Rapid and non-invasive detection of plants senescence using a delayed fluorescence technique

Lingrui Zhang, Da Xing,* Junsheng Wang and Lingling Li

Received 8th December 2006, Accepted 15th February 2007
First published as an Advance Article on the web 6th March 2007
DOI: 10.1039/b617893f

Senescence is a phase of leaf ontogeny marked by declining photosynthetic activity that is paralleled by a decline in chloroplast function. The photosystem II in a plant is considered to be the primary site where delayed fluorescence (DF) is produced. We report here a simple, rapid, and non-invasive technique for detecting plants senescence based on quantitative measurements of DF. In the experimental study, various senescence symptoms induced by age or hormones were examined in the *Catharanthus roseus* L. G. Don plants. Detecting the DF emissions from leaves with a home-made DF biosensor enables DF parameters of *C. roseus* to be produced in a short time. Meanwhile, evaluations of leaves senescence were made from measurements of chlorophyll content, ion leakage, and net photosynthesis rate (Pn) based on the consumption of CO_2 in the tested plants. The results of our investigation demonstrate that the changes in DF intensity of green plants can truly reflect the changes in photosynthetic capacity and chlorophyll content during age-dependent and hormone-modulated senescence. Moreover, the DF intensity negatively correlates with ion leakage in both types of senescence. With proper calibration, DF may provide an important approach for monitoring senescence process *in vivo* and quantitatively evaluating senescence extent. Therefore, a DF technique could be potentially useful for less time-consuming and automated screening of the interesting mutants with genetic modifications that change the plant senescence progress.

Introduction

Senescence is considered to be the final stage in leaf development.[1] During senescence, leaf cells experience dramatic changes in metabolism. The most striking phenotypic change is the yellowing of the leaf caused by the preferential breakdown of chlorophyll and chloroplasts.[2] Because the leaf is the major assimilating organ of plants, the loss of the photosynthetic pigment and the breakdown of the photosynthetic apparatus, attenuating anabolic events such as photosynthesis and protein synthesis,[3] will inhibit plant growth and development, thus limiting yield in crops and reducing the value of ornamental plants.[4] Therefore, detecting plant senescence plays an important role not only in identifying interesting mutants with genetic modifications that change the senescence process, but also in manipulating senescence in agricultural applications.

The initiation and progression of leaf senescence can be regulated by an array of endogenous and external factors.[3,5] It has been established that plant hormones are involved in the regulation of leaf senescence.[6] For example, methyl jasmonate (MeJA) and abscisic acid (ABA) promote leaf senescence, while 6-benzyladenine (6-BA) and other cytokinins delay senescence in monocot plants like oat, rice and wheat.[7] Indeed, senescence is a phase of leaf ontogeny marked by declining photosynthetic and other anabolic capacities.[3,5,8] Thus, traditional evaluation of leaf senescence mainly depends on measuring the photosynthetic capacity and chloroplast function. Some instruments, which can be used to evaluate senescence, especially those based on a gas exchange technique, have been rapidly developed. Most commercial instruments are prone to interference from environmental factors and this limits their applications.[9] At present, a chlorophyll fluorescence kinetic technique is widely used for monitoring the changes in the photochemical efficiency of photosystem (PS) II, and although it is a non-invasive probe for studies of photosynthetic metabolism, some difficulties have been encountered.[10]

Delayed fluorescence (DF) is the phenomenon of photon emission by a living system after its stimulation by visible radiation.[11,12] The mechanism of DF generation has been described in greater detail elsewhere.[9,11,12] DF has many practical applications.[12] It can be used as a sensitive indicator of many stress factors.[9,13–15] Additionally, the energy conversion in photosynthesis can be evaluated by quantifying DF based on the correlation between DF intensity and chlorophyll content (within a limited range).[16] Even the changes in the rate of transport through the phosphate translocator can be clearly manifested in induction kinetics of DF.[17,18] In previous reports we have shown that there is a linear correlation between the DF intensity and net photosynthesis rate (Pn) in detached leaves of spinach (*Spinacia oleracea*) in a bench study.[12] More recently, a lot of contrastive experiments further demonstrated that DF intensity also correlates with Pn in attached leaves of many plant species of soybean (*Glycine max* (L.) Merr.), rice (*Oryza sativa* L.), and maize (*Zea May* L.) even under field conditions.[19] Accordingly, the analysis of DF behaviour can be useful in ascertaining the state of the physiological metabolism.[12]

In this paper, a new biosensor for detecting plant senescence symptoms based on the quantitative measurement of DF has been developed. According to maximum and stable intensities of DF, excitation parameters of DF could be optimized experimentally. Compared with common methods for evaluating plants senescence

MOE Key Laboratory of Laser Life Science & Institute of Laser Life Science, South China Normal University, Guangzhou, 510631, China. E-mail: xingda@scnu.edu.cn; Fax: 86-20-8521-6052; Tel: 86-20-8521-0089; Web: http://laser.scnu.edu.cn/xingda.htm

based on gas exchange techniques and biochemical assays, the developed biosensor is an all-weather and easy-to-use measuring instrument. It utilizes intrinsic DF as the measurement marker and can monitor *in vivo* the changes in plant growth and metabolism with less influence of the environment.[15,19] The current investigation has demonstrated that the DF measured using the biosensor correlates well with typical senescence symptoms such as chlorophyll content, ion leakage, and Pn from commercially available photosynthesis systems during the senescence of *Catharanthus roseus* L. G. Don.

Materials and methods

Plant material and hormone treatments

The plant *C. roseus* was bought from an ornamental plant market. Natural leaves of different ages and nearly same leaf samples at days after leaf emergence (DAE) were obtained by cutting leaves at the approximate middle of the petioles with a sharp scalpel to minimize wounding effects. For dark and hormone treatments, detached leaves at 40 DAE floated on 8 mM Mes buffer (pH 5.8) solution were placed in the dark as control. Different concentrations of 6-benzyladenine (6-BA; Sigma) or methyl jasmonate (MeJA; Sigma) were added in the buffer to incubate leaves for 4 d. 0.4 mM 6-BA or 0.4 mM MeJa (final concentration) was used to incubate leaves for different times. All the experiments were carried out at 22 °C and repeated at least 5 times.

Measurement of photosynthesis rate (Pn)

Pn was measured directly using a commercially available system (LI-6400; LI-COR, Inc., USA) equipped with the standard leaf chamber (2 × 3 cm) and the artificial illumination (irradiated from a modulated tungsten lamp). Pn of leaves at 40 DAE treated with hormones was determined at a leaf chamber CO_2 concentration of 400 ppm after the leaves in the leaf cuvette were irradiated for about 15 min by a saturated irradiation of 1000 μmol photon m^{-2} s^{-1}. The relative humidity (RH) and temperature of the leaf cuvette were about 85% and 22 °C, respectively.

In vivo DF biosensor system and *in vivo* DF measurements

DF emission in the time window from 0.26 to 5.26 s after irradiation was recorded with custom-built DF biosensor system. The diagram of the system is shown in Fig. 1. The technical details of the system have been described elsewhere.[15,19] Here only a brief summary of the essential parts will be presented.

Samples were irradiated by a set of light-emitting diode (LED) (λ = 628 nm, half wave width = 20 nm, single duct output luminous flux = 20 lm). All LEDs were uniformly arrayed along a circumference for homogeneous perpendicular superficial irradiation of the leaves. The irradiance intensity was adjusted by changing the current and controlled within the range between 0 and 3000 μmol photon m^{-2} s^{-1}. DF was monitored at an angle of 0° with respect to the incident LEDs light. After Pn measurement, each sample was immediately placed inside the sample chamber of the system to dark-adapt for 5 min before the irradiation source was turned on. Custom-built humidity, temperature, and CO_2 controllers controlled, respectively, the RH, temperature and

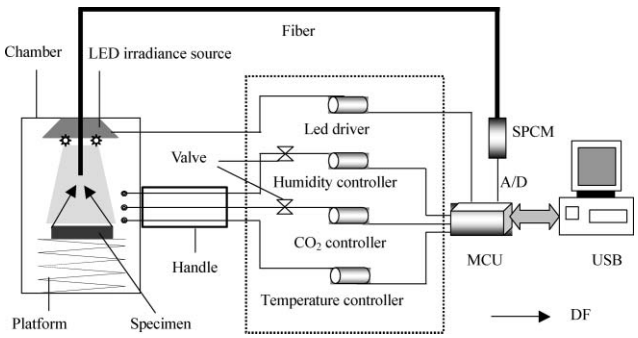

Fig. 1 A diagram of the experimental setup for delayed fluorescence measurements: LED, SPCM and MCU represent the light-emitting diode, single photon counting module, and micro control unit (AT89c55), respectively.

CO_2 concentration of the sample chamber. Immediately after the illumination period, the DF from the sample was collected by an optical fiber bundle and transmitted to an ultra-high-sensitive Single Photon Counting Module (SPCM (MP963, Perkin-Elmer, Wiesbaden, Germany)) with a wavelength detection range of 185–850 nm. A 660 nm long-pass filter was placed in front of the optical fiber to protect the SPCM from scattering irradiation light. The output signal, which had been amplified and discriminated by the SPCM, was collected and processed by a micro control unit (MCU (AT89c55)) in the local control mode. The collected and processed signal could be storied in a memorizer (AT29c020) before further data analysis using a PC. The samples were irradiated by LED light for 0.2 s. The data collection started at 0.26 s upon the completion of the light irradiation and lasted for 5 s because the DF signal was stable at 0.26 s and decreased to nearly zero at 5.26 s.[15,19] The DF intensity was obtained by the integration between 0.26 and 5.26 s in the DF decay dynamics curve and registered as count per second (cps).

DF measurements of natural leaves of different age and detached leaves at 40 DAE treated with hormones were carried out in the same conditions of Pn measurements.

Determination of pigment content and ion leakage

After measurements of the DF intensity and Pn, chlorophyll was extracted from individual leaves by boiling the leaves in 95% ethanol at 80 °C. The chlorophyll concentration per fresh weight of leaf was calculated as described by Lichtenthaler.[20] The membrane ion leakage was determined by measuring electrolytes leaked from leaves. Leaves were immersed in 8 mL of 400 mM mannitol at 22 °C with gentle shaking for 3 h, after which the initial conductivity was measured.[3] The total conductivity was determined after boiling for 10 min in order to kill the tissues and then the electrical conductivity of this solution was recorded in μS min^{-1}.

Results

Changes in DF intensity, chlorophyll content, and ion leakage during age-dependent senescence

Leaf yellowing caused by the loss of chlorophyll is a typical symptom of senescence.[3] The leaf was considered dead when the entire leaf turned yellow.[21] We first examined the phenotype

of individual leaves of *C. roseus* during age-dependent natural senescence. We observed that the *C. roseus* leaf began to turn yellow at 50 DAE, and the entire leaf turned yellow at 70 DAE (Fig. 2a).

The DF intensity, chlorophyll content, and membrane ion leakage were comparatively measured in leaves from 40 DAE (which is the day when the leaves were fully grown) onwards, (Fig. 2b, c).[22] The results showed that the DF intensity and chlorophyll content changed in a similar way during age-dependent natural senescence. At 50 DAE, the DF intensity and chlorophyll content decreased to 93.8 and 90.8% of control, respectively. Then, rapid and consistent declines in DF intensity and chlorophyll content were observed, which were consistent with the rapid leaf yellowing (Fig. 2a). When the entire leaf turned yellow, both DF intensity and chlorophyll content disappeared almost completely (Fig. 2b). By contrast, a rapid increase in ion leakage of leaves from 55 DAE was observed during age-dependent natural senescence, indicating alterations in the permeability of the membranes and a reduction in their ability to retain solutes and water due to senescence. Statistical analyses showed that, there was a positive correlation between DF intensity and chlorophyll content ($R = 0.997$, Fig. 2d) and a negative correlation between DF intensity and ion leakage ($R = -0.996$, Fig. 2e) during age-dependent natural senescence.

Effects of different concentrations of 6-BA and MeJa on DF intensity

Leaf senescence occurs in an age-dependent manner in many species.[3] However, leaf senescence can be modulated by a range of plant hormones, such as ABA, MeJa, 6-BA and ethylene.[7,23,24] Before examining the hormone-modulated senescence symptoms, the effects of different concentrations of 6-BA and MeJa on the DF intensity of detached leaves at 40 DAE were first assessed. As shown in Fig. 3, after the same incubation period of 4 d in the

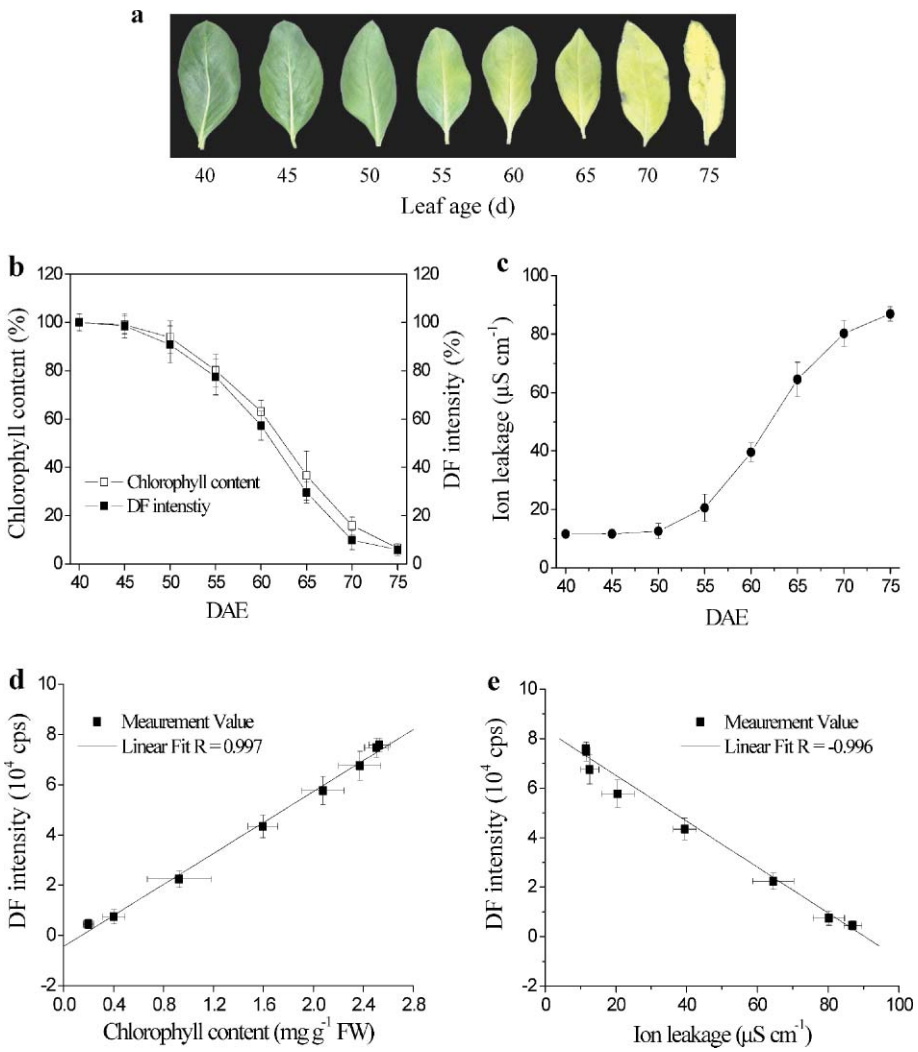

Fig. 2 Age-dependent senescence symptoms in the *C. roseus* leaves. (a) The age-dependent senescence phenotype of *C. roseus* leaves. Photographs show representative leaves at each time point. (b) DF intensity (■), chlorophyll content (□) and (c) ion leakage were examined every 5 d from 40 DAE, when the leaves were just fully grown. The data for chlorophyll content and DF intensity are expressed as a percentage of the content and intensity measured at 40 DAE, respectively, 2.53 mg g^{-1} fresh weight (FW) and 75879.49 cps for chlorophyll content and DF intensity, respectively. (d) The relationship between DF intensity and chlorophyll content ($R = 0.997$, $P < 0.001$). (e) The relationship between DF intensity and ion leakage ($R = -0.996$, $P < 0.001$). Each value is the mean ± SE of five independent leaves.

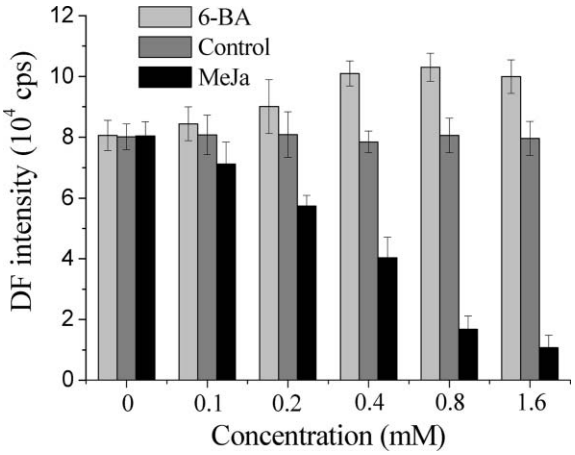

Fig. 3 Effects of different concentrations of 6-BA and MeJa on the DF intensity of *C. roseus* leaves at 40 DAE. Each value is the mean ± SE of five independent leaves.

dark, the DF intensity of leaves increased with the increasing of 6-BA concentration and decreased with the increasing of MeJa concentration. When the concentration of 6-BA was 0.4 mM, the increase in the DF intensity became significant different from the control at $P < 0.01$. Increasing the concentration of 6-BA to 0.8 and 1.6 mM did not result in an improvement for the P values for significant difference. When the concentration of MeJa was 0.2 mM, some yellow spots started to appear on the leaf surface, and the decrease in the DF intensity became significant different from the control at $P < 0.01$. Above 0.4 mM, most leaves lost their green colour and at the same time the DF intensity almost disappeared (Fig. 3).

The dynamics of DF intensity during plant hormone-modulated senescence

Next, we further investigated the dynamics of the DF intensity during plant hormone-modulated senescence. Detached leaves of equal size at 40 DAE, with similar initial DF intensity and weight, were placed in the dark to induce senescence in the presence or absence of 0.4 mM 6-BA or 0.4 mM MeJA. Photographs were taken and the DF intensity was determined every 2 d for a total of 10 d. The photographs of the samples and the temporal profile of the DF intensity from the samples are shown in Fig. 4.

As shown in Fig. 4a, detached leaves in the dark gradually turned yellow during the incubation period of 10 d. The phenotype of leaves showed that dark-induced leaf yellowing could be delayed by treatment with 0.4 mM 6-BA and accelerated by treatment with 0.4 mM MeJa. Dark-induced leaf yellowing began after 4 d of incubation without any treatments, 8 d in the presence of 6-BA, and 2 d in the presence of MeJa (Fig. 4a).

The hormone-modulated senescence process could be clearly indicated by the dynamics of the DF intensity (Fig. 4b). During the first 6 d of incubation with 0.4 mM 6-BA, although there are no visual phenotypic changes in the leaf phenotype, the DF intensity first showed an increasing trend and then began to gradually decrease. Upon treatment with 0.4 mM MeJa, the DF intensity showed a rapid decreasing trend during the whole incubation period of 10 d. After 8 d of incubation, the DF intensity reduced to nearly zero in the leaves treated with 0.4 mM MeJa, whereas

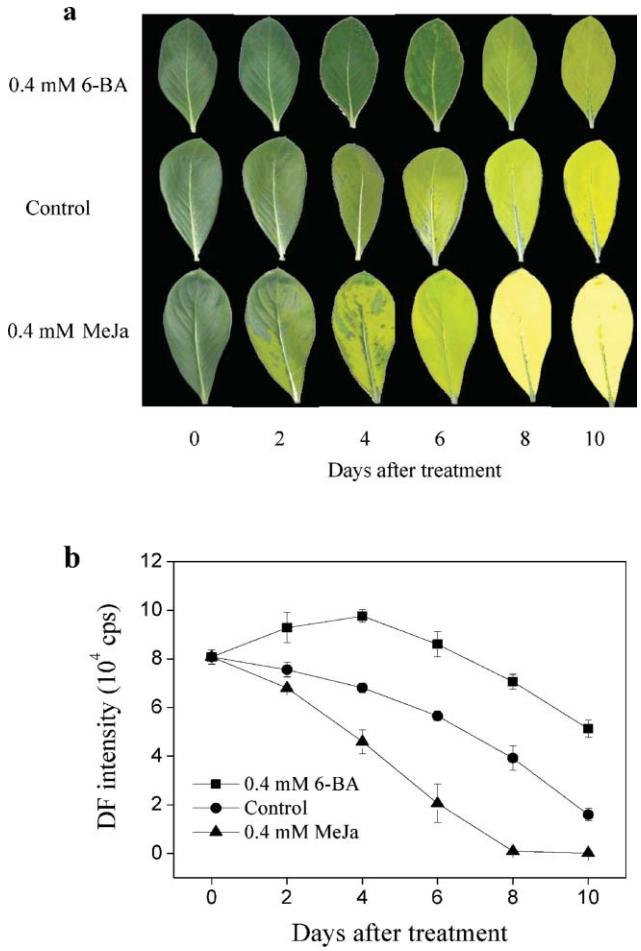

Fig. 4 Plant hormone-modulated senescence symptoms in *C. roseus* leaves. (a) The plant hormone-modulated senescence phenotype of *C. roseus* leaves. Photographs show representative leaves at each time point. (b) The DF intensity was examined every 2 d from 40 DAE. Each value is the mean ± SE of five independent leaves.

the DF intensity just started to decrease in the leaves treated with 0.4 mM 6-BA (Fig. 4b).

DF intensity correlates with Pn, chlorophyll content, and ion leakage during plant hormone-modulated senescence

In order to confirm whether the DF intensity can truly reflect the senescence process and quantitatively evaluate the senescence extent modulated by these hormones, we further examined the typical senescence symptoms of *C. roseus* leaves after treatment with the plant hormones (Table 1) and the relationships between the DF intensity and senescence symptoms (Fig. 5). The results clearly indicated that the leaves showed delayed senescence symptoms during the treatment with 0.4 mM 6-BA but severely accelerated senescence symptoms during the treatment with 0.4 mM MeJa (Table 1). Upon treatment with 0.4 mM 6-BA, both Pn and chlorophyll content first showed an increasing trend during the first 6 d of incubation and then began to gradually decrease after 8 d of incubation. Although ion leakage showed no significant changes during the first 6 d of incubation, it also began to increase after 8 d of incubation, indicating that 6-BA can enhance the activity of the photosynthetic apparatus to some extent.

Table 1 Changes in Pn, chlorophyll content, and ion leakage of *C. roseus* leaves during plant hormone-modulated senescence

	Pn/μmol CO_2 m^{-2} s^{-1}			Chlorophyll content/mg g^{-1} FW			Ion leakage/μS cm^{-1}		
Time/d	6-BA	Control	MeJa	6-BA	Control	MeJa	6-BA	Control	MeJa
0	15.2 ± 0.8	14.7 ± 1.0	15.0 ± 0.2	2.9 ± 0.7	2.8 ± 0.2	2.8 ± 0.55	16.2 ± 0.6	16.2 ± 0.5	16.5 ± 0.2
2	16.5 ± 0.2	14.1 ± 0.7	12.1 ± 0.4	3.1 ± 1.1	2.4 ± 0.2	2.2 ± 0.32	16.1 ± 0.2	18.7 ± 0.3	23.3 ± 0.5
4	18.2 ± 0.8	13.0 ± 0.4	8.0 ± 0.9	3.4 ± 0.1	2.0 ± 0.2	1.7 ± 0.66	16.6 ± 0.7	24.1 ± 1.0	41.1 ± 0.5
6	16.3 ± 0.5	10.5 ± 0.6	5.1 ± 0.6	2.9 ± 1.1	1.5 ± 0.8	1.0 ± 0.84	16.1 ± 0.2	39.2 ± 0.5	60.2 ± 0.7
8	13.1 ± 1.0	8.7 ± 0.1	1.1 ± 0.5	2.4 ± 0.8	0.8 ± 1.1	0.2 ± 0.05	24.1 ± 1.1	55.7 ± 1.1	82.7 ± 1.1
10	11.1 ± 0.6	3.1 ± 0.9	0.1 ± 0.5	2.1 ± 0.5	0.4 ± 0.8	0.01 ± 1.21	30.4 ± 0.4	68.7 ± 1.1	89.7 ± 0.1

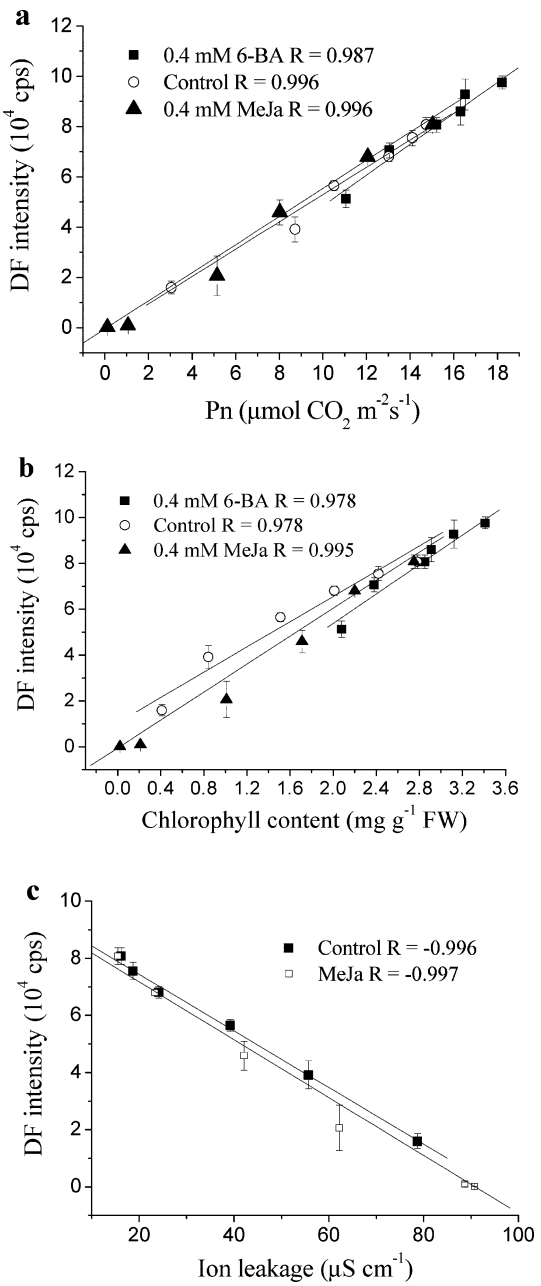

Fig. 5 The relationships between the DF intensity and typical senescence symptoms (data from Fig. 4 and Table 1). (a) The DF intensity positively correlates with Pn. (b) The DF intensity positively correlates with chlorophyll content. (c) The DF intensity negatively correlates with ion leakage. Each value is the mean ± SE of five independent leaves.

By contrast, both Pn and chlorophyll content showed a rapid decreasing trend upon treatment with 0.4 mM MeJa and decreased to nearly zero in the leaves after 8 d of treatment. At the same time, the ion leakage increases to 410.1% of control. Moreover, the ion leakage increases more markedly in the leaves treated with MeJa than in the control during the whole incubation period of 10 d. These data clearly show that the changes in the DF intensity exhibit a trend similar to that of Pn and chlorophyll content, but opposite to that of ion leakage during plant hormone-modulated senescence.

Statistical analyses showed that there was a correlation between the DF intensity and the typical senescence symptoms during hormone-modulated senescence (Fig. 5a–c). The DF intensity not only positively correlates with Pn and chlorophyll content but also negatively correlates with ion leakage.

Discussion

Senescence is a developmental event that leads to the death of a cell, an organ, or organism upon aging.[3] The yellowing process which occurs in leaf senescence is a ubiquitously biological phenomenon in most plant species. It is accepted that the chlorophyll content, photochemical efficiency of PSII and membrane ion leakage are typical senescence-associated physiological markers.[25–27] The changes in the DF intensity that we have measured were consistent with leaf yellowing of *C. roseus* (Fig. 2a, b). Moreover, a positive correlation between the DF intensity and chlorophyll content and a negative correlation between the DF intensity and ion leakage were observed during natural senescence in *C. roseus* leaves (Fig. 2d, e). These findings indicate that the DF intensity can be used to ascertain the senescence process and evaluate the senescence extent induced by age.

Although leaf senescence is regarded as a developmental programmed event, it can be highly modulated by internal factors such as plant hormones, reproduction, and cellular differentiation.[6,8,27] Among these internal factors, plant hormones have been characterized most thoroughly at the molecular and physiological levels.[23,24] The senescence symptoms we examined were the loss of chlorophyll content, decrease in photosynthesis rate, and increase in ion leakage. When compared to the control, the detached leaves of *C. roseus* exhibited a delay in the appearance of these senescence symptoms in the presence of 6-BA but an acceleration in the presence of MeJa (Fig. 4a, Table 1). These phenomena are similar to those observed in Arabidopsis and wheat.[3,7]

Measurements of the DF signal showed that the DF intensity of detached leaves of *C. roseus* increased with the increasing of 6-BA concentration and decreased with the increasing of MeJa

concentration (Fig. 3). Similar changes in the photosynthesis rate and chlorophyll content were also observed (data not shown). More interestingly, delayed or accelerated senescence in detached leaves of *C. roseus* related directly to hormone-induced changes in the dynamics of the DF intensity (Fig. 4). The excellent correlation between the DF intensity and these typical senescence symptoms strongly indicates that the DF intensity can be used to truly reflect the senescence process and quantitatively evaluate the senescence extent modulated by these hormones (Fig. 5a–c).

Leaf senescence is marked by a decline in the chloroplast function and photochemical efficiency of PSII,[3,8] which determine the rate of backward electron transport reactions in the reaction center of PSII and thus the DF intensity.[12,28] During leaf senescence, the chlorophyll protein complexes are decomposed and the integrity of the thylakoid membranes is broken (Fig. 2, Table 1).[8,9] This reduced the ability of P680 in PSII to initiate charge separation and transport to produce Q^-.[9] The DF intensity, directly related to the concentration of Q^- generated in the reaction center of PSII during light illumination,[9] thus, decreases (Fig. 2 and 4). Moreover, the chlorophyll degradation combined with the decreased expression of genes related to photosynthesis and protein synthesis during senescence affects the absorption of both CO_2 and light energy, and therefore reduced the photosynthesis rate and DF intensity (Fig. 4, Table 1).[9,29] Therefore, the DF intensity always displays a highly consistent trend with Pn and chlorophyll content but an opposite trend with ion leakage during age-dependent and hormone-modulated senescence.

In summary, the data presented in this work indicate that the changes in the DF intensity can truly reflect the senescence process and quantitatively evaluate the senescence extent modulated by age or plant hormones. Hopefully, with proper calibration, this DF technique will provide a new powerful method for the rapid and non-invasive monitoring of plant senescence.

Conclusions

This study has demonstrated the effectiveness of the DF technique for a rapid and non-invasive monitoring of plant senescence. The sensitivity of DF towards changes in the photosynthetic metabolism, coupled with the ease and speediness of DF measurements made using the biosensor, makes the DF technique potentially useful for the rapid and non-invasive detection of plant senescence modulated by age or plant hormones. The biosensor presented here, combined with a SPCM and a MCU, enables portable and *in vivo* measurements. Moreover, the low cost, simple and convenient operation, and the less interference from the environment even in field working conditions (achieved by using custom-built humidity, temperature, and CO_2 controllers) make the DF biosensor extremely suitable for practical applications.

Abbreviations

DF, delayed fluorescence; Pn, net photosynthesis rate; MeJA, methyl jasmonate; ABA, abscisic acid; 6-BA, 6-benzyladenine; PSII, photosystem II; DAE, days after leaf emergence; Mes, 2-(*N*-morpholino) ethanesulfonic acid; FW, fresh weight; Q, quinine acceptor of PSII.

Acknowledgements

This work was supported by the National Natural Science Foundation of China (Grant No.: 30670507; 30600128; 30470494), and the Natural Science Foundation of Guangdong Province (Grant No.: 015012).

References

1 N. Ori, M. T. Juarez, D. Jackson, J. Yamaguchi, G. M. Banowetz and S. Hake, Leaf senescence is delayed in tobacco plants expressing the maize homeobox gene *knotted1* under the control of a senescence-activated promoter, *Plant Cell*, 1999, **11**, 1073–1080.

2 H. Gut, C. Ruts, P. Matile and H. Thomas, Leaf senescence in a non-yellowing mutant of Festuca pratensis: Degradation of carotenoids, *Physiol. Plant.*, 1987, **70**, 659–663.

3 H. R. Woo, K. M. Chung, J.-H. Park, S. A. Oh, T. Ahn, S. H. Hong, S. K. Jang and H. G. Nam, ORE9, an F-Box protein that regulates leaf senescence in *Arabidopsis*, *Plant Cell*, 2001, **13**, 1779–1790.

4 J. Sh. Zhai, W. M. Guo, K. Zhou and Q. Zheng, Studies and applications of 1-MCP on delaying ornamental crops senescence, *Acta Horticulturae Sinica*, 2005, **32**, 165–170.

5 Y. H. He and S. S. Gan, A gene encoding an acyl hydrolase is involved in leaf senescence in *Arabidopsis*, *Plant Cell*, 2002, **14**, 805–815.

6 B. F. Quirino, Y. S. Noh, E. Himelblau and R. M. Amasino, Molecular aspects of leaf senescence, *Trends Plant Sci.*, 2000, **5**, 278–282.

7 Q. H. Ma and X. M. Wang, Characterization of an ethylene receptor homologue from wheat and its expression during leaf senescence, *J. Exp. Bot.*, 2003, **54**, 1489–1490.

8 C. M. Smart, Gene expression during leaf senescence, *New Phytol.*, 1994, **126**, 419–448.

9 C. L. Wang, D. Xing, L. Z. Zeng, C. F. Ding and Q. Chen, Effect of artificial acid rain and SO_2 on characteristics of delayed light emission, *Luminescence*, 2005, **20**, 51–56.

10 S. A. Zhang, Discussion on chlorophyll fluorescence kinetics parameters and their significance, *Chin. Bull. Bot.*, 1999, **16**, 444–448.

11 G. Christen, R. Steffen and G. Renger, Delayed fluorescence emitted from light harvesting complex II and photosystem II of higher plants in the 100 ns–5 micros time domain, *FEBS Lett.*, 2000, **475**, 103–106.

12 C. L Wang, D. Xing and Q. Chen, A novel method for photosynthesis measuring using chloroplasts delayed fluorescence, *Biosens. Bioelectron.*, 2004, **20**, 454–459.

13 L. Chaerle and D. Van der straeten, Seeing is believing: imaging techniques to monitor plant health, *Biochim. Biophys. Acta*, 2001, **1519**, 153–166.

14 D. Z. Badretdinov, S. A. Kuznetsova, S. V. Poltev and A. K. Kukushkin, Backward electron transport in photosystem 2 reaction center and temperature dependence of delayed luminescence characteristics, *Bioelectrochemistry*, 2002, **56**, 13–16.

15 L. R. Zhang, D. Xing and J. S. Wang, A non-invassive and real-time monitoring of the regulation of photosynthetic metabolism biosensor based on measurement of delayed fluorescence *in vivo*, *Sensors*, 2007, **7**, 52–66.

16 J. M. Anderson and N. K. Boardman, Fractionation of the photochemical systems of photosynthesis. I. Chlorophyll contents and photochemical activities of particles isolated from spinach chloroplasts, *Biochim. Biophys. Acta*, 1996, **112**, 403–421.

17 A. V. Gushchin and A. K. Kukushkin, Effect of nickel and cadmium ions on delayed luminescence of bean leaf, *Biophysics*, 1997, **42**, 455-460.

18 S. Khuznetsova, T. Bogracheva, C. Hedley, A. Tuleshova and A. Kukushkin, The rate of transport through a phosphate translocator affects delayed luminescence induction: an experiment and a theoretical model, *Annu. Appl. Biol.*, 2001, **138**, 117–121.

19 J. S. Wang, D. Xing, L. R. Zhang and L. Jia, A new principle photosynthesis capacity biosensor based on quantitative measurement of delayed fluorescence *in vivo*, *Biosens. Bioelectron.*, 2007, DOI: 10.1016/j.bios.2006.12.007.

20 H. K. Lichtenthaler, Chlorophylls and carotenoids: pigments of photosynthetic biomembranes, *Methods Enzymol.*, 1987, **18**, 350–382.
21 V. Grbić and A. B. Bleecker, Ethylene regulates the timing of leaf senescence in *Arabidopsis*, *Plant J.*, 1995, **8**, 595–602.
22 R. van der Heijden, D. I. Jacobs, W. Snoeijer, D. Hallard and R. Verpoorte, The catharanthus alkaloids: pharmacognosy and biotechnology, *Curr. Med. Chem.*, 2004, **11**, 607–628.
23 R. A. Weidhase, J. Lehmann, H. Kramell, G. Sembdner and B. Parthier, Degradation of ribulose-1,5-bisphosphate carboxylase and chlorophyll in senescing barley leaf segments triggered by jasmonic acid methyl ester and counteraction by cytokinin, *Physiol. Plant.*, 1987, **69**, 161–166.
24 J. A. D. Zeevaart and R. A. Creelman, Metabolism and physiology of abscisic acid, *Annu. Rev. Plant Physiol. Plant Mol. Biol.*, 1988, **39**, 439–473.
25 L. Fan, S. Zheng and X. Wang, Antisense suppression of phospholipase D retards abscisic acid- and ethylene-promoted senescence of postharvest *Arabidopsis* leaves, *Plant Cell*, 1997, **9**, 2183–2196.
26 S. A. Oh, J.-H. Park, G. I. Lee, K. H. Paek, S. K. Park and H. G. Nam, Identification of three genetic loci controlling leaf senescence in *Arabidopsis thaliana*, *Plant J.*, 1997, **12**, 527–535.
27 H. Thomas and J. L. Stoddart, Leaf senescence, *Annu. Rev. Plant Physiol.*, 1980, **31**, 83–111.
28 D. Z. Badretdinov, E. A. Baranova and A. K. Kukushkin, Study of temperature influence on electron transport in higher plants *via* delayed luminescence method: experiment, theory, *Bioelectrochemistry*, 2004, **63**, 67–71.
29 H. G. Nam, The molecular genetic analysis of leaf senescence, *Curr. Opin. Biotechnol.*, 1997, **8**, 200–207.