Applications of Chlorophyll Fluorescence to Measurement of Plant Vitality

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Abstract

The chlorophyll fluorescence combined with the O-J-I-P transients was examined in the leaves of the crinum plants (*Crinum asiaticum* var. *japonicum* BAK.), in order to satisfy the demand for rapid *in vivo* measurement of vitality and to apply easily to approach questions of economical interest concerning the plant vitality. The photosynthetic efficiency, Fv/Fm, of crinum plants was higher in summer than in winter. In summer, the Fv/Fm values were lower in day time than at dawn and night, suggesting that photosynthetic efficiency is chronically photoinhibited in day time. In winter, there was no prominent diurnal changes of Fv/Fm values. However, based on the O-J-I-P transients, the values of PI_{NO} and SFI_{NO} dramatically increased at noon in summer, and the $\Psi_o/(1-\Psi_o)$ value diurnally fluctuated in winter. These results indicated that indexes such as PI_{NO}, SFI_{NO} and $\Psi_o/(1-\Psi_o)$ can be used as the indicators for *in vivo* measurement of environmental stresses.

Key words : *Crinum asiaticum* var. *japonicum*, plant vitality, chlorophyll fluorescence, O-J-I-P transients

Introduction

Photosynthesis is the process by which plants convert radiant energy into a chemically stable form. The pathway of this energy transduction is complex, involving several physical and chemical mechanisms and many components. The process is initiated when light is absorbed by the antenna molecules within the photosynthetic membrane. The absorbed energy is transferred as excitation energy and is either trapped at a reaction center and used to do chemically useful work, or dissipated mainly as heat and less as emitted radiation - fluorescence (Harbinson and Rosenqvist, 2003). The feature of the emitted fluorescence are basically determined by the absorbing pigments, the excitation energy transfer and the nature and orientation of the fluorescing pigments. However, fluorescence is also affected by the redox state of the reaction centers and of the donors and acceptors of photosystem II (PSII), and is moreover sensitive to a wide variety of photosynthetic events, e.g., intactness of PSII, membrane degradation, electron transport effciency, and so on (Rosenqvist and Kooten, 2003). Although the effect of each factor on fluorescence is often indirect and not easily quantified and distinguished from one another, fluorescence measurements have been successfully used to monitor and characterize a wide variety of photosynthetic events (Lichtenthaler and Rinderle, 1988).

The present paper aims to give informations about the vitality of the plant materials. Furthermore, the O-J-I-P transients are analyzed providing a description of dynamic capacities of the photosynthetic samples. This

procedure satisfies the demand for rapid *in vivo* measurement of vitality, and can be thus easily applied to approach questions of economical or commercial interest concerning the vitality of plants.

Materials and Methods

Plant materials and field sites

The leaves of crinum plants (*Crinum asiaticum* var. *japonicum* BAK.), grown in the beach near their natural habitat, were used in this research from August 2001 to February 2002. The leaves fully developed on the sun-exposed layer were chosen for this research. Air temperature, relative humidity and light intensity of the stand were recorded diurnally during investigation days.

Fluorescence measurements

Chlorophyll fluorescence was measured with a fluorometer (PEA, Hansatech Ltd., King's Lynn, Norfolk, England) with 650 nm of 3,000 μ moles/m^2/s light intensity. After 15 min of the dark adaptation, a light pulse of 1 s with an intensity of 1,500 μmoles/m^2/s was provided by the PEA light source.

The values of 1-qN and 1-qP were obtained from direct fluorescence measurements using two strong light pulses one before and one after 10 s of light adaptation with an intensity of 150 μmoles/m^2/s. A light pulse of 1 s with an intensity of 1,500 μmoles/m^2/s was applied which provoked a fast fluorescence rise (Strasser *et al.*, 2000; Oh *et al.*, 2001).

O-J-I-P transients

The fluorescence transients were induced by a red light (peak at 650 nm) of 3,000 μ moles/m^2/s from the PEA light source, recorded by a PEA fluorometer, and digitized on line with 12 bit resolution from 10 μs to 1 s. The four typical steps called O, J, I and P were shown on a logarithmic time scale (Haldimann and Strasser, 1999).

Results and Discussion

Seasonal fluorescence changes

The fluorescence data of the leaves of crinum plants were measured at dawn (06:00) from summer to winter and discussed on the effects of temperature decrease on the photosynthetic efficiency (Fig. 1).

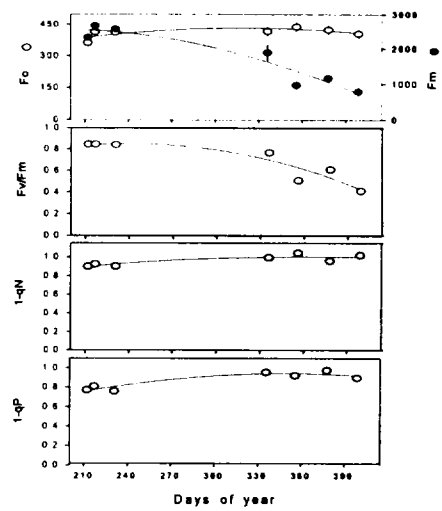

Fig. 1. Seasonal changes of chlorophyll fluorescence parameters (Fo, Fm, Fv/Fm, 1-qN and 1-qP) from leaves of *Crinum asiaticum* var. *japonicum*. Chlorophyll fluorescence was measured at dawn (06:00), and the values represent the averages±SE of 20 indipendent measurements.

The photosynthetic efficiency, Fv/Fm, of crinum plants was higher in summer than in winter. It dramatically decreased in winter depending on temperature drop. This decrease of Fv/Fm in winter was accompanied with the

decrease of Fm and slight increase of Fo. This means that Fv/Fm decrease in winter is a phenomenon similar to the chronical photoinhibition (Long *et al.*, 1994; Oh and Koh, 2004a), which was shown in many plants under high temperature and high light of day time in summer. The values of 1-qP and 1-qN, which are respectively related to photochemical quenching (qP) and non-photochemical quenching (qN), were also calculated from direct fluorescence signals with two stong light pulse. The values of 1-qN were high both in summer and winter; 0.90~0.92 in summer and 0.96~1.0 in winter, suggesting that there was little fluorescence dissipation both in summer and winter (Oh and Koh, 2004a). The values of 1-qP maintained in the range of 0.76~0.80 in summer, contrasted to higher range of 0.90~0.97 in winter. This result indicates that the reaction centers of PS II were more reduced in winter than in summer (Sonoike, 1999).

Diurnal fluorescence changes

The diurnal changes of weather factors and fluorescence were measured from dawn to night (Fig. 2, 3). In summer (Fig. 2), the Fv/Fm values were lower in day time than at dawn and night. The decrease of Fv/Fm in day time was accompanied with the slight decrease of Fm and increase of Fo. This means that crinum plants are chronically photoinhibited in day time in summer (Long *et al.*, 1994; Oh and Koh, 2004b). The values of 1-qP and 1-qN were also lower in day time, suggesting that fluorescence dissipates in day time in summer. In winter (Fig. 3), although Fv/Fm decreased prominently depending on temperature drop, there was no prominent diurnal changes. However, the values of 1-qP were lower in day time than at dawn and night, accompanied with slight decrease of 1-qN. This indicates that there was little dissipation of fluorescence in winter than in summer (Oh and Koh, 2004b).

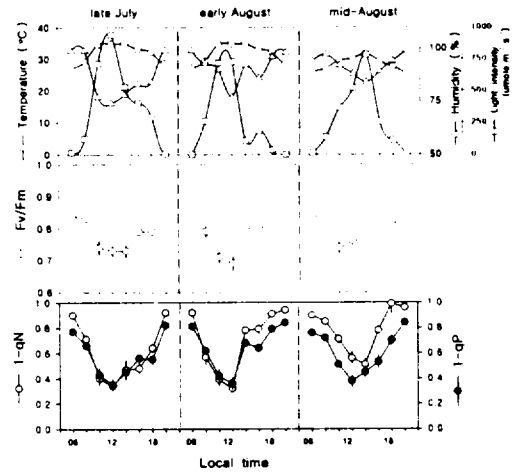

Fig. 2. Diurnal change of environmental factors and chlorophyll fluorescence parameters (Fv/Fm, 1-qN and 1-qP) from leaves of *Crinum asiaticum* var. *japonicum* on the natural habitat in summer season. The values represent the averages± SE of 20 independent measurements.

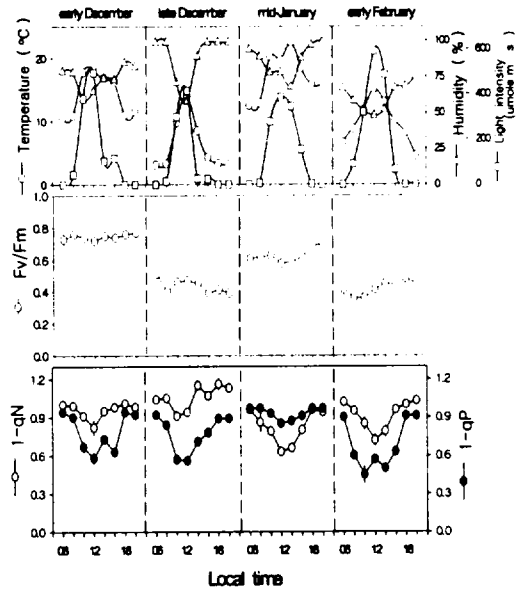

Fig. 3. Diurnal change of environmental factors and chlorophyll fluorescence parameters (Fv/Fm, 1-qN, 1-qP) from leaves of *Crinum asiaticum* var. *japonicum* on the natural habitat in winter season. The values represent the averages±SE of 20 independent measurements.

O-J-I-P fluorescence transients and vitality indexes

The O-J-I-P fluorescence transients and their vitality indexes were applied to follow the responses of the photosynthetic apparatuses in crinum leaves upon the diurnal changes of environmental factors (Fig. 4).

In summer, the crinum leaves showed a typical O-J-I-P fluorescence transients, although the variable Chl a fluorescence decreased when exposed to mid-day environmental conditions. The relative values of vitality indexes, derived from the O-J-I-P fluorescence transients, were plotted using the spider-plot presentations. Of these indexes, PI_{NO} and SFI_{NO} dramatically increased at noon in summer. In early and late winter, the O-J-I-P fluorescence transients were similar each other with slightly low fluorescence in J, I and P steps at noon. However, the transient curve of late winter was very flattened, suggesting that crinum plants were influnced by low temperture during winter (Oh and Koh,2002a). However, their spider-plots showed similar patterns in all vitality indexes. Particularly, the values of $\Psi o/(1-\Psi o)$ diurnally fluctuated both in early and late winter; the $\Psi o/(1-\Psi o)$ values increased at noon. These results indicate that several indexes such as PI_{NO}, SFI_{NO} and $\Psi o/(1-\Psi o)$ can be used as the indicators for *in vivo* measurement of environmental stresses. Furthermore, it suggests that basic fluorescence understanding combined with the O-J-I-P transients is useful to analyze vitality of any plant material, even in any situation.

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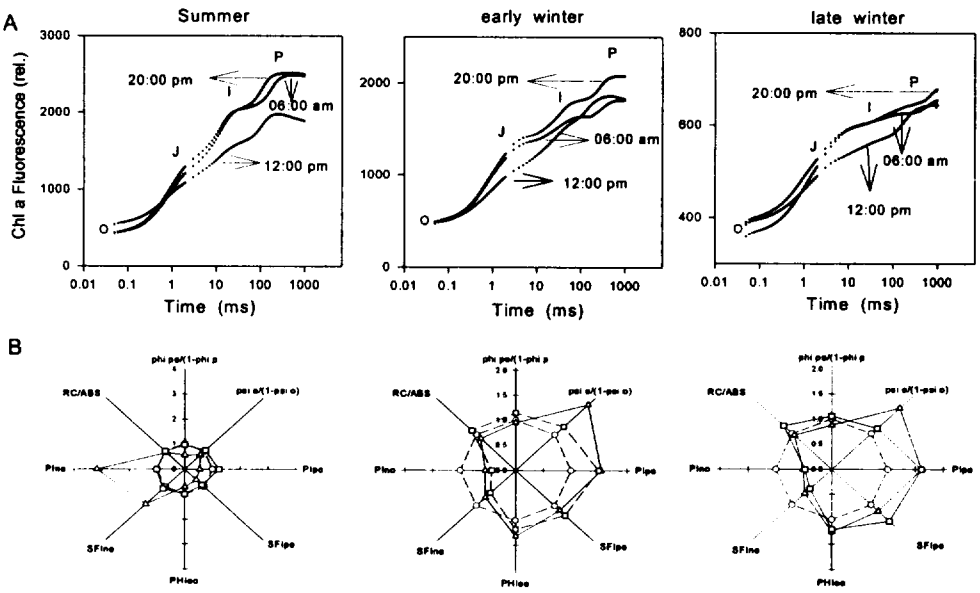

Fig. 4. Fluorescence transients O-J-I-P (A) and indexes (B) quantifying the vitality of PS II from leaves of Crinum plants in summer, early and late winter.

References

Haldimann, P. and R. J. Strasser. 1999. Effects of anaerobiosis as probed by the polyphasic chlorophyll a fluorescence rise kinetics in pea (*Pisum sativum* L.). Photosynthesis Research 62:67-83.

Harbinson, J. and E. Rosenqvist, 2003. An introduction to chlorophyll fluorescence. In: Practical applications of chlorophyll fluorescence in plant biology (DeEll, J. R. and P. M. A. Toivonen, eds.), Kluwer Academic Publishers, Boston, pp. 1-29.

Lichtenthaler, H.K. and U. Rinderle. 1988. The role of chlorophyll fluorescence in the detection of stress conditions in plants. Crit. Rev. Anal. Chem. 19:29-85.

Long, S.P., S. Humpries and P.G. Falkowski. 1994. Photoinhibition of photosynthesis in nature. Annu. Rev. Plnat Physiol. Plant Mol. Biol. 45:633-662.

Oh, S.J., J.G. Koh, E.S. Kim, M.Y. Oh and S.C. Koh. 2001. Diurnal and seasonal variation of chlorophyll fluorescence from Korean fir plants on Mt. Halla. Korean J. Environ. Biol. 19(1):43-48.

Oh, S.J. and S.C. Koh. 2004a. Chlorophyll fluorescence and antioxidative enzyme activity of crinum leaves exposed to natural environmental stress in winter. Korean J. Environ. Biol. 22(1):233-241.

Oh, S.J. and S.C. Koh. 2004b. Photochemical efficiency and psbA gene expression of crinum leaves under natural environmental stress in winter. J. Environ. Sci. 13(4):359-365.

Rosenqvist E. and O. van Kooten, 2003. Chlorophyll fluorescence: a general description and nomenclature. In: Practical applications of chlorophyll fluorescence in plant biology (DeEll, J. R. and P. M. A. Toivonen, eds.), Kluwer Academic Publishers, Boston, pp. 31-77.

Sonoike, K. 1999. The different roles of chilling temperatures in the photoinhibition of photosystem I and photosystem II. J. Photochem. Photobiol. B: Biol. 48:136-141.

Strasser R.J., A. Srivastava, and M. Tsimilli-Michael. 2000. The fluorescence transient as a tool to characterize and screen photosynthetic samples. In: Probing photosynthesis: mechanism, regulation and adaptation (M. Yunus, U. Pathre and P. Mohanty, eds.), Taylor and Francis, London and New York, pp. 445-483.