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Physiological changes and growth promotion induced in poplar seedlings by the plant growth-promoting rhizobacteria *Bacillus subtilis* JS

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Abstract

This study aimed to determine the effects of plant growth-promoting rhizobacteria *Bacillus subtilis* JS on the growth and physiological changes of *Populus euramericana* and *Populus deltoides* × *P. nigra*. Poplar seedlings were treated with *B. subtilis* JS and their growth was monitored for up to 120 d. Three different types of treatments [control, B1 (*B. subtilis*:double-distilled water, 1:100, v/v), and B2 (1:50)] were established. *B. subtilis* JS enhanced seedling height by 62% and total biomass by 37% after 120 d. Physiologically, the photosynthetic rate increased by 54%, and the total chlorophyll (Chl) content, foliage nitrogen and phosphate content were significantly higher after treatment with B2 than that of the control. These results suggest that the total Chl content is directly related to not only the photosynthetic capacity of the foliage but also to the nitrogen content, indicating that the strain JS may promote the growth of poplar.

Additional key words: biofertilization; biomass; fast-growing tree; gas exchange; pigment; root activity.

Introduction

Bacillus subtilis is a gram-positive bacterium (Kunst *et al*. 1997) that can be isolated from several terrestrial and aquatic environments, suggesting that it is broadly adapted to grow in diverse environments within the biosphere (Earl *et al* 2008). *B. subtilis* can form circular or oval spores that endure and survive under unfavorable environmental conditions. It can promote plant growth *via* various mechanisms such as by releasing volatile substances (Song *et al*. 2012, Han 2014). Zou *et al*. (2010) reported that the volatile substance (2-pentylfuran) produced by *B. megaterium* XTBG34 promoted the growth of *Arabidopsis thaliana* L. Similarly, Ryu *et al*. (2003) and Zhang *et al*. (2007*)* reported that volatiles (2R- and 3R-butanediol) produced by *B. subtilis* GB03 increased *A. thaliana* biomass by inducing the phytohormone auxin. Furthermore, antibiotics produced by *B. subtilis* inhibited plant diseases (Asaka *et al*. 1996). Thus, antibiotics or secreted hormones produced by *B. subtilis* can help promote directly or indirectly plant growth (Kloepper *et al*. 2004, Arkhipova *et al*. 2005). *B. subtilis* strain JS was newly isolated from the soil of a *Miscanthus*-growing pot in a greenhouse at the University of Seoul. Even if *Bacillus* group offers a biological solution to the formulation problem (Emmert and Handelsman 1999), little is known about the effects of growth promotion using the strain JS. Among *B. subtilis* strains, JS affected the gene expression of plant growth-promoting rhizobacteria (PGPR)-induced genes in tobacco (*Nicotiana tabacum* L. 'Xanthi'). Moreover, the regulatory role of photosynthesis-related genes upregulated by strain JS *via* diverse plant growth-promoting processes was verified in various plants (Jang 2015, Kim *et al*. 2015a). Solar energy fixation is accomplished by photosynthesis in plants, and this process is related to Chl in leaves (Salisbury and Ross 1992). Therefore, the energy balance of plants can be estimated by their Chl contents. Song *et al*. (2012) reported that emission of *B. subtilis* JS volatiles markedly increased seedling growth in tobacco (*Nicotiana tabacum* L. cv. 'Xanthi'). Shoot fresh mass, lateral root and root hair numbers, and primary root length of Xanthi seedlings

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*Abbreviations***:** B1 – *Bacillus subtilis* JS inoculation with double-distilled water (dilution rate of 1 in 100); B2 – *Bacillus subtilis* JS inoculation with double-distilled water (dilution rate of 1 in 50); Car_T – total carotenoid; Chl_T – total chlorophyll; CFUs – colony-forming units; DDW –double-distilled water; E – transpiration rate; g_s – stomatal conductance; N_{Leaf} – total nitrogen content of leaf; PGPR – plant growth-promoting rhizobacteria; P_{Leaf} – phosphate content of leaf; P_N – net photosynthetic rate; RGR – relative growth rate; ROS – reactive oxygen species; SRCs – short rotation coppice cultures; TF – triphenyl formazan; TTC – triphenyltetrazolium chloride; WUE – water-use efficiency.
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increased after treatment with *B. subtilis* JS. These results suggested that volatiles produced by *B. subtilis* JS played roles as growth elicitors. These volatiles stimulated the expression of genes encoding hormones and Chl *a*/*b*-binding proteins, resulting in plant growth promotion. Volatiles of *B. subtilis* JS are also known to increase the expression of many pathogenesis-related genes and confer disease resistance in plants (Song *et al*. 2012, Kim *et al*. 2015a). Furthermore, they induce the expression of genes associated with the scavenging of reactive oxygen species (ROS), thereby reducing cellular damage caused by ROS-induced stress (Song *et al*. 2012, Kim *et al*. 2015a,b).

Due to its rapid growth and strong sprouting abilities, poplar is an important woody crop used to produce biomass in short rotation coppice cultures (SRCs) (Gordon and Promnitz 1976, Dickmann *et al*. 1996). However, in South Korea, in particular, SRCs are established mostly on soils having poor quality, such as barren lands (arid areas, landfills, swamps, and reclaimed lands). This is because land for plantations is limited, and wood crops need to be planted in large areas, such as reclaimed lands (Park *et al*. 2009). However, some studies have shown that biomass production is limited in reclaimed lands (Yeo *et al*. 2010, Kim *et al*. 2011). If inorganic nutrients or substances, which can improve the growth of the rhizosphere system, are applied, the production of woody biomass can be increased. Among biofertilizers, PGPR can minimize land salinization, while improving the pH, nitrogen (N) availability from nitric acid and ammonia, phosphate (P) content, and exchangeable positive ion amount of the soil (Yadav *et al*. 2000, Vessey 2003, Lee *et al*. 2013).

This study aimed (*1*) to confirm the growth-promoting effects of the PGPR, *B. subtilis* JS, in poplar seedlings, and (*2*) to determine the physiological changes that improved the growth of poplar seedlings treated with *B. subtilis* JS.

Materials and methods

Plant culture: Poplar seedling clones and hybrid clones (*Populus deltoides* × *P. nigra* 'Dorskamp'; *Populus euramericana* 'Eco28', 'I-476', and 'Venziano') were used for the experiment since they are known to grow on reclaimed lands (Shin *et al*. 2012). Seedlings were established in a Venlo-type greenhouse at the University of Seoul, Seoul, Republic of Korea. Seedlings were planted in February 2015 and were investigated from May to September 2015. At the study site, cuttings of poplar were grown for over 60 d (from March to May) for selecting fast-growing tree clones, and their survival rate was over 93%. The experiment was performed in the three different treatment plots (180 × 120 cm) for 120 d (until the end of August 2015). Sixty cuttings (20 in each of the three treatments, with four types of clone × five replicate plants per clone) were used for planting in the pots, which were obtained from 12 cm above the ground (height, 30 cm, Ø 25 cm). Seedling growth assays were performed in small pots (25 × 18 × 30 cm; cavity volume, 3 L). For the pot experiment, soil was sterilized by autoclaving at 121°C for 15 min at 15 psi. Each pot was filled with equal parts of soil from Saemanguem-reclaimed land, located in Gimje City in the Republic of Korea, peat (*Klasman*, *Potground-H*, Germany), and perlite [1:1:1, v(reclaimed land soil)/v(peat)/v(perlite); *see* text table below]. During the experiment, all the pots were kept under natural light (sun light), with monthly maximum and minimum temperatures of 46.2°C and 13.5°C, respectively. The monthly mean humidity was 56.8%.

Characteristics of the soil used for the experiment. pH – potential of hydrogen; EC – electrical conductivity; O.M – organic matter; Avail. P – available phosphorus; T-N – total nitrogen; CEC – cation exchange capacity.

$pH_{[1:5]}$	$EC_{[1:5]}$ [dS m^{-1}]	O.M [%]	Avail. P [mg kg^{-1}]	T-N [%]	CEC [cmol kg^{-1}]	K [cmol kg^{-1}]	Na [cmol kg^{-1}]	Mg [cmol kg^{-1}]	Ca [cmol kg^{-1}]
6.8	0.07	2.0	19.73	0.15	11.39	0.91	1.01	2.01	1.01

***Bacillus subtilis* JS culture and plant treatments**: The *B. subtilis* JS (National Patent Classification Code: KR 1020140028777, *GenBank* accession number CP003492), gram-positive bacterial strain, isolated from the soil used to grow *Miscanthus sinensis* var. *Purpurascens* (Song *et al*. 2012), was used during the experiment. *B. subtilis* JS was stored at −80°C in 15% glycerol for long-term storage and use. Bacteria were cultured as follows. Bacterial strains were streaked on nutrient agar (NA) medium (*Junsei Chemical*, Japan) and cultured at 28°C. A single colony on NA was transferred to 30-mL of nutrient broth (*Difco*, USA) and grown on a reciprocating shaker (110 rpm) at 28°C for 14 h. The bacteria concentration was adjusted to at least 1×10^7 colony-forming units (CFUs) mL^{-1}. For the greenhouse experiment, the 40 of the 60 poplar seedlings (initial cutting length, 12 cm) were drenched with the bacterial culture that was diluted to either 1:100 (B1, 100-fold) or 1:50 (B2, 50-fold) with double-distilled water (DDW; 1 L per pot). The remaining 20 seedlings were used for the control treatment, which were drenched only with DDW. *B. subtilis* JS microbial agent (about 1×10^9 CFU mL^{-1}), diluted with DDW (1 L per pot), was added to the topsoil of each pot from May to August 2015. The application interval was three times a week.

Seedling growth and biomass: The height, diameter, and biomass of each seedling were measured every month from March to September 2015. To investigate the growth rate of poplar seedlings, relative growth rate (RGR) over the study intervals was calculated for each plant as: RGR (cm per day) = (ln M_f − ln M_i)/T (Poorter and Remkes 1990), where M_i and M_f are initial and final growth data (seedling height and diameter), respectively, and T is the time interval (number of days). At the end of the experiment, plants were removed from the soil and harvested, and their roots were washed. The plants were separated into roots (excluding cuttings), shoots, and leaves. Seedling height was measured using a folding ruler for height (*Stabila*, Germany) from the root collar to the end of an apical leaf. Root collar diameter was measured at 5 cm above the ground using an *ABS Diamatic* caliper (*CD-15DC*, *Mitutoyo*, Japan). The fresh mass of each sample was measured immediately after harvest. All samples were oven-dried to a constant biomass in a drying oven at 70°C (*DS-80-2*, *Dasol Scientific*, Republic of Korea) for 96 h prior to estimating the dry mass of the leaves, stems, and branches (Makkonen and Helmisaari 1998). Total biomass was obtained from on these data.

Analysis of plant physiological changes: The net photosynthetic rate (P_N) of the foliage was measured using a red-blue LED light source *Li-6400-02B* (*Li-Cor*, USA) of the *Li-6400* portable photosynthesis system (*Li-Cor*, USA). Irradiance-response curves were generated by placing the leaves sampled at the fourth to sixth point from apex of each poplar clone in *B. subtilis* JS treatments in a leaf chamber at 25°C, 400 μmol(CO_2) mol^{-1}, and 50–60% relative humidity. After this initial preparation, the P_N of seedlings was measured at 10:00 during the experiment period. The leaves were acclimated for 2 min before measurement, at a photon flux density of 1,500 μmol (photon) m^{-2} s^{-1}. Water-use efficiency (WUE; as indicated by water-use efficiency of leaf) was calculated using the

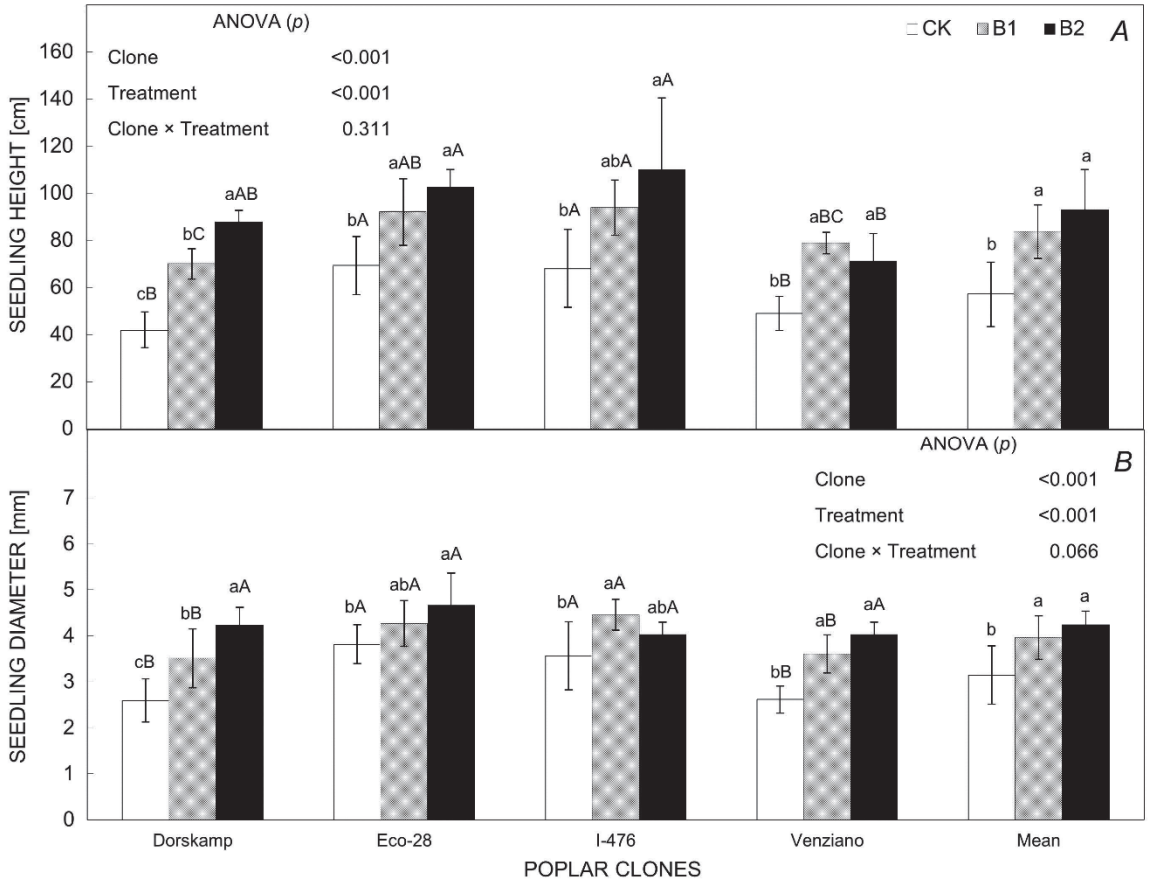

Fig. 1. The effect of *Bacillus subtilis* JS treatment on the seedling height and seedling diameter of *Populus euramericana* (Eco28, I-476, Venziano) and *Populus deltoides* × *P. nigra* (Dorskamp). The poplar seedlings were treated as follows: CK – distilled water as a control; B1 – dilution rate of 1 in 100 (*B. subtilis*:DDW, 1:100, v/v); B2 – dilution rate of 1 in 50 (*B. subtilis*:DDW, 1:50, v/v). Bars indicate standard error. One-way and two-way *ANOVA*s (clone × treatment) were used for statistical analysis by using *SPSS*. *Post hoc* comparisons were performed using the *Tukey*'s post test at a significance level of $p \leq 0.05$. *Different uppercase letters* indicate significant differences among poplar clones ($p \leq 0.05$) of the same treatment; *Different lowercase letters* indicate significant differences among *B. subtilis* JS treatments (B1, B2 and CK) of the same clone ($p \leq 0.05$).

following formula of Cernusak *et al*. (2006): WUE = P_N/transpiration rate (E), where $P_N = u_e(c_e - c_c)/(100 \times S) - c_cE$; u_e: mole flow rate of air entering the leaf chamber [μmol s^{-1}], c_e: mole fraction of CO_2 in the leaf chamber [μmol(CO_2) mol^{-1} (air)], c_c: mole fraction of CO_2 entering in the leaf chamber [μmol(CO_2) mol^{-1}(air)], S: leaf area (cm^2). and E [mmol (H_2O) m^{-2} s^{-1}] = $u_e(w_c - w_e)/S \times 105(1 - w_c/1{,}000)$; u_e: mole flow rate of air entering the leaf chamber (μmol s^{-1}), w_c: mole fraction of water vapor in the leaf chamber [mol(H_2O) mol^{-1}(air)], w_e: mole fraction of water vapor entering the leaf chamber [mmol(H_2O) mol^{-1}(air)], S: leaf area (cm^2).

Chlorophyll (Chl) and carotenoids (Car) were extracted from 0.1 g (fresh mass) leaf discs, sampled three times from each of the *B. subtilis* JS treatments and each clone (in total 36 leaf samples), and subjected to photosynthetic efficiency measurements, with 80% acetone solution in a 10-mL brown vial for one week at 4°C. Absorbance was measured at 663 nm (Chl *a*), 645 nm (Chl *b*), and 470 nm (Car) using a microplate reader (*Epoch*, *Bio-Tek*, USA). Chl content (Chl *a* and *b* and total Chl [Chl_T]) and total carotenoid content (Car_T) were calculated according to the equation reported by Arnon (1949): Chl $a = 12.7 \times A_{663} - 2.69 \times A_{645}$, Chl $b = 22.9 \times A_{645} - 4.68 \times A_{663}$, total Chl $(a + b) = 20.2 \times A_{645} - 8.02 \times A_{663}$, total carotenoids = $(1{,}000 \times A_{470} - 1.82 \times$ Chl $a - 85.02 \times$ Chl $b)/198$, where A_x refers to the absorbance of the extract solution in 1-cm path length cuvette at particular wavelength. Pigment concentration was calculated as mg $g(FM)^{-1}$. The total nitrogen content of leaf (N_{Leaf}) was determined using the Kjeldahl method (Bremner 1996) using an autoanalyzer (*Kjeltec 2300*, *Foss*, Sweden). The phosphate content of the leaf (P_{Leaf}) was determined using the vanadate method (Hanson 1950). Absorbance was measured at 470 nm using a microplate reader (*Epoch*, *Bio-Tek*, USA). The root activity of poplar seedlings treated with *B. subtilis* JS was measured using the triphenyltetrazolium chloride (TTC) method (Yoshida 1966, Hirata 1990). First, fresh fine roots of seedlings were sampled. Then, the root samples were washed under running water until they were free from soil particles. Subsequently, the root samples of each seedling were cut into 2-cm long segments, and the segments were mixed systematically. Next, 500 mg of fresh root samples was weighed and placed in a Thunberg tube containing 10 mL of mixed solution (1% TTC solution, 0.1M sodium phosphate buffer solution, and DDW mixed, at a ratio of 1:4:5). The solution was then evaporated using a suction pump. The roots were incubated in the dark in a shaking water bath (*HB–205SW*, *HanBaek Scientific*, Republic of Korea) at 30°C for 2 h. The reaction was then stopped by adding 2 mL of 2 N sulfuric acid. The root segments were dried, and were ground with 3–5 mL of ethyl acetate and sea sand to extract the formazan from the roots. Finally, the absorbance of formazan was measured using a spectrophotometer at 470 nm using a microplate reader, and a standard curve was generated using the mixed solution (TTC solution, 0.2 mg; sodium hydrosulfite, 15–30 mg, and 99% ethyl acetate) using the microplate reader. The root activity (Hirata 1990) was calculated as follows: relative root activity = mg (created triphenyl formazan, TF) g^{-1}(dry mass of roots, DM) h^{-1}(reaction time with solution).

Statistical analysis: All statistical analyses for different treatments were performed using the *Statistical Package for the Social Sciences* (*SPSS*, Chicago, IL, USA) *version 23* for *Window* software. One-way and two-way analysis of variance (*ANOVA*) was used for statistical analysis. Least significant difference among the mean values was performed using the one-way *ANOVA* test to evaluate the differences between treatments. Two-way *ANOVA* was used to test for interactions between treatments and clones (treatment × clones). *Post hoc* comparisons were performed using the *Turkey's* test at a significance level of $p \leq 0.05$. We examined relationships among parameters using nonlinear regression analyses and coefficients of determination (r^2).

Results

Seedling growth and changes in leaf nitrogen and phosphate contents after PGPR treatment: Poplar clones were grown for 240 d after treatment with *B. subtilis* JS. Seedling height was significantly different between the control (CK) and treatment (B1, B2) groups. The average seedling height and diameter of treated seedlings (B1, 83.7 ± 11.3 cm; B2, 93.0 ± 17.1 cm) were greater than those of the control (CK, 57.1 ± 13.7 cm) seedlings (Fig. 1). After PGPR treatment, the seedling height of the B2 group was approximately 1.6-fold (62.9% increase) higher than that of the CK group (Fig. 1*A*). Seedling height was significantly different across all poplar clones. The average seedling diameter of the treatment group (B2, 4.2 ± 0.3 mm; B1, 3.96 ± 0.47 mm) was greater than that of the control (3.1 ± 0.6 mm). There was an approximately 1.3-fold (35.0% increase) difference in the seedling diameter between the treated (B1, B2) and CK groups (Fig. 1*B*). Furthermore, the overall growth (seedling height and diameter) of Eco28 and I-476 clones was higher than that of other clones.

The height of I-476 and Eco28 clones was greater than the other clones under all conditions (B2, B1, and CK; Fig. 1*A*). The total seedling diameter of the I-476 clone in the B1 and CK groups was the largest one, but the clones in the B2 group were not significantly different (Fig. 1*B*). Furthermore, both height and diameter were influenced by clone and treatment, but the interaction was not significant (Fig. 1).

Among other growth parameters, leaf, shoot, and root DM of all poplar clones across treatments are shown in Table 1. The *B. subtilis* JS treatment caused a significant increase in the mean DM (B2, 59.9 ± 8.1 g; B1, 62.8 ± 7.4 g) compared with that of the control (CK, 43.4 ± 8.7 g). The PGPR, *B. subtilis* JS, treatment increased the leaf, shoot, and root mass by 37.9, 48.9, and 33.1%, respectively. Among all of poplar clones, the DM of Dorskamp was highest in the B1 group (61.7 ± 11.1 g), followed by that in the B2 group (60.3 ± 2.2 g), and it was the lowest in CK (33.9 ± 7.9 g). The DM of Eco28 and I-476 was also the highest in the B1 group (Eco28, 65.0 ± 8.7 g; I-476, 64.1 ± 7.1 g), followed by that in the B2 group (Eco28, 57.0 ± 12.9 g; I-476, 62.2 ± 2.8 g), and the lowest in CK (Eco28, 41.0 ± 3.95 g; I-476, 49.0 ± 8.3 g). However, the DM of Venziano was not significantly different. Therefore, the total DM of Dorskamp, Eco28, and I-476 were significantly higher in each treatment group (B1, B2) compared with controls. The DM of Venziano and I-476 (Venziano, 49. 8 ± 3.7 g; I-476, 49.0 ± 8.3 g) were higher than that of Eco28 (41.0 ± 3.95 g) in the CK group, but Dorskamp was the lowest in this group (33.9 ± 7.9 g). However, there were no significant differences between clones in the B1 and B2 groups. In addition, there were no significant differences between the clones or in the interaction between the clone and treatment (except shoot DM between clones) on leaf, shoot, root, and total DM of all clones (Table 1).

The PGPR treatment increased P_{Leaf} and N_{Leaf} by 78.9 and 39.3%, respectively. The P_{Leaf} and N_{Leaf} of poplar were the highest in the B1 group (N_{Leaf}, 0.79 ± 0.26%; P_{Leaf}, 0.38 ± 0.14%), followed by those in the B2 group (N_{Leaf}, 0.65 ± 0.29%; P_{Leaf}, 0.33 ± 0.07%), and the lowest in the CK group (N_{Leaf}, 0.32 ± 0.15%; P_{Leaf}, 0.24 ± 0.10%). The P_{Leaf} of poplar clones was not significantly different between the CK and B2 groups. However, the P_{Leaf} of the I-476 clone was the highest among all the clones in the B1 treatment (Fig. 2*A*). The N_{Leaf} of poplar clones was not significantly different between the CK and B1 groups, but the I-476 clone in the B2 group higher than the other clone (Fig. 2*B*). Moreover, P_{Leaf} and N_{Leaf} were influenced by treatment, whereas they were not influenced by the clone or the interaction between clone and treatment (Fig. 2).

Effect of *B. subtilis* JS treatment on photosynthetic parameters: The P_N and WUE of poplar seedlings treated with *B. subtilis* JS in September 2015 are shown in Fig. 3. There was a 54.8% increase in P_N in the treated group compared with the CK group. The average P_N of the B2 and B1 groups was 10.62 ± 1.95 μmol m^{-2} s^{-1} and 9.83 ± 0.49 μmol m^{-2} s^{-1}, respectively. However, the average P_N of the CK groups was 6.86 ± 1.48 μmol m^{-2} s^{-1}.

Table 1. The effect of *Bacillus subtilis* JS treatment on the leaf dry mass, shoot dry mass, root dry mass, and total dry mass of *Populus euramericana* and *Populus deltoides* × *P. nigra.* Values are average ± standard errors from five replicates. One-way *ANOVA* test was performed to evaluate the differences between treatments (B1, B2, and CK). Two-way *ANOVA* was performed for each trait to test for variation between *B. subtilis* JS treatments and poplar clones. Different *uppercase letters* in the same column represent significant differences between poplar clones of the same treatment at $p \leq 0.05$. Different *lowercase letters* in the same column represent significant differences between *B. subtilis* JS treatments of the same clone, as determined by *Tukey*'s post test. CK – distilled water as a control; B1 – dilution rate of 1 in 100 (*B. subtilis*:DDW, 1:100, v/v); B2 – dilution rate of 1 in 50 (*B. subtilis*:DDW, 1:50, v/v); DM – dry mass.

Species	Clone	Treatment	Leaf [g^{-1}(DM)]	Shoot [g^{-1}(DM)]	Root [g^{-1}(DM)]	Total [g^{-1}(DM)]
Populus deltoides × *P. nigra*	Dorskamp	CK	10.73 ± 1.22^{bB}	6.03 ± 0.46^{bB}	17.13 ± 7.51^{bA}	33.90 ± 7.93^{bB}
		B1	16.51 ± 0.09^{aA}	10.64 ± 2.45^{aB}	34.57 ± 8.84^{aA}	61.72 ± 11.14^{aA}
		B2	18.07 ± 0.64^{aA}	9.74 ± 2.10^{abC}	32.53 ± 1.50^{aA}	60.34 ± 2.24^{aA}
Populus euramericana	Eco28	CK	11.14 ± 0.61^{aB}	8.30 ± 0.87^{bAB}	21.60 ± 4.5^{aA}	41.04 ± 3.95^{bAB}
		B1	21.32 ± 1.66^{aA}	13.81 ± 1.46^{aAB}	29.87 ± 6.27^{aA}	65.00 ± 8.67^{aA}
		B2	17.98 ± 9.42^{aA}	14.54 ± 1.82^{aB}	24.50 ± 4.50^{aB}	57.02 ± 12.89^{abA}
	I-476	CK	12.08 ± 2.89^{bB}	11.96 ± 4.74^{aA}	24.97 ± 1.50^{aA}	49.00 ± 8.28^{bA}
		B1	19.64 ± 1.97^{aA}	16.02 ± 3.27^{aB}	28.43 ± 2.34^{aA}	64.09 ± 7.05^{aA}
		B2	14.21 ± 5.00^{abA}	18.78 ± 2.40^{aA}	29.20 ± 2.61^{aAB}	62.19 ± 2.75^{aA}
	Venziano	CK	16.50 ± 1.73^{aA}	12.18 ± 1.12^{bA}	21.10 ± 1.73^{bA}	49.78 ± 3.65^{aA}
		B1	22.01 ± 4.93^{aA}	14.43 ± 0.81^{aAB}	23.90 ± 2.12^{abA}	60.35 ± 5.81^{aA}
		B2	19.31 ± 8.56^{aA}	14.21 ± 1.06^{aB}	26.63 ± 3.50^{aAB}	60.15 ± 12.76^{aA}
Populus spp.	Mean	CK	12.61 ± 2.86^{b}	9.62 ± 3.43^{b}	21.20 ± 4.83^{b}	43.43 ± 8.66^{b}
		B1	19.87 ± 3.25^{a}	13.73 ± 2.78^{a}	29.19 ± 6.24^{a}	62.79 ± 7.43^{a}
		B2	17.39 ± 6.17^{a}	14.32 ± 3.72^{a}	28.22 ± 4.17^{a}	59.93 ± 8.12^{a}
ANOVA (*p*)	Clone		0.188	<0.001	0.205	0.372
	Treatment		<0.01	<0.001	<0.001	<0.001
	Clone × Treatment		0.780	0.472	0.083	0.480

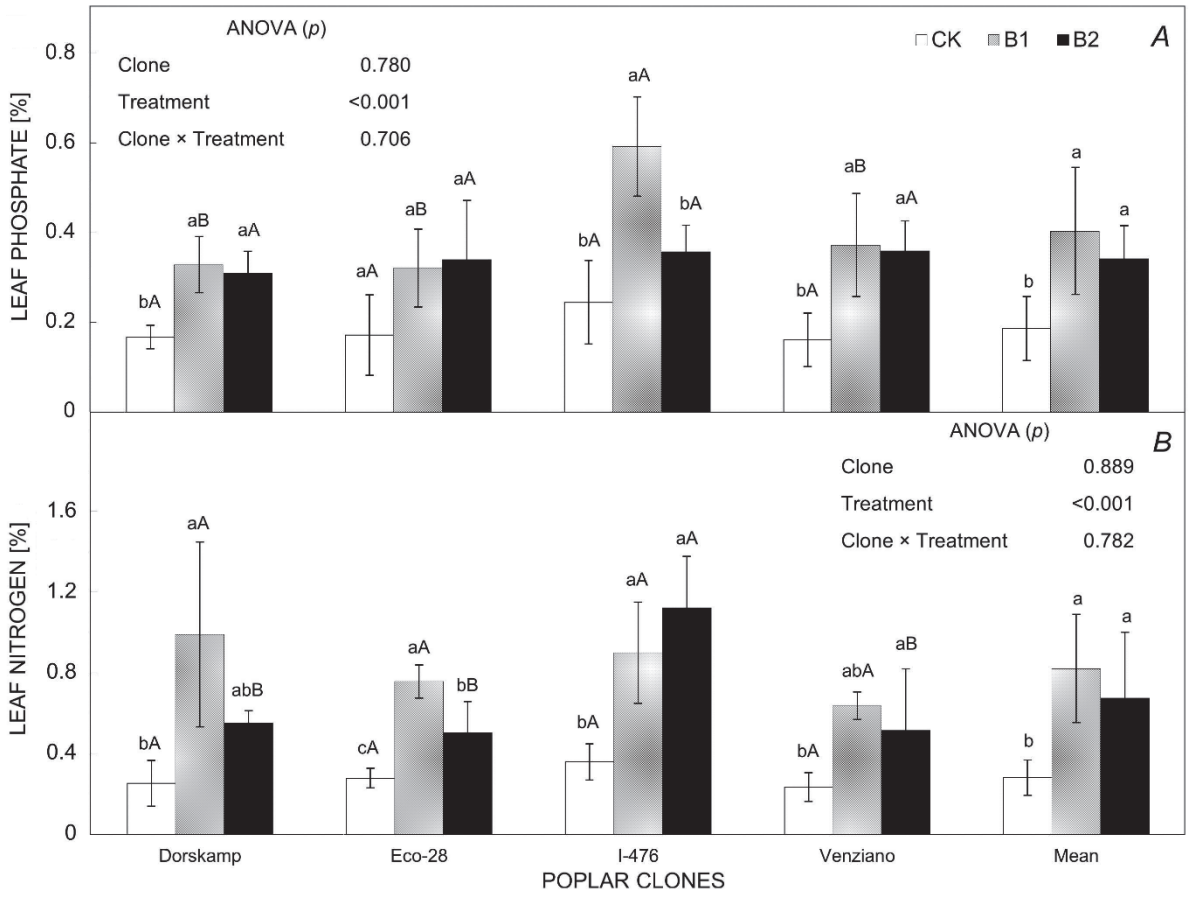

Fig. 2. The effect of *Bacillus subtilis* JS treatment on the leaf phosphate and leaf nitrogen contents of *Populus euramericana* (Eco28, I-476, Venziano) and *Populus deltoides* × *P. nigra* (Dorskamp). The poplar seedlings were treated as follows: CK – distilled water as a control; B1 – dilution rate of 1 in 100 (*B. subtilis*:DDW, 1:100, v/v); B2 – dilution rate of 1 in 50 (*B. subtilis*:DDW, 1:50, v/v). Bars indicate standard error. One-way and two-way *ANOVA*s (clone × treatment) were used for statistical analysis by using *SPSS*. *Post hoc* comparisons were performed using the *Tukey*'s post test at a significance level of $p \leq 0.05$. Different *uppercase letters* indicate significantly differences between poplar clones ($p \leq 0.05$) of the same treatment; different *lowercase letters* indicate significant differences between *B. subtilis* JS treatments (B1, B2 and CK) of the same clone ($p \leq 0.05$).

Thus, the P_N was significantly higher in the treated groups than that in the CK group. The P_N of the Venziano clone in the CK and B1 groups were higher than the other clones. However, P_N was not significantly different between clones in the B2 group (Fig. 3*A*). Similar to P_N, the WUE was the highest in the B2 group, followed by that in the B1 and CK groups (Fig. 3*B*). The *B. subtilis* JS B1 and B2 treatments increased the WUE by 52.9 and 66.6%, respectively. The WUE of the Eco28 clone was the highest one in the CK group. However, it was not significantly different between clones in the B1 and B2 groups (Fig. 3*B*). In addition, P_N and WUE were influenced by clone and treatment, but they were not influenced by their interaction (Fig. 3).

The Chl content of the B1 and B2 groups was significantly greater than that of the CK group from June to August. When compared with the CK group in June, the Chl_T and Car_T of the B2 group increased by 113.3 and 76.4%, respectively (Table 2). The average Chl_T of the B2 group in June and August was 1.92 ± 0.21 mg g^{-1}(FM) and 1.19 ± 0.67 mg g^{-1}(FM), respectively. Furthermore, the Chl_T of the CK group in June and August was 0.90 ± 0.25 mg g^{-1}(FM) and 0.54 ± 0.16 mg g^{-1}(FM), respectively. Among all poplar clones investigated in June and August, the Chl_T of Dorskamp was the highest in the B1 group and B2 group, followed by CK. Chl_T of Venziano was also greater in the B1 and B2 groups compared with CK. Moreover, Chl_T of Eco28 and I-476 in June and August was significantly higher in the B1 and B2 groups compared with CK.

Chl_T of all clones investigated in June was not significantly different in each treatment group. However, Chl_T of I-476 in August was higher than others in B1 and B2 groups, but there were no significant differences between clones in the CK group.

The Car_T of the B2 group in June and August was 0.30 ± 0.04 mg g^{-1}(FM) and 0.25 ± 0.05 mg g^{-1}(FM), respectively. The Car_T of the CK group in June and August was 0.17 ± 0.03 mg g^{-1}(FM) and 0.09 ± 0.02 mg g^{-1} (FM), respectively. Among all poplar clones investigated in June

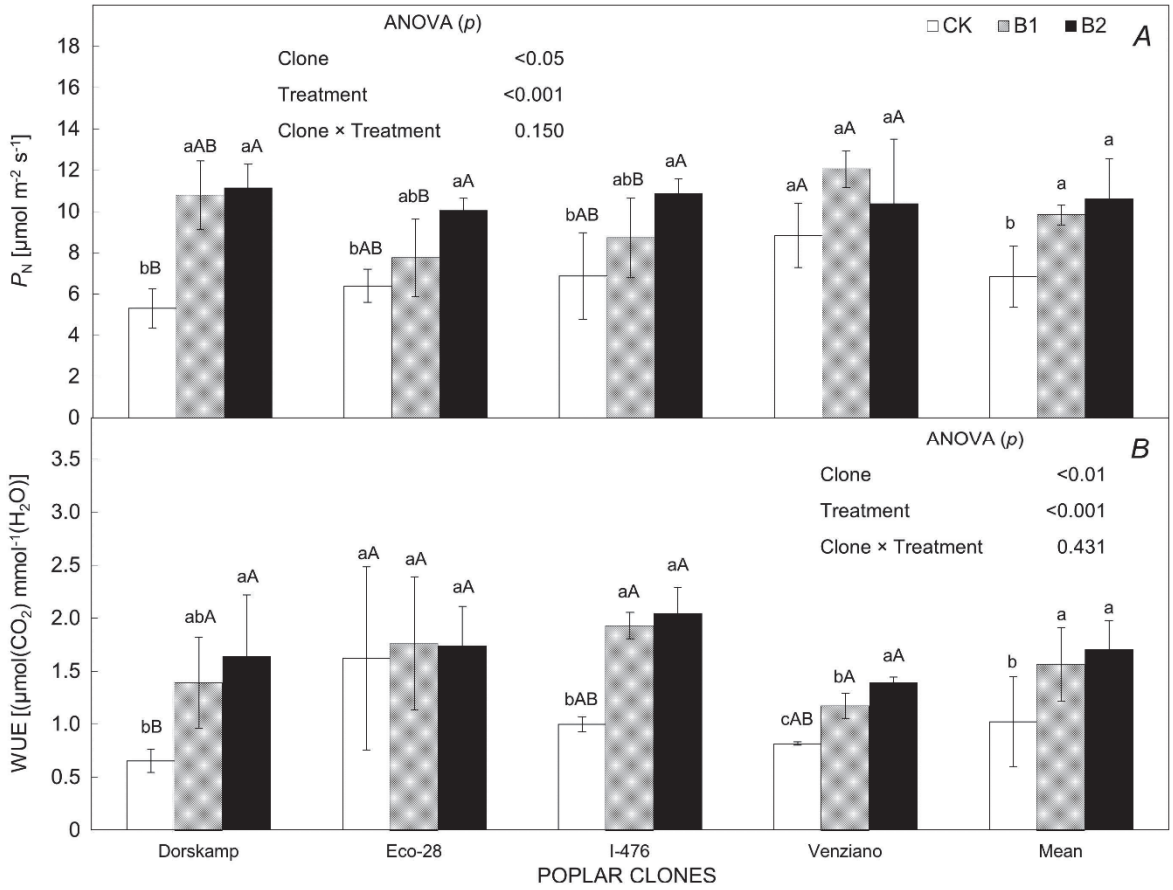

Fig. 3. The effect of *Bacillus subtilis* JS treatment on the net photosynthetic rate (P_N) and water-use efficiency (WUE) of *Populus euramericana* (Eco28, I-476, Venziano) and *Populus deltoides* × *P. nigra* (Dorskamp). The poplar seedlings were treated as follows: CK – distilled water as a control; B1 – dilution rate of 1 in 100 (*B. subtilis*:DDW, 1:100, v/v); B2 – dilution rate of 1 in 50 (*B. subtilis*:DDW, 1:50, v/v). Bars indicate standard error. One-way and two-way *ANOVA*s (clone × treatment) were used for statistical analysis by using *SPSS*. *Post hoc* comparisons were performed using the *Tukey*'s post test at a significance level of $p \leq 0.05$. Different *uppercase letters* indicate significant differences between poplar clones ($P \leq 0.05$) of the same treatment; different *lowercase letters* indicate significant differences between *B. subtilis* JS treatments (B1, B2 and CK) of the same clone ($p \leq 0.05$).

and August, Car_T of Dorskamp was the highest in the B1 and B2 groups compared with CK. Car_T of Venziano was also highest in the B1 and B2 groups compared with CK. Moreover, Car_T of Eco28 and I-476 in June and August was also significantly higher in the B1 and B2 groups compared with CK. In addition, Chl and Car_T were influenced by treatment, but they were not influenced by clone (except Chl investigated in August) or the clone × treatment interaction (Fig. 3).

Growth promotion and root activity after PGPR treatment: The root activity of poplar seedlings, calculated in mg(TF) g^{-1}(DM) h^{-1}, was the highest in the B2 group (83.99 ± 5.51), followed by the B1 (44.8 ± 10.8) and CK (46.64 ± 10.75) groups (Fig. 4). Compared with the CK group, the TF content of the B1 and B2 groups increased by 28.2 and 14.0%, respectively. The root activity of Dorskamp clones in the CK and B1 groups was the highest. Moreover, the Dorskamp and I-476 clones had the higher root activity than the other clones in the B2 group (Fig. 4*B*). In addition, the seedling height, root DM, and total DM, as well as the root activity, were correlated with each other and were higher in the treated than those in the control groups (Table 5). Moreover, the TF and root activity were influenced by clone, treatment, and their interaction (Fig. 4).

Discussion

Shoot growth after inoculation is related to the specific clone: Increased plant size is a fundamental characteristic of growth, with shoot height, diameter of area, dry matter, plant volume, size, and yield being useful proxies to evaluate growth (Salisbury and Ross 1992). The effect of *B. subtilis* strain inoculation enhanced root growth, shoot

Table 2. Effect of *Bacillus subtilis* JS treatment on the total chlorophyll and carotenoid contents of *Populus euramericana* and *Populus deltoides* × *P. nigra.* Mean ± SE (n = 5). Strain concentration of *B. subtilis* JS is 1×10^9 CFU. CK – control seedling; B1 – dilution rate of 1 in 100 (*B. subtilis*: DDW, 1:100, v/v); B2 – dilution rate of 1 in 50 (*B. subtilis*: DDW, 1:50, v/v); FM, fresh mass; Chl_T, total chlorophyll content; Car_T, total carotenoid content. One-way *ANOVA* test was performed to evaluate the differences between treatments (B1, B2, and CK). Two-way *ANOVA* was conducted for each trait to test for variation between *B. subtilis* JS treatments and poplar clones. *Post hoc* comparisons were performed using the *Tukey*'s post test at a significance level of $p \leq 0.05$. Different *uppercase letters* in the same column (treatment) represent significant differences between poplar clones of the same treatment at $p \leq 0.05$; different *lowercase letters* in the same column represent significant differences between *B. subtilis* JS treatments of the same clone, as determined by *Tukey*'s post test.

Species	Clone	Treatment	Chl_T [mg g^{-1}(FM)] June	Chl_T [mg g^{-1}(FM)] August	Car_T [mg g^{-1}(FM)] June	Car_T [mg g^{-1}(FM)] August
Populus deltoides × *P. nigra*	Dorskamp	CK	0.80 ± 0.17^{bA}	0.50 ± 1.25^{bA}	0.15 ± 0.03^{bA}	0.09 ± 0.02^{bA}
		B1	2.07 ± 0.41^{aA}	1.35 ± 3.71^{aB}	0.33 ± 0.06^{aA}	0.23 ± 0.08^{aA}
		B2	2.23 ± 0.36^{aA}	1.59 ± 1.14^{aAB}	0.36 ± 0.04^{aA}	0.25 ± 0.02^{aAB}
Populus euramericana	Eco28	CK	0.61 ± 0.23^{bA}	0.34 ± 1.03^{bA}	0.13 ± 0.04^{bA}	0.06 ± 0.02^{bA}
		B1	1.85 ± 0.26^{aA}	1.52 ± 2.20^{aAB}	0.29 ± 0.06^{aA}	0.23 ± 0.03^{aA}
		B2	1.88 ± 0.04^{aA}	1.28 ± 1.74^{aAB}	0.28 ± 0.01^{aB}	0.22 ± 0.03^{aB}
	I-476	CK	1.17 ± 0.60^{bA}	0.71 ± 3.47^{bA}	0.20 ± 0.09^{bA}	0.11 ± 0.05^{bA}
		B1	1.45 ± 0.53^{aA}	2.03 ± 4.66^{aA}	0.24 ± 0.06^{aA}	0.33 ± 0.10^{aA}
		B2	1.78 ± 0.13^{aA}	1.78 ± 3.83^{aA}	0.27 ± 0.02^{aB}	0.31 ± 0.07^{aA}
	Venziano	CK	1.03 ± 0.25^{bA}	0.60 ± 0.85^{bA}	0.19 ± 0.04^{bA}	0.11 ± 0.03^{bA}
		B1	1.63 ± 0.12^{aA}	1.14 ± 0.34^{aB}	0.25 ± 0.02^{aA}	0.22 ± 0.01^{aA}
		B2	1.78 ± 0.24^{aA}	1.09 ± 3.00^{aB}	0.28 ± 0.03^{aB}	0.21 ± 0.04^{aB}
Populus spp.	Mean	CK	0.90 ± 0.25^{b}	0.54 ± 0.16^{a}	0.17 ± 0.03^{b}	0.09 ± 0.02^{b}
		B1	1.75 ± 0.27^{a}	1.01 ± 0.76^{a}	0.28 ± 0.04^{a}	0.25 ± 0.05^{a}
		B2	1.92 ± 0.21^{a}	1.19 ± 0.67^{a}	0.30 ± 0.04^{a}	0.25 ± 0.05^{a}
ANOVA (p)	Clone		0.372	<0.01	0.192	<0.01
	Treatment		<0.001	<0.001	<0.001	<0.001
	Clone × Treatment		0.480	0.139	0.110	0.572

biomass, and total N content in woody plants and other legumes, according to Utkhede *et al*. (1992), Eşitken *et al*. (2002), Singh *et al*. (2008), and Badizi *et al*. (2016), and our result also showed growth promotion (Fig. 1, Table 1) and increased N content of poplar seedlings (Fig. 2*A*). Interestingly, similar to *Bacillus* strain GB03 in white clover (Han *et al*. 2014), our result indicated that strain JS is a more efficient promoter of shoot growth than root growth in poplar seedlings (Table 1). It has been reported that specific cultivars or clones significantly affected shoot length and diameter of apple seedlings (Khan *et al*. 1998, Bianco *et al*. 2003), and our study showed similar results in poplar seedlings. Aslantaş *et al*. (2007) reported that inoculation with *Bacillus* strains promoted significant tree growth in the field, but growth responses were strain-specific. For example, *Bacillus* strain OSU-142 significantly increased both shoot length and diameter, whereas *Bacillus* M-3 showed no effect on these measures of growth. Different clones significantly affected shoot length and diameter of apple trees (Khan *et al*. 1998, Bianco *et al*. 2003). In our study, the height and diameter of each poplar clone was higher in the CK than B1 and B2 groups until June. However, from July on, each clone was higher in the B1 and B2 groups than that of CK (Tables 3, 4), because the physiological activity of the leaves was the highest in August (Wilson *et al*. 2000, Misson *et al*. 2006). Aslantaş *et al*. (2007) reported *Bacillus* strain inoculation strongly influenced shoot number, length, diameter, and yield during the early stages of growth. In particular, Venziano and I-476 clones were the highest among treatments after July. Among poplar clones, shoot growth data showed clonal specificity, but root growth did not show significant differences between clones. Eco28 clone was the most efficient biomass producer in the B1 and B2 groups compared with CK, whereas the Dorskamp clone was lower than the other clones in all treatments. In general, the I-476 clone was reported to have the lowest biomass production in SRCs (Shin *et al*. 2012). Our results showed I-476 clone was the clone with the greatest biomass production potential in the B2 group. These results suggest that growth and biomass production of poplar seedlings Eco28 and I-476 are more efficient than those of Dorskamp and Venziano after *Bacillus* inoculation treatment. Even if I-476 and Venziano clones showed the lowest growth after the *Bacillus* treatment prior to June, they showed the greatest growth after July. The inoculation modes of strain JS for each clone played a very important role in the effects observed. Thus, PGPR may interact synergistically with the selected clone. However, with the exception of TF and root activity, our results did not show a synergistic, metabolic interaction involving clone and treatment.

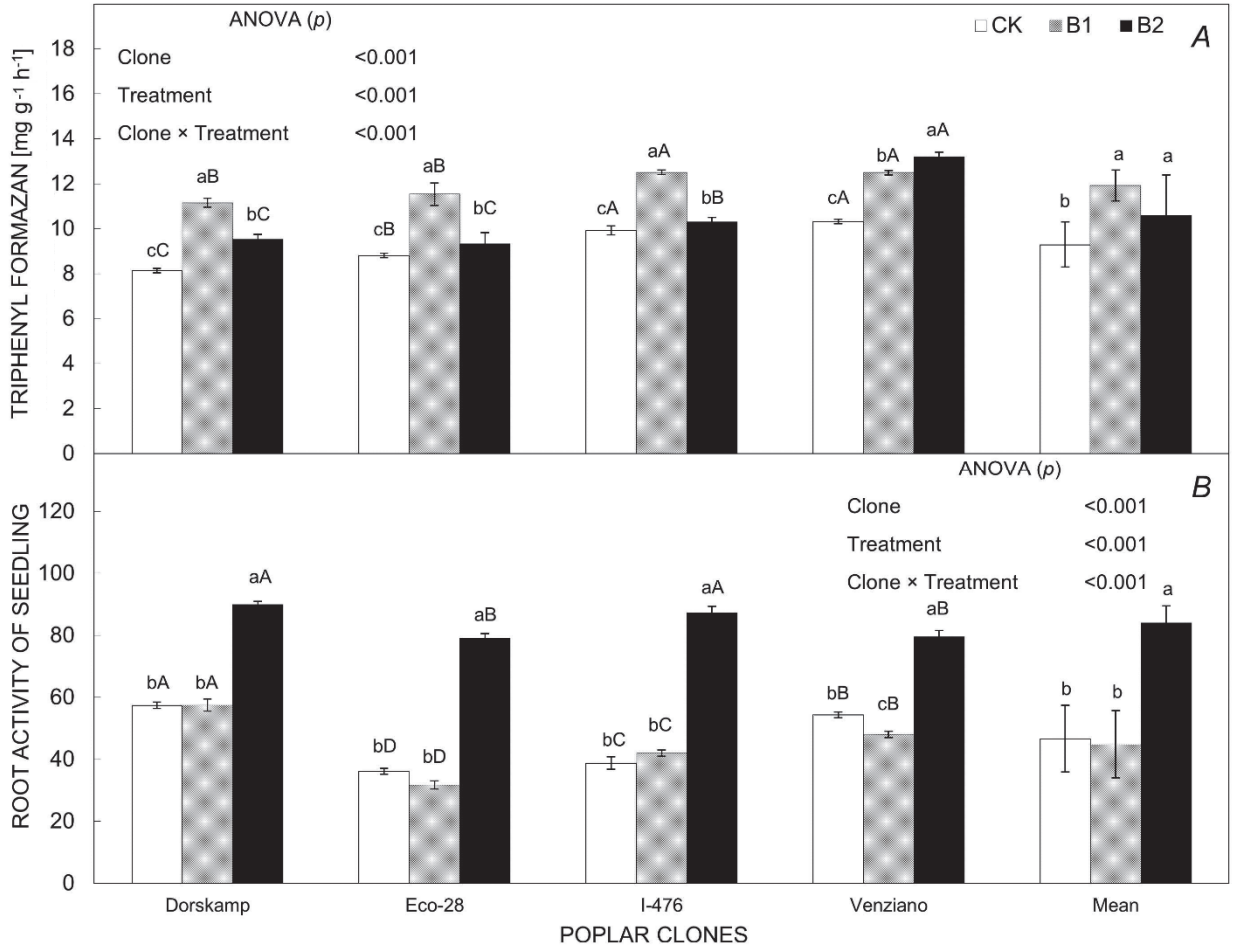

Fig. 4. The effect of *B. subtilis* JS treatment on the triphenyltetrazolium chloride reactivity (triphenyl formazan content) and relative root activity of *Populus euramericana* (Eco28, I-476, Venziano) and *Populus deltoides* × *P. nigra* (Dorskamp) seedlings. The poplar seedlings were treated as follows: CK – distilled water as a control; B1 – dilution rate of 1 in 100 (*B. subtilis*:DDW, 1:100, v/v); B2 – dilution rate of 1 in 50 (*B. subtilis*:DDW, 1:50, v/v). Bars indicate standard error. One-way and two-way *ANOVA*s (clone × treatment) were used for statistical analysis by using *SPSS*. *Post hoc* comparisons were performed using the *Tukey*'s post test at a significance level of $p \leq 0.05$. Different *uppercase letters* indicate significant differences between poplar clones ($p \leq 0.05$) of the same treatment; different *lowercase letters* indicate significant differences between *B. subtilis* JS treatments (B1, B2, and CK) of the same clone ($p \leq 0.05$).

Relationship between N content and photosynthetic parameters on *B. subtilis* JS treatment: Previous studies have demonstrated that hybrid poplar allocates a greater proportion of resources to aboveground biomass when pedospheric N is readily available (Ibrahim *et al*. 1997, Coleman *et al*. 1998). Nitrogen availability in *Populus* modulated parameters that affect carbon gain, including net photosynthesis. This parameter was affected by N-induced changes to leaf maturation and senescence (Cooke *et al*. 2005). Photosynthesis requires the integration of endogenous signals, as well as environmental factors (Ku *et al*. 1977, Leister *et al*. 2005). *B subtilis* could enhance plant photosynthetic activity by increasing leaf photosynthetic activity by rising leaf photosynthetic efficiency, and Chl content (Li *et al*. 2016). In Petri-dish studies, more than ten chloroplast-associated genes in *Arabidopsis* have been shown to be differentially expressed when plants were exposed to volatiles of *B. subtilis* strain as seedlings (Zhang *et al*. 2007), indicating that the promotion of growth may, at least in part, be a consequence of increases in P_N (Han *et al*. 2005, Xie *et al*. 2009). Our results showed that JS inoculation significantly increased N_{Leaf} (Fig. 2), Chl (Table 2), and P_N (Fig. 3) compared with the CK group. The increased P_N after *Bacillus* inoculation has been shown to increase with N_{Leaf} or Chl in bean, coffee, willow (Lima *et al*. 1999, Netto *et al*. 2005, Weih *et al*. 2007), banana (Bandopadhyay 2015), and poplar (this study), whereas contrasting results were reported for sunflower (Ciompi *et al*. 1996). Moreover, N_{Leaf} and P_N of poplar clones showed positive a correlation ($r^2 = 0.21$, $p<0.01$) in all treatments and clones. N_{Leaf} was positively correlated with WUE in deciduous plants, such

Table 3. Changes of effect of *Bacillus subtilis* JS treatment on the seedling height of *Populus euramericana* and *Populus deltoides* × *P. nigra.* Mean ± SE (n = 5). Strain concentration of *B. subtilis* JS is 1×10^9 CFU. RGR - relative growth rate = $\ln(\text{Diameter}_{\text{September}} - \text{Diameter}_{\text{March}})$ 180days^{-1}; CK – control seedling; B1 – dilution rate of 1 in 100 (*B. subtilis*: DDW, 1:100, v/v); B2 – dilution rate of 1 in 50 (*B. subtilis*: DDW, 1:50, v/v). One-way *ANOVA* test was performed to evaluate the differences between treatments (B1, B2 and CK). Two-way *ANOVA* was conducted for each trait to test for variation between *B. subtilis* JS treatments and poplar clones. *Post hoc* comparisons were performed using the *Tukey*'s post test at a significance level of $p \leq 0.05$. *Different uppercase letters* in the same column (treatment) represent significant differences among poplar clones of the same treatment at $p \leq 0.05$. *Different lowercase letters* in the same column represent significant differences among *B. subtilis* JS treatments of the same clone, as determined by *Tukey*'s post test.

Species	Clone	Treatment	Seedling height [cm]						
			March	May	June	July	August	September	RGR [cm d^{-1}]
P. deltoids × *P. nigra*	Dorskamp	CK	12.64 ± 0.98^{aA}	33.30 ± 4.82^{aA}	40.80 ± 6.75^{bB}	39.60 ± 5.97^{bB}	41.88 ± 7.49^{cC}	42.02 ± 7.56^{cB}	0.019 ± 0.002^{cB}
		B1	12.48 ± 0.36^{aA}	28.06 ± 4.89^{aA}	42.18 ± 7.99^{abB}	66.34 ± 6.53^{aB}	69.93 ± 6.20^{bB}	70.01 ± 6.42^{bC}	0.022 ± 0.001^{bB}
		B2	12.78 ± 0.80^{aA}	26.40 ± 3.74^{aA}	54.10 ± 8.06^{aAB}	74.47 ± 5.05^{aA}	86.23 ± 4.28^{aA}	87.92 ± 4.91^{aAB}	0.024 ± 0.000^{aAB}
P. euramericana	Eco28	CK	12.50 ± 0.12^{aA}	28.48 ± 8.74^{aA}	63.08 ± 9.54^{aA}	67.10 ± 10.26^{bA}	69.19 ± 12.30^{bA}	69.32 ± 12.29^{bA}	0.022 ± 0.001^{bA}
		B1	12.24 ± 0.24^{aA}	31.63 ± 4.07^{aA}	64.22 ± 10.24^{aA}	85.20 ± 13.18^{aA}	91.41 ± 13.57^{aA}	92.01 ± 14.12^{aAB}	0.024 ± 0.001^{aA}
		B2	12.48 ± 0.44^{aA}	30.47 ± 3.75^{aA}	64.44 ± 5.48^{aA}	84.78 ± 5.15^{aA}	98.02 ± 5.72^{aA}	102.81 ± 7.37^{aA}	0.025 ± 0.000^{aA}
	I-476	CK	12.38 ± 0.46^{aA}	37.20 ± 2.50^{aA}	59.00 ± 10.11^{aA}	66.52 ± 15.27^{aA}	68.07 ± 16.51^{aAB}	68.11 ± 16.57^{bA}	0.022 ± 0.002^{bA}
		B1	12.40 ± 0.34^{aA}	31.23 ± 4.59^{abA}	57.73 ± 7.68^{aA}	82.10 ± 12.07^{aAB}	91.79 ± 13.51^{aA}	93.92 ± 11.77^{abA}	0.024 ± 0.001^{abA}
		B2	12.28 ± 0.29^{aA}	25.93 ± 5.49^{bA}	52.49 ± 10.88^{aAB}	75.74 ± 12.72^{aA}	88.81 ± 14.16^{aA}	110.06 ± 30.41^{aA}	0.025 ± 0.002^{aA}
	Venziano	CK	12.78 ± 1.40^{aA}	33.50 ± 7.00^{aA}	42.03 ± 8.37^{bB}	45.78 ± 8.41^{cB}	48.10 ± 7.22^{cBC}	49.04 ± 7.31^{bB}	0.020 ± 0.001^{bAB}
		B1	12.36 ± 0.37^{aA}	32.03 ± 2.62^{abA}	53.73 ± 8.06^{aAB}	71.05 ± 5.05^{aAB}	78.47 ± 4.28^{aAB}	78.90 ± 4.91^{aBC}	0.023 ± 0.000^{aAB}
		B2	12.52 ± 0.19^{aA}	24.55 ± 4.38^{bA}	41.60 ± 3.43^{bB}	58.03 ± 5.28^{bB}	66.63 ± 5.83^{bB}	71.39 ± 11.55^{aB}	0.023 ± 0.001^{aB}
Populus spp.	Mean	CK	12.58 ± 0.17^{a}	33.12 ± 3.57^{b}	51.23 ± 11.46^{a}	54.75 ± 14.15^{a}	56.81 ± 13.89^{b}	57.12 ± 13.70^{b}	0.021 ± 0.002^{b}
		B1	12.37 ± 0.10^{a}	30.74 ± 1.82^{ab}	54.47 ± 9.26^{a}	76.17 ± 8.94^{a}	82.90 ± 10.64^{a}	83.71 ± 11.31^{a}	0.024 ± 0.001^{a}
		B2	12.52 ± 0.21^{a}	26.84 ± 2.54^{b}	53.16 ± 9.35^{a}	73.26 ± 11.14^{a}	84.92 ± 13.20^{a}	93.04 ± 17.13^{a}	0.024 ± 0.001^{a}
ANOVA (p)	Clone		0.580	0.685	<0.001	<0.001	<0.001	<0.001	<0.001
	Treatment		0.561	<0.01	0.452	<0.001	<0.001	<0.001	<0.001
	Clone × Treatment		0.972	0.084	0.029	0.054	0.042	0.311	0.035

Table 4. Changes of effect of *Bacillus subtilis* JS treatment on the seedling diameter of *Populus euramericana* and *Populus deltoides* × *P. nigra.* Mean ± SE ($n = 5$). Strain concentration of *B. subtilis* JS is 1×10^9 CFU. *RGR*, relative growth rate = $\ln(\text{Diameter}_{\text{September}} - \text{Diameter}_{\text{May}})$ 120days^{-1}; CK – control seedling; B1 – dilution rate of 1 in 100 (*B. subtilis*: DDW, 1:100, v/v); B2 – dilution rate of 1 in 50 (*B. subtilis*: DDW, 1:50, v/v). One-way *ANOVA* test was performed to evaluate the differences between treatments (B1, B2 and CK). Two-way *ANOVA* was conducted for each trait to test for variation between *B. subtilis* JS treatments and poplar clones. *Post hoc* comparisons were performed using the *Tukey*'s post test at a significance level of $p \leq 0.05$. *Different uppercase letters* in the same column (treatment) represent significant differences among poplar clones of the same treatment at $p \leq 0.05$. *Different lowercase letters* in the same column represent significant differences among *B. subtilis* JS treatments of the same clone, as determined by *Tukey*'s post test.

Species	Clone	Treatment	Seedling diameter [mm] May	June	July	August	September	Net growth	RGR [mm d^{-1}]
P. deltoids × *P. nigra*	Dorskamp	CK	3.04 ± 0.49^{aAB}	5.04 ± 0.82^{aA}	5.29 ± 0.74^{aA}	5.50 ± 0.78^{bA}	5.63 ± 0.69^{bA}	2.59 ± 0.47^{cB}	0.08 ± 0.01^{bC}
		B1	2.98 ± 0.36^{aB}	4.25 ± 0.30^{aB}	5.38 ± 0.37^{aB}	5.82 ± 0.42^{abB}	6.26 ± 0.38^{abB}	3.51 ± 0.64^{bB}	0.10 ± 0.02^{aB}
		B2	2.68 ± 0.38^{aA}	4.37 ± 0.78^{aA}	5.74 ± 0.46^{aA}	6.64 ± 0.47^{aA}	6.91 ± 0.40^{aB}	4.23 ± 0.39^{aA}	0.12 ± 0.01^{aA}
P. euramericana	Eco28	CK	2.62 ± 0.45^{aB}	5.22 ± 1.24^{aA}	6.22 ± 0.88^{aA}	6.49 ± 0.90^{aA}	6.82 ± 0.87^{aA}	3.81 ± 0.42^{bA}	0.11 ± 0.01^{aA}
		B1	2.99 ± 0.32^{aB}	5.39 ± 0.47^{aA}	6.50 ± 0.47^{aA}	7.01 ± 0.35^{aA}	7.25 ± 0.65^{aAB}	4.26 ± 0.50^{abA}	0.12 ± 0.01^{aAB}
		B2	2.80 ± 0.18^{aA}	5.11 ± 0.25^{aA}	6.40 ± 0.51^{aA}	7.13 ± 0.80^{aA}	7.46 ± 0.86^{aAB}	4.66 ± 0.70^{aA}	0.13 ± 0.01^{aA}
	I-476	CK	3.34 ± 0.29^{aAB}	5.58 ± 0.49^{aA}	6.36 ± 0.79^{aA}	6.80 ± 0.82^{aA}	6.90 ± 0.78^{bA}	3.56 ± 0.74^{bA}	0.10 ± 0.02^{aAB}
		B1	3.32 ± 0.48^{aAB}	5.52 ± 0.37^{aA}	6.77 ± 0.77^{aA}	7.35 ± 0.50^{aA}	7.77 ± 0.68^{abA}	4.45 ± 0.34^{aA}	0.12 ± 0.01^{aA}
		B2	3.10 ± 0.53^{aA}	4.72 ± 0.52^{bA}	6.58 ± 0.86^{aA}	7.32 ± 0.88^{aA}	8.51 ± 1.18^{aA}	4.03 ± 0.48^{abA}	0.12 ± 0.01^{aA}
	Venziano	CK	3.80 ± 0.43^{aA}	5.38 ± 0.59^{abA}	5.90 ± 0.44^{aA}	6.25 ± 0.61^{aA}	6.41 ± 0.43^{bA}	2.61 ± 0.29^{bB}	0.08 ± 0.01^{bBC}
		B1	3.83 ± 0.37^{aA}	5.44 ± 0.39^{aA}	6.66 ± 0.64^{aA}	7.09 ± 0.63^{aA}	7.43 ± 0.44^{aA}	3.60 ± 0.41^{aB}	0.11 ± 0.01^{aB}
		B2	2.79 ± 0.24^{bA}	4.66 ± 0.20^{bA}	5.87 ± 0.35^{aA}	6.43 ± 0.14^{aA}	6.89 ± 0.28^{abB}	4.03 ± 0.27^{aA}	0.12 ± 0.01^{aA}
Populus spp.	Mean	CK	3.20 ± 0.59^{ab}	5.30 ± 0.80^{a}	5.94 ± 0.79^{a}	6.26 ± 0.87^{b}	6.44 ± 0.83^{b}	3.14 ± 0.73^{b}	0.09 ± 0.02^{b}
		B1	3.28 ± 0.50^{a}	5.15 ± 0.64^{ab}	6.33 ± 0.78^{a}	6.82 ± 0.75^{ab}	7.18 ± 0.77^{a}	3.96 ± 0.61^{a}	0.11 ± 0.01^{a}
		B2	2.84 ± 0.37^{b}	4.72 ± 0.53^{b}	6.15 ± 0.64^{a}	6.88 ± 0.69^{a}	7.44 ± 0.98^{a}	4.24 ± 0.52^{a}	0.12 ± 0.01^{a}
ANOVA (p)	Clone		<0.001	<0.01	<0.001	<0.001	<0.001	<0.001	<0.001
	Treatment		<0.01	0.010	0.174	<0.01	<0.001	<0.001	<0.001
	Clone × Treatment		0.042	0.365	0.638	0.359	0.307	0.066	0.038

Table 5. Correlation analysis between physiological parameter and growth on *Bacillus subtilis* JS treatments of *Populus euramericana* and *Populus deltoides* × *P. nigra.* * – correlation coefficient is significant at the 0.05 level; ** – correlation coefficient is significant at the 0.01 level.

Factor	Coefficient of correlation	Factor N_{Leaf}	P_{Leaf}	P_N	WUE	Chl	TF	Root activity
N_{Leaf}	Pearson coefficient	-	0.527**	0.464**	0.451**	0.527**	0.425**	0.188
	P	-	0.001	0.005	0.006	0.001	0.010	0.273
	r^2	-	0.277	0.21	0.20	0.27	0.18	0.035
P_{Leaf}	Pearson coefficient	0.527**	-	0.392*	0.569**	0.346*	0.570**	0.074
	P	0.001	-	0.020	0.000	0.039	0.000	0.667
	r^2	0.277	-	0.154	0.324	0.12	0.32	0.006
P_N	Pearson coefficient	0.464**	0.392*	-	0.275	0.594**	0.477**	0.502**
	P	0.005	0.020	-	0.110	0.000	0.004	0.002
	r^2	0.21	0.154	-	0.075	0.35	0.22	0.252
WUE	Pearson coefficient	0.451**	0.569**	0.275	-	0.330*	0.182	0.178
	P	0.006	0.000	0.110	-	0.049	0.287	0.300
	r^2	0.20	0.324	0.075	-	0.109	0.033	0.032
Leaf [g (DM)]	Pearson coefficient	0.283	0.459**	0.443**	0.148	0.388**	0.508**	0.025
	P	0.094	0.005	0.008	0.389	0.19	0.002	0.887
	r^2	0.08	0.21	0.19	0.22	0.15	0.258	0.001
Shoot [g (DM)]	Pearson coefficient	0.497**	0.561**	0.459**	0.426**	0.404**	0.508**	0.180
	P	0.002	0.000	0.006	0.010	0.015	0.002	0.294
	r^2	0.24	0.31	0.21	0.181	0.16	0.258	0.032
Root [g (DM)]	Pearson coefficient	0.439**	0.523**	0.463**	0.349*	0.534**	0.297	0.187
	P	0.007	0.001	0.005	0.037	0.001	0.100	0.274
	r^2	0.19	0.27	0.21	0.122	0.28	0.07	0.035
Total [g (DM)]	Pearson coefficient	0.523**	0.667**	0.599**	0.771**	0.589**	0.542	0.169
	P	0.001	0.000	0.000	0.000	0.000	0.001	0.324
	r^2	0.27	0.44	0.35	0.595	0.34	0.294	0.029
Seedling height	Pearson coefficient	0.236	0.367*	0.560**	0.273	0.523**	0.221	0.396
	P	0.165	0.028	0.000	0.107	0.001	0.196	0.017
	r^2	0.05	0.13	0.31	0.075	0.27	0.049	0.156
Seedling diameter	Pearson coefficient	0.362**	0.474**	0.504**	0.429**	0.490**	0.259	0.436**
	P	0.030	0.004	0.002	0.009	0.002	0.128	0.008
	r^2	0.13	0.22	0.25	0.184	0.23	0.067	0.190

as poplar (Adams *et al*. 2016). WUE also showed a similar pattern in this study. In our study, N_{Leaf} in poplar increased with WUE ($r^2 = 0.20$, $p<0.01$) in all treatments and clones. At the physiological level, WUE can be defined as the ratio of photosynthesis (carbon gain) to transpiration (water loss) (Xu and Hsiao 2004). The WUE of *P. euramericana* Eco28, I-476, and Venziano, and *P. deltoides* × *P. nigra* Dorskamp might be improved by increased P_N and decreased *E* and stomatal conductance (g_s) (data not shown), indicating increased resistance to restrict foliar pathogens and drought. WUE is one of the major factors required for the survival, growth, and vitality of trees (Ni and Pallardy 1991, Tolentino *et al*. 2006). In addition, Li *et al*. (2016) reported that *B. subtilis* could improve WUE of intact leaves in broad beans (*Vicia faba* L. cv. 'Da qing pi') by adjusting the leaf stomatal aperture (width/length) and photosynthetic activity, and this improved WUE was associated with the vitality of *B. subtilis*-triggered stomatal closure. In all treatments and clones, there was a significant positive relationship between N_{Leaf} and shoot ($r^2 = 0.24$, $p<0.01$), root ($r^2 = 0.19$, $p<0.01$), and total ($r^2 = 0.27$, $p<0.01$) biomass yield. Among them, the high N_{Leaf} and P_{Leaf} allowed for increased shoot growth (than root growth) and P_N (Vafadar *et al*. 2014), and our results were consistent with previous reports.

Bacillus subtilis* strain JS affects photosynthetic and biochemical parameters of *Populus: Chl is a vital segment of leaf colors and is critical during photosynthesis. Without adequate pigment contents, plant cannot perform photosynthesis (Mathivanan *et al*. 2017). In our study, JS treatment (B1, B2) significantly enhanced photosynthetic capacity (Fig. 3). Improved growth under *Bacillus* strain treatment is most likely a result of improved nutrition, leading to higher P_N on *Stevia rebaudiana* (Vafadar *et al*. 2014), *P. euramericana,* and *P. deltoides* × *P. nigra* (this study). Overall, Chl *a*, Chl *b*, and Car contents in leaves of poplar clones significantly increased by increasing *B. subtilis* JS inoculation (Table 2), because *Bacillus* caused accumulation of Chl (Mohamed *et al*. 2012). The increased Chl in plant inoculated by *Bacillus* (Table 2) probably resulted in higher P_N and thus improved total biomass (Vafadar *et al*. 2014), and our result supported this suggestion (Fig. 3). There was a significant

relationship between P_N and Chl in June (r^2 = 0.35, p<0.01) although P_N and Chl in August had a weak positive relationship (r^2 = 0.18, p<0.05). It has been demonstrated that Chl assumes a vital role in ATP generation and assurance of fundamental plant constituents (Kochot *et al*. 1998). Chl is one of the important biochemical parameters, and it is used as an index of plant protected capacity (Mathivanan *et al*. 2017). Zhang *et al*. (2007) and Li *et al*. (2016) reported that *B. subtilis* strains augment photosynthetic capacity by increasing P_N and Chl in *Arabidopsis*. The reason for the increase in P_N is attributed to the increased N_{Leaf} and P_{Leaf} after strain JS inoculation. Therefore, N_{Leaf} was attributed to changes in the photosynthetic apparatus and activities after *Bacillus* treatment. Chl *a*, Chl *b*, and Chl_T are indicative of photosynthetic and metabolic activity (Wright and Jones 2006, Hartmann *et al*. 2009). We noted an alteration in the pigment content in poplar seedlings upon treatment with strain JS during the growing season (June and August). Interestingly, the Dorskamp clone had the lowest Chl_T in the CK group, but it showed the highest Chl_T among the B2 group across the growing season. Therefore, we concluded that strain JS inoculation resulted in the greatest increase of Chl_T in the Dorskamp clone. It has been reported that *Bacillus* treatment enhanced the Chl_T of *Catharanthus roseus* (Lenin and Jayanthi 2012) and *Ociumum basilicum* (Heidari *et al*. 2011). Chl content and total biomass increased due to *Bacillus* inoculation (Karami *et al*. 2016). Carotenoids are an accessory pigment in the photosynthetic assimilation of plants. The highest Car content was reported in 75-d-old plants (*Arachis hypogaea* L.) when compared with all other sampling days, whereas the lowest content was recorded in crops grown without *Bacillus* (Mathivanan *et al*. 2017). In *Vigna mungo* (L.) Hepper, a *Bacillus* treatment had a positive effect on plant growth and Car content (Hernandez *et al*. 2014). Similarly, *Bacillus* strain JS treatment on *Populus* had a positive effect on Car_T, especially in the Venziano clone (Table 2). Kim *et al*. (2015b) reported that two photosynthetic genes (Chl *a*/*b*-binding protein and chloroplast sedoheptulose-1,7-bisphosphatase) upregulated by bacterial volatiles of strain JS were classified as related to metabolic processes in *N. tabacum* L. cv. 'Xanthi'. These results likely suggest why P_N of the *B. subtilis* JS-treated plants was significantly higher than that of the CK (Fig. 3*A*). In addition, the two genes are known to be involved in photosynthetic capacity. Among them, the Chl *a*/*b*-binding protein, located in the light-harvesting complex of PSII (Pichersky *et al*. 1987), increased during the growing season by natural light exposure (Kim *et al*. 2015b). Overexpression of chloroplast sedoheptulose-1,7-bisphosphatase results in enhanced photosynthetic efficiency and growth promotion in *N. tabacum* L. 'Xanthi' (Miyagawa *et al*. 2001, Lefebvre *et al*. 2005). The upregulation of Chl *a*/*b*-binding protein expression strongly suggests the activation of the photosynthetic gene due to application of strain JS volatiles on *N. tabacum*, indicating that the bacterial volatiles increased the P_N. These results imply that plant growth promotion by *B. subtilis* JS may be due to the upregulation of enzymes by bacterial volatile organic compounds (Kim *et al*. 2015b). Our results also suggest that the volatiles are capable of promoting growth of *Populus* with activation of photosynthetic genes similar to those observed in *N. tabacum* treated with strain JS. Since the volatiles were from strain JS, we expected a similar result with *Populus*. However, the research relating on upregulation of Chl *a*/*b*-binding protein in *Populus* has not been conducted yet. Therefore, further study should be conducted on the expression of Chl-binding protein in *Populus*.

Correlation between growth promotion and root activity after PGPR treatment: The 2,3,5-triphenyltetrazolium chloride (TTC) test has been used to study the vitality of different plant root tissues (Lassheikki *et al*. 1991, Lindström and Nyström 1987) and to measure respiratory activity (Joslin and Henderson 1984, Stūrīte *et al*. 2005). The PGPR promoted the growth of the plant, increasing the root surface area and general root architecture (Biswas *et al*. 2000, Lucy *et al*. 2004). Root activity is therefore important in studies for plant growth and nutrient dynamics. The colorless TTC is reduced to a red-colored TF due to the dehydrogenase activity of the mitochondrial respiratory chain (Richter 2007). Our results showed that *B. subtilis* strain JS inoculation increased root biomass (Table 1), TF content, and root activity (Fig. 4). In addition, significant positive relationships were found between root activity and root biomass. The root activity (r^2 = 0.03) and TF contents (r^2 = 0.07) increased with increased root biomass, but they were not significantly different. In terms of nutrients absorption, TF contents affected N_{Leaf} and P_{Leaf}; P_N and had positive relationships with N_{Leaf} (r^2 = 0.18, p<0.01), P_{Leaf} (r^2 = 0.32, p<0.01), and P_N (r^2 = 0.22, p<0.01), respectively. TF content showed a positive correlation with aboveground biomass, root biomass, and P_N in 27 soybean cultivars across the growing season (Cui *et al*. 2016), and our study revealed that TF content had a positive correlation with leaf (r^2 = 0.25, p <001) and shoot biomass (r^2 = 0.25, p<0.01). In this work, we evaluated if root activity on poplar seedling could be considered as an indicator of root growth vigor and physiological activities. Cui *et al*. (2016) concluded some signal molecules synthesized in roots are transported into stems and leaves to regulate photosynthesis and growth. These signal molecules include hormones and nutrients that directly adjust gene expression and protein synthesis involved in photosynthesis and growth. As a result, photosynthesis and metabolism may be affected. Seo (2015) investigated whether foliage treatment of strain JS promoted growth and affected physiological changes of three indoor plants,

including *Schefflera arboricola* 'Hong Kong', *Plectranthus tomentosa*, and *Epipremnum aureum*. Of these plants, *E. aureum* showed a slight growth-promoting effect after strain JS foliage spray treatment [DDW: *B. subtilis* JS (1:100, v/v)]. However, there was no significant difference between treated and untreated plants. Furthermore, the JS treatment did not cause a plant growth-promoting effect in the other indoor plants investigated. Thus, *B. subtilis* JS is a soil bacteria that affects growth promotion *via* the rhizosphere rather than other parts of plants. Further, because it might interact with microorganisms and other soil organisms that secrete volatile growth-promoting substances, it promotes growth of plants by affecting the roots. However, in the case of foliar treatment, the growth-promotion effect becomes limited. In conclusion, plant growth promotion and TTC reactivity by the bacterium *B. subtilis* JS offer obvious evidence that soil drench is more effective than other PGPR treatments. However, it remains to be seen what is associated with TF and activity on rhizosphere, and additional investigation (such as matters secreted by strain JS) should be performed.

Conclusions: This study documented that a newly isolated bacterial strain from the soil used to grow *Miscanthus sinensis* var. *purpurascens* at University of Seoul (*B. subtilis* JS) promoted growth and altered the physiology of *P. euramericana* and *P. deltoides* × *P. nigra*, but the magnitude of the responses varied with the strain's concentration and plant clone. Physiological parameters influenced most by treatments, but they were not influenced by the clone or the interaction between the clone and treatment.

These results may be due to the effect of *Bacillus* as biofertilizer, due to increase of the photosynthetic rate, or due to the role of N nutrition in producing growth-promoting substances that resulted in more efficient absorption of nutrients. As these are the main demands for production of photosynthetic pigments, the Chl content increased. These results suggest that *B. subtilis* JS could affect biomass production, increasing P_N, Chl_T, and Car_T content. Nevertheless, future studies are clearly needed in order to further understand crosstalk between upregulation of genes, such as Chl-binding protein, volatile organic compound, and physiological processes in *Populus*.

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