RESEARCH PAPER

The enhancement of cyclic electron flow around photosystem I improves the recovery of severely desiccated Porphyra yezoensis (Bangiales, Rhodophyta)

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Abstract

Porphyra yezoensis, a representative species of intertidal macro-algae, is able to withstand periodic desiccation at low tide but is submerged in seawater at high tide. In this study, changes in photosynthetic electron flow in P. yezoensis during desiccation and re-hydration were investigated. The results suggested that the cyclic electron flow around photosystem I (PSI) increased significantly during desiccation, continued to operate at times of severe desiccation, and showed greater tolerance to desiccation than the electron flow around PSII. In addition, PSI activity in desiccated blades recovered faster than PSII activity during re-hydration. Even though linear electron flow was suppressed by DCMU [3-(3′,4′-dichlorophenyl)-1,1-dimethyleura], cyclic electron flow could still be restored. This process was insensitive to antimycin A and could be suppressed by dibromothymoquinone (DBMIB). The prolonged dark treatment of blades reduced the speed in which the cyclic electron flow around PSI recovered, suggesting that stromal reductants, including NAD(P)H, played an important role in the donation of electrons to PSI and were the main cause of the rapid recovery of cyclic electron flow in desiccated blades during re-hydration. These results suggested that cyclic electron flow in P. yezoensis played a significant physiological role during desiccation and re-hydration and may be one of the most important factors allowing P. yezoensis blades to adapt to intertidal environments.

Key words: Cyclic electron flow, desiccation, photosystem I, Porphyra yezoensis.

Introduction

Cyclic electron flow around photosystem I (PSI) in the thylakoid membranes was discovered >50 years ago (Arnon et al., 1954), and has been shown to occur in higher plants, eukaryotic algae, and cyanobacteria (Asada et al., 1993; Ravenel et al., 1994; Mi et al., 1995). In particular, this pathway has been investigated in Arabidopsis thaliana, Chlamydomonas reinhardtii, and tobacco (Nicotiana tabacum var. Petit Havana) (Hörváth et al., 2000; Joet et al., 2001, 2002; Munekage et al., 2002, 2004; DalCorso et al., 2008; Takahashi et al., 2009; Iwai et al., 2010). In addition, several reports provided strong evidence that the cyclic electron transfer chain around PSI could be organized into supercomplexes that associate PSI, cytochrome b6, and ferredoxin-NADP+ reductase (Carrillo and Valleios, 1983; Joliot and Joliot 2002; Iwai et al., 2010). Recently, it has been reported that cyclic electron flow around PSI was essential for photosynthesis in plants (Munekage et al., 2004). During steady-state photosynthesis, cyclic electron flow contributes to ATP synthesis and adjusts the ATP: NADPH ratio (Arnon et al., 1967; Backhausen et al., 2000;
Kramer et al., 2004a), which is essential for preventing stroma over-reduction (Munekage et al., 2004). More importantly, a number of papers have demonstrated that cyclic electron flow around PSI plays a significant physiological role in plant responses to stresses, such as drought or desiccation (Horváth et al., 2000; Golding and Johnson, 2003; Bukhov and Carpentier, 2004; Gao et al., 2011). However, the above-mentioned model plants have limited desiccation tolerance, although some bryophytes and ferns show some tolerance (Heber et al., 2007). Therefore, it is more appropriate to choose desiccation-tolerant species when undertaking studies on the effects of desiccation on the cyclic electron flow around PSI.

Porphyra spp. inhabit the high intertidal zones of rocky seashores, are periodically submerged in seawater at high tide, and are exposed to air at low tide (Tseng et al., 1983). During exposure to air, especially during the daytime, they are subjected to severe desiccation (Davison and Pearson, 1996). High intertidal Porphyra reportedly demonstrate extreme tolerance to desiccation and can survive a loss of up to 85–95% of their water during desiccation at daytime low tides (Blouin et al., 2011). Hence, Porphyra spp. is an excellent model for investigating the response and tolerance mechanisms of intertidal algae to desiccation. Furthermore, the PSI-driven cyclic electron flow is particularly important in the plant response to drought or desiccation; therefore, as a highly desiccation-tolerant species, Porphyra is an appropriate model for studying the effect of desiccation on cyclic electron flow around PSI in plants.

Most Porphyra blades consist of a single layer of cells during the macroscopic gametophyte blade phase of their life cycle. This feature is quite distinct from higher plants. Porphyra has become an important marine crop worth ~US$1.3 billion per year (Blouin et al., 2011) and has been intensively cultivated in eastern Asian countries, including China, Japan, and South Korea. Porphyra tolerance to desiccation has been widely applied in Porphyra aquaculture. Nets seeded with P. yezoensis blades are raised out of the sea by farmers to expose the blades to air for several hours a day, several times a week. This aerial drying is used to kill harmful seaweeds and strengthen the disease resistance of Porphyra (Lin et al., 2009; Blouin et al., 2011).

To date, there have been several reports on the effect of desiccation on Porphyra photosynthetic performance (Johnson et al., 1974; Smith et al., 1986; Lipkin et al., 1993; Zou and Gao, 2002; Lin et al., 2009; Contreras-Porcia et al., 2011). In addition, reports show that Porphyra blades have developed a high tolerance to desiccation (Sibbald and Vidaver, 1987; Lipkin et al., 1993; Lin et al., 2009; Blouin et al., 2011; Contreras-Porcia et al., 2011). The photosynthetic function of Porphyra blades under severe desiccation can be restored rapidly when re-hydrated, but there is little information available on the response and tolerance mechanisms of Porphyra to desiccation. Little is known regarding the responses of photosynthetic electron flow in Porphyra to desiccation, although this has been documented in lichens and higher plants (Bukhov et al., 2004; Heber and Shuvalov, 2005; Liu et al., 2006).

Recently, it has been reported that an increase in cyclic electron flow around PSI occurred in desiccated green macroalgae, Ulva sp, which are higher green algae with a photosynthetic apparatus similar to that of higher plants (Gao et al., 2011). However, Porphyra is a phylogenetically basal red algae which has special light-harvesting complexes (phycobilisomes) that are similar to those of cyanobacteria. In order to investigate the relationship between the cyclic electron flow around PSI and the desiccation and re-hydration of these lower red algae, P. yezoensis blades were chosen for the study and their physiological responses to desiccation and re-hydration were determined. Furthermore, particular attention was paid to photosynthetic electron flows around PSI and PSII during desiccation and re-hydration.

Materials and methods

Sample collection

Porphyra yezoensis blades were collected in the morning from the intertidal zones of Qingdao (36°0′3″ N, 121°2′0″ E), China, between April and May 2011 when irradiance ranged between 300 μmol photons m⁻² s⁻¹ and 400 μmol photons m⁻² s⁻¹. The blades were rinsed lightly in sterile seawater to remove any sediment or epiphytes and then were cultured in the laboratory at 15 °C and under an irradiance regime of 150–200 μmol photons m⁻² s⁻¹. Healthy blades with similar physiological states were chosen.

Water content determination

The absolute water content (AWC) of the P. yezoensis blades was determined by:

$$AWC = \left(\frac{W_t - W_d}{W_o - W_d}\right) \times 100\%$$

where Wd is the weight of blades dried for 24 h at 80 °C, Wo is the wet weight of the fresh blades after the gentle removal of water from the surface, and Wt is the weight of blades at time t after dehydration. Tissue paper was used to remove water from the surface of the blades in order to avoid the formation of salt crystals. The blades were then naturally dehydrated in the laboratory at 150–200 μmol photons m⁻² s⁻¹ at room temperature. When they reached ~13% AWC, the desiccated blades were re-hydrated in seawater at room temperature.

Chlorophyll fluorescence and P700 measurement

The photosynthetic properties of the blades were measured during desiccation and re-hydration. The chlorophyll fluorescence of PSII and the redox state of P700 were determined concomitantly at room temperature using a Dual-PAM-100 fluorometer (Walz, Effeltrich, Germany) connected to a PC with WinControl software. In order to assess the fluorescence and P700 parameters, the repetitive application of saturation pulses and the automated induction and recovery curve routine in the Dual-PAM software were used. Light from a 620 nm light-emitting diode (LED) and blue actinic light at 100 μmol photons m⁻² s⁻¹ from 460 nm LED arrays were delivered to P. yezoensis blades for 5 min periods by a DUAL-DR measuring head. Additionally, saturating light pulses of 300 ms duration and 10 000 μmol photons m⁻² s⁻¹ were delivered. The P. yezoensis blades were dark adapted for 3–5 min before measurement. F0 was determined and then a saturating flash was applied to the blades in order to detect the maximum fluorescence (Fm). The difference between Fm and F0 was referred to as the variable fluorescence (Fv). The maximum quantum yield was obtained as Fv/Fm (Schreiber, 2004). Fv/Fm was measured when the blades were illuminated. Y(II) is the effective
photochemical quantum yield of PSI (Kramer et al., 2004b). The electron transport rate of PSI (ETRI) was calculated by Dual-PAM software. The $F_r/F_m$ and $F_0$ images of Porphyra yezoensis blades during desiccation and re-hydration were examined with the Maxi version of an IMAGING-PAM M-series (Walz, Effeltrich, Germany).

Together with fluorescence measurement, the saturation pulse method was used to determine P700 parameters (Klughammer and Schreiber, 1994). P700 was measured in the dual-wavelength mode (photodetector set to measure 775 nm and 830 nm pulse-modulated light) (Schreiber and Klughammer, 2008). The maximum P700 signal, $P_m$, was determined by application of the saturation pulse in the presence of far-red light, which was considered as analogous to $F_m$. The zero P700 signal, $F_0$, was determined when complete reduction of P700 was induced after the saturation pulse and cessation of far-red illumination. $F_m$ is the maximum P700 signal induced by combined actinic illumination plus saturation flash. Based on the values of $P_m$, $P_0$ and $P_m$, the $Y(0)$, as well as the quantum yield of non-photochemical energy dissipation in PSI due to donor-side limitation and acceptor-side limitation [Y(ND) and Y(NA), respectively] were calculated by Dual-PAM software and saved in a report file (Pründel et al., 2008). The photosynthetic electron transport rates of PSI (ETRI) were also calculated by Dual-PAM software.

### Inhibitor treatment

To monitor electron flow around PSI and PSII, the inhibitors 3-(3',4'-dichlorophenyl)-1,1-dimethyleurea (DCMU) and dibromothymoquinone (DBMIB) were used to treat Porphyra yezoensis blades at different levels of dehydration before measurement with a Dual-PAM-100. DCMU is known to block electron transport after the primary acceptor in PSII ($Q_A$). Therefore, linear electron flow was diminished or abolished in the presence of DCMU (Jørgensen et al., 2002). With the DCMU treatment, fully hydrated (100% AWC) blades were treated at room temperature with 5 µM DCMU for 1 min and the ETR was subsequently measured. After measurement, blades were treated at room temperature for 1 min in the presence of 80 µM DBMIB, which is known to block the electron transport from plastoquinone (PQ) to the cytochrome b$_6$f complex (Frank and Trebst, 1995; Ivanov et al., 2005; Iwai et al., 2010) and effectively suppresses cyclic electron flow around PSI (Herbert et al., 1990). Subsequently, the blades were measured again. As described above, the photosynthetic performance of blades at 69% and 20% AWC were examined after treatment with the inhibitors.

The blades at 13% AWC were re-hydrated for 5 min in seawater containing 5 µM DCMU before measurement, following which the blades were incubated in 10 µM antimycin A (AA) for 5 min, which specifically inhibits PGR5-dependent PSI cyclic electron transport (Manekage et al., 2002; Shikanai, 2007). Then the ETR was measured. Subsequently, they were incubated for 5 min in 80 µM DBMIB before measurement. The ratios of cyclic electron flow to total electron flow at varying levels of desiccation were derived from:

$$\text{ETRI (treated with DCMU)}/\text{ETRI (no inhibitor)}$$

### Dark treatment

To investigate the influence of starch degradation on photosynthetic electron flow, the Porphyra yezoensis blades were incubated at 15 °C in darkness for 12, 24, 48, and 72 h, respectively. After dark treatment, the blades were immediately treated with 5 µM DCMU in darkness. Then the blades were dehydrated naturally in the presence of DCMU to avoid the influence of linear electron flow on cyclic electron flow around PSI. They were re-hydrated for 5 min in seawater containing 5 µM DCMU when AWC reached ~13%, after which the ETR was measured. In addition, the physiological states of the blades after varying dark treatment times were measured.

### Statistical analysis

The results were expressed as mean values of five independent experiments ± standard deviation (SD). Data were analysed by analysis of variance (ANOVA) using the SPSS 18.0 statistical software. Tukey’s multiple comparison test was used at an α=0.05 significance level to determine any significant differences between different levels of desiccation.

### Results

**Maximum quantum yield ($F_v/F_m$) and minimum fluorescence ($F_0$) of blades during desiccation and re-hydration**

There were large changes in $F_v/F_m$ and $F_0$ during desiccation and re-hydration (Figs 1, 2). $F_v/F_m$ decreased significantly with increasing loss of water from the blades (Fig. 1). Meanwhile, $F_0$ increased steadily as the blade water content dropped between onset of desiccation and 14% AWC where $F_0$ reached its maximum (Fig. 2). When the AWC of the blades decreased to ~14%, the blades were re-hydrated. Clearly, the $F_v/F_m$ increased significantly during re-hydration and was fully restored after 30 min of re-hydration (Fig. 1).

In contrast, there was a decreasing trend in $F_0$ during re-hydration (Fig. 2). In addition, the $F_v/F_m$ and $F_0$ blade images were examined during desiccation and re-hydration with a Maxi-Imaging-PAM (Figs 1A, 2A). The images for...
Quantum yields of PSI and PSII during desiccation and re-hydration

There were large changes in the effective quantum yields of PSI and PSII in the blades during desiccation and re-hydration (Fig. 3). Y(II) increased slightly ($P < 0.05$) from the onset of desiccation down to 70% AWC. During further desiccation, Y(II) declined steadily and decreased to zero when AWC neared 23%. In comparison, Y(I) clearly rose from the onset of desiccation down to 70% AWC, where Y(I) reached a maximum (0.8). There were significant differences ($P < 0.05$) between Y(I) at 100% AWC and 70% AWC. Subsequently, Y(I) decreased steadily to zero as AWC approached ~13%. Upon re-hydration, both Y(I) and Y(II) increased sharply. However, the recovery of Y(I) was more rapid than that of Y(II). Moreover, there was a decreasing trend in Y(I) during further re-hydration.

There were variable tendencies in the quantum yields of non-photochemical energy dissipation in PSI under desiccation and re-hydration (Fig. 4). During desiccation, the Y(ND) decreased rapidly to a level (0) which rapidly recovered during re-hydration. In contrast, the Y(NA) increased significantly and increased to a maximum of ~39%. Interestingly, it declined significantly at moderate levels of desiccation. During further desiccation (39–13% AWC), Y(NA) increased significantly to a maximum of ~0.55. After 5 min of re-hydration, both Y(ND) and Y(NA) were restored to normal levels. However, both showed decreasing trends under further re-hydration.

Electron transport rates of PSI and PSII during desiccation and re-hydration and their response to inhibitors

The electron transport rates of PSI and PSII changed considerably during desiccation and re-hydration (Fig. 5). The ETRII increased slightly from the onset of desiccation.
to 70% AWC, where the ETRII values reached a maximum of ~17%. With further dehydration, the ETRII dropped steadily and decreased to zero when AWC approached 23%. In contrast, the ETRI rose significantly and increased to a maximum of ~34 at 70% AWC. There were significant differences between the ETRI at 100% AWC and at 70% AWC ($P < 0.05$). Subsequently, the ETRI declined gradually and decreased to zero when AWC reached 13%. After a 5 min re-hydration period, the ETRI recovered rapidly to a level even higher than that of the fully hydrated blades (100% AWC). In contrast, the ETRI decreased slightly after a further period of re-hydration. After 30 min of re-hydration, the ETRII had slowly recovered but had still not reached the pre-desiccation rate.

During an investigation of the photosynthetic electron transport in PSI and PSII in *P. yezoensis* blades under desiccation, the ETRI and the ETRII of the blades were determined in the presence of inhibitors at varying levels of desiccation: 100% AWC, 69% AWC, and 20% AWC (Fig. 6 and Table 1). When the fully hydrated blades (100% AWC) were treated with DCMU, the ETRII dropped sharply to zero, suggesting that linear electron flow was effectively inhibited (Fig. 6A). Meanwhile, the ETRI decreased significantly to a relatively low value (2.8), which then decreased immediately to zero upon treatment with DBMIB (Fig. 6A). When the blades at 69% AWC were treated with DCMU, both the ETRI and the ETRII decreased strongly to 5.3 and 0, respectively. Furthermore, the ETRI was effectively suppressed by DBMIB (Fig. 6B and Table 1). When AWC reached 20%, both the ETRII and the ETRI decreased to 0 and 12.8, respectively. Clearly, the ETRI was not affected by DCMU but was greatly reduced by DBMIB. The ratio of cyclic electron flow to total electron flow in desiccated blades at 69% AWC (14.9%) was much higher than that of fully hydrated blades (9.4%; Table 1). At 20% AWC, this increased sharply to 97.7%, suggesting that cyclic electron flow around PSI was dominant at this level of desiccation. All these results suggested that cyclic electron flow around PSI in *P. yezoensis* blades was less sensitive to desiccation than PSII and continued to operate under severe desiccation conditions.

**Changes in electron transport rates in PSI in response to varying durations of dark treatment and to inhibitors during re-hydration**

When AWC reached 13%, the ETRI decreased to its lowest level (0), which rapidly recovered during re-hydration (Fig. 5). More interestingly, the ETRI could still recover to
There were large changes in the responses of the physiological states of blades undergoing varying durations of dark treatment, as reflected in the similar ETRI and $F_v/F_m$ values at 100% AWC. There was a gradual decline in the ETRI when desiccated blades at 13% AWC were re-hydrated in the presence of DCMU while undergoing prolonged dark treatment (Fig. 7B). The ETRI was much lower in the re-hydrated blades that had undergone dark treatment for 72 h compared with those that had not undergone dark treatment (i.e. dark 0 h). There were significant differences in the ETRI between dark treatment times.

### Discussion

The responses of $F_v/F_m$ and $F_0$ to desiccation and re-hydration

There were large changes in $F_v/F_m$ and $F_0$ in *P. yezoensis* blades during desiccation and re-hydration (Figs 1, 2). $F_v/F_m$ is a sensitive indicator of the photosynthetic performance of plants, and decreases significantly when plants are subjected to stresses (Maxwell and Johnson, 2000). The results of this study suggested that in *P. yezoensis*, a lower eukaryotic photosynthetic organism, the response of blade $F_v/F_m$ to desiccation was similar to that of higher plants. However, even following the loss of >85% of their water content, the blades recovered rapidly when re-hydrated, which is quite different from the response in higher plants (with the exception of some bryophytes and ferns). In addition, $F_0$ gradually increased with the loss of water (Fig. 2), whereas, during re-hydration, $F_0$ decreased. It has been proposed that the increase in $F_0$ is a result of the separation of the light-harvesting pigment protein complex from PSII core complexes in isolated chloroplasts under heat-pre-treated conditions (Schreiber and Armond, 1978).

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**Table 1.** The ETRI of blades at varying durations of desiccation (100, 69, and 20%) and the ETRI of blades treated with the inhibitors DCMU and DBMIB at varying levels of desiccation.

The ratio of cyclic electron flow to total electron flow was calculated as ETRI (treated by DCMU)/ETRI (no inhibitor). The data are mean values from five independent replicates.

<table>
<thead>
<tr>
<th>Desiccation levels</th>
<th>ETRI (no inhibitor)</th>
<th>ETRI (DCMU)</th>
<th>ETRI (DBMIB)</th>
<th>Cyclic electron flow/total electron flow</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fully hydrated state (100% AWC)</td>
<td>29.8±1.5 $n=5$</td>
<td>2.8±0.2 $n=5$</td>
<td>0 $n=5$</td>
<td>9.4%</td>
</tr>
<tr>
<td>69% AWC</td>
<td>35.6±0.8 $n=5$</td>
<td>5.3±0.5 $n=5$</td>
<td>0 $n=5$</td>
<td>14.9%</td>
</tr>
<tr>
<td>20% AWC</td>
<td>12.8±0.7 $n=5$</td>
<td>12.5±0.6 $n=5$</td>
<td>0 $n=5$</td>
<td>97.7%</td>
</tr>
</tbody>
</table>

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**Note:**

~10 when desiccated blades, with an AWC of 13%, were treated with DCMU to suppress PSII. The ETRI was not influenced by AA, whereas it was effectively stopped by DBMIB (Fig. 7A). These results indicated that as PSII was suppressed, PSI-driven cyclic electron flow in *P. yezoensis* blades could still be restored during re-hydration and that it was insensitive to AA.

It has been proposed that starch degradation can provide exogenous NAD(P)H, which could donate electrons to intersystem electron carriers (Bukhov et al., 2002; Bukhov and Carpentier, 2004). The *P. yezoensis* blades were treated in darkness to determine the effects of starch degradation on the recovery of cyclic electron flow around PSI during re-hydration. The results showed no obvious changes in the physiological states of blades undergoing varying durations of dark treatment, as reflected in the similar ETRI and $F_v/F_m$ values at 100% AWC. There was a gradual decline in the ETRI when desiccated blades at 13% AWC were re-hydrated in the presence of DCMU while undergoing prolonged dark treatment (Fig. 7B). The ETRI was much lower in the re-hydrated blades that had undergone dark treatment for 72 h compared with those that had not undergone dark treatment (i.e. dark 0 h). There were significant differences in the ETRI between dark treatment times.

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**Figure 7.** (A) Changes to the ETRI and ETRII in desiccated *P. yezoensis* blades (13% AWC) in response to re-hydration in the presence of DCMU. The antimycin A (AA) treatment was performed after the blades had been treated with DCMU and subsequently the blades were treated with DBMIB before further measurement. (B) The influence of dark treatment on the recovery of cyclic electron flow around PSI in desiccated blades (13% AWC). Inset show the $F_v/F_m$ of blades having undergone varying durations of dark treatment. The desiccated blades were re-hydrated in the presence of DCMU.
Alternatively, Yamane et al. (1997) reported that a partly reversible inactivation of the PSII reaction centre at high temperature could lead to an increase in $F_0$. It is known that the main light-harvesting complexes in *Porphyra* are phycobilisomes that are composed of predominantly hydrophilic polypeptides and are highly mobile (Mullineaux et al., 1997; MacColl, 1998). Nevertheless, $F_0$ still increased significantly as the AWC reached 23% and PSII was inactivated. It should be noted that phycobilisomes are essentially immobile under such desiccated conditions. These results suggested that the reversible inactivation of the PSII reaction centre might be the main cause of the increase in $F_0$ in *P. yezoensis* blades during desiccation.

**Cyclic electron flow activity around PSI increased significantly during desiccation**

Clearly, the PSI activity of *P. yezoensis* blades increased significantly ($P < 0.05$; Tukey’s test) during desiccation and was still activated under severe desiccation (19–15% AWC) when linear electron flow had stopped (Fig. 5). Cyclic electron flow around PSI at 69% AWC was much higher than at 100% AWC (Fig. 6A, B). Furthermore, the PSI activity of desiccated blades was not affected by DCMU but was specifically suppressed by DBMIB (Fig. 6C). All these results implied that PSI-driven cyclic electron flow in *P. yezoensis* blades was elevated during desiccation and still continued to operate under severe desiccation. These results were consistent with earlier reports that cyclic electron flow around PSI was stimulated by many stresses such as drought and salt stress (Jeanjean et al., 1993; Joet et al., 2002; Golding and Johnson, 2003; Bukhov and Carpentier, 2004; Gao et al., 2011). Indeed, cyclic electron flow around PSI plays a critical physiological role in plant tolerance to desiccation (Canaiani et al., 1989; Herbert et al., 1990; Heber and Walker, 1992; Horváth et al., 2000; Munekage et al., 2002; Golding and Johnson, 2003). During photosynthesis, cyclic electron flow around PSI could generate a pH gradient across the thylakoid membrane that not only could be used to synthesize ATP, but could also induce thermal dissipation and provide protection for the photosynthetic apparatus (Heber and Walker, 1992; Munekage et al., 2002; Golding and Johnson, 2003; Kramer et al., 2004a). Hence, it is possible that in *P. yezoensis* blades, cyclic electron flow around PSI may contribute substantially to photosynthetic electron flow during desiccation in order to provide ATP and protect the photosynthetic apparatus. This could potentially be one of the most important factors allowing *P. yezoensis* blades to tolerate desiccation stress during low tide.

*The up-regulation of cyclic electron flow around PSI triggered a transient increase in photosynthetic activity at moderate levels of desiccation*

The effective quantum yields of PSI and PSII were significantly affected by desiccation and re-hydration (Fig. 3). PSI activity increased significantly ($P < 0.05$; Tukey’s test) at a moderate level of desiccation (70% AWC), as reflected in increased Y(I). In contrast, PSII activity increased slightly from the onset of desiccation to 70% AWC. A transient increase in photosynthetic capability has been reported in some *Porphyra* spp. (Johnson et al., 1974; Gao and Aruga, 1987; Lipkin et al., 1993). It is unclear what caused the increased photosynthetic capability in *Porphyra* at moderate levels of desiccation.

The quantum yields of non-photochemical energy dissipation in PSI increased significantly during desiccation as reflected by Y(ND) and Y(NA). Y(ND) and Y(NA) show the quantum yield of non-photochemical energy dissipation in PSI reaction centres due to a shortage of electrons and electron acceptors, respectively (Pfündel et al., 2008). The increase in Y(ND) correlated with the functional breakdown of PSII (Fig. 3). The activity of PSII, which provides electrons by water splitting, was rapidly suppressed at severe levels of desiccation. Consequently, the rates of linear electron flow were low and even stopped, which ultimately resulted in elevated Y(ND). These results suggested that both donor-side and acceptor-side electron flow were affected during desiccation. There was a slight decrease in Y(ND) at moderate levels of desiccation (Fig. 4). More interestingly, as AWC approached 61%, Y(NA) declined significantly but increased steadily under further desiccation—a phenomenon also found in desiccated *Ulva* spp. (Gao et al., 2011). Cyclic electron flow around PSI increased significantly at 70% AWC (Fig. 5 and Table 1), which could lead to the effective downstream transport of electrons. The transient increase in Y(I) and the decline of Y(NA) at moderate levels of desiccation demonstrated that the redox rate of P700 was elevated. These results suggested that up-regulation of cyclic electron flow around PSI at moderate levels of desiccation led to increased PSI activity in *P. yezoensis* blades and ultimately resulted in a transient increase in photosynthetic activity.

*The recovery of cyclic electron flow around PSI as linear electron flow was suppressed*

After only 5 min of re-hydration, the ETRI of the desiccated blades at 13% AWC rapidly recovered to former levels (Fig. 5), suggesting that PSI recovery was much faster than that of PSII. Furthermore, this study found that when the blades at 13% AWC (where ETRI had decreased to zero) were treated with DCMU, the ETRI could still be restored to ~10 (Fig. 7A). These results suggested that as PSII was suppressed, the PSI-driven cyclic electron flow in desiccated *P. yezoensis* blades could still be restored. Thus, the recovery of cyclic electron flow around PSI in the desiccated blades was independent of linear electron flow. It remains unclear how cyclic electron flow around PSI was restored during re-hydration if there was no electron donation from PSII.

These results indicated that prolonged dark treatment of blades was accompanied by a gradual decline in the ETRI when blades at 13% AWC were re-hydrated in DCMU. Therefore, this study suggests that the dark treatment (floridean starch degradations) of *P. yezoensis* blades, which caused a decrease in the pool of stromal reductants including NAD(P)H, profoundly influenced the recovery of
PSI-driven cyclic flow in the desiccated blades. There is strong evidence that exogenous NADPH and NADH can donate electrons to intersystem electron carriers (Asada et al., 1992, 1993; Endo et al., 1997; Bukhov et al., 2001). For example, in the mesophyll chloroplasts of maize, the pool of stomatal reductants, such as NADPH, is capable of rapid electron donation to P700\(^+\) (Asada et al., 1993) and the NADPH and NADH produced are mainly generated from starch degradation in the chloroplasts (Bukhov et al., 2002; Bukhov and Carpentier, 2004).

The cyclic electron flow around PSI was insensitive to AA but was effectively suppressed by DBMIB (Fig. 7A). Two pathways of cyclic electron flow around PSI have been proposed, including the PGR5-dependent cyclic flow, which could be specifically inhibited by AA, and the chloroplastic NAD(P)H dehydrogenase (NDH)-dependent cyclic flow (Shikanai, 2007; Endo et al., 2008). The results of this study suggested that the NDH-dependent cyclic flow played a significant physiological role in the recovery of cyclic electron flow around PSI in P. yezoensis blades. Furthermore, the results showed that the ETRI increased significantly when the blades were exposed to AA for >15 min. Indeed, AA is also a known inhibitor of the respiratory cytochrome bc1 complex and may cause accumulation of NADH in mitochondria, which could then be transported into chloroplasts (Rochaix, 2011). This would cause increased cyclic electron flow around PSI. These results further support the notion that stomatal reductants, such as NAD(P)H, donate electrons to intersystem electron carriers. Published papers, together with these results, suggest that stomatal reductants, such as NAD(P)H, which could accumulate by florian starch degradation, may contribute substantially to the rapid recovery of PSI-driven cyclic electron flow in re-hydrated blades. This could generate a pH gradient and provide ATP for a variety of processes in the chloroplast and also protect the photosynthetic apparatus.

The photosynthetic performance of P. yezoensis blades changed significantly during desiccation and re-hydration. The activity of PSI-driven cyclic electron flow was elevated significantly during desiccation and continued to operate under desiccated conditions, demonstrating much greater tolerance to desiccation stress than PSI. The PSI in desiccated blades was restored faster than PSII during re-hydration. Most importantly, as PSI was suppressed, the PSI-driven cyclic electron flow of the desiccated P. yezoensis blades at 13% AWC could still be rapidly restored when re-hydrated and was also insensitive to AA. Moreover, the recovery of cyclic electron flow around PSI was influenced by the dark treatment duration, suggesting that stomatal reductants, including NAD(P)H, played an important role in the donation of electrons to PSI and was mainly responsible for the rapid recovery of cyclic electron flow around PSI in the desiccated blades during re-hydration. Overall, this study suggests that cyclic electron flow around PSI in P. yezoensis blades plays a significant physiological role during desiccation and re-hydration. This may be one of the most important factors allowing P. yezoensis blades to adapt to intertidal environments.

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**References**


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