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Stress-Induced Alteration of Chlorophyll Fluorescence Polarization and Spectrum in Leaves of *Alocasia macrorrhiza* L. Schott

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Abstract The value of intrinsic chlorophyll fluorescence polarization, and the intensity in emission spectrum were investigated in leaf segments of *Alocasia macrorrhiza* under several stress conditions including different temperatures (25–50°C), various concentrations of NaCl (0–250 mM), methyl viologen (MV, 0–25 μM), SDS (0–1.0%) and $NaHSO_3$ (0–80 μM). Fluorescence emission spectrum of leaves at wavelength regions of 500–800 nm was monitored by excitation at 436 nm. The value of fluorescence polarization (*P* value), as result of energy transfer and mutual orientation between chlorophyll molecules, was determined by excitation at 436 nm and emission at 685 nm. The results showed that elevated temperature and concentrations of salt (NaCl), photooxidant (MV), surfactant (SDS) and simulated SO_2 ($NaHSO_3$) treatments all induced a reduction of fluorescence polarization to various degrees. However, alteration of the fluorescence spectrum and emission intensity of F_{685} and F_{731} depended on the individual treatment. Increase in temperature and concentration of $NaHSO_3$ enhanced fluorescence intensity mainly at F_{685}, while an increase in MV concentration led to a decrease at both F_{685} and F_{731}. On the contrary, NaCl and SDS did not cause remarkable change in fluorescence spectrum. Among different treatments, the negative correlation between polarization and fluorescence intensity was found with $NaHSO_3$ treatments only. We concluded that *P* value being measured with intrinsic chlorophyll fluorescence as probe in leaves is a susceptible indicator responding to changes in environmental conditions. The alteration of *P* value and fluorescence intensity might not always be shown a functional relation pattern. The possible reasons of differed response to various treatments were discussed.

Keywords Chlorophyll fluorescence polarization · Fluorescence spectrum · Leaves · *Alocasia macrorrhiza* · Stresses

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Introduction

Wild plants are usually subjected to various detrimental growth factors in the nature and frequent activities of human being [1]. Various kinds of environmental stresses, such as low or high temperature, drought, salinity and oxidation, are the main abiotic factors restricting the production of crops and forest plants. Membrane systems on the surface of a cell and its suborganelles are sensors responding to changes of environmental conditions. It has been reported that alteration in chemical and physical properties of membrane resulted in the modification of membrane lipids and membrane proteins, as well as the expression of genes involved in the acclimation of cells to ambient factors [2].

Chloroplast is the major target of many environmental stresses [3]. Photosystem II (PSII) consisted of pigments and proteins in thylakoid membrane of chloroplasts are particularly susceptive to the change of environmental factors. The intrinsic chlorophyll *a* fluorescence measurement has been an active field in photosynthetic research and is being applied widely in plant stress physiology and ecology to provide numerously valuable information of plant response, adaptation and injury to different ambient conditions [5]. In general, the feature of fluorescence

spectrum and emission/quenching intensity of PSII were studied frequently in the concerned stress researches using in vitro experiments. However, to our knowledge, less report so far has been involved in the change in chlorophyll fluorescence polarization under various stress conditions, especially from the in vivo experiment [3, 4]. Fluorescence polarization is a new and powerful technique for characterizing macromolecular association [6], it could produce more information of the interaction between chlorophyll molecules during excitation energy transfer. Therefore, a description of the feature of chlorophyll fluorescence polarization in leaves might be of benefit to elucidate the alteration of energy transfer at pigment molecular level in thylakoid membrane of photosynthetic machinery in vivo when suffered from unfavorable conditions.

The present study covers a range of responses to high temperature, photooxidation, salinity, surfactant and $NaHSO_3$ in leaves of a wild herb plant, *Alocasia macrorrhiza*, using chlorophyll fluorescence polarization and fluorescence spectrum technique.

Materials and methods

Plant material

Alocasia macrorrhiza L. Scott is an understory perennial herb, occurring in subtropical nature rainforest and monsoonal forest. It is able to adapt the sunny condition but is more preferred to deep shade, hot and humid area [7]. The tested plants can be found commonly in the campus of South China Botanical Garden. The young and fully expanded leaves on top of seedlings were harvested and excited as leaf segments before use.

Stress treatment

Short term temperature treatment: Leaf segments were immersed into distilled water in the dark at 25, 30, 35, 40, 45 or 50°C for 30 min, respectively. Temperature was controlled by a microthermostat (HB-8, China).

NaCl treatment: The leaf segments were incubated in 0, 100, 150, 200, 250 or 300 mM of NaCl solutions at 25°C and a PFD of 20 μmol $m^{-2}s^{-1}$ for 30 h. The photon flux density was measured using a Lor quantum meter (USA).

Photooxidation treatment: Methyl viologen (MV, Sigma, USA) treatments with 0, 10, 15, 20 or 25 μM were carried out for 24 h using same temperature and light conditions as in NaCl treatment.

Surfactant treatment: An anionic surfactant, sodium dodecyl sulfate (SDS), at 0, 0.2, 0.4, 0.6, 0.8 or 1.0% was used to incubate the leaf segments for 24 h.

$NaHSO_3$ treatment: Leaf samples were treated by $NaHSO_3$ 20, 40 or 80 mM for 24 and 48 h, respectively. The samples were maintained as described above.

After each treatment, leaf materials were washed with distilled water to remove the residual reactant in leaf surface and kept on the wet paper in Petri dish to prevent the leaf from water loss.

Fluorescence spectrum and polarization determination

Chlorophyll fluorescence measurement was performed with leaf segments directly at room temperature by a fluorescence spectrophotometer (LS 55, Perkins-Elmer Inst. USA). Leaf disc 1 cm in diameter was sealed in a solid sample compartment (LS Series front surface accessory, 1 cm diameter). The emission spectrum in the wavelength (λem) range of 500–800 nm was rescored by an exciting wavelength (λex) at 436 nm. Fluorescence polarization was determined using a polarization attachment. The λex and λem were 435 nm and 685 nm, respectively. Fluorescence polarization value (P value) was given automatically by the instrument. The equation is $P=(I_{vv}-GI_{vh})/(I_{vv}+GI_{vh})$, where I_{vv} is the intensity when both excitation and emission polarizers are in vertical position (excitation and emission); I_{vh} represents the intensity with the polarizors at vertical and horizontal position (excitation and emission); $G=(I_{hv}/I_{hh})$ is the Grating factor (instrumental correction factor), I_{hv} is the intensity with polarizors horizontal and vertical, while I_{hh} is the intensity with the polarizors both at horizontal position.

Statistical analysis

The data in Figs. 1, 2, 3, 4, and 5 were the means±SE from three to four individual measurements of different leaf segments. Statistical analysis was conduced by a t-test. Significance levels quoted are at $P<0.05$ with different letters on the curves of fluorescence polarization.

Results

Heat-induction

Two typical peaks, at 685 nm (F_{685}) and at 731 (F_{731}), were observed in the emission spectra of chlorophyll fluorescence at different temperature (ex=436 nm, Fig. 1a). These two emission peaks have been already observed at room temperature and were assigned to PSII [8]. Our data show that fluorescence intensity of F_{685} changed slightly at temperature around 25–35°C, but became important as temperature elevated up to above 40°C. The levels of F_{685} at 45 and 50°C reached to 198 and 310% of that at 25°C. F_{731} level showed similar trend as F_{685}, but was at less

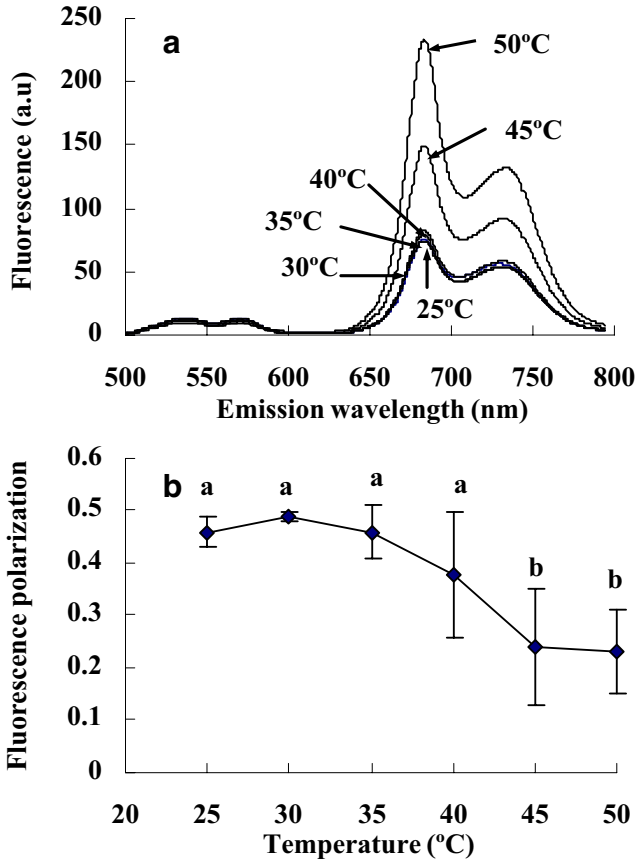

Fig. 1 Changes in chlorophyll fluorescence spectra (**a**) and fluorescence polarization (**b**) in leaves of *Alocasia macrorrhiza* induced by elevated temperature. Values are means of three to four individual leaf sample measurements. Different *small letters* above the data point of *P* value indicate statistically significant differences at $P<0.05$.The same below

increasing extent with temperature elevation. It was 162 and 234% of that at 25°C, respectively. Hence, the F_{685}/F_{731} ratio increased with increasing temperature (data not shown). The enhanced emission of fluorescence suggested that heat causes the reduction of excited light energy transferring into chemical energy. F_{685} is originated from CP43 of PSII [9], thus, the higher F_{685}/F_{731} ratio induced by high temperature might reflect an obvious alteration of the core antenna within PSII. And the enhanced fluorescence emission was inferred by the depression of electron rate in the reducing end of PSII.

The change in pattern of chlorophyll fluorescence polarization value was somewhat different from that of fluorescence intensity (Fig. 1b). *P* value did not show a significant change by temperature of 25–40°C treatment, then diminished rapidly when treated temperature was higher than 40°C. Upon increasing temperature from 25 to 50°C, *P* value reduced to only a half of that of the control (25°C). This obvious decrease thus confirmed the alteration of the energy transfer feature among chlorophyll molecules [10] by severe heat stress.

Salt-induction

Salt stress was known to disturb the normal physiological processes and chloroplast ultrastructure at various levels [11, 12]. The extent of the effect by salt ions depends on their concentration and plant tolerance. There are no reports describing about the change in chlorophyll fluorescence polarization and yield of fluorescence emission in higher plants induced by various concentrations of salt at leaf level [13, 14]. In Fig. 2a, we found that the types of fluorescence spectra and the intensities of F_{685} and F_{731} were apparently not altered by NaCl concentration around 100–250 mM. The polarization value of F_{685} was 0.47±0.04 in control leaves but decreased to 0.28±0.06–0.38±0.08 in NaCl treated leaves (Fig. 2b). The decrement of *P* value was about 20–40%. Among the given NaCl concentrations, *P* value was the lowest in leaves treated by 100 mM of NaCl, then increased to a relatively steady level at NaCl concentration between 150 and 300 mM.

Mohanty et al. [13] reported that salt induced changes of the fluorescence emission in cells of an intact algal,

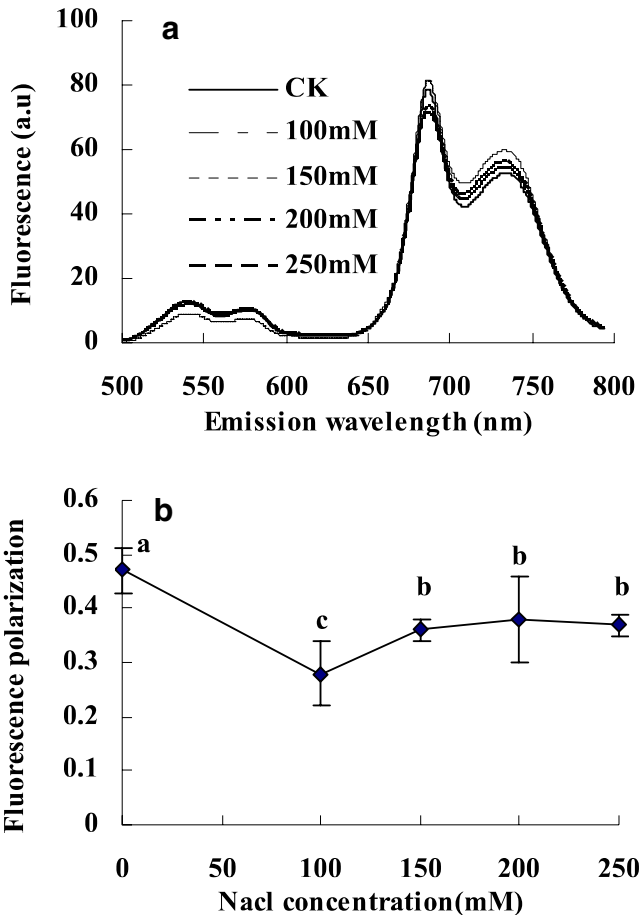

Fig. 2 Changes in chlorophyll fluorescence spectra (**a**) and fluorescence polarization (**b**) in leaves of *Alocasia macrorrhiza* induced by various concentrations of NaCl

Chlorella pyrenoidosa, involved a combination of osmotic change and ionic change. The effect is mainly ionic of low concentration of salt (0.1 M), but is largely osmotic at very high salt concentration (0.4 M). Thus, the differential *P* values in leaves of *Alocasia* caused by different concentrations of NaCl might also be related to the major ionic or osmotic role.

Photooxidation-induction

Methyl viologen (MV) is a redox-active compound of bipyridyl herbicides and is now being used as a potential oxidant forming the toxic superoxide radical [15] in the researches of oxidative stress in plants. The fluorescence spectra of *Alocasia* species changed pronouncedly by the addition of MV in the light (Fig. 3a). F_{685} reduced gradually and F_{731} became completely loss with the increase of MV concentration. The fluorescence intensity of F_{685} at which the leaves were treated with 25 μM MV was significantly lowered to 18.9% of that of the control. The suppression of F_{731} was stronger than that of F_{685} due to the MV action. These low intensities of chlorophyll fluorescence implied the low capacities of energy trapping and transferring caused by internal photooxidative damage of PSII.

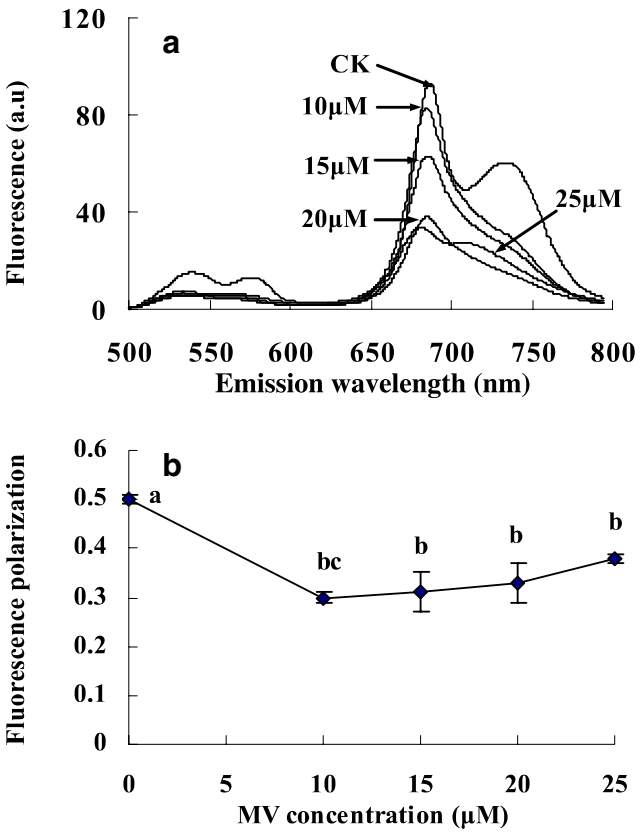

Fig. 3 Changes in chlorophyll fluorescence spectra (**a**) and fluorescence polarization (**b**) in leaves of *Alocasia macrorrhiza* induced by MV-photooxidation

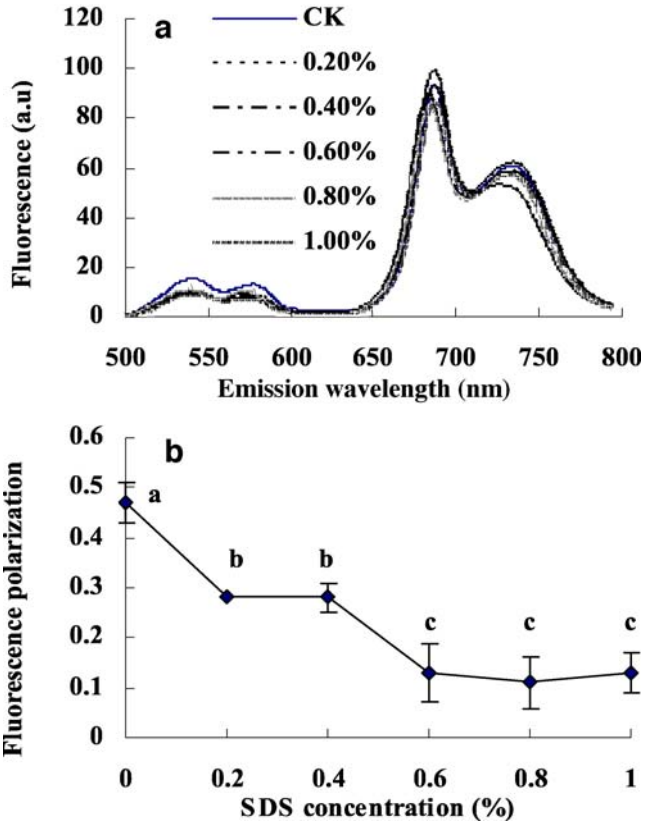

Fig. 4 Changes in chlorophyll fluorescence spectra (**a**) and fluorescence polarization (**b**) in leaves of *Alocasia macrorrhiza* induced by SDS

Fluorescence polarization decreased firstly at low MV concentration (10 μM), then showed a slightly increase as MV concentration increasing. Nevertheless, over a range of MV concentration from 10 to 25 μM, *P* values were still significantly less than that of the control (Fig. 3b). Such changing tendency was similar to that described in Fig. 2b with NaCl treatment.

SDS-induction

SDS has been widely applied in the research of biomembrane. It plays an important role on protein conformation and pigment solubilization of membranes [16, 17]. In this paper, SDS was selected to study its effect on chlorophyll fluorescence emission and polarization of PSII at leaf level.

Chlorophyll fluorescence emissions of F_{685} and F_{731} were slightly reduced by SDS treatment (Fig. 4a), implying that the ability of energy transfer of PSII was less inhibited by 0.2–1.0% of SDS. Data presented in Fig. 4b illustrated the changing pattern of fluorescence polarization values as a function of SDS concentrations. A marked drop in polarization value was seen at SDS concentration of 0.6%, which was 27.6% of the control level, and then it stood on a similar value when SDS concentration was increased

forward to 1.0%. Taking the data from Fig. 4a and b together, it can be seen that the alterations of *P* value and emission spectrum induced by SDS are not generated synchronously. Change in fluorescence intensity might lag behind that in *P* value in this case.

$NaHSO_3$-induction

Bisulfate (HSO_3^{-1}) is the water-soluble intermediate of SO_2 metabolism in plant cells. It is toxic but could be finally transformed into sulfate through the oxidation in the light [18]. An enhancement of F_{685} emission was observed by $NaHSO_3$ treatment. It increased by 21–150% over control level in response to $NaHSO_3$ concentration from 20 to 80 mM. Moreover, F_{731} emission peak became unnoticeable under 40–80 mM of $NaHSO_3$ (Fig. 5a).

Reversely, *P* values exhibited a continuous decline with increasing $NaHSO_3$ concentration and treatment time. A rapid reduction of *P* value was observed at a given concentration of 80 mM. The decrement was 59.3 and 38.8% more compared to control one, respectively, after 1 and 2 days treatment.

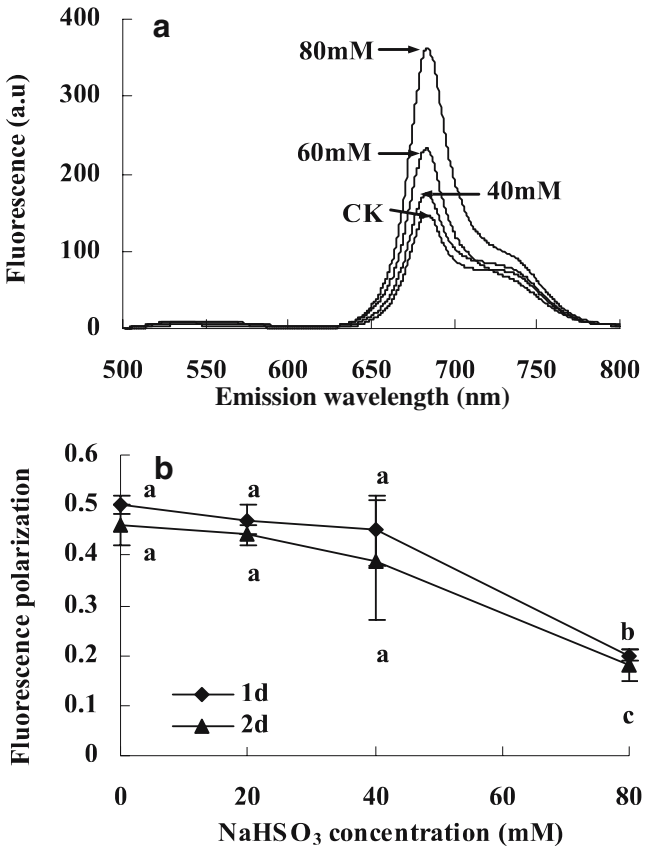

Fig. 5 Changes in chlorophyll fluorescence spectra (**a**) and fluorescence polarization (**b**) in leaves of *Alocasia macrorrhiza* induced by $NaHSO_3$

Discussion

The intrinsic chlorophyll polarization reflects energy transfer between different pigment molecules and their degree of mutual orientation [19]. Change in polarization value of PSII fluorescence depends on the spillover efficiency of the excitation energy from PSII to PSI. Measurement of polarization degree could be used to monitor the changes in energy migration due to alteration in pigment–pigment interaction [10, 14]. In the present study, the obvious alteration of chlorophyll *P* values was found to accompany with different feature of fluorescence spectra in leaves of *Alocasia macrorrhiza* under various stress conditions. Low polarization value of chlorophyll demonstrates an energy transfer between different chlorophyll molecules and their degree of mutual orientation in living plants [20]. The pronounced decrease in *P* value in *Alocasia* leaves upon incubation with high temperature, NaCl, MV, SDS and $NaHSO_3$ suggested that the interrelationship between chlorophyll molecules has been interfered and that the migration of the excitation energy among the antenna chlorophylls of PSII has been increased [10], and hence the spillover of the excitation energy from PSII to PSI might be low. Therefore, the sensitivity of chlorophyll *P* value to the changes of environmental factors is considered as one of the response/adaptation/damage indicator of PSII.

A negatively related affect between *P* value and fluorescence emission intensity was observed by treating the leaves with $NaHSO_3$ (r=−0.8887 and −0.9302 for 2 and 1 days treatment) and high temperature (45–50°C) in our experiments. Interestingly, no similar results were found from NaCl, MV and SDS treatments. Whitmarsh and Levine [21] reported that low chlorophyll polarization accompanied with high fluorescence yield in a unicellular alga, and the *P* value decreased as the fluorescence lifetime increased. Nevertheless, our results elucidated that the relationship between *P* value and fluorescence intensity might not be correlated directly.

Recently, Behera et al. [4] and Deo et al. [3] evidenced that an increase of chlorophyll fluorescence polarization (Ex 600/Em 685) was accompanied with malondialdehyde accumulation in isolated chloroplasts of primary wheat leaves or senescing cotyledons of clusterbean under longer term (5–15 days) of water stress. The different data (*P* value decrease under various stresses) presented here might be due to the discrepant experimental species and conditions such as the short term treatment, in vivo measurement using leaf segment and different excitation wavelength (Ex 436/Em 685) in our study.

Differential chlorophyll fluorescence intensities in *Alocasia* leaves under several conditions were considered to be resulted from distinct extent of modification of PSII responding to the

respective treatment, because the different degrees of binding LHCP to pigment complexes were assessed to induce the variability in chlorophyll fluorescence intensity and P various seedling types of pumpkin cotyledons [22]. An enhancement of F_{685} intensity in *Alocasia* leaves at elevated temperature is in agreement with earlier works using *Arabidopsis* chloroplasts [23] and similar to Fo increase in rice leaves of our previous work [24]. The rise of chlorophyll fluorescence indicated an inhibition of excitation energy transfer from LHCP antenna to PSII reaction center. It was ascribed to the separation of LHCP from the PSII core [23], the reduction in numbers of open PSII centers and in electron transfer efficiency, as well as the accelerated oxidative damage induced by high temperature [24]. Whereas the rising trend of fluorescence emission induced by $NaHSO_3$ might be largely due to the depression of excitation energy transfer between chlorophyll molecules and within PSII by oxidative toxicity of HSO_3^{-1}. MV mediated the generation of superoxide radical which could sequentially derivate other activated oxygen species H_2O_2 and hydroxyl free radical in chloroplasts. It led to the oxidative degradation of chlorophyll and protein, the inactivation of PSII, and the disruption of membrane [15, 25]. The inducted quenching of F_{685} and F_{731} by MV was consistent with the decrement of Fm, qP and Φ_{PSII} associated with an increment of qN in our previous study on some woody species under MV photooxidative condition [26]. Clearly, the effect of MV on the quenching of chlorophyll fluorescence in this case was an expression of oxidative injury in PSII, resulting in photoexcitation energy loss through the free radical reaction.

Salt stress has been corroborated causing less energy migration among PSII units [27], induced the susceptibility of PSII to photoinhibition [28], the state II transition [12], the swelling of thylakoids [11] and cellular oxidative stress [29]. However, as can be seen from Fig. 2a, the feature of fluorescence emission spectra were not altered much by NaCl concentration up to 250 mM, indicating that the fluorophore concentration of PSII was still maintained near the initial state. When combined the data of less decreasing *P* value in Fig. 2b together, it is likely that these responses are mainly involved in a regulation of PSII to NaCl stress, and *Alocasia macrorrhiza* may tolerate the NaCl concentration as high as 250 mM. Whereas the fact that F_{685} and F_{731} intensities remained almost unaltered with an apparently lasting reduction of fluorescence polarization after SDS incubation might be resulted from a mechanism differed from that of NaCl. In addition to being a protein denaturant to break the hydrogen bond and hydrophobic bond in protein, SDS was reported to inhibit the photochemical function of PSII [30, 31], to solubilizate the thylakoid membrane and to release the pigments, especially chlorophyll b, from the pigment–protein complexes [16]. It could also increase leakage of calcium from a liposome membrane [32]. Therefore, we speculate that the molecular structure of some surface proteins and the rotational movement of chlorophyll molecules in *Alocasia* species were probably modified to certain extent by the tested SDS concentrations, but part of the free chlorophyll releasing from solubilizated thylakoid still did not affect the fluorescence emission much.

Membrane system is shown to be involved in various signal transduction pathways. Incorporating an exogenous lipid fluorescence probe, such as DPH (1, 6-diphenyl-1, 3, 5-hexatriene) into membrane lipids and detecting the change in polarization degree is the common technique to monitor membrane fluidity involved chloroplast membrane. It has been elucidated that the decrease in polarization of the exogenous fluorescence probe signified the increase in membrane fluidity by in vitro experiments using barley thylakoid [1], yeast [33, 34] and animal cell [35]. Elevated temperature and NaCl stress had been evidenced to decrease *P* value and increase plasma membrane fluidity in yeast cells [33, 34] or pea thylakoid [36] by exogenous lipophilic fluorescence probe. A remarkable increase in thylakoid membrane fluidity labeling with DPH was also found in spinach thylakoid treated by SDS in our experiment (data not show), which is consistent with the decrease of chlorophyll *P* value in leaves after SDS treatment in the present study. Chlorophylls are fluorescent and are bound to proteins as pigment–protein complexes embedded in the thylakoid membrane. An alteration of chlorophyll fluorescence polarization is probably related with the change in property of membrane. The susceptibility of chlorophyll fluorescence polarization in response to several stress factors in the present in vivo experiment led us to question whether presence of any relationship between chlorophyll fluorescence polarization and membrane fluidity, and the possibility of exploring the thylakoid membrane fluidity indirectly but simply with chlorophyll fluorescence polarization measurement. To solve this problem, more detailed works are expected to be conducted in the future.

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References

1. Maiseyenkava YA, Pshybytko NL, Kabashnikova LF (2005) Greening barley seedlings under high temperature. Gen Appl Plant Physiol 31:3–14
2. Vigh L, Maresca B, Harwood JL (1998) Does the membrane's physical state control the expression of heat shock and other genes? Trends Biochem Sci 23:369–374
3. Deo PM, Biswal UC, Biswal B (2006) Water stress-sensitized photoinhibition in senescing cotyledons of clusterbean: Changes

in thylakoid structures and inactivation of photosystem 2. Photosynthetica 44:187–192
4. Behera RB, Mishra PC, Choudhury NP (2002) High irradiance and water stress induce alterations in pigment composition and chloroplast activities of primary wheat leaves. J Plant Physiol 159:967–973
5. Adams WW III, Demming-Adams B (2004) Chlorophyll fluorescence as a tool to monitor plant response to the environment. In: Papageorgiou GC, Govindjee S (eds) Chlorophyll a fluorescence: a signature of photosynthesis. Advances in photosynthesis and respiration, vol 19. Springer, Dordrecht, pp 583–604
6. Checovich WJ, Bolger RE, Burke T (1995) Fluorescence polarization—a new tool for cell and molecular biology. Nature 375:254–256
7. Chow WS, Qian L, Goodchild DJ, Anderson JM (1988) Photosynthetic acclimation of *Alocasia macrorrhiza* (L.) G. Don. Aust J Plant Physiol 15:107–122
8. Andrizhiyevskaya EG, Chojnicka A, Bautista JA, Diner BA, van Grondelleand R, Dekker JP (2005) Origin of the F_{685} and F_{695} fluorescence in photosystem II. Photosynth Res 84:173–180
9. Govindjee S (1995) Sixty-years since Kautsky: chlorophyll *a* fluorescence. Aust J Plant Physiol 22:131–160
10. Yamamoto Y, Barber J (1981) Measurement of degree of chlorophyll fluorescence polarization in relation to the regulation of excitation energy transfer between photosystem 1 and II in pea chloroplasts. Biochim Biophys Acta 637:224–230
11. Yamane K, Kawasaki M, Taniguchi M, Miyake H (2003) Differential effect of NaCl and polyethylene glycol on the ultrastructure of chloroplasts in rice seedlings. J Plant Physiol 160:573–575
12. Liu XD, Shen YG (2006) Salt shock induces state II transition of the photosynthetic apparatus in dark-adapted *Dunaliella salina* cells. Environ Exp Bot 57:19–24
13. Mohanty P, Govindjee S, Wydrzynski T (1974) Salt-induced alteration of the fluorescence yield and of emission spectra in *Chlorella pyrenoidosa*. Plant Cell Physiol 15:213–224
14. Paliwal R, Singhal GS (1987) Fluorescence polarization of trypsin digested photosystem II membranes. Photosynth Res 12:83–90
15. Chia LS, McRae DG, Thompson JE (1982) Light-dependence of paraquat-initiated membrane deterioration in been plants. Evidence for the involvement of superoxide. Physiol Plant 56:492–499
16. Yang CM, Hsu JC, Lu YK, Yin MH (1996) Pigment solubilization of the chloroplast thylakoid membranes by a surfactant. Botanical Bulletin of Academia Sinica 37:121–126
17. Moosovi-Movahedi AA (2005) Thermodynamics of protein denaturation by sodium dodecyl sulfate. Journal of the Iranian Chemical Society 2:189–196
18. Rennenberg H (1984) The fate of excess sulfur in higher plants. Annu Rev Plant Physiol 35:121–153
19. Becker JF, Breton J, Geacintov NE, Trentacosti F (1976) Anisotropy of photosynthetic membranes and the degree of fluorescence polarization. Biochim Biophys Acta 440:531–544
20. Arnold W, Meek ES (1956) The polarization of fluorescence and energy transfer in grana. Arch Biochem Biophys 60:82–90
21. Whitmarsh J, Levine RP (1974) Excitation energy transfer and chlorophyll orientation in the green alga *Chlamydomonas reinhardi*. Biochim Biophys Acta 368:199–213
22. Behera LM, Choudhury NK (1997) Changes in the chlorophyll fluorescence characteristics of chloroplasts from intact pumpkin cotyledons, caused by organ excision and kinetin treatment. Photosynthetica 34:161–168
23. Kunst L, Browse J, Somerville C (1989) Altered chloroplast structure and function in a mutant of Arabidopsis deficient in plastid glycerol-3-phosphate acyltransferase activity. Plant Physiol 90:846–853
24. Lin ZF, Peng CL, Lin GZ, Zhang JL (2005) Thermostability of photosynthesis in two new chlorophyll b-less rice mutants. Sci China Ser C Life Sci 48:139–147
25. Peng CL, Lin ZF, Lin GZ (2000) Superoxide production rate and photosynthetic feature in leaves of some plant species under photooxidation. Acta Phytophysiol Sin 26:81–87
26. Lin ZF, Peng CL, Lin GZ (1999) Membrane injury and PSII inactivation in some subtropical woody plants induced by photooxidation. Acta Bot Sin 41:871–876
27. Wong D, Govindjee S (1979) Antagonistic effects of mono- and divalent cations on polarization of chlorophyll fluorescence in thylakoids and changes in excitation energy transfer. FEBS Lett 97:373–377
28. Lu C, Qiu N, Lu Q, Wang B, Kuang T (2002) Does salt stress lead to increased susceptibility of photosystem II to photoinhibition in halophyte *Suaeda salsa* grown outdoors? Plant Sci 163:1063–1068
29. Gueta-Dahan Y, Yaniv Z, Zilinskas BA, Ben-Hayyim G (1997) Salt and oxidative stress: similar and specific responses and their relation to salt tolerance in citrus. Planta 203:460–469
30. Lin ZF, Li XP, Lin GZ, Peng GL (1997) Effects of protein modifiers, denaturants and active oxygen on photosystem II photoinactivation of spinach leaves. Journal of Tropical and Subtropical Botany 5:59–64
31. Apostolova EL, Ivanov AG (1996) Influence of Triton X-100 on the structure and functions of pea thylakoid membranes. J Plant Physiol 145:239–469
32. Tanaka K, Takeda T, Nakamura M, Yamamura S, Miyajima K (1989) Interactions of mixed surfactant solution with liposome membrane. Colloid Polym Sci 267:520–524
33. Khaware RK, Koul A, Prasad R (1995) High membrane fluidity is related to NaCl stress in *Candida membranefaciens*. Biochem Mol Biol Int 35(4):875–880
34. Swan TM, Watson K (1997) Membrane fatty acid composition and membrane fluidity as parameters of stress tolerance in yeast. Can J Microbiol 43:70–77
35. Hachisuka H, Nomura H, Mori O, Nakano S, Ohubo K, Kusuhara M, Karashima M, Tanikawa E, Higuchi M, Sasai Y (1990) Alterations in membrane fluidity during keratinocyte differentiation measured by fluorescence polarization. Cell Tissue Res 260:207–210
36. Barber J, Ford RC, Mitchell RAC, Millner PA (1984) Chloroplast thylakoid membrane fluidity and its sensitivity to temperature. Planta 161:375–380