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A microbial endophyte enhanced growth of switchgrass under two drought cycles improving leaf level physiology and leaf development

Bingxue Wang¹, John R. Seiler¹ and Chuansheng Mei²

¹Department of Forest Resources and Environmental Conservation, Virginia Tech, Blacksburg, VA, 24061, USA

² Center for Sustainable and Renewable Resources, Institute of Advanced Learning and Research, Danville, Virginia, 24540, USA

Correspondence Author: Bingxue Wang

E-Mail: wangbx@vt.edu

Tel: 1-540-838-5522

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Abstract

Beneficial microbial endophytes increase productivity and induce stress tolerance, which could contribute to developing a low input and sustainable bioenergy crop production system. We tested for the first time, the effects of microbial endophytes on growth and leaf-level physiology of switchgrass (*Panicum virgatum*) under a moderate drought preconditioning and a successive severe drought stress. *Burkholderia phytofirmans* strain PsJN-inoculated switchgrass plants were 23.6 % taller ($p < 0.0001$) and possessed 116% more tillers ($p < 0.0001$) during the moderate drought. Pre-drought conditioned PsJN-inoculated switchgrass had higher photosynthetic rates (Ps) at all levels of water stress; whereas, control switchgrass only benefited from pre-drought when leaf water potential (LWP) dropped below -1 MPa. Both PsJN inoculation and drought stress accelerated the leaf senescence and the strain promoted tillering/height ratio, which indicated more rapid development. In sum, enhancement of drought tolerance of switchgrass by PsJN was indicated by this study.

1. Introduction

Global consumption of energy tripled during the last 4 decades with heavy reliance on unsustainable fossil energy. This led to interest in bioenergy crops, which are mainly based on food crops (Solomon, 2010) and future biofuel crops (e.g. switchgrass, *Miscanthus* and poplar). The US government policy is to displace 30% of present petroleum consumption with 910 million tons of biomass creating a sustainable biofuel scenario (Downing et al., 2011). Switchgrass (*Panicum virgatum* L.) maintains a relatively high growth rate with low water and

nutrient requirements, which enables it to be grown on marginal lands unsuitable for farming, thereby avoiding direct competition with food crop production on fertile land. A variety of biotic and abiotic stresses have negative effects on plant growth and crop yields. Drought stress has been predicted to impact more than 50% of arable lands by 2050 (Vinocur and Altman, 2005) and has a direct effect on forage production worldwide (Frank et al., 1996). Improving productivity and drought stress tolerance will help to economically produce switchgrass and positively affect the biofuels industry.

Beneficial symbiotic microbes promote growth and improve stress resistance of plants through increasing available nutrients, physiology changes and hormone regulations (Compant et al., 2010; Compant et al., 2005a; Glick, 2004; Mei and Flinn, 2010). In addition, these symbionts provide environmental and economic benefits to sustainable biofuel crop system by reducing fertilizer and pesticide use (Bakker et al., 2012; Compant et al., 2005a; Mehnaz and Lazarovits, 2006; Mei and Flinn, 2010; Weekley et al., 2012). Since switchgrass is naturally associated with a diverse group of fungi (Kleczewski et al., 2012) and bacteria (Gagne - Bourgue et al., 2013), utilizing beneficial microbial as ‘bio-fertilizer’ and ‘bio-pesticide’ would be a natural and promising way to improve switchgrass productivity. A few studies reported enhanced growth of switchgrass due to symbiotic association (Ghimire and Craven, 2011; Ker et al., 2012; Kim et al., 2012; Kleczewski et al., 2012), but physiological mechanisms behind these symbiotic microbes induced growth benefits were not studied.

The well-studied bacterial endophyte *Burkholderia phytofirmans* strain PsJN was originally isolated from onion roots, which also inhabited the vegetative organs of grapevine (Compant et al., 2005b), switchgrass (Kim et al., 2012) and maize (Naveed et al., 2014). *B. phytofirmans* can improve production and/or enhance stress tolerance in a variety of plants such

as *Arabidopsis* (Poupin et al., 2013; Sun et al., 2009; Zuniga et al., 2013), potato (Bensalim et al., 1998; Nowak et al., 1998), tomato (Sharma and Nowak, 1998), grapevine (Barka et al., 2006; Fernandez et al., 2012b), maize (Naveed et al., 2014) and switchgrass (Kim et al., 2012) by manipulating plant hormones, root growth, photosynthetic rates (Ps) and carbohydrate metabolism. The presence of a 1-aminocyclopropane-1-carboxylate (ACC) deaminase, IAA metabolic pathway and quorum sensing are necessary for PsJN's plant growth promotion effects (Sun et al., 2009; Zuniga et al., 2013). PsJN enhanced chilling resistance, drought stress resilience and verticillium wilt resistance in grapevine (Barka et al., 2006; Fernandez et al., 2012b), maize (Naveed et al., 2014) and tomato (Sharma and Nowak, 1998), respectively. Higher biomass, photosynthetic rates, chlorophyll content and photochemical efficiency were found in PsJN-inoculated grapevine and maize under chilling stress and drought stress (Barka et al., 2006; Naveed et al., 2014), respectively. Trehalose metabolism also participated in PsJN induced chilling tolerance of grapevine (Fernandez et al., 2012b). Previous results demonstrated that PsJN increased 'Alamo' switchgrass seedling biomass production by 50% (Kim et al., 2012). However, the effect of PsJN on drought tolerance of switchgrass has not been studied. How endophyte inoculation influences the relationship between photosynthetic rates and leaf water potential or growth and development during drought is not known. Studying physiological changes associated with PsJN inoculation under drought stress would help develop a low input and sustainable switchgrass production system (Mei and Flinn, 2010).

In this study, we investigated physiological changes, including those associated with drought tolerance, in response to inoculation with the bacterial endophyte PsJN. Our hypothesis are: 1) PsJN inoculation enhances drought tolerance in switchgrass by inducing more rapid development and leaf senescence. 2) a mild, drought preconditioning treatment primes

switchgrass for later drought stress by modifying development and leaf-level physiology, and 3) PsJN inoculation increases drought tolerance of switchgrass by allowing a maintenance of photosynthesis to lower potentials.

2. Materials and Methods

Plant material and growth condition

Switchgrass (*Panicum virgatum* L.) cv. Alamo seeds were purchased from Warner Brother's Seed Co. (Lawton, OK). These seeds were surface-sterilized by soaking in 70% ethanol for 2 min and rinsing 3X with distilled water. Then they were de-husked by immersing in 60% H₂SO₄ with stirring for 30 min, washed 3X with distilled water, and sterilized with 0.4 M sodium hypochlorite containing 0.1% Triton 100 for 30 min followed by 5X rinse with sterile

deionized water. Finally the sterilized seeds were then germinated in petri-dishes in a growth incubator at 25°C, under white fluorescent light ($67 \mu\text{mol m}^{-2} \text{s}^{-1}$) and a 16 h photoperiod. After 6 days of germination, switchgrass seedlings were soaked in *Burkholderia phytofirmans* strain PsJN suspension (0.5 of OD₆₀₀) and control seedlings were soaked in PBS buffer for one min.

After 3 weeks, plants were transferred to 20 4-gallon pots filled with a soil mix composed of 2/3 Miracle-Gro Potting Mix (Scotts Miracle-Gro Company, Marysville, Ohio) and 1/3 Arabidopsis growing media (Lehle Seeds, Round Rock, Texas) with 3 plants/pot in a greenhouse under a temperature of 25°C and a 16-h photoperiod on April 2nd 2012. Ten pots were planted with PsJN inoculated Alamo seedlings and ten pots were planted with uninoculated control seedlings.

Bacterial endophyte culture conditions

B. phytofirmans strain PsJN-GFP was obtained from Dr. Angela Sessitsch (Austrian Institute of Technology, Seibersdorf, Austria). PsJN-GFP cultures were streaked on King's B (KB) solid medium as previously described in (Pillay and Nowak, 1997). Inoculum was produced by transferring one loop of PsJN from 2-day-old cultures to 5 ml KB broth in a 15-ml culture tube, followed by incubation at 28°C on a shaker (220 rpm) overnight. Five ml of the overnight PsJN cultures were added to 45 ml KB broth in a 250-ml Erlenmeyer flask and grown to 0.7 OD₆₀₀. Bacterial cells were collected by centrifugation at 3,500 rpm for 7 min at 4°C, then re-suspended in PBS buffer (10 mM NaH₂PO₄ containing 0.8% NaCl, pH 6.5), after which the OD₆₀₀ was adjusted with PBS buffer to 0.5.

Two drought cycles

The drought stress experiment consisted of a mild, drought preconditioning cycle followed by a more severe drought cycle. After transplanting, each pot initially received 1300 ml water/week from April 2nd 2012 to April 16th 2012 (simulating average growing season precipitation for central Virginia). The first drought cycle (mild drought preconditioning) started two-weeks after transplanting. Five pots of inoculated seedlings and five pots of non-inoculated seedlings were randomly chosen and received only 425 ml water/week while the rest of pots remained well watered. Following this 40-day water reduction (April 16th 2012 to May 25th 2012) all plants were rehydrated to field capacity for 10 days (May 25th 2012 to June 4th 2012). The second severe drought cycle started after full hydration on June 4th 2012. All plants received no further water until photosynthesis (Ps) reached zero, which was monitored by a LI-6400 portable photosynthesis system (LI-COR Inc., Lincoln, NE, USA) each day at ambient relative humidity. Immediately, the pots were rehydrated to field capacity. The plants were harvested individually when the photosynthetic rates recovered and became stable. Harvesting of switchgrass was finished on July 3rd 2012.

Experimental measurements

During the first, mild drought preconditioning cycle, the height of plants in each pot was measured every 3 or 4 days. The average height of all plants in the pot was used in statistical analyses. Relative growth rates (RGR) were calculated as the following equation: $RGR = \ln[(Height_{D_{n+1}} - Height_{D_n}) / (D_{n+1} - D_n)]$. D_n and $Height_{D_n}$ indicated age and average height of switchgrass in the same pot at the n th measurement, respectively. D_{n+1} and $Height_{D_{n+1}}$ indicated age and average height of switchgrass in the same pot at the $(n+1)$ th measurement, respectively. Tiller number of each plant was recorded every 3-4 days from the 4th week of the

experiment. At the end of the experiment, the height of each plant was also recorded and averaged for each pot. Leaf senescence was measured by counting the number of dead leaves in each plant from the end of the first drought preconditioning cycle to the beginning of the second drought stress.

During the second, severe drought cycle, gas exchange was measured every day on a fully expanded leaf at the top of the canopy with a LI-6400 portable photosynthesis system (LI-COR Inc., Lincoln, NE, USA) at ambient relative humidity and the following settings: 3000 $\mu\text{mol m}^{-2} \text{s}^{-1}$ photosynthetic photon flux density (PPFD), 400 $\mu\text{mol mol}^{-1}$ reference CO_2 concentration, 25 °C block temperature, and a flow rate of 385 $\mu\text{mol sec}^{-1}$. During the gas exchange measurement, a leaf from the same pot was excised at the leaf collar and plant water potential was determined immediately using a pressure chamber (PMS Instrument Co., Corvallis, OR). At the end of rehydration, aboveground plant parts were harvested and dried in an oven at 65 °C for 48 h.

Experimental design and Analysis

A completely randomized design with two factors and repeated measures was used in this experiment. Five replicates were assigned to each treatment. Differences in biometric parameters between different treatments were investigated with a Student t-test or two-way ANOVA and considered significantly different when $p < 0.1$ at each date. Biomass indexes were analyzed using repeated measurements two-way ANOVA (analysis of variance), where treatments were the between subjects effects and time was the within subject effect. Polynomial regression was used to investigate the effects of PsJN inoculation and drought pre-conditioning on leaf gas exchange regressed on leaf water potential. Linear regression was used to investigate the effects of PsJN inoculation and drought pre-conditioning on tiller number (log transformed)

regressed on plant height. All statistical analyses were performed with JMP Pro 10 (SAS Institute, Cary, NC).

3. Results

Growth and yield

Prior to the start of drought preconditioning, PsJN inoculation resulted in increased leaf area, leaf number and plant height by 32% ($p<0.0001$), 25% ($p<0.0001$) and 50% ($p<0.0001$), respectively (Table 1).

PsJN-inoculated plants continued to have greater height during the mild drought preconditioning regardless of water treatment ($p<0.0001$, Table 2, Figure 1). However, there was a significant statistical interaction ($p=0.0764$) between PsJN inoculation and drought preconditioning on height where the growth enhancement by PsJN inoculation was more evident

in the drought preconditioning treatment. PsJN-inoculated switchgrass was 31.9% taller than control plants under drought conditioning and only 24.3% taller than control plants when well-watered (Table 2). Mild drought preconditioning significantly increased the height of PsJN-inoculated plants by 6.4% ($p=0.0314$) but did not affect the height of control plants ($p=0.7257$, Figure 1, Table 2). Drought preconditioning began to significantly arrest the height growth of control switchgrass by 14.1% ($p=0.0797$, Figure 1, Table 2) since the first rehydration while the growth enhancement benefits of PsJN inoculation on height vanished ($p=0.6636$). The negative effects of the drought preconditioning on height of control plants lasted to the 2nd drought cycle ($p=0.0031$, Figure 1), during which all treatments had water withheld. Nevertheless, the height of PsJN-inoculated switchgrass was not influenced by drought preconditioning, since no difference in height was found during 1st rehydration and 2nd drought stress period.

Figure 1 Effect of drought pre-conditioning on mean ($\pm$ S. E.) height of control and PsJN inoculated switchgrass during 1st drought (drought pre-conditioning, A), 1st rehydration (B) and 2nd drought and rehydration (C). Asteroids indicated significant differences between pre-watered and drought pre-conditioning using test slice of repeated measure ANOVA (n.s indicates not significant, * $p < 0.1$, ** $p < 0.01$). The corresponding p-value was also showed on the panels.

Relative growth rates gradually decreased as switchgrass aged ($p < 0.0001$, Table 2, Figure 2). PsJN-inoculation decreased relative growth rate by 20.2% ($p = 0.012$) during the drought preconditioning treatment; however, PsJN-inoculated switchgrass plants were still larger. After the 1st rehydration, PsJN inoculation did not affect relative growth rate. Preconditioning increased the relative growth rates of switchgrass by 120% ($p = 0.0923$) for both infected and uninfected plants. PsJN-inoculated switchgrass had 84.2% more tillers than control plants during the drought preconditioning cycle ($p < 0.0001$, Table 2, Figure 2). However, this effect of PsJN inoculation on tiller number did not extend to the following rehydration ($p = 0.6709$) and 2nd drought cycle ($p = 0.8678$). There was no significant statistical interaction ($p = 0.6808$) between PsJN inoculation and drought preconditioning in terms of tiller number.

Because PsJN's growth promotion benefits disappeared by the end of the study and pre-drought conditioned switchgrass caught up during the second drought cycle, there was no difference between any treatments at final harvest in terms of height, tiller number and aboveground biomass (Table 3).

Figure 2. Effects of PsJN inoculation and mild drought preconditioning on relative growth rates (RGR) and tiller number of switchgrass. Asteroids indicated significant differences between PsJN inoculated plants and control at each time period using student t-test (*p < 0.1; **p < 0.01, ***p < 0.001). Black arrows indicated the beginning of drought stress and grey arrows indicated the beginning of rehydration

Development and senescence

Tiller number was strongly correlated with plant height ($p < 0.0001$, Figure 3) and PsJN inoculation ($p = 0.0446$) and drought preconditioning ($p = 0.0388$) modified the correlation between height and tiller number. For switchgrass at the same height, PsJN-inoculated plants had 18.2% more tillers than non-inoculated plants, and drought pre-conditioned plants had 16.7% more tillers than pre-watered plants on average. There was no significant statistical interaction between pre-drought treatment and PsJN inoculation ($p = 0.9175$) for tiller number/height ratio. Nevertheless, PsJN's beneficial effects on tiller number/height ratio were more evident when switchgrass was shorter but gradually disappeared when switchgrass grew taller (Figure 3). In contrast, drought preconditioning did not affect tiller number/height ratio at the beginning but gradually came into effect as switchgrass grew taller (Figure 3).

Figure 3 Tiller number related to height (cm) as influenced by PsJN inoculation and two drought stress cycles

Panel A models: Control (closed circles and solid lines) $\text{Log}(y)=-2.663+0.040x$ ($R^2=76.6\%$, $p<0.0001$), PsJN (open circles and dashed lines) $\text{Log}(y)=-1.93+0.034x$ ($R^2=68.6\%$, $p<0.0001$). $P_{PsJN}<0.1$ indicates a significant change in slope due to PsJN inoculation.

Panel B models: Pre-watered (closed circles and solid lines) $\text{Log}(y)=-2.260+0.036x$ ($R^2=76.4\%$, $p<0.0001$), Pre-conditioning (open circles and dashed lines) $\text{Log}(y)=-2.749+0.043x$ ($R^2=74.2\%$, $p<0.0001$). $P_{Pre-conditioning}<0.1$ indicates a significant change in slope due to drought pre-conditioning

Both drought treatment ($p < 0.0001$) and PsJN inoculation ($p < 0.0001$) increased leaf senescence and this effect lasted to the following rehydration (Table 2, Figure 4). There was a significant statistical interaction between drought stress and PsJN inoculation ($p < 0.0001$) on senescent leaf numbers during the first drought preconditioning cycle. Leaf senescence was greatest (2.7 leaves per plant) when plants were inoculated with PsJN under drought preconditioning. Following the second severe drought stress there were no differences in number of senesced leaves due to PsJN inoculation or previous drought hardening.

Figure 4 Effects of PsJN inoculation and preconditioning on leaf senescence. Grey arrow indicated the start of rehydration, and black arrow indicated the start of drought stress. Error bars were $\pm$ SE of the means. Asteroids indicated significant differences between treatments at each time period using ANOVA (* $p < 0.1$).

Gas exchange and leaf water potential

At the beginning of the final, severe drought cycle and, at leaf water potentials above -1.0 MPa there was no relationship between leaf water potential and photosynthesis ($p=0.5064$, Figure 5). At leaf water potentials above -1.0 MPa, drought preconditioning increased photosynthetic rates of PsJN-inoculated switchgrass by 26.4% ($p=0.0024$) but had no effect on the control plants (Figure 5).

At leaf water potentials below -1 MPa, there was a strong correlation between Ps and leaf water potential ($R^2=82.6\%$, $p<0.0001$, Figure 5). Pre-conditioned switchgrass maintained higher Ps ($p=0.0045$) under lower leaf water potentials (Figure 5). PsJN inoculation did not induce significant changes in Ps ($p=0.3784$) but higher mean photosynthetic rates were observed. There was no significant statistical interaction between PsJN inoculation and drought preconditioning for photosynthetic rates at leaf water potentials below -1 MPa.

Figure 5 Effect of PsJN inoculation and drought preconditioning on the relationship between photosynthetic rate and leaf water potential during second drought cycle.

Panel A: Photosynthetic rates related to leaf water potential as influenced by PsJN inoculation and drought preconditioning treatment combinations. The vertical dashed line indicated leaf water potential equals -1MPa. When leaf water potential >-1 MPa, Photosynthetic rates were not correlated with leaf water potential ($R^2=0.06\%$, $p=0.5064$). When leaf water potential <-1 MPa, Photosynthetic rates were significantly correlated with leaf water potential ($R^2=82.6\%$, $p<0.0001$). Black lines indicated Control plants and grey lines indicated PsJN inoculated plants. Solid lines indicated pre-watered plants and dashed lines indicated drought pre-conditioned plants.

Panel B: The effects of PsJN inoculation and drought preconditioning on photosynthetic rates when leaf water potential >-1 MPa. Error bars were ± 1 SE and different letters indicated significant differences ($p < 0.1$) between inoculation and pre-drought hardening treatment combinations

Panel C: The effects of PsJN inoculation and drought preconditioning on photosynthetic rates when leaf water potential <-1 MPa. Error bars were ± 1 SE and different letters indicated significant differences ($p < 0.1$) between inoculation and pre-drought hardening treatment combinations

4. Discussion

Burkholderia phytofirmans strain PsJN has promoted the productivity of crops such as potato, grapevine and maize under stresses (Barka et al., 2006; Bensalim et al., 1998; Fernandez et al., 2012a; Naveed et al., 2014). During the preconditioning period, PsJN inoculation enhanced switchgrass cv. Alamo growth of both drought pre-conditioned and well-watered switchgrass, which is consistent with the results of Kim et al. (2012) that PsJN inoculation increased the biomass of switchgrass under various conditions. During the preconditioning period, 2/3 reduction of watering did not inhibit height growth and tillering of control switchgrass until the end of the drought cycle, probably because this reduction was too mild to impact the growth since the water consumption of the small seedlings was low. This is similar to reports on crop yield under mild drought (Erice et al., 2010). The demand for water rose as plants grew larger and the preconditioning treatment started to slow the growth of control switchgrass at the beginning of 1st rehydration and this negative effect extended into the next drought stress cycle when all the treatments were under severe drought. The relative growth rates were higher in the preconditioning group during the final severe drought cycle, which might be attributable to the priming effects of the preconditioning treatment. PsJN inoculation reduced the negative effects of 1st drought on growth, which might result from the priming effects of PsJN before drought stress. Enhanced height and tiller growth of endophyte inoculated switchgrass were observed in the pre-drought stress group compared to control under the same stress, especially at a younger stage. In our study on PsJN's effects on young switchgrass seedlings, significantly higher root biomass was observed in PsJN inoculated seedlings (Wang et al., 2015), which could explain enhanced tolerance to drought in PsJN inoculated plants (Naveed et al., 2014). PsJN inoculation also silenced negative effects of drought stress on plants through manipulating IAA and other hormones (Sun et al., 2009; Zuniga et al., 2013) and regulating

branching (tillering) in grasses (McSteen, 2009). However, similar to the whole life cycle analysis of PsJN's effects on *Arabidopsis* (Poupin et al., 2013), the growth advancement effects of PsJN gradually vanished with time as the plants grew larger and the relative growth rate of PsJN-inoculated switchgrass became lower than that of the control group. Thus, the aboveground biomass was not enhanced by PsJN inoculation at final harvesting. In a previous study of bacterial endophyte's effects on poplar, the aboveground biomass was also not influenced by endophytes but the underground biomass was increased (Rogers et al., 2012). The root growth promoting effects by PsJN helped plants to access more water and nutrient resources (Bensalim et al., 1998; Naveed et al., 2014; Wang et al., 2015), which played an important role in the symbiosis. In a greenhouse study, this benefit may be limited by the size of containers. Although the growth promotion effects vanished at the end, PsJN induced early growth of switchgrass may help switchgrass to compete with weeds and facilitate establishment, which is a bottleneck for switchgrass production. Better productivity of PsJN-inoculated switchgrass has been observed in a field trial (Lowman et al., 2015).

Although much attention has been devoted to the growth benefits brought by bacterial endophytes, rarely are its effects on development measured (Lowman et al., 2015; Poupin et al., 2013). Increased leaf senescence (Poupin et al., 2013) and advanced growth stage (Lowman et al., 2015) were associated with PsJN inoculation, which implied more rapidly development. Induced by plant aging and environmental stresses, leaf senescence is a highly regulated physiology process and contributes to the survival under drought stress for several plant species (Munn-Bosch and Alegre, 2004). In this study, both PsJN inoculation and drought stress accelerated the leaf senescence and functioned independently, which may play an important role in the survival of switchgrass. Although previous studies showed increased tillering due to

endophytes (Clay, 1987; Ker et al., 2012; Kim et al., 2012), none investigated if the increase in tiller number was simply due to increased plant size or altered development. However, even for switchgrass at the same height, both PsJN and drought preconditioning changed tiller number/height ratio, which indicated altered development and allocation of biomass.

Several studies investigated the response of woody species (Guarnaschelli et al., 2003; Ladjal et al., 2000; Ruiz-Sánchez et al., 2000; Vilagrosa et al., 2003; Zwiazek and Blake, 1989) and herbaceous species (Agnew and Carrow, 1985; Elmi and West, 1995; Jiang and Huang, 2000, 2001) to drought preconditioning, which improved resistance of plants against freezing (Hoffman et al., 2012), heat shock (Jiang and Huang, 2000, 2001; Ladjal et al., 2000), transplanting shock (Duryea and McClain, 1984; Landis et al., 1999) and drought stress (Ruiz-Sánchez et al., 2000; Vilagrosa et al., 2003). Pre-conditioned plants maintained higher water status, higher photosynthetic rates, stomatal conductance and root dry weight during the subsequent stresses (Jiang and Huang, 2000; Vilagrosa et al., 2003), which probably resulted from greater osmotic adjustment (Elmi and West, 1995; Jiang and Huang, 2001; Zwiazek and Blake, 1989). However, none studied the relationship between photosynthetic rates, and leaf water potential of pre-conditioned plants. During the final severe drought when all the switchgrass plants were completely cut-off from water, photosynthetic rates were not correlated to leaf water potential when leaf water potential was above -1 MPa. However, once leaf water potential dropped below this threshold, photosynthetic rates significantly decreased and was strongly correlated with lower leaf water potential. This threshold leaf water potential (-1 MPa) was very close to the leaf osmotic potential at full turgor (-1.04 MPa) of well-watered switchgrass we measured in another study (data not shown), which indicates that osmotic potential and turgor loss plays a role in the decline of photosynthetic rates in response to water stress. At lower leaf

water potential, preconditioned and PsJN plants maintained higher photosynthetic rates. This may have been the result of osmotic adjustment in pre-conditioned plants. Previous studies also reported osmotic adjustment in Kentucky bluegrass which was associated with accumulation of ion solutes and water soluble carbohydrates (Jiang and Huang, 2001). Both preconditioning drought (Clay, 1987) and endophyte inoculation (Elmi and West, 1995) adjusted osmotic potential in grass species, therefore osmotic adjustments might contribute to the interplay of PsJN inoculation and drought stress on photosynthetic rates.

5. Conclusion

PsJN inoculation enhanced height and tiller growth of switchgrass during a drought preconditioning and later severe drought stress. Drought preconditioning also primed switchgrass for the following severe drought stress, and this priming effect was magnified in PsJN-inoculated switchgrass. PsJN inoculation also advanced development and increased photosynthetic rates under both well-watered and stressed condition, which might participate in PsJN induced drought stress tolerance of switchgrass.

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Tables

Table 1 Effect of Ps JN endophyte inoculation on switchgrass leaf area (cm²), leaf number and height (cm) before the start of mild drought preconditioning

	Leaf Area (cm ²)	Leaf No.	Height (cm)
Control	3.1± 0.1(10)	4.0± 0.1(10)	14.2± 0.9(10)
PsJN	4.1± 0.1(10)	5.0± 0.1(10)	21.3± 0.5(10)
p-value	<0.0001	<0.0001	<0.0001

Each value was presented as mean±SE (n). Probabilities were given for the results of student t-test for each parameter. Student t-tests were performed with JMP Pro 10 (SAS Institute, Cary, NC)

Table 2 Effects of PsJN endophyte inoculation and drought stress on RGR, tiller numbers, and number of dead leaves

		Height (cm)	RGR	Tiller	Dead leaves
1 st Drought (Pre-conditioning)	Pre-conditioned Control	57.7±1.6 (60) ^c	0.043±0.003(60) ^{ab}	1.3±1.1(40) ^b	1.9±0.1(15) ^b
	Pre-conditioned PsJN	76.1±0.9 (60) ^a	0.037±0.003(60) ^b	2.8±1.1(40) ^a	2.7±0.1(15) ^a
	Pre-watered Control	57.5±1.4 (60) ^c	0.048±0.003(60) ^a	1.4±1.1(40) ^b	0.1±0.1(15) ^d
	Pre-watered PsJN	71.5±1.4 (60) ^b	0.038±0.003(60) ^b	2.2±1.1(40) ^a	1.5±0.1(15) ^c
	Days	<0.0001	<0.0001	<0.0001	p =0.0005
	PsJN	<0.0001	p=0.0120	<0.0001	<0.0001
	Preconditioning	p=0.2008	p=0.3680	p=0.5808	<0.0001
	PsJN* Preconditioning	p=0.0764	p=0.5619	p=0.2028	<0.0001
1 st Rehydration	Pre-conditioned Control	96.2±5.1(15) ^b	0.011±0.013(15) ^a	5.4±1.0(15) ^a	1.8±0.2(15) ^b
	Pre-conditioned PsJN	104.0±2.6(15) ^{ab}	0.023±0.009(15) ^a	6.7±0.6(15) ^a	3.4±0.3(15) ^a
	Pre-watered Control	112.8±4.1(15) ^a	0.002±0.012(15) ^a	6.8±0.9(15) ^a	0.5±0.1(15) ^c
	Pre-watered PsJN	108.8±3.0(15) ^{ab}	0.010±0.007(15) ^a	6.4±0.8(15) ^a	1.6±0.4(15) ^b
	days	p=0.5403	p =0.0323	p=0.0064	p =0.1312
	PsJN	p =0.4930	p =0.7829	p=0.6709	<.0001
	Preconditioning	p =0.0138	p =0.1404	p=0.4001	<.0001
	PsJN* Preconditioning	p =0.3407	p =0.2739	p=0.3972	p =0.3474
2 nd Drought & Rehydration	Pre-conditioned Control	107.8±5.8(10) ^c	0.101±0.024(5) ^a	11.1±2.4(10) ^a	1.2±0.2(5) ^a
	Pre-conditioned PsJN	109.2±3.0(10) ^{bc}	0.099±0.049(5) ^a	12.2±1.0(10) ^a	2.5±0.6(5) ^a
	Pre-watered Control	126.7±4.6(10) ^a	0.058±0.019(5) ^a	11.0±1.9(10) ^a	2.7±1.2(5) ^a
	Pre-watered PsJN	118.6±3.6(10) ^{ab}	0.032±0.020(5) ^a	10.5±2.3(10) ^a	2.1±1.5(5) ^a
	Days	p =0.0675		p =0.0398	
	PsJN	p =0.4302	p =0.6474	p =0.8678	p =0.7384
	Preconditioning	p =0.0019	p =0.0923	p =0.6181	p =0.6006
	PsJN* Preconditioning	p =0.2671	p =0.6953	p =0.6808	p =0.3538

Means followed by the same letter (s) in a column for each parameter did not differ significantly at $\alpha=0.1$. Data were means $\pm$ standard error (replicates number). Probabilities were given for the results of a repeated measure two-way ANOVA for each parameter

Table 3 Effects of PsJN inoculation and two drought stress cycles on height, tiller No. and aboveground biomass at final harvest.

	Height (cm)	Tiller	Aboveground Biomass (g)
Control	133±6(10) ^a	15±3(10) ^a	46.5±10.0(10) ^a
PsJN	127±3(10) ^a	15±2(10) ^a	45.6±7.8(10) ^a
Pre-watered	133±4(10) ^a	14±3(10) ^a	48.0±10.1(10) ^a
Pre-conditioned	127±5(10) ^a	15±2(10) ^a	44.1±7.7(10) ^a
PsJN	P=0.3768	P=0.8730	P=0.9440
Preconditioning	P=0.3304	P=0.8392	P=0.7643
PsJN* Preconditioning	P=0.6776	P=0.7659	P=0.3607

Means followed by the same letter (s) in a column for each parameter did not differ significantly at $\alpha=0.1$. Data were means $\pm$ standard error (replicates number) for the main effects of PsJN and preconditioning. Probabilities were given for the results of two-way ANOVA for each parameter.