPADA V1100D*, Shanghai, China) and soluble sugar content was calculated as: Total soluble sugar = (standard curve value × dilution ratio)/sample mass. The results were expressed as mg g^{-1}(DM).

Anatomical structure of leaves: The samples for anatomical structure of soybean leaves were taken after one month of treatment. Leaf segments (5 × 5 mm) without major veins were cut from each middle leaflet of three latest fully expanded leaves with a surgical scissors. The segments were fixed in a formaldehyde (FAA) solution (38% FAA/glacial acetic acid/70% alcohol, 5:5:90, v/v/v) at 4°C. The fixed segments were dehydrated in a graded alcohol and *n*-butyl alcohol series, embedded in paraffin, and cut by rotary microtome (*RM2235*, *Leica Microsystems Ltd*., Germany) at thickness of 10 µm. Light microscopy (*Nikon Eclipse50i*, Japan) was carried out with a 10-µm thick transverse section of the leaf stained with 0.5% safranine solution.

Chloroplast ultrastructure: The segments (0.5 × 0.5 cm) of soybean leaves were prefixed with a mixed solution of 3% glutaraldehyde, then the segments were post-fixed in 1% osmium tetroxide, dehydrated in acetone series, infiltrated, and embedded in *Epox 812*. The semi-thin sections were stained with methylene blue and ultrathin sections were cut with diamond knife, stained with uranyl acetate and lead citrate. Sections were examined with a transmission electron microscope (*H-600IV*, *Hitachi*, Japan).

Elemental analyses were performed to investigate Ti mobilization and accumulation in soybean plants. The concentration of Ti in the roots, stem, and leaves were analyzed by an inductively coupled plasma emission spectrometer (ICP-OES/ICP-MS, *Aurora M90, Bruker,* Bremen, Germany). The plant samples were dried, weighed (0.1 g), placed in a glass vial, and then digested by 2 ml of HNO_3 and 0.5 mL of H_2O_2 at a temperature of 150°C until the solution turned whitish; the solution was then evaporated and reduced to 1 ml. The digested samples were diluted by 10 mL of 2% nitric acid and then filtered through 0.2-µm nylon filter, followed by 0.02-µm membrane filter paper. The final filtered solution was diluted three times before analysis by ICP-MS. The results were expressed as mg kg^{-1}.

Statistical analysis: All the data recorded for every trait were analyzed using *Statistix 8.1*. An analysis of variance (*ANOVA*) was used to determine the treatment effects on the measured variables. The least significance difference (LSD) test was performed to compare means at 5% probability level. *OriginPro 8.0* was used for the graphical presentation of the data.

Results

Leaf area (LA) and leaf thickness (LT): In our study, the highest LA (56.7 cm^2) and LT (208.6 µm) were measured under NL, while the lowest LA (29.6 cm^2) and LT (131.8 µm) were found under SC under control conditions. The Ti application had a considerable effect on LA and LT, as did the light and shade conditions. LA and LT of T_1 treatment significantly increased and the maximum LA (73.9 cm^2) and LT (411.2 µm) were obtained, The interactive effect showed that under NL and SC, T_1 treatment had the highest leaf area of 73.9 cm^2 and 38.5 cm^2. Interactive effects of Ti concentrations and light conditions for LA and LT were found to be significant (Fig. 1*A*,*B*). Overall, the T_1 increased LA and LT by 27 and 70%, respectively, compared with control treatment. In addition, anatomical structure of leaf revealed that Ti significantly affected the leaf thickness under NL and SC (Fig. 2).

Dry matter (DM): The Ti application and different light conditions showed a significant effect on plant and leaf dry matter (DM) accumulation. Similarly, different light conditions changed the pattern of DM distribution between leaves and stem. The maximum (0.9 g per plant) leaf dry matter (LDM) and (1.3 g per plant) plant dry matter (PDM) were noted under NL, whereas minimum (0.3 g per plant) LDM and (0.5 g per plant) PDM were measured under SC. However, LDM and PDM increased as the Ti content increased from T_0 to T_2. In NL, the highest LDM (1 g per plant) and PDM were noticed under treatment T_2, whereas the lowest LDM (0.5 g per plant) and PDM (0.8 g per plant) were recorded in T_0 treatment. Interactive results for different light conditions and Ti concentrations were found significant (Fig. 1*C,D).* On average, LDM and PDM increased by 31 and 29% in T_2 than T_0 treatment, respectively, suggesting that LDM and PDM were directly proportional with the increase in the leaf size.

Chl content: In this experiment, light conditions caused significant differences in Chl contents. As shown in Table 1, Chl content of soybean leaves in NL (30.4) was significantly higher than those of SC (26.1). Additionally, the Chl content significantly increased with the increase in Ti contents. The highest (31.2) and the lowest (24.5) Chl content was determined in treatment T_2 and T_0, respectively. The interactive effect of Ti concentrations and different light conditions was found significant. Relative to control treatment (T_0), soybean plants under T_2 reached 27% higher Chl content.

Photosynthetic characteristics: Different light conditions and Ti concentrations showed a significant impact

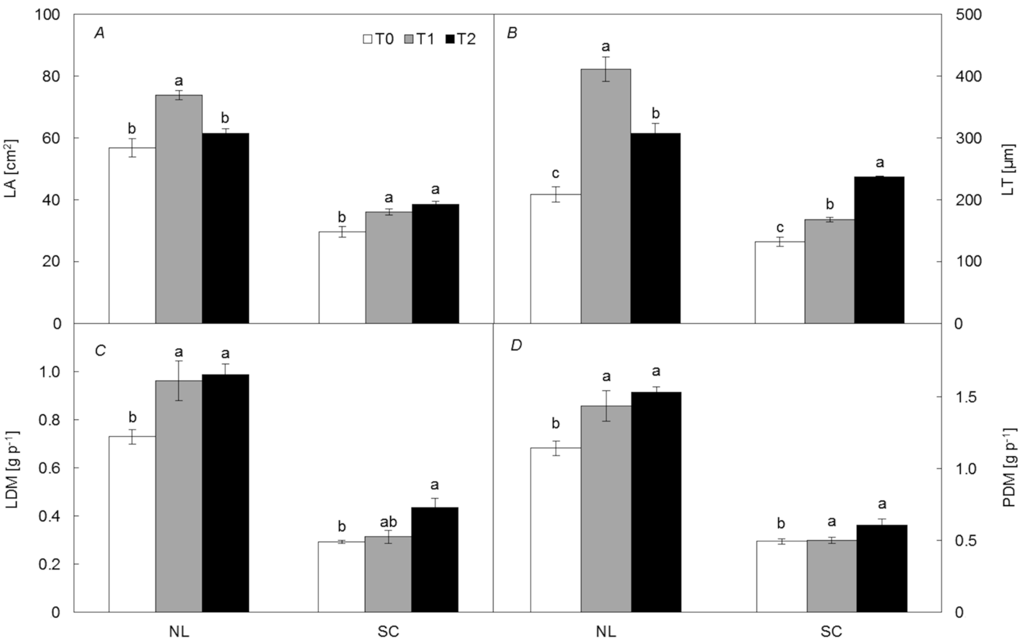

Fig. 1. Effect of titanium (Ti) on aerial parts of soybean seedlings under normal light (NL) and shade conditions (SC). T_0, T_1, and T_2 refer to 0, 12.5, and 25 mg(Ti) L^{-1}, respectively. (*A*) leaf area (LA); (*B*) leaf thickness (LT); (*C*) leaf dry mass (LDM); and (*D*) plant dry mass (PDM). Values are means ± SE, *n* = 3. Different *lowercase letters* indicate a significant difference ($p<0.05$) between treatments.

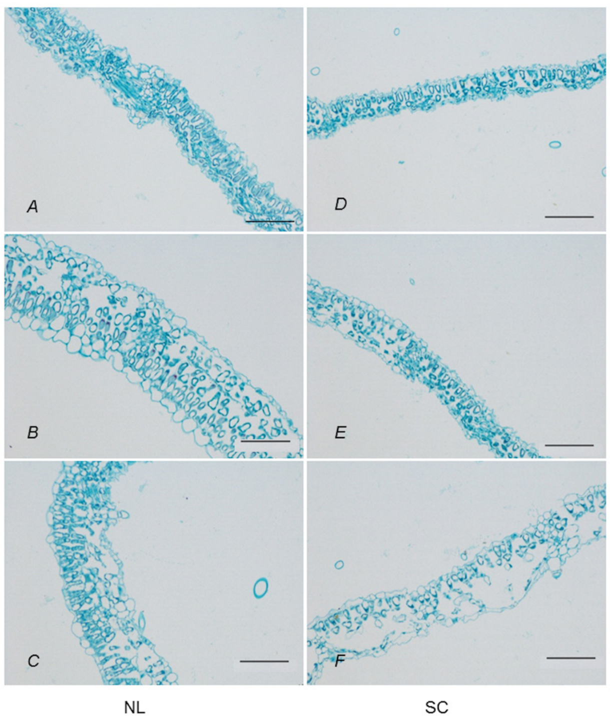

Fig. 2. Leaf anatomy of soybean plants under normal light (NL) (*A–C*) and shaded conditions (SC) (*D–F*) treated with 0 mg(Ti) L^{-1} (T_0) (*A*,*D*); 12,5 mg(Ti) L^{-1} (T_1) (*B*,*E*); and 25 mg(Ti) L^{-1} (T_2) (*C*,*F*). Bars indicate 100 µm in length.

on photosynthetic parameters (P_N, E, g_s, and C_i). The maximum P_N [14.3 µmol(CO_2) m^{-2} s^{-1}], E [3.9 mmol(H_2O) m^{-2} s^{-1}], and g_s [0.19 mol(H_2O) m^{-2} s^{-1}] were recorded under NL compared to the values under SC, where following values were measured: P_N of 10.3 µmol(CO_2) m^{-2} s^{-1}, E of 2.9 mmol(H_2O) m^{-2} s^{-1}, g_s of 0.10 mol(H_2O) m^{-2} s^{-1}, and C_i 297.6 µmol(CO_2) mol^{-1}. Ti application significantly increased the P_N, g_s, and E, and the highest P_N (14.4), g_s (0.2), and E (3.6) were recorded under T_2, while the lowest P_N (10.1) and E (3.3) were noticed in control (T_0), and the lowest g_s (0.1) was noticed in T_1 treatment. On the contrary, Ti application significantly decreased the C_i of soybean plants, maximum (320.8) and minimum (271.0) C_i was noticed under T_0 and T_2 treatment, respectively. Interactive effect of Ti concentrations and different light conditions was found significant for all photosynthetic parameters (Table 1). Overall, treatment T_2 increased P_N by 42% compared to T_0, suggesting that P_N increased as the LA and Chl content increased by Ti application.

Chl fluorescence parameters: In our experiment, the Chl fluorescence was changed significantly during the experimental period in response to Ti application and different light conditions. There was not a significant difference for $\Phi_{(PSII)}$ under both light conditions. However, Ti application significantly enhanced $\Phi_{(PSII)}$ in T_2 treatment under SC, where the maximum $\Phi_{(PSII)}$ was recorded (Fig. 3*A*). Different light conditions and Ti application considerably affected $\Phi_{(P)}$, $\Phi_{(f,D)}$, $\Phi_{(NPQ)}$, and q_p. Compared with SC, the $\Phi_{(P)}$, $\Phi_{(f,D)}$, $\Phi_{(NPQ)}$, and q_p under NL were higher by 32, 13, 10, and 16%, respectively (Fig. 3*B–E*). Similarly, under NL, Ti application significantly increased

Table 1. Effect of Ti application on Chl content and photosynthetic parameters of soybean plants under normal light (NL) and shade conditions (SC). T_0, T_1, and T_2 refer to 0, 12.5, and 25 mg(Ti) L^{-1}, respectively. Data are means, $n = 3$. Values which do not share the *same letters* in the column differ significantly at $p \le 0.05$. P_N – net photosynthetic rate, g_s – stomatal conductance, C_i – intercellular CO_2 concentration, E – net transpiration rate, * – significant.

Environmental conditions (Ec)	Chl (SPAD value)	P_N [µmol(CO_2) m^{-2} s^{-1}]	g_s [mol(H_2O) m^{-2} s^{-1}]	C_i [µmol(CO_2) mol^{-1}]	E [mmol(H_2O) $m^{-2}s^{-1}$]
NL	30.417^a	14.258^a	0.1917^a	278.58^b	3.9833^a
SC	26.100^b	10.355^b	0.1031^b	297.67^a	2.9575^b
LSD (0.05)	1.4606	0.3166	0.0363	13.259	0.2200
Treatment					
T_0	24.475^c	10.145^c	0.1628^a	320.75^a	3.5513^a
T_1	29.100^b	12.375^b	0.1136^b	272.63^b	3.2625^a
T_2	31.200^a	14.400^a	0.1658^a	271.00^b	3.5975^a
LSD (0.05)	1.4167	0.4178	0.0447	17.127	0.5350
Interaction (Ec × T)	*	*	*	*	*

the $\Phi_{(P)}$, $\Phi_{(f,D)}$, and q_p of soybean plants, and the maximum of $\Phi_{(P)}$, $\Phi_{(f,D)}$, and q_p were 0.11, 0.3, and 0.45, respectively, under T_1 treatment, while minimum $\Phi_{(P)}$, q_p, and $\Phi_{(f,D)}$ were measured in SC (Fig. 3*B–D*). In addition, the highest value (0.65) of $\Phi_{(NPQ)}$ was noted under treatment T_2 and the lowest value was recorded in T_1 treatment (Fig. *3E*). Moreover, significant differences in Chl fluorescence parameters were noticed in the interaction of different light conditions and Ti concentrations. Overall, these results are indicating that changes in photosynthetic rate under different light conditions were directly associated with the changes in Chl fluorescence parameters.

Total soluble sugar (TSS): To investigate the differences in leaf TSS of soybean in response to Ti concentrations, the TSS of leaves under NL and SC for T_0, T_1, and T_2 were analyzed after seven days of Ti application (foliar). The different light conditions significantly affected the TSS content in soybean leaves, and the maximum TSS content (34.6 mg g^{-1}) was measured in leaves under NL, whereas shade significantly decreased the TSS. The TSS increased as Ti concentrations increased from T_0 to T_2. The maximum TSS (32.2 mg g^{-1}) was observed in treatment T_2 and minimum TSS (23.3 mg g^{-1}) was noticed under control treatment (T_0). Furthermore, the interaction of Ti concentrations and different light conditions for TSS showed significant differences (Fig. 4). On average, TSS of soybean plants under T_2 increased by 38% than that of T_0 treatment.

Chloroplast ultrastructure of soybean: In this study, changes in the ultrastructure of chloroplasts occurred (Fig. 5). The different light conditions and Ti concentrations significantly changed the shape, size, and number of chloroplasts. Under NL, the leaves under T_2 treatment had thicker grana (G) stacks compared to T_1 and T_0 (Fig. 5*C*). The number of starch grains (S) increased in T_1 and T_2, while the larger starch grains were observed in leaves grown under NL. Under SC, the starch grains and grana stack of T_0 were smaller in size as compared to T_1 and T_2 plants (Fig. 5*D*). However, T_1 demonstrated more and larger starch grains as compared to T_2 and T_0 (Fig. 5*E*).

Ti accumulation and distribution in soybean: In both light conditions, the highest Ti accumulation was noted in soybean plants under NL, whereas it decreased sharply in SC (Fig. 6*D*). Differences between Ti concentrations revealed that soybean plants under T_1 accumulated 30% more Ti than that in T_0 treatment. Interestingly, relative to the shade, soybean plants under NL showed 222.6% higher Ti accumulation. Moreover, the distribution pattern of Ti in different plant organs is presented for all Ti treatments and different light conditions (Fig. 6*A–C*). Large fluctuations were observed for Ti content in leaf, stem, and roots under different light conditions. The maximum accumulation of Ti was observed in roots and stem followed by leaves. The highest Ti content in roots (138.3 mg kg^{-1}), stem (32.4 mg kg^{-1}), and leaf (40.9 mg kg^{-1}) were recorded under NL. However, under SC, the highest Ti accumulation was noticed in leaf (27.7 mg kg^{-1}), roots (28.9 mg kg^{-1}), and stem (18.7 mg kg^{-1}). The Ti treatments increased the stem, leaf, and root Ti content, and the highest Ti content in roots (83.9 mg kg^{-1}), stem (25.2 mg kg^{-1}), and leaf (34.3 mg kg^{-1}) was noticed under treatment T_2, respectively. Relative to control (T_0), under NL, T_2 treatment increased Ti content by 29.8% while under SC T_1 treatment increased Ti content by 85.4% (Fig. 6*D*).

Discussion

In multiple cropping systems, retardation of photosynthesis due to shade stress results in low agricultural productivity. Crop growth can be improved by enhancing their light-use efficiency under shade conditions (Qu *et al.* 2017). In previous studies, it has been reported that the Ti application can play a favorable role in improving crop growth and seed yield by increasing the crop resistance to abiotic stress (Feizi *et al.* 2013, Choi *et al.* 2015). In current study, the effects of Ti on growth parameters of soybean plants grown under different light conditions were investigated. Leaf

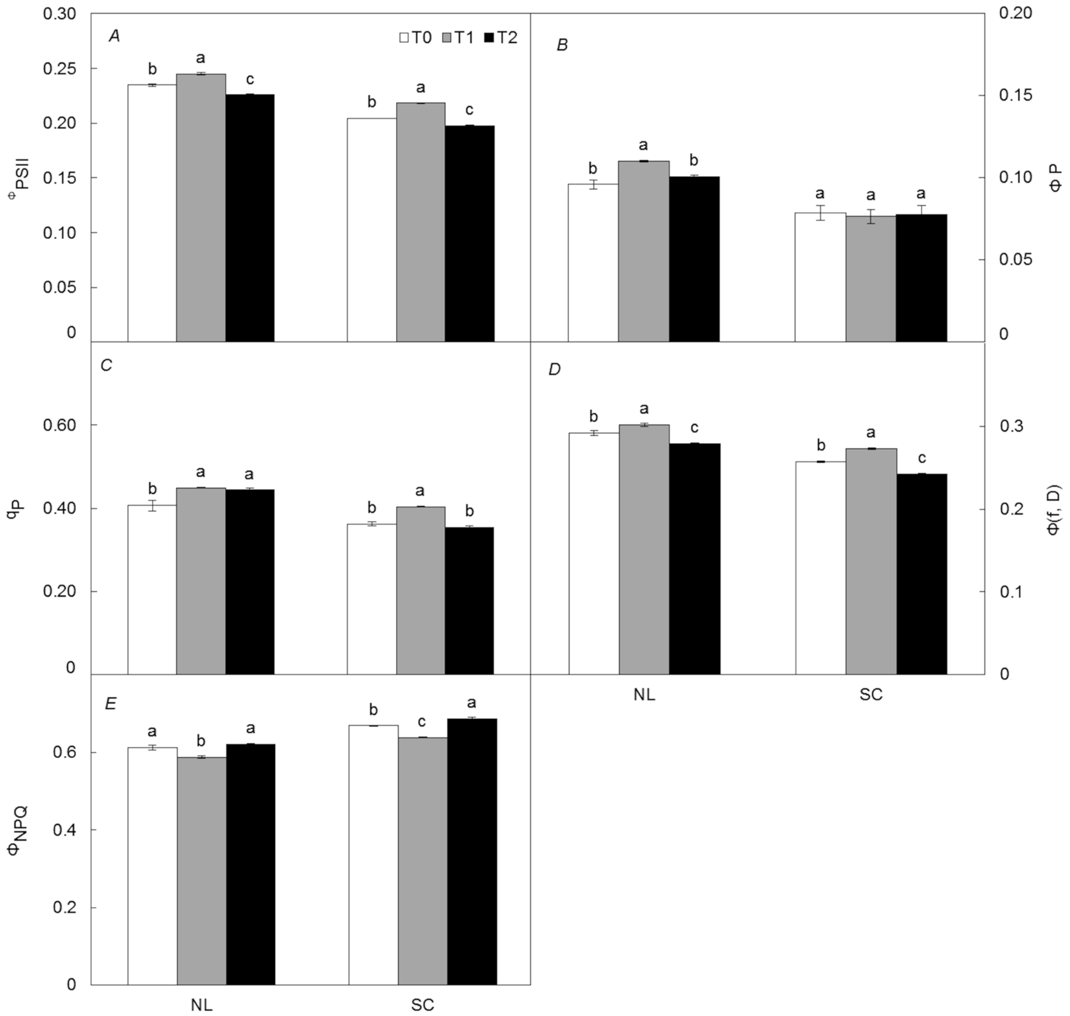

Fig. 3. Effect of titanium (Ti) on soybean chlorophyll fluorescence parameters under normal light (NL) and shade conditions (SC). T_0, T_1, and T_2 refer to 0, 12.5, and 25 mg(Ti) L^{-1}, respectively. Values are means ± SE, *n* = 3. Different *lowercase letters* indicate a significant difference ($p<0.05$) between treatments. $\Phi_{(PSII)}$ – maximum quantum yield of PSII photochemistry, $\Phi_{(P)}$ – effective quantum yield of PSII, q_p – coefficient of photochemical quenching, $\Phi_{(f,D)}$ – quantum yield of constitutive nonregulatory nonphotochemical quenching, $\Phi_{(NPQ)}$ – quantum yield of light induced regulatory nonphotochemical quenching.

is the main light-harvesting plant organ and its anatomy influences the photosynthetic capacity (Bielczynski *et al.* 2017). Similarly, it was found that Ti improved the leaf area, leaf thickness, Chl content, and total plant biomass of soybean under shade stress. Foliar application of Ti significantly improved soybean growth by mitigating the adverse effects of shade. However, Ti can induce toxicity depending on its application dosage (Ruffini Castiglione *et al*. 2016). A higher rate of Ti application suppresses the plant growth and root elongation, and is toxic (Ruffini Castiglione *et al*. 2011).

Furthermore, foliar application of Ti increased the Chl content in tomato and cucumber leaves (Servin *et al.* 2013, Raliya *et al.* 2015). In the present study, it was found that the lower concentration of Ti could improve leaf morphological characteristics. However, under stress conditions (shade), the higher concentration would be beneficial to minimize the detrimental effect of abiotic stress. Under shade conditions, improving light-use efficiency is critical. Previous research has shown that the soybean seedlings intercropped with maize exhibited significantly downregulated P_N (–38.3%), E (–42.7%), and g_s (–55.4%) due to low available light. (Su *et al.* 2014). In our experiment, T_2 treatment significantly increased the P_N by increasing the g_s and E of soybean plants as compared to the T_0 (Table 1). The C_i decreased with the increasing content of Ti under different light conditions. These results suggested that the increase in P_N could be due to the increase in g_s after Ti application. In this study, higher g_s, 0.22 and 0.14 μmol(H_2O) m^{-2} s^{-1} under light and shade, respectively, indicated that the changes in P_N were closely associated with stomatal opening.

Increased photosynthetic capacity is always accompanied with high quantity of electrons passing through PSII (Yao *et al.* 2017). Chl fluorescence is one of the main

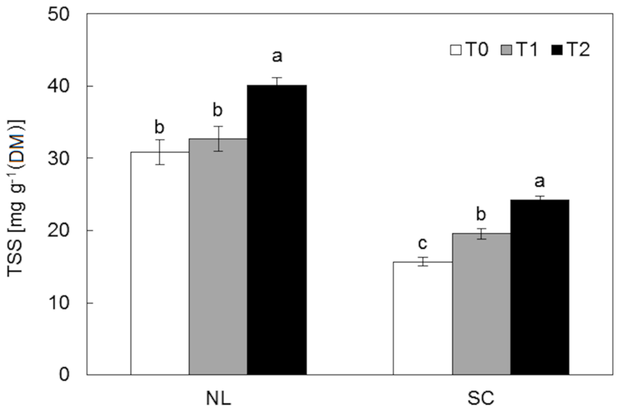

Fig. 4. Effect of titanium (Ti) on total soluble sugar (TSS) content of soybean leaves under normal light (NL) and shade conditions (SC). T_0, T_1, and T_2 refer to 0, 12.5, and 25 mg(Ti) L^{-1}, respectively Values are means ± SE, *n* = 3. Different *lowercase letters* indicate a significant difference (p<0.05) between treatments.

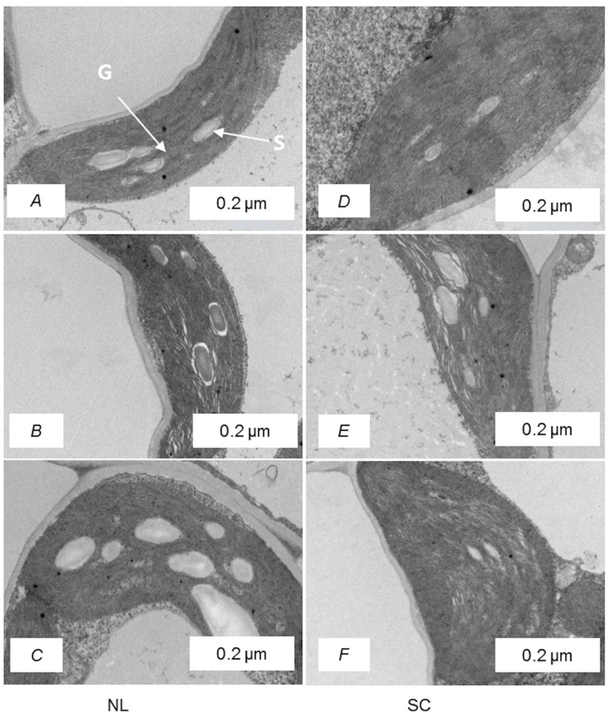

Fig. 5. Chloroplast ultrastructure of soybean under normal light (NL) (*A–C*) and shade conditions (SC) (*D–F*) treated with 0 mg L^{-1} (T_0) (*A*,*D*); 12,5 mg(Ti) L^{-1} (T_1) (*B*,*E*); and 25 mg(Ti) L^{-1} (T_2) (*C*,*F*). G – grana, S – starch grain.

indicators of photosynthetic regulation and plant responses to environmental conditions because of its sensitivity and convenience (Dai *et al.* 2009). Former studies have reported that a decrease in plant growth under SC was due to lower energy absorbed by the leaf and subsequently translocated to PSII (Huang *et al.* 2011, Yao *et al.* 2017). In present study, similar results were obtained as $\Phi_{(P)}$ and $\Phi_{(f,D)}$ were significantly lower in soybean plants under SC compared to NL. Compared with T_0 and T_2, treatment T_1 significantly increased the $\Phi_{(P)}$, $\Phi_{(f,D)}$, q_P, and $\Phi_{(NPQ)}$ under SC and NL, whereas, the higher concentration of Ti (T_2) decreased the Chl fluorescence. It reveals that Ti enhanced the efficiency of PSII that could enhance the photosynthesis by improving the energy transport from PSII to PSI under both conditions (NL and SC).

Soluble sugar content is the direct expression of the strong photosynthesis. Plants translocate sugar from photosynthesizing leaves to storage cells which indicates the physical fitness of the plants (Amiard *et al.* 2005). In present study, shade had a negative impact on the sugar content of soybean leaves (Fig. 4). Similarly, former studies have reported that cloudy days and low light conditions reduced the soluble sugar content in leaves (Lichtenthaler *et al.* 1981). However, in this experiment, foliar application of Ti considerably improved the sugar content in plants under SC. Under NL and SC, T_2 demonstrated significantly the higher soluble sugar content as compared to T_1 and T_0 (Fig. 4). Similar results have been reported that titanium oxide improved the soluble sugar content of tomato (Nishizawa *et al*. 2008) and strawberry (Choi *et al*. 2015).

Chloroplast ultrastructure controls the photosynthetic performance of crops under changing environmental conditions (Shao *et al.* 2014). In our study, the number of chloroplasts and grana decreased significantly under shade conditions (Fig. 5*D*). The T_1 and T_2 treatments improved the chloroplast ultrastructure by increasing the number of chloroplasts, thylakoid granal and stromal lamellae under light and shade conditions, which suggests the beneficial effect of Ti application on photosynthetic apparatus. Furthermore, the improved structure of chloroplasts by Ti applications suggested that it might develop the shade-tolerant mechanism in soybean plants, especially under shade conditions, such as intercropping and relay intercropping. Carvajal *et al.* (1994) proposed that Ti increased iron activity in cell chloroplast and cytoplasm and resulted in increasing plant photosynthetic activity.

It was reported that Ti application increased the effectiveness of iron in chloroplasts where most of photosynthetic activity occurs (Jaberzadeh *et al.* 2013).

The total Ti accumulation by soybean plants increased by applying Ti at the rate of 25 mg L^{-1} (T_2) in NL, however, the level of accumulation varied between light and shade conditions (Fig. 6*D*). Our results are similar with previous findings that Ti accumulation in plants increased with increasing concentration of Ti, *e.g.*, tomato plant accumulated 40 to 50 mg(Ti) kg^{-1} (Raliya *et al.* 2015). In addition, Ti distribution in different plant parts was considerably changed under both conditions. The Ti content in leaves, stems, and roots of soybean plants was significantly higher under NL than that at SC. In support of our findings, previous study found distinct Ti distribution in different plant organs under different light environments (Conway *et al.* 2015).

Conclusion: Through foliar application, Ti can enter into plant cells by gas-uptake mechanisms and alleviate the detrimental effect of shade stress on plants. Ti improves

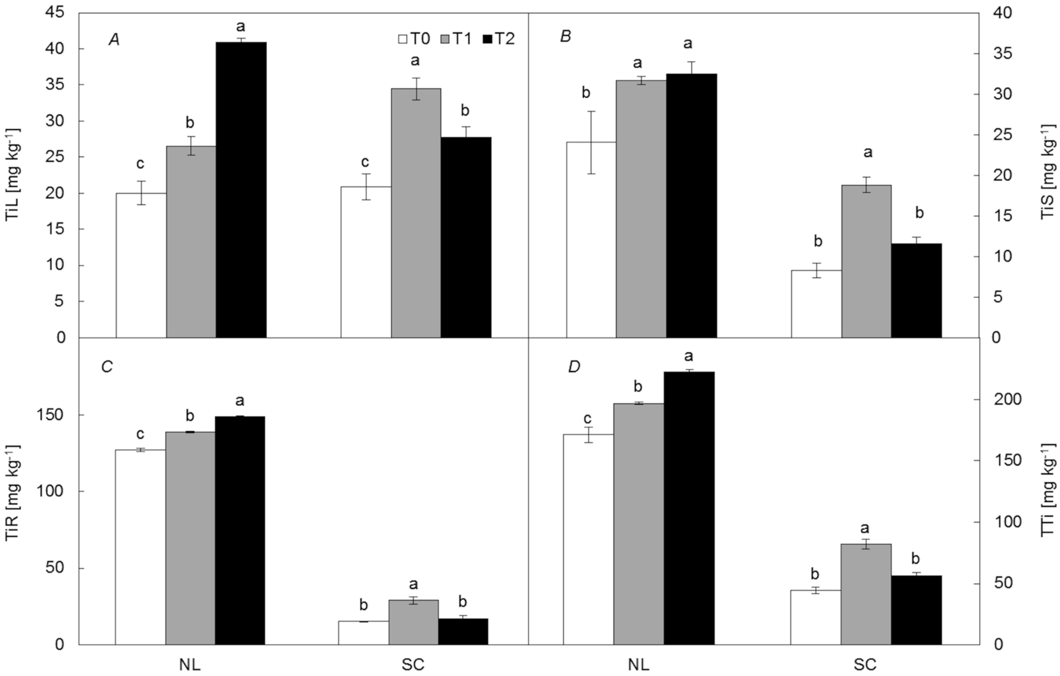

Fig. 6. Titanium accumulation in soybean plants treated with 0 mg(Ti) L^{-1} (T_0); 12,5 mg(Ti) L^{-1} (T_1); and 25 mg(Ti) L^{-1} (T_2) under normal light (NL) and shade conditions (SC). TiL (*A*), TiS (*B*), TiR (*C*), and TTi (*D*) represent the Ti uptake in leaves, stem, roots, and total titanium accumulation in soybean plants, respectively. Values are means ± SE, *n* = 3. Different *lowercase letters* indicate a significant difference ($p<0.05$) between treatments.

photosynthesis through increasing leaf area, Chl content, Chl fluorescence parameters, such as improving efficiency of $\Phi_{(P)}$, $\Phi_{(f,D)}$, q_p, and $\Phi_{(NPQ)}$, chloroplast structure, and soluble sugar content under NL and SC. Once the Ti ions are acquired, they are distributed through the entire plant by the vascular network. In order to further explore the mode of Ti action under shade stress, it would be necessary to examine the activity of key enzymes in photosynthesis and their gene expression. Furthermore, bioaccumulation of Ti may affect the edible parts of soybean plants. Therefore, fundamental research with respect to Ti-plant interaction and seed quality are needed to ensure its precise application in agriculture.

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