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

C. AMALRIC*, H. SALLANON**,***, F. MONNET **, A. HITMI**, and A. COUDRET**

Institut Universitaire de Technologie, Département de Génie Biologique,
Rue de l’Ecole Normale, 15000 Aurillac, France *
Laboratoire de ‘Biotechnologies Environnement Santé’, Université d’Auvergne,
Rue de l’Ecole Normale, 15000 Aurillac, France **

Abstract

The symbiotic association of endophyte fungus, Neotyphodium loli, 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 (gs), 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 CO2 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 gs, P_N, and photorespiratory electron transport rate.

Additional key words: endophyte; Lolium perenne; Neotyphodium loli; 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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***Author for correspondence; fax: (33) 04 71 45 57 51; e-mail: sallanon@cicsun.univ-bpclermont.fr
Abbreviations: C_i: internal CO2 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 phytopatology 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 μmol m⁻² s⁻¹ photosynthetically active radiation at the pot surface, day/night temperature of 23±2/18±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** ($\psi_w$) 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 $\psi_w$ 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 μmol m⁻² s⁻¹). Actinic irradiation was initiated at 400 or 1200 μmol m⁻² 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 $O_2$: CO₂ 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 μmol mol$^{-1}$ and photon flux density 400 μmol m$^{-2}$ 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 ($\Phi_{PS2}$) 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).

![Graph showing Fv/Fm and $\Phi_{PS2}$](image)

Fig. 1. Maximal photochemical efficiency ($F_v/F_m$) (A) and actual photochemical efficiency ($\Phi_{PS2}$) (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, $\psi_w$ (Table 1), maximal photochemical efficiency of dark adapted leaves ($F_v/F_m$), and actual photochemical efficiency of PS2 ($\Phi_{PS2}$) measured at 20 % or 2 % O$_2$ (Fig. 1), gs, $E$, 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 ± SD, n = 15). Values followed by the same letter were not significantly different with the Mann and Whitney’s test.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Water-stressed</th>
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<tbody>
<tr>
<td></td>
<td>E+</td>
<td>E-</td>
</tr>
<tr>
<td>Leaf dry mass [g]</td>
<td>7.2 ± 1.9 a</td>
<td>7.8 ± 1.3 a</td>
</tr>
<tr>
<td>Tiller number</td>
<td>78 ± 22 a</td>
<td>73 ± 23 a</td>
</tr>
<tr>
<td>Water potential [MPa]</td>
<td>-0.7 ± 0.21 a</td>
<td>-0.65 ± 0.3 a</td>
</tr>
</tbody>
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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 \( \upsilon_w \), \( F_v/F_m \), \( \Phi_{PS2} \), \( g_s \), and \( E \) (Table 1, Figs. 1 and 2). \( \upsilon_w \) 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 \( \Phi_{PS2} \) 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.](image)

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$_4$ 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 $\Phi_{PS2}$ 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 $\Phi_{PS2}$ and apparent quantum yield of CO$_2$ assimilation in non-photorespiratory conditions. In photorespiratory and non-photorespiratory conditions, the measure of $\Phi_{PS2}$ allows to estimate the flow rate of photosynthetic electron transport [\text{\mu mol(e$^-$) m$^{-2}$ 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.](image)

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.

### References


