Composition and characteristic differences in photosynthetic membranes of two ecotypes of reed (*Phragmites communis* L.) from different habitats

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Abstract

As compared with the swamp reed (SR) ecotype of *Phragmites communis* growing in the desert region of northwest China, plants of the dune reed (DR) ecotype from the same region possessed lower chlorophyll (Chl) content in leaves, and less thylakoids and grana stacks in chloroplasts. Tube gel electrophoresis without stain showed that the contents of Chl-protein (Pro) components related to photosystem 2 (PS2) were markedly lower in the DR thylakoid membranes than in the SR thylakoid membranes, while the contents of Chl-Pro components associated with PS1 were almost the same in both types. SDS-PAGE analysis indicated that the content of polypeptides of the light-harvesting Chl a/b complex of PS2 (LHC2) was lower in the DR thylakoids. Besides, the conformation of LHC2 within the DR thylakoid membranes was also altered as indicated by circular dichroism spectra. Hence in the DR, reduced energy harvesting by declining the size of LHC2 might be responsible for the down-regulated PS2 activity. Chl fluorescence parameters, Fv/Fm and quantum efficiency of PS2 (ΦPS2), were lower in the DR leaves than in the SR ones. However, non-photochemical quenching coefficient (qN) was greater in DR than that in SR, implying other energy dissipation way exists in the DR photosynthetic membranes.

Additional key words: chlorophyll fluorescence; circular dichroism spectra; ecotypes; electrophoresis; LHC2; natural drought; photosystems 1 and 2; proteins.

Introduction

The ability of plants to respond to long-term changes in their environment is crucial for determining their resistance to stress, and is a significant factor in their habitat performance (Walters and Horton 1999). Since growth depends on the activity of the photosynthetic system, this has inevitably been subject to intense selection pressure of environment—either on the ability to produce photosynthetic machinery or to photosynthesise more efficiently (Fitter and Hay 1981). Chloroplast functions such as light harvesting, excitation energy transferring and transforming, water splitting, and phosphorylation during photosynthetic process are carried out by the Chl-Pro complexes with definite molecular arrays and conformations embedded in thylakoid membrane. So the changes in the components and organisation of photosynthetic membranes may lead to a re-modelling in the efficiency of radiant energy utilisation and adjustment of photosynthetic mechanisms.

In recent years, much attention has been paid to changes in the responsive adjustment of photosynthetic membranes to various biotic and abiotic stresses. Identification of PS2 as the primary damage site in thylakoids has led to considerable attention focused upon PS2 with respect to environmental stress effects on photosynthesis. The two photosystems, PS1 and PS2, especially PS2, are affected by water stress, which leads to lowered electron transport through them (Matorin *et al.* 1982, Havaux *et al.* 1986, Valladares and Pearcy 1997). Exposed to drought treatment, both wheat and maize seedlings exhibited lowered PS2 activities in studies of our institute (He *et al.* 1995, Hao *et al.* 1996).

As one of the most abundant Chl-Pro complexes of thylakoid, LHC2 connected with reaction centre of PS2 accounts for almost half the Chl and one-third of protein of the membranes (Kühlbrandt and Wang 1991). Besides functioning as an accessory antenna of PS2 in higher plants and green algae, LHC2 is also involved in the stacking of thylakoid, the regulation of energy distri-
tion between the two photosystems, and the dissipation of excessive excitation energy (Bassi et al. 1990, Baker 1991, Kühlbrandt and Wang 1991, Ruban et al. 1993). The components of LHC2 are easily affected by different environmental stresses, such as temperature, water deficit, irradiance, and mineral nutrition. Under artificial water stress treatments, changes in the contents of LHC2 and internal antennae Chl-Pro complexes were observed in wheat cultivars (Lu et al. 1995, Wei et al. 2000), pea (Giardini et al. 1996), maize (Hao et al. 1996), etc. As a major thylakoid component, great flexibility of LHC2 in response to changes in environmental conditions suggests that LHC2 may possess very important physiological functions and contribute to adjustment of photosynthetic mechanisms under adverse conditions.

All photosynthetic processes on the thylakoid membranes need a perfect co-operation between PS1 and PS2. Only if excitation energy is felicitously distributed and electron flow is well balanced between the two photosystems, can a maximum photosynthetic efficiency be ensured (Haehnel 1984). However, it is not clear what changes happen in photosynthetic membranes subject to long-term selection pressure of environment. And little is known about how the components of PS1 and PS2 in thylakoid membranes are adjusted and well fitted together, and thus give optimum photosynthetic performance of plants in response to long-term natural drought habitat.

*Phragmites communis* Triniius is a typical hydrophyte. In the long-term adaptation to natural drought and saline habitats, this reed species in desert regions of Northwest China has evolved four different ecotypes with genetic difference (Cheng et al. 2001). Studies on these reed ecotypes conducted in our laboratory have proved some differences in photosynthetic characteristics exist in response to drought and saline habitats (Wang et al. 1998, Zheng et al. 2000). Our previous investigation on the swamp reed and the dune reed also showed great differences in chloroplast electron transport rate and photo-phosphorylation (Zhu et al. 2001). In order to reveal the possible adaptation mechanism responsible for the DR's photosynthetic performance in drought dune habitat, we investigated the leaf chlorophyll (Chl) fluorescence, chloroplast ultrastructure, and compositional and biochemical properties of thylakoid membranes of the common swamp reed and arid-resistant dune reed.

**Materials and methods**

**Plants and sampling site:** Two ecotypes of *P. communis* materials, swamp reed and dune reed, and the sampling site were described in detail in Zhu et al. (2001) except that the sampling date was during June 22 to 25, 2001. The second fully expanded leaves from the apices were collected from randomly selected plants at midday and frozen in liquid N2 at once until the samples were used for extraction. Fresh leaves were used for preparing leaf discs and electron microscope samples.

**Leaf pigment content and Chl fluorescence:** While sampling, Chl fluorescence of the second fully expanded leaves was determined at midday (10:00 to 12:00), on June 22, 2001, with a FMS-2 portable fluorescence monitoring system (Hansatech, UK). The leaves were dark adapted for 20 min prior to the measurement. Applied saturating flash and actinic irradiances were 10 000 μmol (photon) m−2 s−1 for 2 s and 300 μmol (photon) m−2 s−1 for 20 s, respectively. Measurement processes and calculations of Chl fluorescence parameters, Fv/Fm = (Fm − F0)/(Fm − F0) and ΦPS2 = (Fm′ − F0)/Fm′, followed the instructions of User Manual for Hansatech Fluorescence Monitoring System FMS2.

Leaf discs (diameter 0.5 cm) were punched from a large number of leaves with a borer, then mixed and placed in a moist chamber. 20 discs were immersed in airtight tube containing 10 cm³ of 80 % acetone and kept in dark till all discs were completely decoloured. Pigment contents were measured by the method of Lichtenthaler and Wellburn (1983).

Transmission electron microscopy: The second fully expanded leaves from the apices were collected and cut midway between the leaf margin and midrib to 1×1 mm. A total of at least three leaves from different plants were analysed. These leaf pieces were immediately fixed in 2 % glutaraldehyde buffered with 0.2 M phosphate buffer solution (PBS, pH 7.2). After fixation for about 20 h, the samples were washed with the same PBS and post-fixed with 2 % OsO₄ overnight at 4 °C. After serial dehydration with ethanol, the segments were embedded in Epon 812. Ultra-thin sections were cut on a LKB ultramicrotome with glass knives. Sections were stained with uranyl acetate followed by lead citrate and observed using a Hitachi H-600 transmission electron microscope at 75 kV.

**Thylakoid membranes** were prepared from leaf tissues pulvrised in liquid nitrogen and homogenised in pre-chilled isolation buffer (50 mM potassium phosphate, 10 mM KCl, 0.33 M sucrose, pH 7.2). The homogenate was filtered through four layers of cheesecloth and centrifuged at 2 000×g for 10 min. The pellet of chloroplasts was suspended in the above medium without sucrose, and centrifuged at 4 000×g for 20 min. after which the resulting pellets were washed twice with 1 mM Na₂EDTA (pH 8.0) and once with 50 mM Tricine (pH 8.0) (Dunkley and Anderson 1979). The thylakoid membrane pellets were suspended in 50 mM Tricine (pH 8.0), frozen in liquid N₂, and stored at −70 °C until analysis. The concentrations of Chl and protein in the thylakoid aliquots were determined according to Sgherri et al. (2000) except
that Chl determination was carried out following the method of Lichtenthaler and Wellburn (1983).

Circular dichroism (CD) analysis of thylakoid membranes: Micro-organisation of Chl a/b light-harvesting complex in thylakoids was analysed by CD spectra at room temperature with the JASCO J-20C automatic CD apparatus (Japan) within the wavelength range 600-800 nm (Barzda et al. 1994). Fresh isolated thylakoid membranes were suspended in 50 mM Tricine (pH 8.0) on the basis of 20 g Chl m⁻³. Each sample was analysed under the following conditions: vertical scale, 10 m° cm⁻¹; optical path length, 0.5 cm; time constant, 4 s; spectral band width, 4 mm.

Fully-denaturing SDS-PAGE and non-denaturing tube gel electrophoresis: Two polyacrylamide gel electrophoresis procedures were used. Separation of Chl-Pro complexes was performed on tube gels under non-denaturing conditions using the method of Anderson et al. (1978) with some modification as reported by Li and Lin (1995). Thylakoid membranes were solubilised in a solution containing 0.3 M Tris-HCl (pH 8.8), 10 % glycerol (v/v), and 1 % sodium dodecyl sulphate (m/v), and mass ratio of Chl to SDS of the solubilisation buffer was 1 : 10. Non-solubilised material was removed by centrifugation at 10 000 × g for 2 min and the supernatant was quickly loaded onto 8 % disc polyacrylamide gels [50 µg Chl per tube]. The gels were run at 4 °C and constant current of 2 mA per tube for 4-5 h. The relative content of certain thylakoid peptides on Chl basis (7 µg) was determined using the SDS-PAGE system of Laemmli (1970) modified by the addition of 6 M urea to the gel. Concentrations of the acrylamide resolving gel and stock gels were 4.00 and 13.75 %, respectively. Gels were stained with Coomassie Brilliant Blue.

The results of PAGE were photographed with the Gel-Pro Imaging System (version 3.0), and the signal intensity was analysed densitometrically using a Shimadzu CS-910 double wavelength thin-layer scanner (Shimadzu, Japan).

Results

Alteration in leaf pigment composition and analysis of Chl fluorescence: DR leaves, which are slightly yellowish-green relative to the SR leaves, contained less pigments than the SR did (Table 1). On the basis of leaf area, contents of Chl and carotenoids in the DR leaves were about 70 and 80 % of those in the SR leaves. Chl a/b ratios in the leaves of reed ecotypes were 2.86 (SR) and 3.34 (DR) suggesting that Chl b decreased more than Chl a in the DR leaves in comparison with that of SR (Table 1).

Table 1. Pigment composition [g m⁻²] and chlorophyll (Chl) fluorescence of two reed ecotypes grown in different habitats. Means ± SD of three independent experiments with 20 leaf discs each for pigment contents and 11-13 measurements of different leaves for Chl fluorescence. SR – swamp reed, DR – dune reed.

<table>
<thead>
<tr>
<th></th>
<th>SR</th>
<th>DR</th>
<th>DR/SR [%]</th>
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<tbody>
<tr>
<td>Chl α</td>
<td>1.085 ± 0.073</td>
<td>0.768 ± 0.057</td>
<td>70.74</td>
</tr>
<tr>
<td>Chl β</td>
<td>0.378 ± 0.021</td>
<td>0.230 ± 0.013</td>
<td>60.94</td>
</tr>
<tr>
<td>Chl a+b</td>
<td>1.463 ± 0.087</td>
<td>0.998 ± 0.069</td>
<td>67.97</td>
</tr>
<tr>
<td>Car</td>
<td>0.579 ± 0.038</td>
<td>0.455 ± 0.040</td>
<td>78.58</td>
</tr>
<tr>
<td>Chl a/b</td>
<td>2.86 ± 0.17</td>
<td>3.33 ± 0.11</td>
<td>116.43</td>
</tr>
<tr>
<td>Chl/Car</td>
<td>2.54 ± 0.20</td>
<td>2.20 ± 0.18</td>
<td>86.61</td>
</tr>
<tr>
<td>Fv/Fm</td>
<td>0.81 ± 0.04</td>
<td>0.74 ± 0.05</td>
<td>91.36</td>
</tr>
<tr>
<td>ΦPS2</td>
<td>0.67 ± 0.02</td>
<td>0.53 ± 0.02</td>
<td>79.10</td>
</tr>
<tr>
<td>qN</td>
<td>0.75 ± 0.06</td>
<td>0.82 ± 0.09</td>
<td>109.33</td>
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The efficiency of excitation energy capture by 'open' PS2 reaction centres is defined by the ratio of variable to maximal fluorescence, Fv/Fm. Close correlation with other measures of quantum efficiency of photosynthesis is in a wide range of species has resulted in widespread use of Fv/Fm as a screening parameter for stress response. As shown in Table 1, this parameter in DR was lower (0.74) than in SR (0.81). Similar situation was true for ΦPS2, quantum efficiency of PS2 photochemistry. Thus the activity of PS2 decreased in DR more than in SR. However, non-photochemical fluorescence quenching coefficient (qN) in the DR leaves was higher than that in the SR ones, suggesting that the DR possessed a higher radiation-less dissipation of excitation energy than the SR (Table 1).

Chloroplast ultrastructure in mesophyll cells: The presence of grana stacks is a predominant ultrastructural characteristic of thylakoids in higher plants. In parallel with lower contents of photosynthetic pigments, there were less thylakoids and less grana stacks in the DR chloroplast than that in the SR one (Fig. 1). This indicated that the chloroplast ultrastructure in mesophyll cells of the DR was altered in response to long-term natural drought as compared with that of the SR.

CD spectra of thylakoid membranes: A common feature of the structure pattern of LHC2 aggregates is the large proportion of α-helices contained in the protein component. Such proteins can assemble to multimeric liquid-crystal-like structure exhibiting large CD signals influenced by optical properties of the helical host molecule, pitch length, and integrating statement of the pigment molecules embedded with protein molecules (Faludi-Daniel and Mustard, 1983). The CD signal shape and magnitude for the DR were distinctly different from those for the SR at four major characteristic bands of LHC2 CD spectrum (Fig. 2). With the exception of the positive band peaking between 662 and 665 nm in the DR
spectrum, two signal bands associated with Chl \( a \) molecule in LHC2 emerged around 685 nm (+) and 675 (-) nm were either extremely weak or blue-shifted. The

![Fig. 1. Electron micrographs of mesophyll chloroplast from the swamp reed (SR) and from the dune reed (DR). Samples were stained with uranyl acetate and lead citrate.](image1)

macrohelicity in the LHC2 complex (Garab et al. 1988). This result suggests that some differences exist in the conformation of the LHC2 complex within thylakoid membranes between the two ecotypes of reed growing in different habitats.

![Fig. 2. CD spectra of isolated thylakoid membrane from the two reed ecotypes (swamp reed ... dune reed ... ) at room temperature. 20 g Chl m\(^{-2}\) optical path length 0.5 cm.](image2)

**Pigment-protein complexes and polypeptides of thylakoid membranes:** Pigment-protein complexes of thylakoid membranes in two ecotypes were separated and analysed by tube gel electrophoresis under non-denaturing conditions. The analysis (Fig. 3A) showed that SR and DR possessed a similar Chl-Pro complex pattern, but the contents of each complex differed. The relative contents of CP1 and CP1a, two Chl-Pro complexes associated with PS1 in the DR, reached 107 and 86 % of the SR ones, respectively. However, both the light-harvesting antenna complexes and reaction centre complexes associated with PS2 in the DR thylakoid membranes decreased, especially LHCP\(^1\), LHCP\(^3\) and CP2. Gel densitometric scanning further revealed that the reduction scales in the LHCP\(^1\), LHCP\(^3\), and CP2 in the DR thylakoid membranes were 43, 51, and 68 % relative to the SR, respectively.

Consistent with the results of the tube gel electrophoresis, fully denaturing SDS-PAGE analysis indicated that the contents of the polypeptides associated with LHC2 within the 20-30 kDa molecular mass range and PS2 reaction centre polypeptides of 40-50 kDa also decreased in the DR as compared with the SR (Fig. 3B). However, the content of the polypeptide of CF\(_1\) (66 kDa), which can be removed from the thylakoid membranes by washing membranes twice with 2 M NaBr (data not shown), was greater in the DR thylakoid membranes, being 116 % of that in the SR as indicated by the gel densitometric scanning.
Discussion

For many plants, changes in the composition of the photosynthetic apparatus form an important part of the response to growth conditions (Walters and Horton 1999). In adaptation to the arid dune habitat, both the composition and photosynthetic characteristics of photosynthetic membranes in the DR changed as compared with the SR. In Zhu et al. (2001), we reported that the electron transport rates of whole-chain and PS2 in the DR chloroplasts were lower than those in the SR, while its electron transport rate for PS1 exhibited more than 90% activity of that in the SR. The assay of Chl fluorescence in the present investigation indicated that both \( F_{/}/F_m \) and \( \Phi_{PS2} \) of the DR were lower than those of the SR (Table 1). This further confirmed that the efficiency of excitation energy captured by PS2 reaction centres and quantum efficiency of PS2 photochemistry in the DR leaves were affected under drought habitat. Lower photosynthetic pigments in leaves, less chloroplasts in the mesophyll cells, and less grana stacks in the chloroplasts of the DR showed that the composition of thylakoid membrane was also changed (Fig. 1, Table 1).

The thylakoid membrane is composed of discrete macromolecular complexes that function in concert to carry out photosynthetic electron transport and phosphorylation reactions. One of the most important macromolecules, LHC2, is very important for the dissipation of excessive excitation energy and regulation of energy distribution between PS1 and PS2. Nevertheless, the present electrophoresis results indicated that in comparison with the SR thylakoid membranes, the DR showed decreased contents of Chl-Pro complexes associated with LHC2 (Fig. 3A) whose apoproteins were encoded by the nuclear-encoded \( cab \) genes. And the conformation of LHC2 within the DR thylakoid membranes was also markedly different from that in the SR (Fig. 2). Nuclear-encoded plastid genes may be regulated more at transcriptional level under stress conditions (Karpinski et al. 1994). However, due to a significantly elevated level of steady-state transcript of the \( cab \) gene in the DR thylakoid membranes (Wang et al. 1998), it seems that lower content of the LHC2 may not be due to a reduced transcription or accumulation of its mRNA. Stephen and William (1984) suggest that the accumulation of LHCPs within the thylakoid membranes depends not only upon the light-induced accumulation of LHCP mRNA but also on the accumulation of Chl \( a \) and Chl \( b \), both playing crucial roles in stabilising the pigment-proteins in thylakoid membranes. Experiment that pigments induce folding of light-harvesting Chl \( a/b \)-binding protein indicated the monomer of the apoprotein of LHC2 could not fold correctly, and also failed to form trimer for lack of sufficient Chl \( a \) and Chl \( b \), and therefore were degraded by protease (Paulsen et al. 1993). Studies with the "chlorina-\( f_2 \) mutant of barley further proved that although Chl \( b \) is

Fig. 3. Unstained polyacrylamide tube gel showing chlorophyll (Chl)-protein complexes of thylakoids (A) and fully denaturing SDS-PAGE of thylakoid polypeptides (B) isolated from swamp reed (SR) and dune reed (DR). (A) Each tube was loaded with solubilised thylakoid membranes corresponding to 50 \( \mu \)g Chl. (B) Equal amounts of Chl (7 \( \mu \)g) were separated on 13.75% polyacrylamide slab gel containing 6 M urea and stained with Coomassie Brilliant Blue.
not needed for the synthesis, transport, processing, or thylakoid insertion of the polypeptides which bind with this photosynthetic pigment, it is important in stabilising these thylakoid membrane polypeptides and hence in regulating the accumulation of these proteins (Bellemare et al. 1982). Considering the lower pigment contents in the DR leaves (Table 1), we suggest that the decreased accumulation of Chl a/b-protein complexes in the DR thylakoid membranes might result from the lower contents of pigments. The most possible explanation is that the contents of pigments in the DR leaves, especially Chl b, decrease in response to dune habitat, which in turn affects the stabilisation and conformation of Chl a/b-containing complexes in the thylakoid membranes. Of course, other possible regulations, such as protein translation and the process of Chl integration with protein per se, can not be excluded. Since LHC2 is closely associated with grana formation in thylakoids, less grana stacks in the DR thylakoid might originate from the lack of LHC2.

The Chl-Pro components related to the DR PS1 were little affected (Fig. 3A) and the DR thylakoid membranes exhibited a greater content of CF1 than the SR membranes (Fig. 3B). These results supplied further explanation for consistent increases in activities of thylakoid-binding ATPase and cyclic photophosphorylations in DR chloroplasts as reported by Zhu et al. (2001). Cyclic electron transport could support a large trans-thylakoid photon gradient when access of CO2 to the photosynthetic apparatus was reduced (Harbinson and Foyer 1991). And increase in the degree of energisation of the thylakoids would enhance the dissipation rate of excitation energy by non-radiative processes and consequently decrease qPS2 (Rees and Horton 1990). Similar situation might be true in the DR leaves with lower intercellular CO2 concentration (Wang et al. 1998) and larger cyclic photophosphorylation (Zhu et al. 2001).

In thylakoids, a good correlation has been established between the content of Chl a/b light-harvesting complex of grana and the characteristic large CD signal of thylakoids (Faludi-Daniel and Mustárdy 1983, Barzda et al. 1994, Garab et al. 1988). PS1 is much more effective than PS2 to drive a 40-80% decrease in the CD signal. This decrease is ascribed to a partial loss of macrohelicity in the light-harvesting Chl a/b-protein complex, in response to a proton gradient generated most effectively by PS1 (Garab et al. 1988). Decreased signals of CD spectra in the DR thylakoid membranes (Fig. 2) further confirmed the existence of larger trans-thylakoid proton gradient in the DR chloroplast. Although the molecular mechanism and the site of the non-photochemical quenching of PS2 excitation energy have not been definitively resolved, trans-thylakoid proton gradient may well be a prerequisite for occurrence of such non-photochemical quenching (Baker 1991). Walters and Horton (1991) also suggested a number of physiological processes contributed to qPS2, but the major part of qPS2 occurred in response to the build-up of the thylakoid ΔpH that resulted from electron transport.

Under natural conditions, plant photosynthesis is always radiant energy-saturated, which easily results in photoinhibition or even photodamage. This situation is more severe under stress. However, plants have evolved multiple mechanisms to protect their photosynthetic apparatus from the damage by high irradiance reducing photon harvesting or dissipation of the excess of excitation energy. The content of LHC2, which is considered one of the dissipation sites of excess excitation energy, decreased in the DR photosynthetic membrane (Fig. 3A) as the main light-harvesting component. This change would decline the size of LHC2 in the DR plants that would harvest less photons, and thus would prevent them from photodamage. Larger quantity and quality of thylakoid-bound ATPase (Fig. 3B and Zhu et al. 2001) and increased cyclic photophosphorylation (Zhu et al. 2001) in the DR leaves could form a larger trans-thylakoid proton gradient, and thus would be important in controlling PS2 activity and facilitating radiation-less energy dissipation under natural drought habitat accompanied by high temperature and irradiance (Foyer et al. 1990, Genty et al. 1990, Krause and Weis 1991). Nowadays, xanthophyll cycle-dependent thermal dissipation is believed to be a primary mechanism of radiation-less energy dissipation. In our recent comparative investigation on this aspect between SR and DR, greater activities of enzymes involved in xanthophyll cycle and increased contents of antheraxanthin and zeaxanthin in the DR leaves also coincided with the above conclusion (our unpublished data).

In summary, when reed, a hydrophilic plant, is adapted to long-term drought habitat accompanied with high temperature and irradiance, composition and structure of its photosynthetic membrane and photosynthetic characteristics evidently alter as compared with the swamp ecotype. In order to avoid serious damage of excessive excitation energy on PS2 under water deficit, the plant decreases the size of light-harvesting pigment complexes through adjusting the synthesis of photosynthetic pigments and thus down-regulating its PS2 activity. On the other hand, it enhances the function of PS1 by improving its cyclic electron transport and photophosphorylation (Zhu et al. 2001), which ensures that the DR forms a large trans-thylakoid proton gradient and hence is facilitated by a higher radiation-less energy dissipation of PS2. It is the perfect co-operation of the two photosystems that ensures an optimum photosynthetic performance for DR plants in drought habitat. In this sense, we suggest it may be more appropriate to consider the decreased activity of PS2 in the DR leaves as a protective down-regulation rather than a damage.
References


