In Vivo Measurement of Plant Vitality by the Fluorescence Transient

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Abstract

The chlorophyll fluorescence combined with the O-J-I-P transients were 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 dramatically decreased depending on temperature drop in winter. In summer, the Fv/Fm values was 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 fluctuations of Fv/Fm values. However, based on the O-J-I-P transient, PI_{No} and SFI_{No} dramatically increased at noon in summer, and $\psi o/(1-\psi o)$ diurnally fluctuated in winter. These results indicated that vitality 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.

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. 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 PSII, and is moreover sensitive to a wide variety of photosynthetic events, e.g., proton translocation, thylakoid stacking and unstacking, ionic strength, and the mid point potential of cyt b-559, to name a few. 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.

The first significant realization of the relationship between primary reactions of photosynthesis and Chl a fluorescence came from Kautsky and Hirsh (1931). Since this first realization, our knowledge on the Chl a fluorescence combined with O-J-I-P transients has increased tremendously due to its significance for basic biophysical research as well as applied research:

1) Testing of productivity in agriculture as a function of
   - Style of culture (sustainable agriculture etc.)
   - Regulator (herbicides, pesticides, hormones etc.)
   - Selection of cultivar (transgenic etc.)
   - Drought, heat, cold, light, salt stress etc.
2) Testing of the behavior of a commercialized product.
   - Freshness, taste, color and consistency of vegetables, flowers and fruits as a function of storage and home conditions; moreover, the decision for the optimal moment for such products to be put on the shelves in the supermarket can be made by utilizing the fluorescence techniques.
3) Testing greenhouse conditions concerning light, temperature etc.; economic optimization.
4) Testing the formation and ripening of fruits from the flower to the commercial product by means of residual chlorophyll fluorescence.
5) Testing environmental conditions influenced by pollution.
6) Testing the behavior of ecosystems upon global changes (e.g. of CO_2, O_3, temperature, volatile organic compounds, UV).

During these decades the investigations became more and more thorough utilizing the

advancement in the instrumentaion, and the number of publications on this topic has rapidly increased.

The present paper aims to give informations about the vitality of the plant material. Furthermore, the fluorescence transient is analyzed providing a description of dynamic capacities of the photosynthetic sample. 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 fully developed leaves from 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 were measured with a fluorometer (Plant Efficiency Analyzer (PEA), Hansatech Ltd., King' s Lynn, Norfolk, England) with 650 nm of 3,000μmoles/m^2/s light intensity. After 15 min of the dark adaptation, a light pulse of 1 s with an intensity of 1,500μ moles/m^2/s 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 150μmoles/m^2/s. A light pulse of 1 s with an intensity of 1,500μmoles/m2/s was applied which provoked a fast fluorescence rise (Strasser *et al.*, 2000; Oh *et al.*, 2001).

O-J-I-P Transient

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

RESULTS AND DISCUSSION

Analysis of Seasonal Fluorescence Fluctuation

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).

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, 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

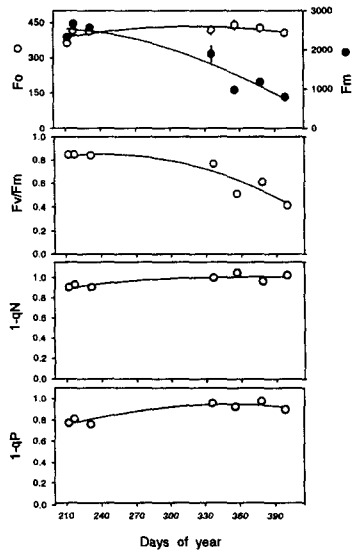

Figure 1. Seasonal variations of chlorophyll fluorescence parameters (Fo, Fm, Fv/Fm, 1-qN and 1-qP) from leaves of *Crinum asiaticum* var. *japonicum*. Chlorophyll fluorescence was measured at dawn (06:00), and the values represent the averages±SE of 20 indipendent measurements.

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. 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.

Analysis of Diurnal Fluorescence Fluctuations

The diurnal fluctuations of weathering factors and fluorescence were measured from dawn to night (Fig. 2,3). In summer (Fig. 2), the Fv/Fm values was 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. The values of 1-qP and 1-qN were also lower in day time, suggesting that fluorescence dissipates both in day time in summer. In winter (Fig. 3), although Fv/Fm decreased prominently depending on temperature drop during winter, there was no prominent diurnal fluctuations. 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.

The 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

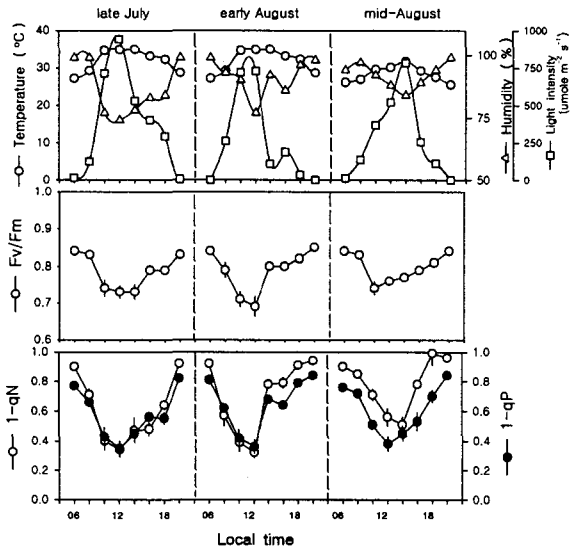

Figure 2. Diurnal variation of environmental factors and chlorophyll fluorescence parameters (Fv/Fm, 1-qN and 1-qP) from leaves of *Crinum asiaticum* var. *japonicum* on the natural habitat in summer season. The values represent the averages ± SE of 20 independent measurements.

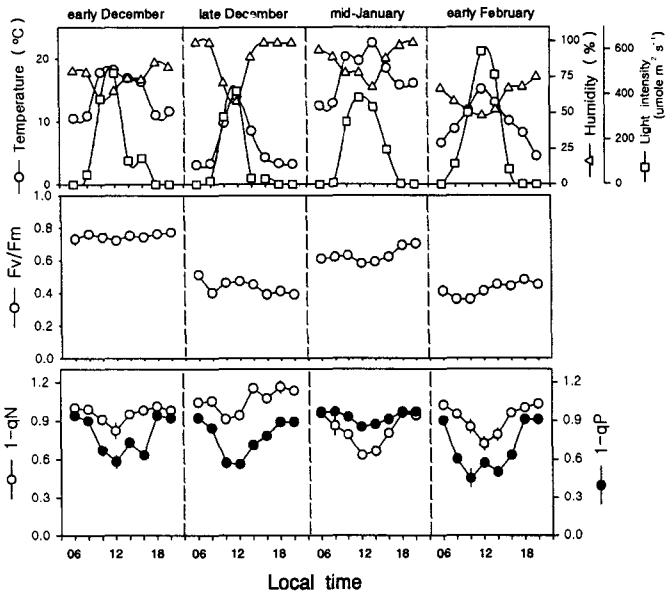

Figure 3. Diurnal variation of environmental factors and chlorophyll fluorescence parameters (Fv/Fm, 1-qN, 1-qP) from leaves of *Crinum asiaticum* var. *japonicum* on the natural habitat in winter season. The values represent the averages±SE of 20 independent measurements.

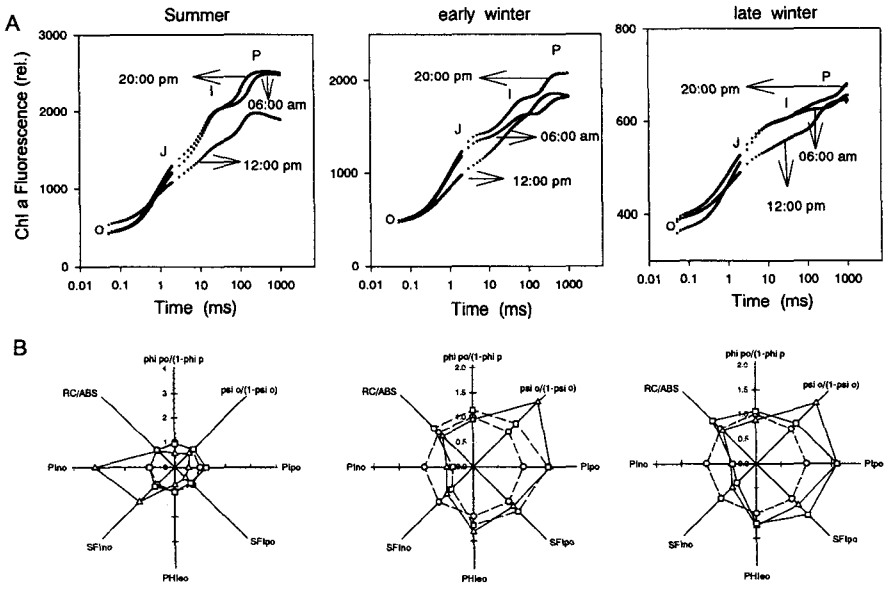

Figure 4. Fluorescence transients O-J-I-P and spider plots of selected parameters quantifying the behaviour of PS Ⅱ ± from leaves of Crinum plants in summer, early and late winter. (A) fluorescence transients O-J-I-P, (B) Vitality indexes.

response of the photosynthetic apparatus in crinum leaves upon the diurnal changes of rnvironmental 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 expressions, derived from the O-J-I-P fluorescence transients, were plotted using the spider-plot presentations. Of the vitality 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 very 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 influenced by low temperature during winter. 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 PINO, SFINO 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 are a useful tool to analyze vitality of any plant material, even in any situation.

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