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Photosynthetic pigments and photosynthetic products of endophyte-infected and endophyte-free *Lolium perenne* L. under drought stress conditions

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Abstract Endophyte-infected (EI) seeds of *Lolium perenne* L. were used to attain endophyte-free (EF) population by heating the seeds at 43°C for 15 min and then 57°C for 25 min. Relative water content (RWC), chlorophyll, soluble sugar and starch content of EI and EF populations under normal and drought stress conditions were compared to investigate the effect of endophyte infection on the host plant. Under severe stress, RWC of EI leaf was significantly higher than that of EF leaf, i.e. EI plants took more advantages over EF plants in water-holding ability. Under mild stress, endophyte could enhance soluble sugars in host plants to improve their osmotic ability. With stress intensification, the improvement of endophyte no longer existed, and more photosynthetic products (such as starch) accumulated in EI plants to survive through the undesirable conditions. In the next spring, EI populations will recover more rapidly than EF populations. The biomass of a population is closely related to its photosynthesis. Under severe stress, EI population significantly accumulated more biomass than EF population. As far as photosynthetic pigments were concerned, contents of Chla, Chlb and Car of EI plants were close to those of EF plants, which suggested that endophyte infection didn't alleviate photosynthetic pigments from being destroyed by drought stress, and endophyte might improve photosynthesis ability of its host plant in other ways.

Keywords endophyte, *Lolium perenne* L., relative water content (RWC), soluble sugar, starch

1 Introduction

In the past, much research work was done on the beneficial effect of endophyte infection on biotic stress resistance of grasses. When abiotic stress such as drought is regarded, most research works focus on several agronomy indexes while little work focuses on physiological response of endophyte-infected grass to drought stress. Especially, the mechanism by which endophyte enhances the drought-resistance of the host plant was hypothesized. Even for the widely studied growth characteristics such as tiller number and productivity, the results are rather different. Some results show that the effect of endophyte infection is positive (Read and Camp 1986; West et al., 1993; Elmi and West 1995), some indicate the effect is neutral (Belesky et al., 1989; Belesky and Fedders 1995), while others report that the effect is negative (Assuero et al., 2000; Faeth and Sullivan 2003). The reason for the differences is that the interaction between grass and endophyte is related not only to the genotype and life history but also to the environmental conditions of the host plant and the corresponding endophyte (Cheplick 1997; Amalric et al., 1999; Johnson-Cicalese et al., 2000; Ahlholm et al., 2002; Faeth et al., 2002). Thus, Belesky and Fedders (1996), and Cheplick and Cho (2003) considered that the beneficial effect of endophyte infection on host plant is based on specific endophyte-plant associations and environmental conditions, so it is unreasonable to extrapolate such beneficial effect appearing in one specific association to other associations. In this study, *Lolium perenne* cv SR4000 infected by *Neotyphodium lolii* was chosen as experimental material. Relative water content (RWC), chlorophyll, soluble sugar and starch content of endophyte-infected (EI) and endophyte-free (EF) populations under normal and drought stress conditions were compared in order to investigate different adaptations of the EI and EF populations as well as the effect of endophyte infection on

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the host plant.

2 Materials and methods

2.1 Materials

The experimental material in this study is *Lolium perenne* cv SR4000. EI seeds of *Lolium perenne* L. were used to attain EF population by heating the seeds at 43°C for 15 min and then 57°C for 25 min. The experimental seedling was bred in winter in 1998, and then experimental populations were constructed twice with ramets in April 1999 and April 2000 respectively.

2.2 Drought stress treatment

2.2.1 Design of experiments

EI and EF normally grown plants were transplanted into the soil column system designed for the experiment. Each system was comprised of a cylinder-shaped sleeve filled with soil and fixed in the ground. The system was 25 cm in diameter and 60 cm in depth. These columns were placed in the holes dug in the plot. The top 0~25 cm layer was filled with a compound of soil, sand and biological fertilizer (soil : sand : biological fertilizer = 18:10:1). Each column contained approximately 14.3 kg oven-dried soil. Field capacity and permanent wilting point were 18.53 % and 8.31 % respectively. Eighteen column systems were made as required (9 for EI and 9 for EF).

The experimental populations were treated by cyclic drought stress and three stress levels were designed, *i.e.* control, mild and moderate levels. The stress treatment period was from Sep. 29, 2000 to Nov. 7, 2000, and sustained for 40 days. In this period, the control set was watered once per six days. The mild stress set was watered

on the 10th, 25th and 40th day respectively and the moderate stress set was watered only once at the end of the period. When watered, all populations were watered to field capacity. When the stress period ended, the shoots were harvested, leaving a stubble height of about 4 cm. The remaining plant parts lived through the winter in their natural condition. During the next spring, the regeneration characters of each population were investigated

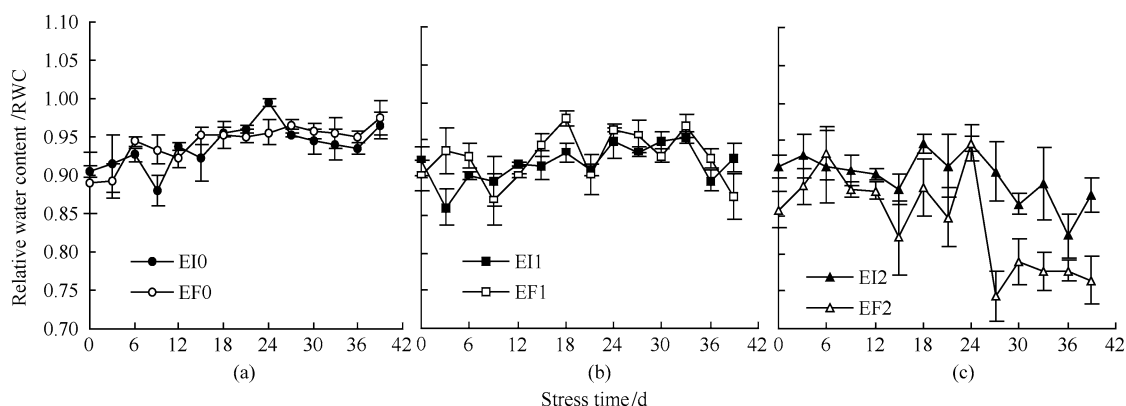
2.2.2 Measurements and statistical analysis

A newly expanded leaf, or the second leaf down which had recently fully expanded, was taken for the determination of RWC, chlorophyll, soluble sugar and starch content. The leaf samples were taken between 2:00 and 2:30 p.m. The sampling work was carried out at three-day intervals. RWC was calculated by weighing method. Chlorophyll content was measured according to the method reported by Lin et al.(1999), and soluble sugar and starch content were measured according to the reference (Plant Physiology Teaching and Research Group of East China Normal University 1980). All data were analyzed using one-way and two-way analysis of variance (ANOVA) using programs of the SPSS statistical package.

3 Results

3.1 RWC of EI and EF leaves

The changes of RWC of EI and EF leaves were in close relation with soil moisture (Fig. 1). Under control condition, RWC of both EI and EF leaves were maintained above 90%; under mild drought stress, it decreased slightly. Under moderate stress, however, it decreased significantly as the time goes on.



(a): Control; (b): Mild stress; (c): Moderate stress, the same below
Fig. 1 Relative water content of EI and EF leaves under different water conditions

When RWC of EI and EF leaves were compared, it showed that RWC of EI leaves was close to that of EF leaves under control and mild stress, while at the latter stage of moderate stress, RWC of EI leaves was significantly higher than that of EF leaves, which suggested that EI plants took more advantages over EF plants in water-holding ability.

3.2 The contents of Chl and Car in EI and EF leaves

The contents of Chla, Chlb and Car under the three treatments were similar (Fig. 2). Both mild stress and moderate stress did not result in significant decrease of the contents of these three pigments. In the whole stress period,

the contents of all these three pigments had a trend of decreasing with time. When decrease rates were considered, Chla decreased fastest while Car decreased the slowest.

As for the contents of these three pigments in EI and EF leaves under different stress treatments, it could be found that the contents of these three pigments in EF leaves were all higher than those in EI leaves under control condition (Fig. 2a). With the stress intensified, the contents of these three pigments in EI leaves were higher than those in EF leaves in several measure days (Fig. 2b). Under moderate drought stress, in most measure days, the contents of these three pigments in EI leaves were higher than those in EF leaves (Fig. 2c). All these showed that endophyte infection could increase the contents of photosynthetic pigments in the host plants with the intensification of drought stress.

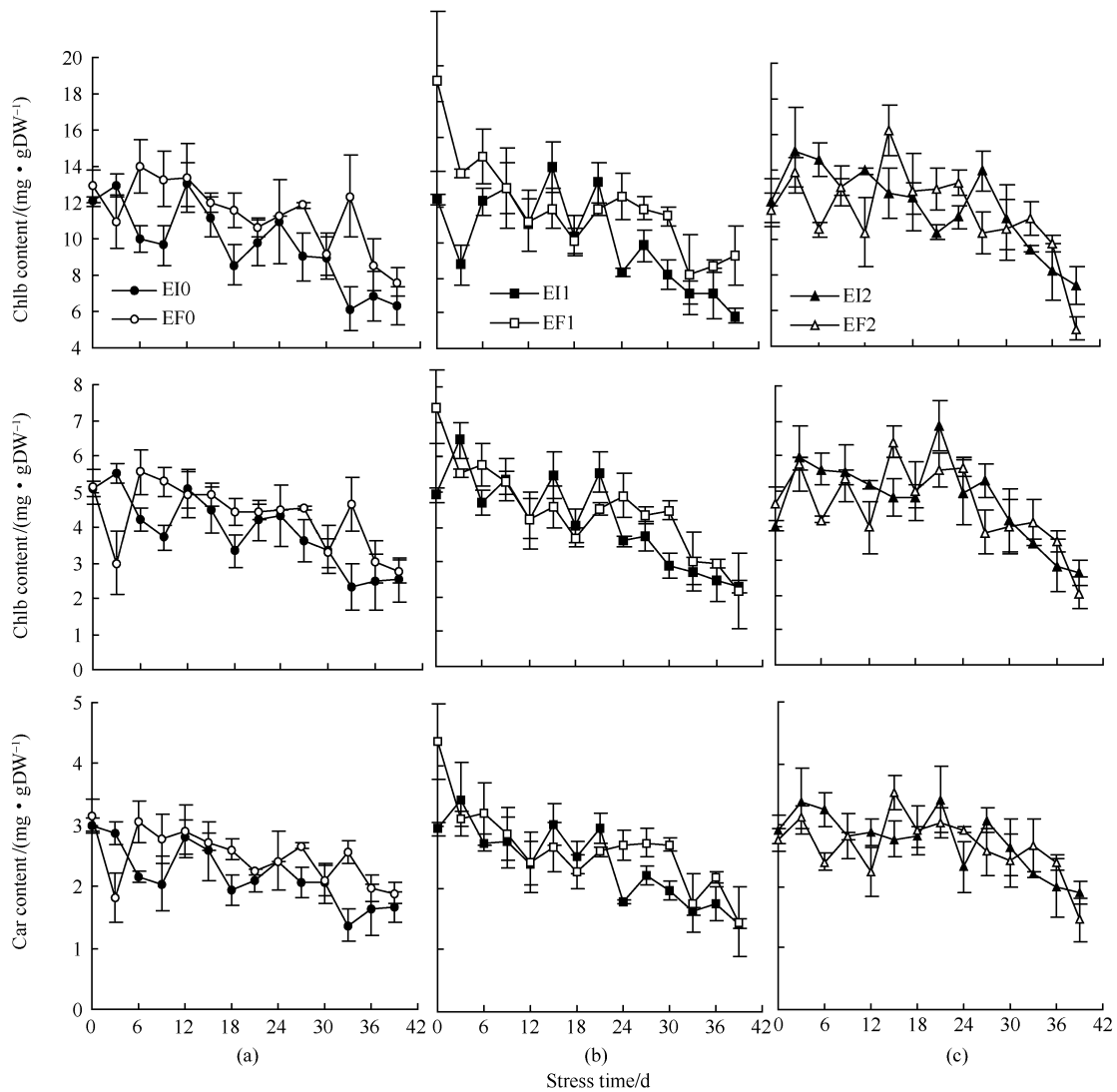


Fig. 2 Contents of chlorophyll a, b and carotenoid of EI and EF leaves under different water conditions

3.3 Contents of soluble sugar and starch in EI and EF leaves

Drought stress had some effect on the contents of soluble sugar and starch in EI and EF leaves (Fig. 3). With the intensification of drought stress, the content of starch decreased greatly. As for the content of soluble sugar, it slightly increased under mild drought stress, and then gradually decreased with the intensification of drought stress. When the contents of soluble sugar and starch in EI

and EF were compared, it could be found that their change patterns were similar under control condition. Under mild drought stress, the contents of soluble sugar and starch in EI leaves were significantly higher than those in EF leaves in almost all measure days. With the intensification of drought stress, the contents of both starch and soluble sugar decreased rapidly, but the content of soluble sugar in EI leaves decreased faster.

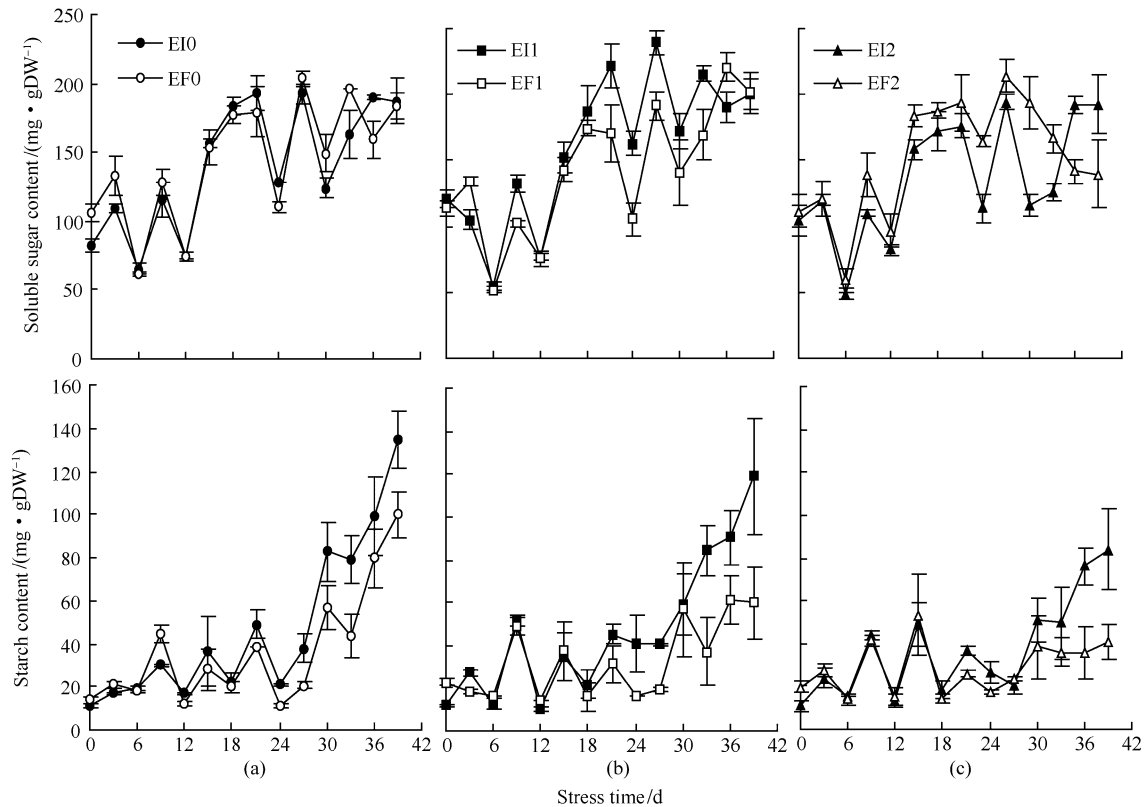


Fig. 3 Contents of starch and soluble sugar of EI and EF leaves under different water conditions

3.4 Above-ground biomass of EI and EF populations

At the end of the stress period, the above-ground tissues of the experimental populations were harvested and the biomass was measured (Fig. 4). The results showed that drought stress could decrease the above-ground biomass of the populations, but the change extent varied due to different stress levels and endophyte infection status. For EI populations, although it appeared that the biomass was negatively affected by drought stress, the difference, however, was not significant between control and stressed populations under either mild or moderate stress. As for the biomass of EF populations, it decreased significantly with the intensification of drought stress, *i.e.* the biomass was significantly lower under moderate drought stress than that under control.

When EI and EF populations were compared, they both didn't show significant differences under control and mild

stress conditions. Under moderate stress, however, the biomass of EF population was lower than that of EI population significantly.

3.5 Regeneration of EI and EF populations

After the aboveground tissues were harvested, the remaining plant parts were left to survive in winter in their natural condition. During the next spring, the regeneration characters of each population were investigated (Fig. 5). The results showed that the tiller number of all stressed populations were significantly lower than that of the control populations, however the stress levels and the endophyte infection status suggested that drought stress had a negative effect on the regeneration of the experimental populations.

As for EI populations, although the tiller number obviously decreased due to drought stress, the difference

was not significant because of the great fluctuation among replicates. When EF population was regarded, the more intense the drought stress was, the worse the regeneration was. And there were significant differences in tiller number among populations treated with three different drought levels. When compared with EF populations, the tiller number of EI populations during regeneration was higher only under moderate drought stress.

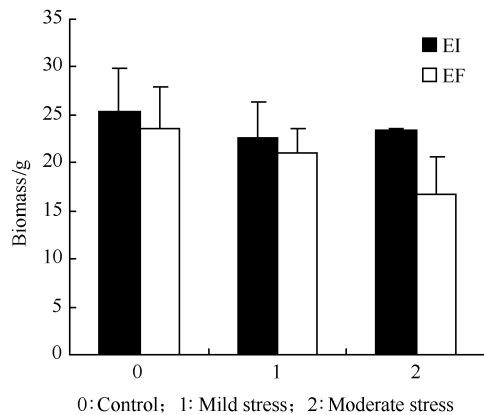


Fig. 4 Upland biomass of EI and EF population at the end of stress experiment

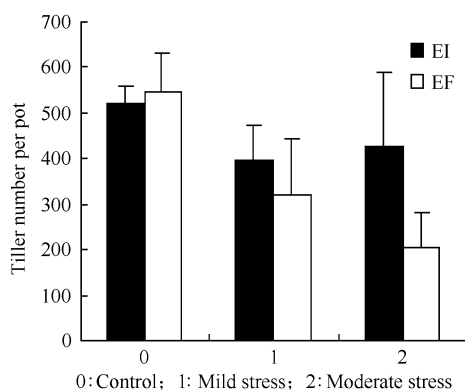


Fig. 5 Tiller number per pot of EI and EF population

4 Discussion

Under drought stress, many adaptive responses may be involved for plants (Xu and Yu 1992), in which osmotic adjustment is an important pathway. As for some drought-resistant plant species, special solutes can be accumulated in the cytoplasm, by which the pressure potential of the cells can be kept when the water potential decreases.

Soluble sugar is a kind of osmotic adjustment substance. It is considered to be an adaptive response to plants to increase soluble sugar content under drought stress. Recently, Karsten reported that under drought stress, the concentration of simple sugar and sucrose increased in both tall fescue and *Lolium perenne*'s sheath to reduce osmotic potential, and the osmotic adjustment ability of

Lolium perenne is greater than that of tall fescue (Karsten and MacAdam 2001). Drought resistant ability of EI plants may be enhanced by both carbohydrate accumulation under normal condition and carbohydrate transportation under drought stress. It is reported that with sufficient water supply, some EI tall fescue symbiosis accumulated more non-structure carbohydrate in their tillers than EF plants (Hill et al., 1990). Once drought stress comes, these carbohydrates can be redistributed quickly, which may play a very important role in the osmotic adjustment of host plants. With regard to the influence of endophyte on carbohydrate accumulation, there were many different results reported. Hardy's research (Hardy et al., 1996) showed that with sufficient water supply the soluble sugar content in tall fescue (Kentucky-31) leaves was not affected by endophyte, which was consistent with Hill's research on the same breed under drought stress condition (Hill et al., 1996). Richardson (Richardson et al., 1992) found that EI plants accumulated more glucose and fructose in leaves and sheaths of tall fescue (genotype CB1) than EF plants. However, investigation on five genotypes of tall fescue suggested that the concentration of non-structure carbohydrate in leaves of three genotypes was EI < EF, in one genotype EI > EF and there were no significant differences in the rest ones (Hill et al., 1990). The results indicated that under the same experimental conditions, responses of host plants infected with the same endophyte varied not only with different host species and breeds, but also with different genotypes in the same breed.

Our study on *Lolium perenne* showed that soluble sugar contents in breed SR4000 were higher in EI than in EF under mild drought stress, but they both were close under control and moderate stress. This suggested that endophyte infection could enhance soluble sugar content in host plants and thus improve their osmotic adjustment ability under mild drought stress. With drought stress intensification, such benefit from endophyte did not exist, and then more photosynthetic products (such as starch) were accumulated in host plants in order to get through undesirable conditions. EI population regenerated more quickly after moderate drought stress, which was coincided with the viewpoint of Malinowski et al. (2000) that endophyte infection may be helpful in sustaining physiological process of host plants at a cost of growth decrease under drought stress. Once the suitable conditions occur, EI host plant may regenerate quickly.

Net photosynthetic ability of plants is indicated directly by the biomass. In our studies, biomass of EI population was significantly higher than that of EF population under moderate drought stress, and the net photosynthetic ability of EI population was hardly affected by drought stress. This conclusion was consistent with that of Richardson et al. (1993). However, as far as photosynthetic pigments were concerned, contents of Chl a, Chl b and Car of EI plants were close to those of EF plants, which indicated that endophyte infection did not alleviate the damage of photosynthetic pigments caused by drought stress. Bonnet

et al. (2003) drew the same conclusion in their studies on endophyte-infected *Lolium perenne* under Zn stress. All these indicated that endophyte may improve photosynthetic ability of host plants in some other pathways.

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