Natural leaf senescence: probed by chlorophyll fluorescence, CO₂ photosynthetic rate and antioxidant enzyme activities during grain filling in different rice cultivars

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Published online: 27 September 2012

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Abstract Natural leaf senescence was investigated in four rainfed lowland rice cultivars, FR 13A (tolerant to submergence), Sabita and Sarala (adapted to medium depth, 0–50 cm stagnant flooding) and Dengi (conventional farmers' cultivar). Changes in the levels of pigment content, CO₂ photosynthetic rate, photosystem II photochemistry and anti-oxidant enzyme activities of flag leaves during grain-filling stage were investigated. Chlorophyll content, photochemical efficiency of photosystem II and CO₂ photosynthetic rate decreased significantly with the progress of grain-filling. Likely, the activities of antioxidant enzymes namely, superoxide dismutase, catalase, guaiacol peroxidase and ascorbate peroxidase decreased with progress of grain-filling. A substantial difference was observed among the four cultivars for the sustainability index (SI) of different photosynthetic parameters and antioxidant enzyme activities; SIs of those parameters, in general, were lower in low yielding cultivar FR 13A compared to the other three cultivars. Among the four cultivars Sabita gave maximum grain yield. Yet, SI of Pn was greater in Sarala and Dengi compared to the Sabita. SIs of electron transport (ETo/CS), maximal photochemical efficiency (Fv/Fm), area above Fo and Fm, catalase and ascorbate peroxidase were also greater in Sarala and Dengi. The data showed that among the different Chl a fluorescence parameters, PI could be used with

greater accuracy to distinguish slow and fast senescence rice cultivars during grain-filling period. It was concluded that maintaining the vitality of rice plants during grain-filling gave guarantee to synthesize carbohydrate, however greater yield could be realized provided superior yield attributing parameters are present.

Keywords Antioxidant enzymes · Flag leaf · Photosynthetic characteristics · Natural leaf senescence · Rice

Abbreviation

AOX	Active oxygen species
APX	Ascorbate peroxidase
Area	Area above Fo and Fm
Chl	Chlorophyll
CAT	Catalase
CS	Cross-section
DAH	Days after heading
Dio/CS	Dissipation of heat per excited CS at Fo level
ETo/CS	Electron transport in a PS II per excited CS at Fo level
Fo	Minimal fluorescence of dark-adapted leaves
Fm	Maximal fluorescence of dark-adapted leaves
Fv	(Fm-Fo) Variable fluorescence
Fv/Fm	Maximum quantum yield of primary photochemistry of PS II
GPX	Guaiacol peroxidase
LSD	Least significant difference
PI	Performance index
Pn	CO ₂ photosynthetic rate
PS	Photosystem
RC/CSo	Number of reaction centres per excited CS at Fo level
SOD	Superoxide dismutase

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Introduction

The ripening period in rice is characterized by grain growth both in size and weight and senescence of leaves. The relationship between rate of leaf senescence and grain growth is complex. Grain growth along with leaf senescence is influenced by climatic conditions, varietal types and many other factors (Yamazaki and Kamimura 2001; Huang et al. 2004; Zhao et al. 2007; Damanik et al. 2010; Zhang et al. 2010b). Natural leaf senescence is faster in *Indica* than in *Japonica* types and in warm regions than in cool regions. Leaf senescence during grain-filling induces alterations in the structural and functional ability of the chloroplasts, which result in decreased CO₂ photosynthetic rate. This reduction in photosynthesis during senescence is associated with the breakdown of protein and pigment content and degradation of membrane lipids (Quirino et al. 2000; Chen et al. 2003; Falqueto et al. 2010; Shu-Kun et al. 2010). Chloroplasts are also the organelles most exposed to active oxygen species toxicity because they function at conditions of high oxygen concentration and light intensity (del Río et al. 1998; Gara et al. 2000; Sarkar and Panda 2009). If the excess energy is not dissipated efficiently, active oxygen species are generated, which reflects the severe damage to the photosynthetic apparatus and accelerates the progress of leaf senescence (Hung and Kao 2004; Panda et al. 2006; Šraj-Kržič et al. 2009).

Photosynthesis in rice plants during the grain-filling period contributes 60–100 % of the final grain carbon content (Yoshida 1981; Wada et al. 1993; Takai et al. 2005). The rest is made up from remobilized storage carbohydrate in culms and leaf sheaths laid down before heading. Among the leaves of a rice plant, the flag leaves were the primary contributor to the accumulation of dry matter in grains as leaf senescence starts from the lower leaves and extends upward with maturity (Falqueto et al. 2009). The senescence of leaves becomes evident due to the higher demand of nitrogen in continuance of grain-filling. Leaf blades are the major source of remobilized N and account for 60–90 % of the mobilized N in rice panicle (Mae 1997; Murchie et al. 2002). An in-dispense situation arise where an optimum synchronization is required between loss of nitrogen from leaf blades so that leaf could maintain greater photosynthetic activities and simultaneously grain-filling processes run smoothly in achieving the greater yield (Yoo et al. 2007). The photosynthetic apparatus especially photosystem II (PS II), is very sensitive to different environmental conditions. *In vivo* chlorophyll fluorescence has been used frequently in the past as a convenient and non-intrusive method to study the photosynthetic performances of different species to different environmental condition such as light intensity, temperature, drought, submergence, and chemical influences and reflects the kinetic process of PS II closure (Strasser and Tsimilli-Michael 2001). A procedure

for quantification and behaviour of PSII activity by O-J-I-P fluorescence transients, known as the JIP-test, was developed by Strasser et al. (1995). The analysis of the fluorescence transient according to the JIP-test leads to the calculation of several phenomenological and biophysical expressions of PS II (Strasser et al. 1995; Sarkar and Panda 2009). Most of the studies on the changes in Chl fluorescence in rice with senescence were conducted mainly at vegetative stage under different abiotic stresses.

In lowland and deepwater ecosystems, submergence is a major constraint to rice production. Traditional cultivars adapted to these ecosystems are generally low-yielding but sensitive to photoperiod, allowing them to avoid submergence stress at the time of flowering (Sarkar and Reddy 2006; Sarkar et al. 2009). The grain-filling period of photosensitive cultivars generally go together with gradual decline of temperature and increment of cloud free sunny weather. So far many studies have been conducted on natural leaf senescence in rice mainly with photo-insensitive *Indica* and *Japonica* rice cultivars (Wada et al. 1993; Zhang and Kokubun 2004; Zhang et al. 2006; Falqueto et al. 2009), however, studies on natural leaf senescence with photosensitive *Indica* rice cultivars are inadequate. The present study elucidates the difference of the response of photosynthesis and antioxidant enzymes activities during the grain-filling period in the flag leaves among four rice cultivars which differ in grain produce capacity.

Materials and methods

Plant materials and growth conditions

The experiment was conducted during wet season with four rice cultivars at the Central Rice Research Institute (CRRI), Cuttack, India. The cv FR 13A, low yielding traditional as well as tolerant to submergence having *Sub1*QTL (Neeraja et al. 2007), Sabita and Sarala (adapted to medium depth stagnant flooding, 0–50 cm) are high-yielding varieties released by All India Coordinated Rice Improvement Programme for rainfed lowlands and Dengi is a local cultivar adapted to the rainfed lowland conditions. The experiment was performed in alluvial sandy clay loam soil of the Mahanadi River delta (pH 6.8, organic C 0.85 %, total N 0.01 %, available P 25 kg/ha, and available K 130 kg/ha) during the wet seasons of 2006 and 2007. Seeds of the 4 cultivars were sown in a nursery seedbed and 25-d-old seedlings were transplanted @ 3 seedlings per/hill in lines that were 20 cm apart and with 15 cm between hills. The experiment was performed in a randomized complete block design with four replications. Chemical fertilizers were added as N:P:K at 60:40:30 kg/ha, respectively. P and K were applied as basal and nitrogen was added in 3 equal splits, as basal, and then at 30 and 90 days after transplanting.

The date of flowering, was last week of October for FR13A and Sabita, 1st week of November for Sarala and 2nd week of November for Dengi. As the grain-filling period progressed, the maximum and minimum temperature, and total rainfall decreased while total sunshine (hour/day) increased in both the years. The average maximum and minimum temperature for the month of October during two years was 29.8–30.3 °C and 24.3–24.5 °C, respectively and for the month of November was 28.7–29.5 °C and 18.0–19.4 °C, respectively. Total rainfall varied between 279.4 and 401.7 mm and 26.8 and 30.8 mm for the month of October and November, respectively. The sunshine (hour/day) was 5.4–5.5 and 8.1–9.3 respectively for the month of October and November.

Photosynthetic CO₂ fixation rate and chlorophyll a fluorescence kinetics

Measurements of CO₂ photosynthetic rate were made on fully expanded flag leaves of five different plants in each plot. The plants were tagged based on the heading date before measuring the photosynthetic rate. An open system photosynthetic gas analyzer was used (model TPS 1, PP Systems, USA) and measurements were made under ambient environmental conditions. After measuring photosynthetic rate, the same intact leaves were used for the measurement of chlorophyll fluorescence using a plant efficiency analyzer (Handy PEA, Hansatech Instruments Ltd., Norfolk, UK). To measure the Chl a fluorescence transients, leaves were maintained in darkness for 30 min and data were recorded from 10 µs up to 1 s with data acquisition every 10 µs for the first 300 µs, then every 100 µs up to 3 ms and later every 1 ms. The signal resolution was 12 bits (0 to 4000 fluorescence units per excited leaf cross section determined by the opening in the leaf clip). In each cultivar at a specific growth period the total numbers of measurements were fifteen (5 x 3 plots) for CO₂ photosynthetic rate and chlorophyll fluorescence parameters. The maximal intensity of the light source, providing an irradiance saturating pulse of 3000 µmol photons/m²/s, was used. Different chlorophyll fluorescence parameters such as F_o, F_m, quantum yield of primary PSII photochemistry (F_v/F_m), area above F_o and F_m (Area), number of reaction centre per cross section (RC/CS_o), dissipation per cross section (D_{io}/CS), electron transport per cross section (E_{To}/CS) and overall performance index (PI) calculated according to the equations of the JIP test (Strasser and Strasser 1995; Strasser et al. 1997).

Determination of flag leaf chlorophyll content and antioxidant enzyme activities

After measurement of the CO₂ photosynthetic rate and chlorophyll fluorescence, the same leaves were pooled together,

finely chopped and mixed properly and used for chlorophyll and antioxidant enzymes activities. To determine chlorophyll content 100 mg of finely chopped fresh leaves were placed in a capped measuring tube containing 25 ml of 80 % acetone and placed inside a refrigerator (4 °C) for 48 h (Sarkar 1998). Chlorophyll concentration was measured spectrophotometrically as described by Porra 2002.

To measure different antioxidant enzymes activities a 500 mg leaf sample was homogenized in 10 ml of 0.1 M potassium phosphate buffer, pH 7.8, containing 1 % insoluble polyvinylpyrrolidone. The extract was centrifuged at 4 °C at 15000 g for 30 min, and the supernatant was used for enzyme activity assay. The activities of superoxide dismutase (SOD, EC 1.15.1.1) was assayed according to Giannopolitis and Ries (1977) with modification suggested by Choudhury and Choudhury (1985), catalase (CAT, EC 1.11.1.6) and guaiacol peroxidase (GPX, EC 1.11.1.7) was measured following the procedure of Ram et al. 2000, ascorbate peroxidase (APX, EC 1.11.1.11) was assayed following Nakano and Asada (Nakano and Asada 1981).

Grain yield and yield-attributes determination

Grain yield was calculated based on a harvested area of 9 m² at the center of each plot and expressed in tonnes/ha. At maturity, twenty representative hills were collected for the measurements of panicle number/m², panicle weight (g/panicle), percentage sterility [(sterile grain/total grain)*100] and harvest index (grain weight/total biomass).

Statistical analysis

Differences between the various parameters assessed in these trials were compared by ANOVA using CROPSTAT (International Rice Research Institute, Manila, Philippines). Means were compared by the least significance difference test provided the *F* test was significant. Associations among different traits were examined by simple correlation and regression analysis using CROPSTAT software. Sustainability index (SI) of different antioxidant enzymes and photosynthetic parameters was calculated as the ratio of (the values at 15 and 20 DAH)/(the values at 0 DAH).

Results

Yield and yield-attributes

Among the four rice cultivars grain yield production was significantly higher in Sabita (5.53 tonnes/ha) followed by Sarala and Dengi and lowest was in FR 13A (Fig. 1f). The numbers of panicles/m² were greater in Sarala followed by Dengi, whereas 1000-grain-weight was greater in Sabita

Fig. 1 Grain yield and yield attributing parameters of four rainfed lowland rice cultivars. Bar represents least significance difference (LSD) at $*p < 0.05$

followed by FR13A (Fig. 1e). The numbers of sterile spikelets were significantly higher in FR13A compared to the other three cultivars (Fig. 1c). The differences in sterile spikelet numbers were also non-significant among these three cultivars. Likely, harvest index was also significantly lower in FR 13A compared to the other three cultivars (Fig. 1d).

Chlorophyll content, chlorophyll fluorescence parameters and CO₂ photosynthetic rate in flag leaves

The chlorophyll content of flag leaf of all the rice cultivars decreased from 0 day of heading up to 25 days of heading with the progression of leaf senescence as well as the progress of grain filling (Fig. 2a). The minimal fluorescence (F_o) significantly decreased only after 25 days of heading where as F_v/F_m did not change much during the progression of grain filling. The reduction in F_m, Area and PI was higher in FR 13 A compared to other cultivars during the grain filling period (Fig. 2). The other fluorescence parameters like RC/CS_o and ETo/CS decreased with the progression of grain filling, on contrary the DI_o/CS was increased from 5 days of heading up to 25 days of heading in all the cultivars (Fig. 3). Among all the Chl fluorescence parameters studied the PI

was found to be more sensitive and significantly decreased with the progression of flag leaf senescence during grain filling. The high sensitivity of the Performance index on an absorption basis (PI_{ABS}) is due to its construction which includes three or four (used in PI_{total}) independent information, such as the fractions : Chlorophyll as RCs of PS II per total chlorophyll, maximal Trapping per Absorption, Maximal electron transport beyond Q_A- per maximal Trapping= maximal Q_A reduction rate and maximal fraction of electron transport through PS I measured as reduction (RE) of end electron acceptors per photons absorbed in PS II by the given light conditions. The CO₂ photosynthetic rate was more decreased in compared to Chl content and Chl fluorescence parameters namely, F_m, Area, RC/CS_o and ETo/CS during grain filling. The decrease of CO₂ photosynthetic rate was more in FR 13A compared to other cultivars (Fig. 3d).

Antioxidant enzyme activities in flag leaves

To explain the difference of antioxidative enzyme activity between the four diverse lowland cultivars and to understand how the antioxidant protective mechanism might be functioning during grain filling, we measured the levels of

Figure 1 consists of six line graphs (A-F) showing various physiological and growth parameters over 25 days after heading for three genotypes: 100% WT (open circles), 100% WT × 100% WT (filled squares), and 100% WT × 100% WT (open squares). The x-axis for all graphs is 'Days after heading' (0, 5, 10, 15, 20, 25). The y-axis for each graph represents a different parameter.

- (A) Chlorophyll (mg / g fresh leaf):** The y-axis ranges from 0 to 4. The 100% WT genotype (open circles) shows the highest chlorophyll levels, while the 100% WT × 100% WT genotype (filled squares) shows the lowest. The 100% WT × 100% WT genotype (open squares) shows intermediate levels. The LSD $\cdot p < 0.05 = 0.56$.
- (B) Fo (a. u.):** The y-axis ranges from 0 to 600. The 100% WT genotype (open circles) shows the highest Fo values, while the 100% WT × 100% WT genotype (filled squares) shows the lowest. The 100% WT × 100% WT genotype (open squares) shows intermediate levels. The LSD $\cdot p < 0.05 = 24$.
- (C) Fm (a. u.):** The y-axis ranges from 0 to 3000. The 100% WT genotype (open circles) shows the highest Fm values, while the 100% WT × 100% WT genotype (filled squares) shows the lowest. The 100% WT × 100% WT genotype (open squares) shows intermediate levels. The LSD $\cdot p < 0.05 = 107$.
- (D) Fv/Fm ratio:** The y-axis ranges from 0.3 to 0.9. The 100% WT genotype (open circles) shows the highest Fv/Fm ratio, while the 100% WT × 100% WT genotype (filled squares) shows the lowest. The 100% WT × 100% WT genotype (open squares) shows intermediate levels. The LSD $\cdot p < 0.05 = 0.031$.
- (E) Area (a. u.):** The y-axis ranges from 0 to 50000. The 100% WT genotype (open circles) shows the highest area, while the 100% WT × 100% WT genotype (filled squares) shows the lowest. The 100% WT × 100% WT genotype (open squares) shows intermediate levels. The LSD $\cdot p < 0.05 = 2288$.
- (F) Performance Index (a. u.):** The y-axis ranges from 0 to 2.5. The 100% WT genotype (open circles) shows the highest performance index, while the 100% WT × 100% WT genotype (filled squares) shows the lowest. The 100% WT × 100% WT genotype (open squares) shows intermediate levels. The LSD $\cdot p < 0.05 = 0.14$.

with the progression of leaf senescence (Fig. 4). The reduction in the activities of CAT was greater compared GPX and APX in flag leaf during grain filling.

Fig. 4 Different antioxidant enzyme activities namely, SOD, APX, GPX and CAT with progress of grain-filling in four rice cultivars. Bar represents least significance difference (LSD) at $*p < 0.05$. FR13A –△–, Sabita –■–, Sarala –▲–, Dengi –□–

Relationship among different physiological and biochemical parameters

Different antioxidant enzyme activities, Chl fluorescence parameters and Chl contents showed highly significant relationship with CO_2 photosynthetic rate, and as expected DfO/CS , which exhibited a negative correlation (Table 1). All the photosynthetic parameters showed significant positive trends with antioxidative enzymes.

Sustainability index (SI) of different physiological and biochemical parameters

The sustainability index (SI) of different photosynthetic parameters and antioxidative enzyme activities of the four

rice cultivars after 15 and 20 d of heading in comparison to data taken at 0 d of heading stage was shown in Table 2. The SI of DfO/CS in the flag leaves of the four rice cultivars was more than 1 whereas the value of SI of the PI_{ABS} was found to be lower in all the cultivars after 20 relatively to 15 days after heading (Table 2). The SIs of Pn , PI , ETo/CS , CAT , APX and SOD were less in low yielding cv. FR 13A compared to the other three cultivars.

Discussion

Photosynthesis as the primary process by which plants use light energy to drive the synthesis of organic compounds, is essential for plant growth. Leaf photosynthesis comprises

Table 1 Correlation coefficients (r values) among different photosynthetic parameters and antioxidant enzyme activities in flag leaf of rice during grain filling

Parameter	APX	GPX	CAT	Chl	Fo	Fm	Fv/Fm	Area	PI	ETo/CS	DfO/CS	RC/CS	Pn
SOD	0.928	0.917	0.915	0.881	0.644	0.898	0.870	0.931	0.918	0.938	-0.649	0.918	0.917
APX	–	0.877	0.923	0.852	0.538	0.799	0.754	0.771	0.877	0.961	-0.664	0.839	0.855
GPX		–	0.908	0.827	0.561	0.819	0.757	0.787	0.913	0.854	-0.662	0.868	0.850
CAT			–	0.871	0.571	0.836	0.810	0.786	0.875	0.865	-0.683	0.864	0.901
Chl				–	0.732	0.923	0.853	0.860	0.802	0.887	-0.627	0.932	0.879
Fo					–	0.838	0.639	0.627	0.496	0.680	-0.232	0.799	0.678
Fm						–	0.926	0.839	0.794	0.910	-0.575	0.970	0.921
Fv/Fm							–	0.826	0.781	0.889	-0.645	0.888	0.890
Area								–	0.804	0.861	-0.659	0.851	0.817
PI									–	0.922	-0.762	0.849	0.905
ETo/CS										–	-0.718	0.926	0.942
DfO/CS											–	-0.602	-0.699
RC/CS												–	0.922

'r' values more than 0.368 signify the highly significant relationship at $*p < 0.01$

Table 2 Sustainability Index (SI) of different antioxidant enzymes and photosynthetic parameters after 15 and 20 days after heading in comparison to data taken at heading stage

Parameters	FR13A		Sabita		Sarala		Dengi	
	15	20	15	20	15	20	15	20
SOD	0.560	0.346	0.650	0.507	0.718	0.486	0.609	0.536
APX	0.364	0.299	0.533	0.367	0.456	0.405	0.552	0.440
CAT	0.220	0.186	0.391	0.218	0.422	0.303	0.482	0.268
GPX	0.383	0.291	0.444	0.221	0.428	0.265	0.460	0.279
Fo	1.090	0.994	0.919	1.114	0.860	0.645	0.968	0.776
Fm	0.691	0.587	0.703	0.616	0.821	0.506	0.805	0.592
Fv/Fm	0.870	0.844	0.928	0.812	0.989	0.934	0.952	0.925
RC/CS	0.630	0.596	0.623	0.586	0.707	0.406	0.730	0.460
DIO/CS	2.111	1.544	1.269	1.946	1.253	1.308	1.167	1.196
ETo/CS	0.368	0.328	0.678	0.565	0.828	0.599	0.843	0.595
PI	0.082	0.059	0.535	0.229	0.452	0.333	0.470	0.215
Chlorophyll	0.541	0.474	0.559	0.464	0.627	0.339	0.648	0.203
Pn	0.396	0.275	0.679	0.339	0.586	0.476	0.709	0.460

SI = (the values at 15 and 20 DAH)/(the values at 0 DAH)

several processes, like light harvesting, PSII photochemistry, and CO₂ assimilation. The O-J-I-P fluorescence transient analysis, so-called JIP-test that counts the stepwise shift of energy through PSII at the reaction centre (RC) level as well as the excited leaf cross-section (CS) level (Strasser et al. 1995). The initial stage of photosynthetic activity of a reaction centre complex is regulated by three functional steps namely absorption (ABS) of light energy flux, trapping flux (TR), conversion of trapped excitation energy to electron transport (ET). Combining these three fluxes as well as the derived specific rate of primary photochemistry (TR per RC = Q_A reduction rate per RC) a multi parametric expression of over all performance index (PI) of PS II was calculated (Strasser et al. 2000). The PI was significantly decreased with the progression of senescence in flag leaf, on contrary DIO/CS increased (Fig. 2). The Fv/Fm ratio remained unchanged. Fv/Fm depends only from the fluorescence extrema Fo and Fm and not of the trajectory between these values. Therefore Fv/Fm is not a sensitive parameter to assess the senescence index in rice (Van Heerden et al. 2004; Oukarroum et al. 2009; Panda et al. 2008; Falqueto et al. 2009). The decline in the value of Fm = ABS kF/kN can reflect a decrease in the ability of PS II to reduce the primary acceptor Q_A (Calatayud and Barreno 2001). Due to the decrease in the absorption rate or/and due to the increase in the rate constant for non photochemical events, the trend in the decrease in the values of Fm was almost similar in all the rice cultivars (Fig. 2). The relative area above the fluorescence curve Sm = Area/Fv between Fo and Fm represents the relative electron acceptor pool size from Q_A to NADP per PS II that includes Q_A and Q_B and PQ (Joliot and Joliot 2002), decreased in greater extent in FR 13A compared to other cultivars. Electron transport in a PS II cross section (ETo/CS) that represents the re-oxidation of reduced Q_A via

electron transport per cross section from PS II to PS I also decrease in greater extent in FR 13A. Among the other three cultivars both Dengi and Sarala maintained greater electron acceptor pool size of PS II. The electron carrier pool-size per PS II reaction center, as well as CO₂ photosynthetic rate, compared to Sabita was bigger in Sarala and Dengi (Table 1). The decrease in area, ETo/CS suggested that damage of acceptor side of PS II occurred during natural senescence of flag leaves, which was more pronounced in the cultivar FR 13A (Figs. 2 and 3). The present investigation showed that the maintenance of CO₂ photosynthetic rate during the progress of grain filling was strongly associated with PS II photochemistry and levels of chlorophylls.

In rice cultivars, importance of photosynthesis during grain-filling period is well documented. Greater photosynthetic rate in the flag leaves during grain-filling can or did improve grain yield in the reported experiments. Delayed flag leaf senescence has been proposed to improve rice grain yield (Horton 2000). The delayed leaf senescence, could sustain longer photosynthetic competence during the grain filling stage and maintain the supply of assimilates to the grain. The newly developed rice hybrid 'LiangYouPeiJiu' with superhigh-yield property showed a slower rate of chloroplast senescence and a higher overall photosynthetic performance index PI_{rel}, gave greater yield compared to the parents (Zhang et al. 2010a). The present results indicated that the differences in leaf senescence were existed in different rice cultivars and the cultivars maintained greater integrity of chloroplast structure and function and photosynthesis gave greater yield (Fig. 1). High yielding improved cultivars had greater sustainability of photosynthesis than in the traditional low yielding cultivar (Sasaki and Ishii 1992; Zhang and Kokubun 2004). Yielding ability of rainfed lowland rice cultivars depends on different yield attributes like

panicle number, 1000-grain weight, number of ear bearing tillers and fertile spikelet numbers (Sarkar and Das 2003; Sarkar et al. 2009). Due to the few number of sufficient spikelet's even the stay green cultivars failed to produce sufficient filled-grain. Instead the newly synthesized carbohydrate started to move to the stem and deposited there (Peng et al. 2005; Fu et al. 2009). The probability to improve rice yield was more with the cultivars that possessed sufficient numbers of spikelets and maintained greater chloroplast structural and functional ability during grain filling stage.

Senescence of leaves is an active process which allows redistribution of their nutrients as the grain matures. On another hand, senescence is an oxidative process that involves the overproduction of active oxygen species (AOS). Cellular damage caused by AOS, via lipid peroxidation, is generally considered to be a major contributor to the senescence syndrome (del Río et al. 1998). The antioxidant enzymes namely, superoxide dismutase, catalase, peroxidase and the ascorbate-glutathione cycle enzymes play a vital role in detoxifying the active oxygen species and help the plant to continue the growth (Asada 1996). With the progress of senescence loss of the activities of SOD, APX, GPX and CAT were faster in low yielding traditional and submergence tolerant cv. FR 13A (Fig. 4). During normal plant growth when the photon intensity is within the range of the utilizing capacity of chloroplasts for CO₂-fixation, the generation rate of AOS is very low, and these molecules are effectively scavenged. Several researchers observed that during the time of fast growth as well as the plant with greater biomass producing capacity showed greater activities of antioxidant enzyme activities to prevent the excess production of AOS (Gara et al. 2000; Hideg et al. 2002; Zhao et al. 2007). The association of different antioxidant enzymes with Pn and other photosynthetic parameters were showed highly significant positive correlation (Table 1). The significant association was due to the overall decrease of leaf function during natural senescence of flag leaves (Yamazaki and Kamimura 2001; Zhao et al. 2007; Falqueto et al. 2009).

The results show that the fast Chl a fluorescence transient measurement provides a non-invasive and rapid method for investigating PS II functional and structural integrity under natural leaf senescence in rice. The overall performance index (PI) obtained through the JIP-test was found as highly sensitive compared to other photosynthetic parameters. Maintenance of better photosynthetic activity along with better antioxidant capacity of flag leaf during grain filling minimizes senescence. Rice cultivars with better yield attributing and slow senescence kind had an edge to produce superior grain yield compare to the cultivars with fast senescence and/or poor yield attributing parameters.

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