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Differential expression of photosynthesis-related genes of reed ecotypes in response to drought and saline habitats

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Abstract

Three ecotypes of reed (*Phragmites communis* Trinius), swamp reed (SR), dune reed (DR), and heavy salt meadow reed (HSMR), growing in desert regions of northwest China were simultaneously investigated in their natural state for gas exchange patterns and the expression of three photosynthesis-related genes, *cab* (the gene for the light-harvesting chlorophyll *a/b* binding protein, LHC), *psbA* (the gene for the reaction centre D1 protein of photosystem 2, PS2), and *16S rDNA* (the gene for plastid 16S rRNA). Stomatal conductance (g_s) and intercellular CO_2 concentration (c_i) were markedly lower in the two terrestrial ecotypes (DR and HSMR) as compared to SR, paralleling a similar observed depression in net photosynthetic rate (P_N). However, DR with the lowest measured g_s and c_i still exhibited a higher P_N compared to HSMR. These results suggest that both stomatal and non-stomatal factors account for the comparatively low carbon assimilation in the terrestrial ecotypes. An increase in the expression of photosynthesis-related genes was observed in DR compared to SR, whereas the reverse situation was true in HSMR. The expression of photosynthesis-related genes may contribute to reed plants' photosynthetic capacity per leaf area under natural water deficits, but the levels of photosynthesis-related gene expression are not directly correlated with reed plants' general ability for survival and adaptation under water deficient conditions.

Additional key words: *cab*; *16S rDNA*; net photosynthetic rate; *Phragmites communis*; *psbA*; stomatal conductance.

Introduction

Both drought and salinity in the soil can result in water deficit in plant tissues, which, in turn, can lead to injury to growth and development. The effects of these two stresses on plants are common to those caused by osmotic stress, but soil salinity brings about ion toxicity in addition. Since decline of photosynthetic productivity is

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the primary feature of plants subjected to dry and saline environments, many studies have focused upon the molecular basis of plants' adaptation to drought and salinity with respect to photosynthesis-related processes (Cheeseman 1988, Baker 1991, Chaves 1991). Artificial drought treatments induce a marked decrease in mRNA complementary to nuclear-encoded photosynthetic genes in tomato leaves (Bartholomew *et al.* 1991); a similar effect can be readily reversed by rehydration of wilted shoots in *Arabidopsis thaliana* (Williams *et al.* 1994). In salt-tolerant alfalfa cell lines, the increased accumulations of transcripts of both nuclear- and chloroplast-encoded photosynthetic genes have been established, which may be the basis for their salt tolerance (Winicov and Button 1991). Whether or not a similar modulation of photosynthesis gene expression is involved in the adaptation of plants to drought and salinity under natural conditions remains to be elucidated (Baker 1991).

Quantitative analysis of the physiological basis of ecotypic differentiation is a powerful tool for ecophysiological study (Lange *et al.* 1981). Reed is a hydrophilous grass of *Gramineae*, but has evolved various ecotypes that exhibit great resistance or tolerance to adverse terrestrial habitats and environmental stresses, such as drought, salinity, low temperature, *etc.* (Haslam 1969, Van Der Toorn and Mook 1982, Matsushita and Matoh 1991, Wang *et al.* 1995). In the present work, two terrestrial reed ecotypes growing in drought and saline habitats in the desert regions of northwest China were compared with the common swamp reed ecotype for their respective gas exchange patterns and the expression of three photosynthesis-related genes encoded by nuclear and plastid genomes.

Materials and methods

Plants and gas exchange measurement: Three reed ecotypes (*Phragmites communis* Trinius), referred to as SR, DR, and HSMR according to the traits of their respective habitats, were selected for study at the Desert Research Institute of Academia Sinica's Research Area (39° 31'-58' N, 100° 4'-36' E; elev. 1300 m) (Wang *et al.* 1995). The region is a typical desert landscape. The mean annual precipitation is 118 mm, while the annual potential evaporation is 2392 mm. Air temperature is characterized by large daily fluctuations, the annual maximum and minimum temperatures being 39 and -27 °C, respectively. The relative humidity usually does not exceed 50 %. Since all sampling sites are located within a narrow area (about 6.5 km^2), the examined reed ecotypes, although found in varying soil water and salt states, share similar meteorological conditions.

During the same day in August, the gas exchange parameters of the third leaves of mature shoots of the three ecotypes were examined individually with a portable *LI-6000* photosynthesis system (*Li-Cor*, Lincoln, USA) in their native habitats where they formed monospecific stands. P_N, g_s, and atmosphere or intercellular CO_2 concentrations (c_a, c_i) were directly obtained. The leaves of these reed ecotypes were simultaneously collected at midday, and quick-frozen on site in liquid nitrogen.

RNA extraction, electrophoresis, and Northern analysis: Leaf tissue pulverized in liquid nitrogen was homogenized in a lysis buffer, which consisted of 8 M guanidine hydrochloride, 20 mM morpholinoethanesulfonic acid, 20 mM EDTA, and 50 mM mercaptoethanol (pH 7.0). Total RNA was extracted from the homogenate essentially according to Logemann *et al.* (1987). Total RNA (10 μg) was separated by electrophoresis through 1.2 % agarose gels containing 2.2 M formaldehyde (Sambrook *et al.* 1989). Gels were stained with ethidium bromide and examined under UV radiation to ensure equal loading of samples. The separated RNA was transferred to nitrocellulose filter (*Schleicher and Schüll*, Dassel, Germany) and fixed at 80 °C for 2 h.

The transcripts of the following photosynthesis-related genes encoded by nuclear and plastid genomes *cab*, *psbA*, and *16s rDNA* were probed. The probe DNAs cloned in *pBR322* plasmid vectors were kindly provided by Y.S. Zhu (Zhu *et al.* 1985). Plasmid DNA was isolated as described by Holmes and Quigley (1981). The recombinant DNAs were digested and the inserts separated by electrophoresis. The inserts were then recovered from the agarose by melting the agarose at 65 °C, followed by phenol, phenol-chloroform, and chloroform extraction and ethanol precipitation. These probes were labelled by nick translation system according to manufacturer's specifications (*Promega*, Madison, USA) with [α-^{32}P]-dCTP (*Amersham*, Arlington Heights, USA).

The blot was hybridized according to Sambrook *et al.* (1989). Prehybridization was performed by overnight incubation at 42 °C in solution containing 50 % deionized formamide, 6×SSC (1×SSC contains 150 mM NaCl and 15 mM sodium citrate, pH 7.0), 5×Denhardt's solution (1 kg m^{-3} each of polyvinylpyrrolidone, bovine serum albumin, and *Ficoll 400*), 0.5 % sodium dodecyl sulfate (SDS), and 100 g m^{-3} denatured salmon sperm DNA in hybridization oven on an incubator shaker. The hybridization was carried out in the identical solution plus heat-denatured probes at 42 °C for 24 h.

The blot was extensively washed after hybridization: 30 min in 2×SSC/0.1 % SDS at room temperature, another 30 min in 0.1×SSC/0.1 % SDS at room temperature, then 30 min in 0.1×SSC/0.1 % SDS at 55 °C. The washed blot was exposed to X-ray film at -70 °C for 12-48 h, using intensifying screens for autoradiography.

Western analysis of thylakoid membrane polypeptides: Thylakoid membranes were prepared from leaf tissues pulverized in liquid nitrogen. The grinding medium contained 0.3 M sucrose, 0.05 M phosphate, and 0.01 M KCl (pH 7.2). The homogenate was filtered through three layers of *Miracloth*, and the filtrate centrifuged at 2000×*g*. The pellets were then washed once in 1 mM EDTA (pH 8.0) and twice in 50 mM Tricine (pH 8.0). The concentrations of chlorophyll (Chl) in the thylakoid membrane preparation were determined spectrophotometrically in 80 % acetone as described by Arnon (1949).

The relative contents of certain thylakoid peptides on Chl basis were determined immunologically by Western blotting analysis. For polyacrylamide gel electrophoresis, the samples were solubilized in 3.3 % SDS, 2.7 M urea, 0.7 % mercaptoethanol (final concentrations), and heated in boiling water for 2 min.

Polypeptides of thylakoid membranes were separated on 12 % acrylamide slab gels using a high-molarity Tris buffer system without urea (Fling and Gregerson 1986). After completion of the electrophoretic run, polypeptides were immediately transferred from the gel onto nitrocellulose filters (*Schleicher and Schüll*) by electroblotting according to Sambrook *et al.* (1989). The blots were blocked with 2 % nonfat milk-powder in 10 mM Tris/HCl (pH 8.0), 150 mM NaCl, and 0.05 % (m/v) *Tween-20* (TBST-buffer), then the first antibody was allowed to react overnight at room temperature in TBST. In order to visualize the immunoreaction, the second antibody coupled to horseradish peroxidase was allowed to react in TBST for 1 h after washing the filter with TBST. The antibody against LHC2 (the LHC of PS2) was a generous gift from R. Bassi (Università Degli Studi Di Verona, Verona, Italy). The antibody against the D1 protein was kindly provided by I. Ohad (Hebrew University, Jerusalem, Israel).

Results

Characteristics of gas exchange: Even though both terrestrial reed ecotypes grow under similar osmotic stress, there is a marked difference between their responses of growth and development to their respective habitats. The HSMR, which in general is incapable of forming inflorescence, produces short and small shoots with apparent chlorotic leaves. The DR, on the other hand, still propagates itself by sexual and asexual means under drought and produces taller shoots than the HSMR (Wang *et al.* 1995). The present work indicates that the two terrestrial ecotypes differ with respect to their photosynthesis performance.

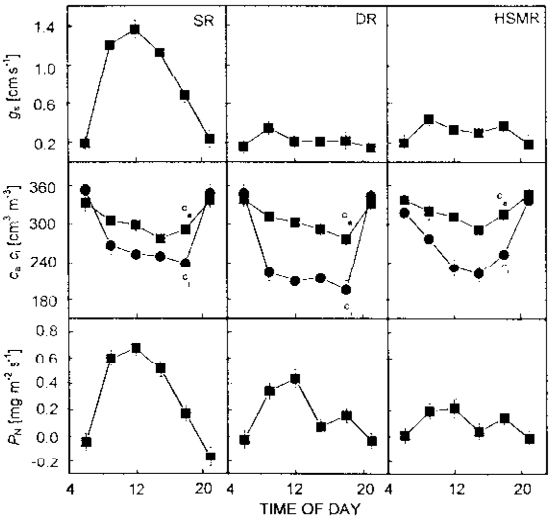

Fig. 1. Diurnal courses of stomatal conductance (g_s), atmosphere- and intercellular CO_2 concentrations (c_a, c_i), and net photosynthetic rate (P_N) in the third leaves of three reed ecotypes (SR, swamp reed; DR, dune reed; HSMR, heavy salt meadow reed). Values are means of 6 leaves. Bars indicate ± SE, where these exceed the sizes of symbols.

The decrease in P_N in both terrestrial ecotypes was pronounced compared to the SR (Fig. 1). Concomitantly with the depression of P_N, g_s of the two terrestrial ecotypes was also reduced, consistent with the observed decreases in the c_i. This indicated that stomatal closure contributed to the limiting of carbon assimilation in the two terrestrial ecotypes. However, stomatal factors alone were probably not the sole components accounting for the depression of photosynthesis in the two ecotypes. Although the DR exhibited a decreased g_s during the entire diurnal photosynthesis period compared to the HSMR, leading to a parallel decrease in c_i, P_N was markedly higher in DR than in HSMR.

Expression of photosynthesis-related genes: The *cab* nuclear gene family consists of several members coding for LHC2. The DR had a significantly elevated level of steady-state transcript of the gene compared to the SR (Fig. 2*A*). In contrast, the transcript level of the gene was lower in the HSMR than in the SR. The result indicated an alteration of *cab* expression among these reed ecotypes because equal amounts of total RNA were loaded in each lane. To determine the responses of photosynthesis-related genes encoded by the plastid genome in these terrestrial ecotypes, transcripts for *psbA* encoding the D1 protein of reaction centre of PS2 were also measured by the Northern blotting analysis (Fig. 2*B*). Compared to the SR, the DR showed a marked increase in the accumulation of *psbA* mRNA; the reverse situation was true for the HSMR. Likewise, the transcript abundance of the plastid *16S rDNA* encoded by plastid genome showed an almost identical difference among the three reed ecotypes (Fig. 2*C*).

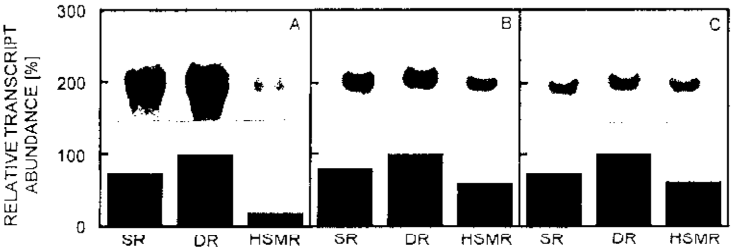

Fig. 2. Northern blotting analysis for *cab* (*A*), *psbA* (*B*), and *16S rDNA* (*C*) transcripts (*insets*) in the third leaves of three reed ecotypes (SR, swamp reed; DR, dune reed; HSMR, heavy salt meadow reed). Each lane was loaded with 10 μg of total cellular RNA. Northern blotting and hybridization with gene clones for *cab*, *psbA*, and *16S rDNA* are described in Materials and methods. The relative abundance is estimated by densitometric scanning of Northern blots. Maximum value in each blot was taken as 100 % and other value were plotted accordingly.

Northern analysis revealed a similar pattern of transcript accumulation of photosynthesis-related genes encoded by both nuclear and plastid genomes in these reed ecotypes. Nevertheless, the additional processes of regulation such as recruitment onto polysomes, translation, and protein stability could also influence the photosynthesis-related gene expression (Mayfield *et al.* 1995). Therefore, further quantitative analysis of the LHC2 and D1 proteins on a per Chl basis was performed among these reed ecotypes using the Western blotting analysis. In comparison with SR, LHC2 accumulated at an increased level in DR, while a reduction in the amount of the protein was observed in HSMR (Fig. 3*A*). Apparently, this was consistent with the Northern analysis results (Fig. 2*A*). From the transcript measurements it was expected that the highest accumulation of D1 protein would be observed in the DR. However, the amount of D1 protein in DR was slightly lower than in SR, suggesting that post-transcriptional processes might be involved in the regulation of *psbA* expression in DR (Fig. 3*B*). In the HSMR, the D1 protein level was even lower, which might be correlated with its poor *psbA* transcript pool. Thus, both transcriptional and post-transcriptional processes are important regulatory elements for *psbA* expression in these reed ecotypes.

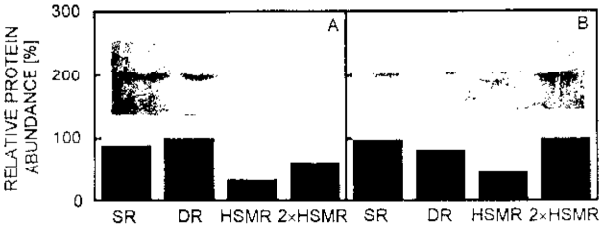

Fig. 3. Immunoblot analysis of thylakoid LHC2 (*A*) and D1 (*B*) proteins (*insets*) on chlorophyll basis in the third leaves of three reed ecotypes (SR, swamp reed; DR, dune reed; HSMR, heavy salt meadow reed). Equal (8 μg chlorophyll) or indicated double (16 μg chlorophyll) amount of sample was applied to each lane. Western blotting and immunodetection are described in Materals and methods. The relative quantities of LHC2 and D1 proteins were estimated by densitometric scanning of the immunoblots. Maximum value in each blot was taken as 100 % and other values were plotted accordingly.

Discussion

Considerable results in the literature show that the repression of photosynthesis gene expression occurs in relation to a wide range of artificial stresses (Burgess and Taylor 1987, Lütz *et al.* 1992, Bredenkamp and Baker 1994, Dannehl *et al.* 1995). Exposed to drought treatments, wheat and maize seedlings exhibited a similar response in our laboratory (He *et al.* 1995, Hao *et al.* 1996).

Both terrestrial reed ecotypes can survive in adverse habitats, but there are marked differences between their growth: the DR possesses a higher photosynthetic productivity compared to the HSMR (Wang *et al.* 1995). This is apparently associated with differences in their photosynthetic competence, demonstrated by gas exchange analysis (Fig. 1). Under normal and moderate water deficits, photosynthesis is usually modulated mainly by stomatal control of CO_2 diffusion. With long-term or more severe water deficits, changes occur in metabolic functions and/or in whole plant behaviour (Chaves 1991). Gas exchange measurements indicate that both the stomatal and nonstomatal responses are involved in determining the photosynthetic competence of the two terrestrial ecotypes. In various plant species nonstomatal inhibition of carbon assimilation has been established under natural and artificial water deficits (*e.g.*, Briggs *et al.* 1986, Di Marco *et al.* 1988, Ziska *et al.* 1990), which could be related to the different aspects of photosynthesis such as ribulosebisphosphatase activity, photophosphorylation, photosynthetic electron transport, *etc.* (Keck and Boyer 1974, Vapaavuori 1986, Moran *et al.* 1994). The expression of photosynthesis-related genes almost necessarily affects photosynthesis under normal conditions, and the LHC2 and D1 proteins in particular are very susceptible to various stresses. Our results suggested that the expression of photosynthesis-related genes in these terrestrial reed ecotypes could, at least to some extent, be responsible for their photosynthetic performance in drought and saline habitats.

The investigation of *cab* transcript accumulation and LHC2 abundance in the present paper suggests that transcript accumulation is a major determinant for the expression of the gene in these reed ecotypes. The results also support the view that nuclear-encoded plastid genes are regulated more at transcriptional level under stress (Karpinski *et al.* 1994). But both transcriptional and post-transcriptional processes seem to be involved in the expression of plastid-encoded *psbA* and *16S rDNA* in these reed ecotypes. The DR possesses the highest transcript level of *psbA* among the three reed ecotypes, but the steady-state abundance of D1 protein in this ecotype is somewhat lower than in the SR. This may be due to a possible accelerated turnover of the D1 protein in DR under drought. The rapid degradation of D1 proteins *in vivo* has been observed even under normal conditions, which can be enhanced by various stresses (Baker 1991). Based on the regulation of gene expression, particularly on the increased accumulation of transcripts, the DR exhibited similar or higher levels of LHC2 and D1 proteins in drought habitat compared to the SR. In contrast, the HSMR showed a reduction in the accumulation of *cab*, *psbA*, and *16S rDNA* transcripts and corresponding decreases in the amounts of LHC2 and D1 proteins in response to long-term salt stress. Water deficits induced by drought and salinity have a different impact on the expression of photosynthesis-related genes in reed plants. In a salt-tolerant alfalfa cell line, a markedly increased accumulation of transcripts of chloroplast- and nuclear-encoded photosynthesis genes has been observed and correlated with cellular salt tolerance (Winicov and Button 1991). However, to date the salt tolerance in cultured cells rarely leads to increased salt tolerance in regenerated plants. Thus, the relationship between the basis of salt adaptation in cultured cells and in whole plants is still not understood (Dracup 1991). As the P_N generally paralleled the expression of *cab*, *psbA*, and *16S rDNA* in the DR and HSMR examined in this study, the expression of photosynthesis-related genes might contribute to reed plants' photosynthetic capacity per leaf area under natural water deficits. However, the two terrestrial ecotypes exhibiting differential expression of photosynthesis-related genes can still grow steadily in their respective habitats, suggesting that the levels of expression of photosynthesis-related genes are not directly correlated with reed plants' general ability for survival and adaptation under water deficit.

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