Quantification of the photosynthetic performance of phosphorus-deficient *Sorghum* by means of chlorophyll-*a* fluorescence kinetics

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Chlorophyll fluorescence induction curves have been used as a sensitive tool for screening the photosynthetic performance of plants. Experimental treatments involving nitrate supply and chilling stress have been shown to affect fluorescence induction curves and other measures of photosynthesis. We have investigated the photosynthetic performance of *Sorghum bicolor* supplied with Long Ashton growth solution containing standard (20 μmol mol^{-1}) or low (5 μmol mol^{-1}) phosphorus. The JIP-test based on the chlorophyll fluorescence induction curve was used as a non-destructive method to measure the relative proportions of energy dissipated by different processes (termed energy fluxes) in the light reactions. The various energy fluxes or derived parameters were compared to find the measures that were most sensitive to the experimental conditions. Plant response to treatments was first evident in selected chlorophyll fluorescence parameters, particularly performance index (PI_{ABS}); plants with increased PI_{ABS} manifested higher electron transport activity and dissipated less energy as heat, possibly as a result of their better phosphorus status, leading to more functional reaction centres. Observed changes in fluorescence were correlated to changes in gas exchange and biomass. Standard phosphorus treatments significantly increased biomass, leaf area, photosynthetic and respiratory rates, carboxylation efficiencies and levels of ribulose biphosphate regeneration rates, relative to plants with low supplies of nutrients.

Introduction

Nutrient limitations affect many aspects of plant physiology including photosynthesis and hence also plant growth and performance. Phosphorus deficiency has important effects on the regeneration of NADP,[1] starch synthesis, the transport of sugars across the chloroplast membrane, energy metabolism and ATP production.[2] Phosphorus deficiencies also affect the quantum and carboxylation efficiencies of photosynthesis,[3] rates of electron transport and regeneration of the primary CO_2 acceptor, ribulose biphosphate (RuBP).[4] It is therefore evident that phosphorus supply will affect plant photosynthetic performance, so that phosphorus treatments may be used to assess techniques for quantifying photosynthetic performance and growth.

Photosynthesis and particularly Photosystem II (PSII) behaviour can be evaluated by means of the fast kinetics of the chlorophyll-*a* (chl-*a*) fluorescence emitted by dark-adapted leaves of plants upon illumination with saturating light. Resultant transients have several inflection points that have been termed O, J, I and P and are related to biophysical and biochemical events of the light reactions (see Methods for details). This method for the analysis of such fluorescence transients has been termed the JIP-test[5,6] and has been used to demonstrate the beneficial role of mycorrhizal symbiosis,[7–10] the effects of inoculation with mixtures of AM fungi and nitrogen-fixing bacteria on PSII activity,[11] and plant sensitivity to different environmental conditions (such as light intensity,[12–16] temperature,[17,18] drought,[19] and chemical agents[20]). Measurements can be carried out quickly, are non-invasive and can be applied under field conditions for the rapid screening of many samples.

Additionally, photosynthetic performance at the level of CO_2 assimilation may be assessed by the construction of light[21] and CO_2 response curves.[22] The aim of this study was to use the JIP-test to assess the effects of two different levels of soil phosphorus application on the photosynthetic characteristics of *Sorghum bicolor* and to demonstrate that the early observed effects on chlorophyll fluorescence correlated to both altered gas exchange and biomass production.

Materials and methods

Sand preparation and growth conditions

River sand was acid-washed with 1% HCl, rinsed with water, sterilized and placed into 1-litre pots. Three sorghum seeds were planted in each pot, which were placed in a growing-tunnel with average night–day temperatures of 19–29°C and midday photo flux densities attaining 1800 μmol m^{-2} s^{-1}. The pots were mist irrigated daily with rainwater for 15 minutes. Once a week all the pots were watered with Long Ashton nutrient solution containing a standard concentration of nutrients, and a standard concentration of phosphorus (20 μmol mol^{-1} P, supplied as NaH_2PO_4)[23] or a low concentration of phosphorus (5 μmol mol^{-1}, supplied as NaH_2PO_4). Low and standard phosphorus treatments are designated as LP and SP, respectively. At 8 weeks, plants were thinned to one plant per pot.

Plant harvesting and data collection

At 4, 8 and 12 weeks after planting, replicate plants from each treatment were harvested. One day prior to the harvests, fluorescence induction curves were constructed for 15, 20 and 7 replicates of each treatment, at 4, 8 and 12 weeks, respectively. Harvested plants were used to determine plant biomass and leaf area. For 8-week-old plants in each treatment, three replicate light and CO_2 response curves were constructed.

Biomass and leaf area

Total plant leaf areas were calculated from correlations of measured leaf area to the products of leaf length and width. Such correlations were based on the measurement of 58 randomly selected leaves and had an r^2 value of 0.97 ($P < 0.0001$). Shoots and roots were separated and oven-dried at 70°C to constant weight and used to calculate biomass.

Light and CO_2 response curves

Light and CO_2 response curves were constructed using a LI-6400 photosynthesis system (LI-COR, Inc., Lincoln, Nebraska). Such curves measure the response of photosynthesis to increasing incident light intensity or CO_2 concentration; the resultant monomolecular responses can be used to determine parameters that quantify the performance of photosynthetic carbon assimilation.[22] For the construction of light and CO_2 responses, leaf temperatures and water vapour partial pressures were 30°C and 16 μmol mol^{-1}, respectively. For light responses, ambient CO_2 concentrations were 360 μmol mol^{-1} and for CO_2 responses light intensity was 1500 μmol m^{-2} s^{-1}.

Gas exchange behaviour was recorded when the sum of the

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Table 1. Selected JIP-test parameters, their biophysical or biochemical meanings and how they are calculated from original data (F_M, F_O, F_{300} and F_J) extracted from fluorescence transients.[26]

Specific energy fluxes expressed per reaction centre (RC)			
Observed rate of QA reduction	M_O	=	$(F_{300} - F_O)/(F_M - F_O)/0.25$ ms
Maximum rate of QA reduction	TR_0/RC	=	$(M_0/V_J) = M_O/(F_J - F_O)/(F_M - F_O)$
Rate of electron transport beyond QA^-	ET_0/RC	=	$(TR_0/RC)\ (1 - V_J)$
Rate of photon absorption	ABS/RC	=	$(TR_0/RC)/[(F_M - F_O)/F_M]$
Rate of heat dissipation	DI_0/RC	=	$(ABS/RC) - (TR_0/RC)$
	V_J	=	$(F_J - F_O)/(F_M - F_O)$
Efficiencies (or flux ratios)			
Efficiency that reduced QA results in electron transport	ET_O/TR_O	=	$(ET_0/RC)/(TR_0/RC)$
Proportion of electron transport to energy dissipation as heat	ET_O/DI_O	=	$(ET_0/RC)/(DI_0/RC)$
Maximum efficiency with which an absorbed photon results in QA reduction	TR_O/ABS	=	$(TR_0/RC)/(ABS/RC) = (F_M - F_O)/F_M$
Efficiency with which an absorbed photon results in electron transport beyond QA^-	ET_O/ABS	=	$(ET_0/RC)/(ABS/RC)$
Density of reaction centres			
Functional reaction centres per cross-sectional leaf area	RC/ABS	=	$(RC/TR_0)\ (TR_0/ABS)$
Performance index			
Compound function of light energy absorption, efficiency of QA reduction and conversion of excitation energy to electron transport	PI_{ABS}	=	$[RC/ABS]\ [(TR_O/ABS)/(F_O/F_M)]\ [(ET_O/TR_O)/\ V_J]$

The subscript '0' indicates the quantification of PSII behaviour at the onset of fluorescence induction.

coefficients of variation for the difference in CO_2 and H_2O between reference and analysis sources, and the change in flow rate, was less than 1%. The Li-6400 equipment changed the flow rate to control the water vapour concentration surrounding the leaf. Gas exchange parameters were calculated according to the equations of Farquhar and von Caemmerer[22] and individual response curves were fitted according to the methods of Causton and Dale.[25] Gas exchange parameters were extracted to compare plants from different treatments.

Fluorescence measurements

Fluorescence transients were measured with a Plant Efficiency Analyser (PEA, Hansatech, U.K.) and subsequently assessed according to the JIP-test.[26] Measurements were made on the second leaf, counting the apical meristem as leaf one, to ensure that leaves at the same stage of development were being measured at each harvest. Measurements were performed at night after at least 6 hours of darkness.

The JIP-test

The JIP-test was performed on all measured fluorescence transients and is based on a simple model of how photon flux absorbed by the photosynthetic antenna pigments (ABS) is dissipated as heat (DI) and fluorescence, or channelled as trapping flux (TR) to the reaction centres to be converted to redox energy by reducing plastoquinone (Q_A) to Q_A^-. Q_A^- is then re-oxidized to Q_A and creates electron transport (ET) that leads to CO_2 fixation.[27] These fluxes are expressed as specific energy fluxes (per reaction centre) or as proportions of one another (flux ratios or yields). Fluorescence values at time intervals corresponding to the steps O–J–P were recorded and used as original data in the JIP-test,[26] including: the maximum fluorescence intensity (F_M), the fluorescence intensity at 50 μs (F_O), 300 μs (F_{300}), and 2 ms (F_J). The derivation of specific energy fluxes and flux ratios has been comprehensively explained by Strasser and co-workers.[5,6,12,26] Table 1 lists JIP-test parameters, their biophysical or biochemical meanings and how they are calculated from original data (F_M, F_O, F_{300} and F_J) extracted from fluorescence transients. Examples of such transients, as affected by phosphorus supply, are given in the results.

In addition to the specific energy fluxes and flux ratios or yields (presented in this paper), it is possible to calculate phenomenological energy fluxes (expressed per leaf cross section) and various other vitality indices (see Strasser *et al.*[26] for details).

Fluorescence signals are expessed in arbitrary units and the JIP-test parameters on a relative basis

Statistical analyses

Differences in above-ground biomass and performance index (PI_{ABS}) between sampling date, treatment and for their interaction were compared by two-way ANOVA. Subsequently, fluorescence and other data collected from an individual sampling date were subject to one-way ANOVA at a critical P level of 0.05. If the data did not meet homogeneity or normality requirements, they were transformed using natural logarithm, square root or arcsine transformations. *Post hoc* comparisons were made where appropriate using Scheffé tests.

Results

Biomass and leaf area

The above-ground biomass was significantly different between sampling dates ($F = 4.38$, $P < 0.0001$), treatments ($F = 19.21$, $P < 0.0001$) and interactions ($F = 9.46$, $P < 0.005$). The differences between SP and LP plants increased over time, were not significantly different at 4 and 8 weeks, but became significantly different by 12 weeks (Fig. 1). Similarly, by week 12, the below-ground biomass and leaf area of the SP plants were significantly higher than those of LP plants (data not shown).

Chlorophyll fluorescence

Average chlorophyll-*a* fluorescence transients and data extracted at set time intervals (F_O, F_{300}, F_J and F_M) for 4-, 8- and 12-week-old SP and LP plants are given in Fig. 2. It is evident from this figure that the shape of the transients is altered by phosphorus supply at 8 and 12 weeks, but not at 4 weeks. F_O, F_{300}, F_J and F_M data extracted for individual replicates were used to

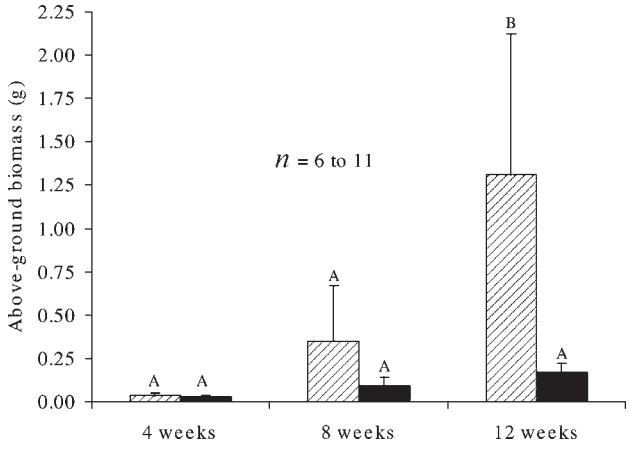

Fig. 1. Mean above-ground biomass for LP (solid) and SP (cross-hatch) plants at 4, 8 and 12 weeks. The number of replicates is shown on the figures and the vertical bars indicate standard deviations. Values with the same letter are not significantly different at the 95% probability level (ANOVA and *post hoc* Scheffé multiple-range test).

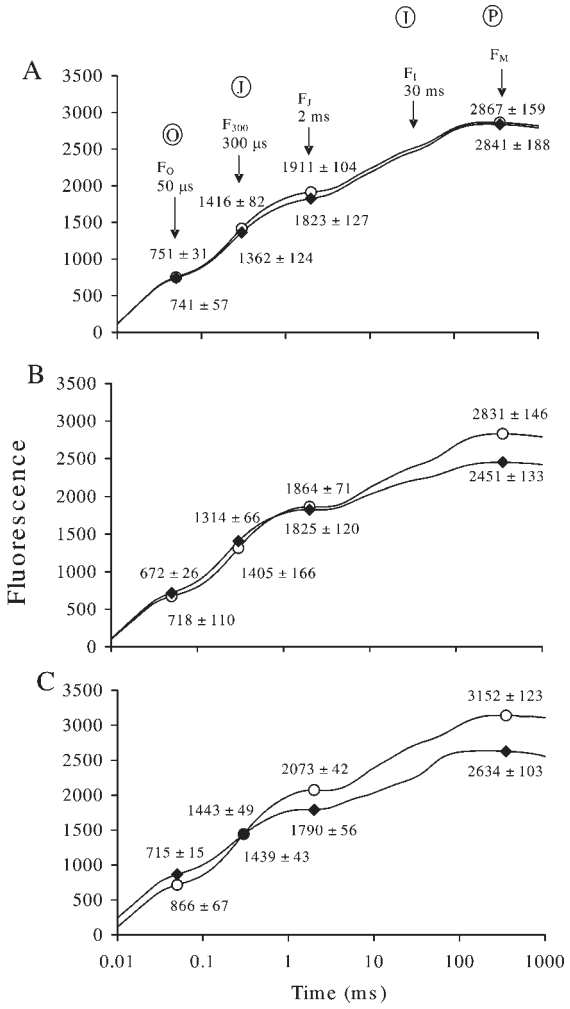

Fig. 2. Mean chlorophyll-*a* fluorescence induction curves for SP (open symbols) and LP plants (filled symbols) at (**A**) 4, (**B**) 8 and (**C**) 12 weeks. The average (± standard deviation) values for fluorescence extracted at time intervals 50 µs (F_O), 300 µs (F_{300}), 2 ms (F_J) and the maximal fluorescence intensity (F_M) are given above for SP plants and below transients for LP plants. The convention of naming inflection points O, J, I and P is indicated on the figure.

calculate derived JIP-test parameters (Table 2) according to the formulae in Table 1. These parameters describe the changes in the shapes of the transients and relate them to altered biophysical and biochemical processes in PSII.

The performance index (PI_{ABS}) of SP and LP plants at 4, 8 and 12 weeks is presented in Fig. 3. PI_{ABS} was significantly different between sampling dates ($F = 4.33$, $P = 0.017$), treatments ($F = 30.68$, $P < 0.0001$) and interactions ($F = 23.24$, $P < 0.0001$). The differences between SP and LP plants increased over time, were not significantly different at 4 weeks, but became significantly different by 8 weeks. To explain altered PI_{ABS} values, a detailed analysis of selected fluorescence and photosynthetic parameters is presented for the 8-week-old plants when PI_{ABS} first became significantly different.

The significantly higher PI_{ABS} values of the SP plants at 8 weeks was the result of significantly higher quantum yields (TR_0/ABS), the efficiency with which a trapped electron can move beyond Q_A^- in the electron transport chain (ET_0/TR_0), and the quantum yield of electron transport (ET_0/ABS; Table 2). Heat dissipation (DI_0/RC and DI_0/ABS) by the LP plants was significantly higher than that of the SP plants. Energy absorption and trapping per reaction centre (ABS/RC and TR_0/RC) and not electron transport per reaction centre (ET_0/RC) were significantly greater in LP plants than in the SP plants. Furthermore, the ratio of electron transport to energy dissipation (ET_0/DI_0) was higher in SP plants,

Table 2. Selected JIP-test parameters calculated for 8-week-old SP and LP plants.

	Treatment	
	SP	LP
TR_0/ABS	0.76 ± 0.05	0.69 ± 0.15
ET_O/TR_O	0.45 ± 0.03	0.36 ± 0.06
ET_0/ABS	0.76 ± 0.03	0.71 ± 0.06
DI_0/RC	0.67 ± 0.05	1.09 ± 0.44
DI_0/ABS	0.24 ± 0.02	0.30 ± 0.14
ABS/RC	2.83 ± 0.14	3.57 ± 0.68
TR_0/RC	2.15 ± 0.10	2.48 ± 0.25
ET_0/RC	0.96 ± 0.05	0.87 ± 0.12
ET_0/DI_0	1.43 ± 0.13	0.80 ± 0.34
RC/ABS	0.76 ± 0.04	0.70 ± 0.13

Abbreviations: TR_0, QA reduction (energy trapping); ET_0, electron transport; and DI_0, heat dissipation. Parameters are expressed on a reaction centre (RC) basis or as ratios of absorbed energy (ABS). TR_0/ABS: the maximum efficiency with which a photon results in QA reduction; ET_0/TR_0: the efficiency with which reduced QA results in electron transport; ET_0/ABS: the efficiency that an absorbed photon results in electron transport beyond Q_A^-. With the exception of ET_0/RC, all parameters were significantly different between the SP and LP plants at the 95% probability level (ANOVA and *post hoc* Scheffé multiple-range test). $n = 20$.

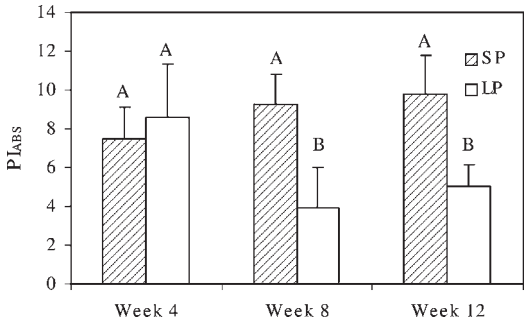

Fig. 3. Performance index (PI_{ABS}) for 4-, 8- and 12-week-old SP and LP plants. Values with the same letter are not significantly different at the 95% probability level.

while the reaction centre density (RC/ABS) in the LP plants decreased significantly.

Gas exchange

At 8 weeks, the photosynthetic rates under light saturation, the light compensation point, dark respiration rates, carboxylation efficiencies and rates of RuBP regeneration for the SP plants were significantly higher than the corresponding values for LP plants (Table 3). The quantum efficiencies were not significantly different between treatments.

Discussion

In this study, the chl-*a* fluorescence transients according to the JIP-test were analysed in order to quantify the PSII behaviour/activity in *Sorghum* supplied with low or standard soil phosphorus. A similar application of the JIP-test has proved successful in screening alfalfa for enhanced electron transport activity after inoculation with mycorrhizal fungi and nitrogen-fixing bacteria[11] and has been used to access plant sensitivity to different environmental conditions.[12–16, 18,20] As in recent studies by van Heerden,[26,27] this study correlated JIP-test fluorescence parameters to both leaf gas exchange and plant growth.

Results showed that plants responded to higher phosphorus supply by producing greater biomass and leaf area. SP plants increased in PSII performance as indicated by both altered CO_2 assimilation and fluorescence emissions. Enhanced PSII performance was the result of an increased number of reaction centres, as indicated by a greater density of reaction centres (RC/ABS) and that these photosystems trapped energy more efficiently (TR_0/ABS), displayed increased electron transport efficiency

Table 3. Photosynthetic parameters from light and CO_2 response curves for SP and LP plants.

	Treatment	
	SP	LP
Light saturated photosynthetic rate (μmol m^{-2} s^{-1})	30.43 ± 1.62	9.82 ± 1.71
Quantum efficiency (mmol mol^{-1})	0.09 ± 0.02	0.07 ± 0.02
Light compensation point (μmol m^{-2} s^{-1})	32.86 ± 4.37	11.68 ± 5.92
Dark respiration rate (μmol m^{-2} s^{-1})	2.84 ± 0.84	0.82 ± 0.52
Carboxylation efficiency (μmol m^{-2} s^{-1})	2.50 ± 1.35	0.80 ± 0.53
RuBP regeneration rate (μmol m^{-2} s^{-1})	25.10 ± 2.50	15.76 ± 3.23

With the exception of quantum efficiency, all parameters were significantly different between the SP and LP plants at the 95% probability level (ANOVA and *post hoc* Scheffé multiple-range test). $n = 3$.

(ET_0/ABS) and dissipated less energy as heat or fluorescence (DI_0/RC and DI_0/ABS). Tsimilli-Michael *et al.*[11] reported a similar enhanced PSII performance for alfalfa inoculated with arbuscular mycorrhizal fungi, as indicated by increased performance index and electron transport, and attributed this to a rise in the number of functional reaction centres and in leaf chlorophyll content.

Like PSII performance, photosynthetic rates under light saturation, carboxylation efficiencies and the rates of RuBP regeneration were higher for the SP than for the LP plants. The results may indicate that the LP plants, because of a phosphorus deficiency, have a reduced capacity for electron transport and hence impaired NADPH and ATP production.[3,4] In addition, the lower values of the parameters measured for the LP plants may have resulted from reduced availability of RuBP for the Calvin cycle.[4]

In LP plants but not in SP plants, PI_{ABS} declined over time, possibly due to increasing phosphorus deficiency. This deficiency resulted in a reduction in the amount of phosphorus-containing intermediates of the electron transport chain. Such reductions impair electron transport capacity and the utilization of absorbed and trapped photosynthetic energy. Energy that cannot be used is dissipated as heat.[30] In addition to this, a reduced capacity for electron transport may raise free-radical formation,[31] contributing to the inactivation of reaction centres.[32]

The differences between the fluorescence parameter, PI_{ABS}, of the variously treated plants were significant 4 weeks before differences in growth and biomass were evident. Measuring fluorescence parameters is a means of detecting early changes in plant performance. In this study the changes detected translated into both altered gas exchange and biomass production. Furthermore, the measurement of fluorescence parameters gives some explanation of the observed effects on photosynthetic gas exchange. The technique can therefore be used as a tool for quickly and non-destructively measuring the effect of nutrients or other factors on plant performance.

1. Marschner H. (1995). In *Mineral Nutrition of Higher Plants,* pp. 131–135. Academic Press, London.
2. Rao I.M., Fredeen A.L. and Terry N. (1990). Leaf phosphate status, photosynthesis, and carbon partitioning in sugar beet. III. Diurnal changes in carbon partitioning and carbon export. *Plant Physiol.* **92**, 29–36.
3. Lauer M.J., Pallardy S.G., Bleins D.G. and Randall D.D. (1989). Whole leaf carbon exchange characteristics of phosphate deficient soybeans. *Plant Physiol.* **91**, 848–854.
4. Fredeen A.L., Raab T.K., Rao I.M., and Terry N. (1990). Effects of phosphorous nutrition on photosynthesis of *Glycine max.* (L.) Mer. *Planta* **181**, 399–405.
5. Strasser R.J. and Govindjee (1992). The F_0 and the O-J-I-P fluorescence rise in higher plants and algae. In *Regulation of Chloroplast Biogenesis,* ed. J.H. Argyroudi-Akoyunoglou, pp. 423–426. Plenum Press, New York.
6. Strasser B.J. and Strasser R.J. (1995). Measuring fast fluorescence transients to address environmental questions: The JIP test. In *Photosynthesis: From Light to Biosphere,* ed. P. Mathis, vol. V, pp. 981–984. Kluwer Academic, Dordrecht.
7. Romano A., Strasser R.J., Eggenberg P. and Martins-Loucao M.A. (1996). Mycorrhization of cork oak micropropagated plantlets: its vitality during acclimatization measured with fast fluorescence techniques. In *Mycorrhizas in Integrated Systems from Genes to Plant Development,* eds C. Azcon-Aquilar, J.M. Barea, pp. 378–376. European Commission (EUR 16728), Luxembourg.
8. Koves-Pechy K., Biro B., Voros I., Takacs T., Osztoics E. and Strasser R.J. (1998). Enhanced activity of microsymbiont-alfalfa system probed by the fast fluorescence rise OJIP. In *Photosynthesis: Mechanisms and effects,* vol. IV, ed. G. Garab, pp. 2765–2768. Kluwer Academic, Dordrecht.
9. Calantzis C., Rivera-Becerril F., Gianinazzi-Pearson V., Gianinazzi S. and Strasser R.J. (1999). Mycorrhiza buffer cadmium-induced stress in pea plants: vitality probing by fast fluorescence transient O-J-I-P. In *Arbuscular Mycorrhizas and Plant Health under Abiotic Stress.* COST-ACTION 8.38 Meeting. Nancy, France.
10. Calantzis C., Trouvelet S., van Tuinen D., Gianinazzi-Pearson V., Gianinazzi S. and Strasser R.J. (2000b). A non-destructive method for evaluating the mycorrhizal status of micropropagated vine root-stock. In *Mycorrhiza and root research in Switzerland, Bull. SGPW/ SSA* No. 14, 8.
11. Tsimilli-Michael M., Eggenberg P., Biro B., Koves-Pechy K., Voros I. and Strasser R.J. (2000). Synergistic and antagonistic effects of arbuscular mycorrhizal fungi and *Azospirillum* and *Rhizobium* nitrogen fixers on the photosynthetic activity of alfalfa, probed by the chlorophyll *a* polyphasic fluorescence transient O-J-I-P. *Appl. Soil. Ecol.* **15**, 169–182.
12. Tsimilli-Michael M., Kruger G.H.J. and Strasser R.J. (1995). Suboptimality as driving force for adaptation: a study about the correlation of excitation light intensity and the dynamic fluorescence emission in plants. In *Photosynthesis: From Light to Biosphere,* ed. P. Mathis, vol. V, pp. 981–984. Kluwer Academic, Dordrecht.
13. Tsimilli-Michael M., Kruger G.H.J. and Strasser R.J. (1996). About the perpetual state changes in plants approaching harmony with their environment. *Archives des Sciences* (Geneva) **49**, 173–203.
14. Srivastava A. and Strasser R.J. (1996). Stress and stress management of land plants during a regular day. *J. Plant Physiol.* **148**, 445–455.
15. Srivastava A. and Strasser R.J. (1997). Constructive and destructive actions of light on the photosynthetic apparatus. *J. Sci. Ind. Res.* **56**, 133–148.
16. Kruger G.H.J., Tsimilli-Michael M. and Strasser R.J. (1997). Light stress provokes plastic and elastic modifications in structure and function of photosystem II in camellia leaves. *Physiol. Plant.* **101**, 65–277.
17. Srivastava A., Guisse B., Greppin H. and Strasser R.J. (1997). Regulation of antenna structure and electron transport in PSII of *Pisum sativum* under elevated temperature probed by the fast polyphasic chlorophyll a fluorescence transient: OKJIP. *Biochem. Biophys.* **A 1320**, 95–106.
18. Strasser B.J. (1997). Donor side capacity of photosystem II probed by chlorophyll a fluorescence transients. *Photosynth. Res.* **52**, 147–155.
19. Van Rensburg L., Kruger G.H.J., Eggenburg P. and Strasser R.J. (1996). Can screening criteria for drought resistance in *Nicotiana tabacum* L. be derived from the polyphasic rise of the chlorophyll a fluorescence transient (OJIP)? *S. Afr. J. Bot.* **62**, 337–341.
20. Ouzounidou G., Moustakas M. and Strasser R.J. (1997). Sites of action of copper in the photosynthetic apparatus of maize leaves: kinetic analysis of chlorophyll fluorescence, oxygen evolution, absorption changes and thermal dissipation as monitored by photoacoustic signals. *Aust. J. Plant Physiol.* **24**, 81–90.
21. Bjorkman O. (1981). Responses to different quantum flux densities. In *Encyclopedia of Plant Physiology,* new ser., eds O.L. Lange, P.S. Nobel, C.B. Osmond and H. Zeigler, **12A**, 57–107.
22. Farquhar G.D. and Caemmerer von S. (1982). Modelling of photosynthetic response to environmental conditions. In *Encyclopedia of Plant Physiology,* new ser., eds O.L. Lange, P.S. Nobel, C.B. Osmond and H. Zeigler, 12A, 549–587.
23. Hewitt E.J. (1966). *Sand and Water Culture Methods Used in the Study of Plant Nutrition.* Commonwealth Agricultural Bureaux, Farnham, Hants, U.K.
24. Lichtenhaler H.K. (1987). Chlorophylls and carotenoids: pigments of photosynthetic biomembranes. *Methods Enzymol.* **148**, 349–382.
25. Causton D.R. and Dale M.P. (1990). The monomolecular and rectangular hyperbola as empirical models of the response of photosynthetic rate to photon flux density, with applications to three *Veronica* species. *Ann. Bot.* **65**, 389–394.
26. Strasser R.J., Srivastava A. and Tsimilli-Michael M. (2000). The fluorescence transient as a tool to characterise and screen photosynthetic samples. In *Probing Photosynthesis: Mechanisms, regulation and adaptation,* eds M. Yunus, U. Pathre, P. Mohanty, chap. 25, pp. 445–483. Taylor & Francis, London.
27. van Heerden P.D.R., Strasser R.J. and Kruger G.H.J. (2004). Reduction of dark chilling stress in N_2-fixing soybean by nitrate as indicated by chlorophyll *a* fluorescence kinetics. *Physiol. Plant.* **121**, 239–249.
28. van Heerden P.D.R., De Villiers M.F, Van Staden J. and Kruger G.H.J. (2003). Dark chilling increases glucose-6-phosphate dehydrogenase activity in soybean leaves. *Physiol. Plant.* **119**, 221–230.
29. Strasser R.J. and Tsimilli-Michael M. (2001). Stress in plants, from daily rhythm to global changes, detected and quantified by the JIP-test. *Chimie Nouvelle* (SRC) **75**, 3321–3326.
30. Osmond C.B., Badger M.R., Maxwell K., Bjorkman O. and Leegood R. (1997). Too many photons: photorespiration, photoinhibition and photooxidation. *Trends Plant Sci.* **2**, 119–120.
31. Møller I.M. (2001). Plant mitochondria and oxidative stress: electron transport, NADPH turnover, and metabolism of reactive oxygen species. *Ann. Rev. Plant Physiol. Plant Mol. Biol.* **52**, 561–591.
32. Asada K. (1999). The water–water cycle in chloroplasts: scavenging of active oxygen and dissipation of excess photons. *Ann. Rev. Plant. Physiol. Plant. Mol. Biol.* **50**, 601–639.