

Gas exchange and chlorophyll fluorescence in symbiotic and non-symbiotic ryegrass under water stress

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Abstract

The symbiotic association of endophyte fungus, *Neotyphodium lolii*, and ryegrass improves the ryegrass resistance to drought. This is shown by a 30 % increase in the number of suckers in infected plants (E+), compared to plants lacking endophyte (E-), and by a higher water potential in the E+ than E- plants. The E+ plants have higher stomatal conductance (g_s), transpiration rate, net photosynthetic rate (P_N), and photorespiratory electron transport rate than the E- plants. The maximal photochemical efficiency (F_v/F_m) and the actual photochemical efficiency (Φ_{PS2}) are not affected by the endophyte fungus. The increase in P_N of the E+ plants subjected to water stress was independent from internal CO_2 concentration. An increased P_N was observed in E+ plants also in optimal water supply. Hence the drought resistance of E+ plants results in increased g_s , P_N , and photorespiratory electron transport rate.

Additional key words: endophyte; *Lolium perenne*; *Neotyphodium lolii*; photosynthesis; stomatal conductance; transpiration rate; water potential.

Introduction

Symbiotic associations with endophyte fungi have been obtained in economically important species *Lolium perenne*, *Festuca arundinacea*, *Festuca pratensis*, and *Festuca rubra*. These fungi complete their entire cycle within the vegetative and

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Abbreviations: C_i : internal CO_2 concentration; Chl: chlorophyll; E+: endophyte plants; E-: non-endophyte plants; E : transpiration rate; F_v/F_m : maximal photochemical efficiency; Φ_{PS2} : actual photochemical efficiency; g_s : stomatal conductance; P_N : net photosynthetic rate; PS: photosystem; ψ_w : water potential.

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reproductive tissues of the grass and are non-pathogenic, but the grass-fungus interactions are responsible for toxicity to many grazing animals (Bacon and Siegel 1988). Endophyte enhances host tolerance to drought, flood and nutrient stresses, and resistance to insects, fungal pathogen, and plant parasitic nematodes (Bacon 1993, Latch 1993). The role of endophyte in protecting ryegrass from drought was confirmed by West *et al.* (1993) and Ravel *et al.* (1997). Endophytic plants may conserve water more efficiently through leaf rolling (Arachevaleta *et al.* 1989) or osmotic adjustment and stomatal closure (Elmi and West 1995). In this work, we studied the effect of ryegrass endophyte *Neotyphodium* on growth, chlorophyll (Chl) fluorescence, and gas exchanges of ryegrass under water stress.

Materials and methods

Plants: Perennial ryegrass, *Lolium perenne* L. cv. Appolo, plants either infected with (E+) or free (E-) from *Neotyphodium lolii* (formerly *Acremonium lolii*) were used. The seeds were obtained from the laboratory of phytopathology of the Institut National Agronomique, Paris Grignon, France. The endophyte-free tillers were produced by exposing symbiotic seeds to a systemic fungicide to eradicate the fungal component (Raynal, personal communication). Plants were grown in plastic pots (one plant per pot) in a mixture of compost and "vermiculite" (2 : 1, v/v). Pots were watered daily to 90 % water holding capacity. Liquid fertiliser was applied every two weeks. Plants were grown in growth chamber, with a 16-h photoperiod, irradiance of 400 $\mu\text{mol m}^{-2} \text{s}^{-1}$ photosynthetically active radiation at the pot surface, day/night temperature of $23 \pm 2 / 18 \pm 2$ °C, and relative humidity of 60 ± 5 %. A water stress treatment was imposed 10 weeks after planting by withholding water during 7 d. Well-watered control pots were maintained during the treatment near field capacity.

Leaf water potential (ψ_w) was measured at the end of the dry cycle. Leaf discs (0.5 cm diameter) were cut and placed immediately in thermocouple psychrometer sample chambers (Wescor C-52; Wescor, Logan, UT, USA). The ψ_w was measured after an equilibration period of 1 h using a Wescor HP 115 microvoltmeter.

Chl fluorescence emission from the upper surface of attached leaves was measured with a portable pulse amplitude modulation fluorometer (Hansatech Instruments, Norfolk, U.K.). The initial fluorescence, F_0 , was obtained after a 30 min dark adaptation. The maximal fluorescence, F_m , was obtained with a saturating flash (1 s, 13 000 $\mu\text{mol m}^{-2} \text{s}^{-1}$). Actinic irradiation was initiated at 400 or 1200 $\mu\text{mol m}^{-2} \text{s}^{-1}$. Saturating flashes were fired every 50 s to determine maximal fluorescence during actinic exposure (F_m'). These conditions were maintained until a steady state of variable fluorescence was achieved. Maximal photochemical efficiency in dark adapted leaves (F_v/F_m , where $F_v = F_m - F_0$) and actual photochemical efficiency of photosystem (PS) 2 electron transport under actinic radiation ($\Phi_{PS2} = (F_m' - F_s)/F_m'$) (Demmig and Björkman 1987, Genty *et al.* 1989) were calculated.

P_N , g_s , transpiration rate, and electron transport rate to O_2 : CO_2 exchange was

measured using a portable infrared gas analyser (LI-6400, LI-COR, Lincoln, USA) connected to an assimilation chamber. Photosynthetic measurements were made at CO_2 concentration of $400 \mu\text{mol mol}^{-1}$ and photon flux density $400 \mu\text{mol m}^{-2} \text{s}^{-1}$ (FLS2 radiation source; Hansatech Instruments, Norfolk, U.K.).

Leaf temperature, chamber temperature, chamber relative humidity, and flow rate were also monitored during each measurement. The period for one measurement ranged from 10 to 15 min. The assimilation chamber is designed so that optic fibres used to measure the fluorescence may be introduced without disturbing the photosynthesis measurements. P_N and the actual quantum efficiency (Φ_{PS2}) were determined simultaneously on the same leaf surface at two different partial pressures of O_2 (2 and 20 kPa) using gas mixture bottles.

Statistical analysis: The effects of treatments (symbiotic association and water stress) were tested ($p = 0.05$) using analysis of variance. All experimental values were averages of six independent experiments. Means were compared with the Mann and Whitney test's at the 0.05 confidence level.

Results and discussion

Plant growth, expressed as leaf dry mass and tiller production, was significantly reduced during the water stress (Table 1). Influence of endophyte upon tiller production was observed and resulted in a 30 % increase of the tiller number in the water stressed E+ compared to the water stressed E- plants. No difference was detected in leaf dry masses (Table 1).

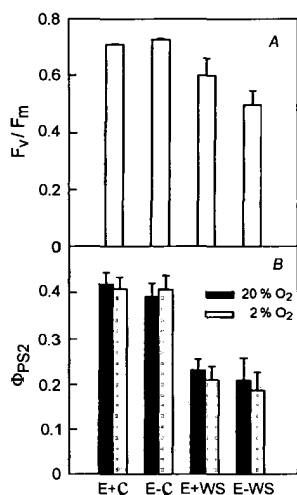


Fig. 1. Maximal photochemical efficiency (F_v/F_m) (A) and actual photochemical efficiency (Φ_{PS2}) (B) of photosystem 2 in symbiotic and non-symbiotic (A) control (E+C and E-C) and water stressed (E+WS and E-WS) ryegrass plants.

In well watered plants, ψ_w (Table 1), maximal photochemical efficiency of dark adapted leaves (F_v/F_m), and actual photochemical efficiency of PS2 (Φ_{PS2}) measured at 20 % or 2 % O_2 (Fig. 1), g_s , E , and internal CO_2 concentration (C_i) (Fig. 2) were

Table 1. Leaf dry mass, tillers number and leaf water potential of symbiotic (E+) and non-symbiotic (E-) control and water-stressed ryegrass plants (means \pm SD, $n = 15$). Values followed by the same letter were not significantly different with the Mann and Whitney's test.

	Control E+	E-	Water-stressed E+	E-
Leaf dry mass [g]	7.2 \pm 1.9 a	7.8 \pm 1.3 a	4.1 \pm 1 b	5.0 \pm 1.4 b
Tiller number	78 \pm 22 a	73 \pm 23 a	68 \pm 19 b	52 \pm 7 c
Water potential [MPa]	-0.7 \pm 0.21 a	-0.65 \pm 0.3 a	-1.6 \pm 0.4 b	-2.1 \pm 0.35 c

similar in E+ and E- leaves. The only parameter influenced by endophyte without imposing water deficit was P_N which was increased in infected plants at 20 or 2 % O_2 . Such differences were also reported by Belesky *et al.* (1987).

Water stress induced a decrease in ψ_w , F_v/F_m , Φ_{PS2} , g_s , and E (Table 1, Figs. 1 and 2). ψ_w decreased from -0.7 MPa in control plants to -1.6 and -2.1 MPa in E+ and E- plants, respectively. The influence of endophyte was also observed in the response of Chl fluorescence parameters and gas exchanges under water stress. F_v/F_m was reduced from 0.708 to 0.602 in E+ plants and from 0.727 to 0.501 in E- plants, respectively. The decreases in P_N , E , and g_s were more pronounced in E+ than in E- plants (Fig. 2). Only two parameters were not influenced by endophyte: the decrease in Φ_{PS2} following water stress and the increase in C_i (Figs. 1 and 2). The mechanisms

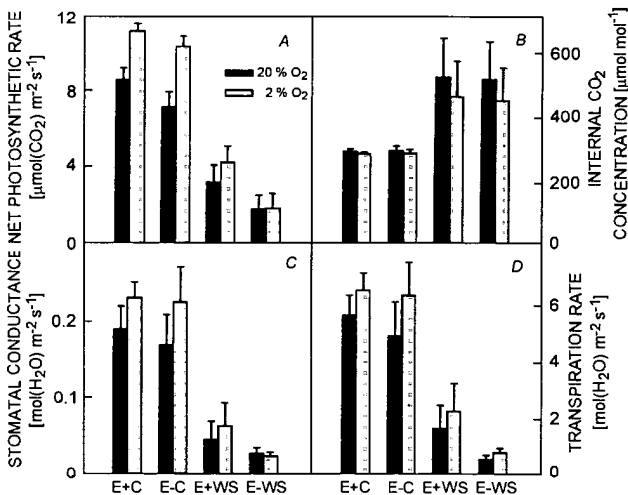


Fig. 2. Net photosynthetic rate (A), internal CO_2 concentration (B), stomatal conductance (C), and transpiration rate (D) in symbiotic and non-symbiotic well watered (E+C and E-C) and water stressed (E+WS and E-WS) ryegrass plants.

that have been proposed to explain the drought resistance within the symbiosis are diverse and include (1) greater root growth that increases the ability to extract water, (2) osmotic adjustment, and (3) stomatal closure. In some cases, g_s and E of symbiotic water-stressed plants were lower than those of non-symbiotic water stressed plants (Zang and Davies 1987). On the contrary, in our study, E+ plants had higher g_s than E- plants. A decrease in g_s can lead to reduced P_N by decreasing C_i .

However, under stress the C_i was higher than in the absence of stress in both E+ and E- plants. Although, photosynthetic processes in C_3 plants are resistant to desiccation (Cornic and Briantais 1991), effects of water stress on F_v/F_m and Φ_{PS2} have been observed. This is probably due to the fact that ryegrass is a plant that accepts large dehydration without dying and is able to rehydrate when water conditions improve.

The reduction in oxygen concentration from 20 to 2 % resulted in an enhancement in P_N , g_s , and E in control plants (Fig. 2). The only significant difference linked to the presence of endophyte concerned P_N . Under stress, the results are comparable with those obtained under 20 % O_2 . A beneficial effect of endophyte was seen on g_s , E , and P_N . As shown by Genty *et al.* (1989), a linear relationship is obtained between Φ_{PS2} and apparent quantum yield of CO_2 assimilation in non-photorespiratory conditions. In photorespiratory and non-photorespiratory conditions, the measure of Φ_{PS2} allows to estimate the flow rate of photosynthetic electron transport [$\mu\text{mol}(e^-)\text{m}^{-2}\text{s}^{-1}$] (Cornic and Briantais 1991). Using the leaf P_N values, it is possible to estimate the rate of electron transport to O_2 by the difference from the calculated total electron transport rate. It was expressed in % of total electron transport rate (Fig. 3).

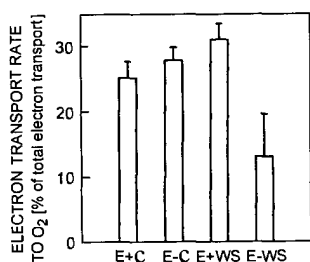


Fig. 3. Electron transport rate to O_2 expressed in % of total electron transport rate, in symbiotic and non-symbiotic well watered (E+C and E-C) and water stressed (E+WS and E-WS) ryegrass plants.

The electron transport rate to O_2 that represents 25.0 and 26.5 % of the total photosynthetic electron transport rate in E+ and E-control plants, was enhanced in water-stressed E+ plants (31 %) and reduced in water-stressed E-plants (13 %) (Fig. 3). The implication of photorespiration in drought resistance has already been described (Coudret *et al.* 1985, Heber *et al.* 1996). The fairly high photorespiration rate could be one of the mechanisms involved in the stress resistance of infected plants.

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