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A comparative chlorophyll *a* fluorescence study on isolated cells and intact leaves of *Bouteloua gracilis* (blue grama grass)

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Abstract

Bouteloua gracilis (blue grama grass) is one of the most drought and grazing tolerant plants in the short-grass ecosystem. To obtain information on their photosystem activities, we measured the fast (< 1 s) chlorophyll *a* fluorescence transient (the OJIP curve) from their leaves, and isolated cells grown photoautotrophically in suspension in a culture medium, or with added sucrose. One of our goals was to study the effect of different sucrose concentrations (0, 0.15, 0.3, and 3%) on PSII activity in isolated cells. Our results on cells suspended in culture medium, using the JIP-test, showed a decrease in PSII activity at increasing sucrose concentrations, while the photoautotrophic cells showed an optimal PSII activity, close to that of the leaves. Further, our data on cells grown in 0, 0.15, and 0.3% sucrose, but with added CO_2, and measured while the cells were deposited on filter paper, indicate that supplementary CO_2 can increase the PSII activity in the presence of sucrose, although further research is necessary to understand these results.

Additional key words: kinetic parameters of the O-J, J-I, and I-P phases; osmotic stress; performance index.

Introduction

In photoautotrophic plant cell cultures, isolated cells grow independently, and are suitable for physiological and biochemical studies; further, they are particularly useful in investigating various aspects of photosynthesis. Comparative studies between isolated cells and the intact plants have shown not only similarities, but differences

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Abbreviations: Area (as related to chlorophyll *a* fluorescence induction curve) – area between the OJIP transient and the horizontal line at maximum fluorescence, F_M; Chl – chlorophyll; ChlFI – chlorophyll *a* fluorescence induction; DCMU (diuron) – 3-(3,4-dichlorophenyl)-1,1-dimethylurea; F_K, F_J (fluorescence at the J level), F_I (fluorescence at the I level), and F_P (fluorescence at the P level) – chlorophyll *a* fluorescence at 0.3, 2, 30 ms, and at the peak P of chlorophyll *a* fluorescence induction, respectively; F_M – maximum Chl *a* fluorescence; F_O – minimum Chl *a* fluorescence (fluorescence at the O level); F_V – the (maximum) variable fluorescence, which is $F_M – F_O$; OEC – oxygen-evolving complex; OJIP transient – the Chl *a* fluorescence transient from F_O to F_P; PQ – plastoquinone; PSI – photosystem I; PSII – photosystem II; Q_A and Q_B – primary and secondary plastoquinone electron acceptors of the photosystem II.

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[a] Since 2019, Govindjee (who had earlier published under one name only) is publishing under his new formal name: Govindjee Govindjee. This article honors Reto J. Strasser at his 75th birthday for his outstanding and unique contributions in the use and analysis of chlorophyll *a* fluorescence towards understanding photosynthesis; *see* also Govindjee *et al*. (2019).

between them (Rogers *et al.* 1987, Chang *et al.* 1997, García-Valenzuela *et al*. 2005). Unlike the cells in intact leaves, those under photoautotrophic cultures require elevated concentrations of CO_2 for growth, have different amounts of CO_2 fixation enzymes, and incorporate a larger portion of CO_2 *via* phosphoenolpyruvate carboxylase (Widholm 1992). However, despite those differences, information obtained from photoautotrophic cell cultures has contributed to our understanding of processes in intact plants (García-Valenzuela *et al.* 2005).

Blue grama grass (*Bouteloua gracilis* (Kunth) Lag. ex Griffith), also known as mosquito grass, is a perennial bunchgrass that is widely distributed throughout the central grassland region of North America. It is widely recognized as an important species in a short-grass ecosystem, and is thought to be the most drought and grazing tolerant plant species in the short-grass community. Due to its economic importance and drought-resistance characteristics, this grass has been the subject of intensive ecological and physiological research (Majerus 1975, Brown and Trlica 1977, Monson *et al*. 1986, Aguilera and Lauenroth 1993, Moreno-Gómez *et al*. 2012). The first tissue culture system of blue grama grass was developed by Aguado-Santacruz *et al*. (2001); it was the first report of a chlorophyll (Chl)-containing cell suspension from the family Poaceae.

The cell line used by Aguado-Santacruz *et al*. (2001) is now considered a model for analyzing cellular mechanisms. Considering drought-resistance characteristics and economic importance of the blue grama grass, investigations on the photosynthetic activity of their isolated cells is expected to be of high significance. Since Chl *a* fluorescence is a highly sensitive signature of photosynthesis (Govindjee 1995, 2004; Kalaji *et al.* 2014, 2017), we have used in this study the fast (up to 1 s) Chl *a* fluorescence induction (ChlFI) curves, also known as the OJIP transients (Strasser and Govindjee 1991), where O is for the origin (the minimum fluorescence, F_O), J and I are for two intermediary fluorescence inflections (F_J at 2 ms and F_I at 30 ms), and P is for the peak (F_P). The OJIP transient, from a 5–30 min dark-adapted photosynthetic sample, is measured during illumination with continous light; we note that under saturating light, the P level attains its maximum level (F_M). These curves are especially useful in the study of PSII activity in plants, algae, and cyanobacteria (Papageorgiou and Govindjee 2004, Kalaji *et al.* 2016, Stirbet *et al.* 2018, 2019), since Chl fluorescence from PSI is much lower than that from PSII, and is essentially constant during illumination (Govindjee 1995, 2004; however, *see* Lazár 2013). The rise in Chl fluorescence from the O to the P level, during illumination, has usually been related to the reduction of the electron acceptor Q_A (a one-electron acceptor plastoquinone molecule, which is tightly bound to the D2 protein of PSII) and, in its oxidized state, shown to be a quencher of Chl *a* fluorescence (Duysens and Sweers 1963, Stirbet and Govindjee 2012). After initial 'fast' light reactions in PSII, Q_A is reduced, which then transfers an electron to Q_B, a plastoquinone molecule that is weakly bound on the D1 protein of PSII; in contrast to Q_A, which is a one-electron acceptor, Q_B is a two-electron acceptor. Once Q_B has been fully reduced by the addition of two electrons (after two 'light' reactions), and two protons (one from a nearby amino acid and another from a bicarbonate ion, bound to a non-heme iron; Shevela *et al*. 2012), a plastoquinol (Q_BH_2) molecule is formed, which is then released in the thylakoid membrane, and is replaced by a new plastoquinone (PQ) molecule from the PQ pool (Velthuys and Amesz 1974).

In this work, we have succeded in establishing a photoautotrophic cell culture of blue grama grass. There has been no earlier report on photoautotrophic cell culture from any species in *Graminaceae*; as far as we know, ours is the first one on photoautotrophic cell suspension culture from a grass, and the second for monocots (the first was for *Asparagus officinalis* (Chaumont and Gudin 1985). Experiments on green cells of blue grama grass exposed to different concentrations of polyethylene glycol (PEG 8000) have demonstrated that their Chl content increases with osmotic stress (García-Valenzuela *et al.* 2005). On the other hand, a question arises from these results: Is the increased Chl concentration under osmotic stress accompanied by an increase in photosynthetic activity? To answer this question, we have used fast Chl *a* fluorescence induction (OJIP) curves to investigate certain aspects of the PSII activity in isolated cells of blue grama grass, grown under photoautotrophic conditions, and have compared these with those from cells grown in the presence of different concentrations (*i.e.*, 0.15, 0.3, and 3%) of sucrose, as well as with the intact leaves of the plant. Further, since photoautotrophic cell cultures were shown to need higher CO_2 concentrations for growth, we have also examined the effect, on the PSII activity, of added CO_2 to the cultures of photoautotrophic cells, as well as to the cells containing sucrose – lower than 3%; in this experiment, we have deposited the cells on filter paper, and measured their ChlFI kinetics.

Materials and methods

Plant material: The blue grama grass plants (*Bouteloua gracilis* (Kunth) Lag. ex Griffith) were grown in pots, under greenhouse conditions, where they were exposed to the average maximum and minimum temperatures of 31 and 15°C, respectively; the average maximum PPFD received by the plants at noon was 550 μmol(photons) m^{-2} s^{-1}.

Plant cell culture: A cell line of blue grama grass was obtained by the method developed by Aguado-Santacruz *et al.* (2001). This cell line was grown in suspension in Murashige and Skoog (MS) culture medium supplemented with 1 mg L^{-1} 2,4-D (2,4-dichlorophenoxyacetic acid), 2 mg L^{-1} BAP (6-benzylaminopurine), and 40 mg L^{-1} adenine, and with 0.15, 0.3, and 3% sucrose, and without sucrose (0%) (*i.e.*, under photoautotrophic condition).

In one experiment, cells were grown in flasks containing 25 mL of culture medium, under ambient CO_2 conditions. These cell cultures were kept at 30 ± 1°C on a shaker (90 rpm) under continuous white light [80 μmol(photons) m^{-2} s^{-1}] provided by fluorescent tubes (*Eco Lite*) of 14 W. In another experiment, cells were grown in the presence of a supplementary CO_2 source, except when 3% sucrose was used. We used a two flask system (125 mL each) connected with a rubber tube (adapted from Flores *et al.* 1993): one of the flasks had 25 mL of the respective cell culture, as described by Aguado-Santacruz *et al.* (2001), while the second flask contained carbonate/bicarbonate buffer (3 M $K_2CO_3/KHCO_3$) that provided 3% CO_2.

Chl *a* fluorescence induction measurement: The fast Chl *a* fluorescence transients (*i.e.*, the OJIP curves) were recorded in all the samples, at room temperature, with a *Plant Efficiency Analyzer* (*PEA*) fluorometer (*Hansatech Instruments Ltd.*, UK). The PPFD of the red actinic flash ($\lambda_{excitation}$= 650 nm) was 3,000 µmol(photons) m^{-2} s^{-1}, and the fluorescence signal ($\lambda_{emission}$ > 700 nm) was recorded for one second, at an acquisition rate of 10 µs for the first 2 ms, and every 1 ms thereafter.

Details of measurements on three different types of samples of blue grama grass follow.

(*1*) Leaves: The fast Chl *a* fluorescence transient was measured on four young, fully expanded leaves of potted blue grama grass plants, after 1-h dark adaptation.

(*2*) Cells in suspension: We used 28-d old cells, grown under ambient CO_2 conditions, and suspended in culture medium with 0, 0.15, 0.3, and 3% sucrose; these suspensions were dark-adapted for 30 min prior to fluorescence measurement. Measurements were made in five replicates, with one Erlenmeyer flask for one replicate.

(*3*) Cells deposited on filter paper: 28-d old cells, grown in the presence of supplementary CO_2, as well as the cells grown in 3% sucrose without added CO_2, were collected from each flask and placed on a piece of filter paper to form a thin layer, then a leaf clip was placed on it to provide darkness for 30 min. The filter paper was moistened by capillary with the same culture medium and placed in a Petri dish to prevent moisture loss; here also, we had five replicates, each Erlenmeyer flask serving as a single replicate.

Analysis of chlorophyll fluorescence transients

Normalization and subtraction of Chl fluorescence induction data: The OJIP transients were plotted on a logarithmic time scale, in order to observe clearly the O-J and J-I phases, which are not visible on a linear time scale. Further, in order to compare fluorescence curves from different samples, all data were normalized to F_O, the minimum fluorescence. We also used double normalization, *i.e.*, normalization between two fluorescence values, such as F_O and F_M, to obtain relative variable fluorescence, $V_{OP} = (F_t - F_O)/(F_M - F_O)$, where F_t is fluorescence at time t. Besides the F_O and F_M, we also used the F_J and F_I to calculate double normalized transient curves: (*1*) $W_{OJ} = (F_t - F_O)/(F_J - F_O)$; (*2*) $W_{JI} = (F_t - F_J)/(F_I - F_J)$; and (*3*) $W_{IP} = (F_t - F_I)/(F_M - F_I)$. Furthermore, in order to emphasize dissimilarities between double normalized curves characterizing two different samples, we have calculated difference curves: *e.g.*, $\Delta W_{OJ} = [(F_t - F_O)/(F_J - F_O)]_{Sample1} - [(F_t - F_O)/(F_J - F_O)]_{Sample2}$.

Analysis of O-J, J-I, and I-P phases of Chl fluorescence transient: The graphs of V_{OP}, W_{OJ}, and W_{JI} (from 0 to 2 ms; 2 to 30 ms; and 30 ms to t_{Fm}, respectively) were used to compare the amplitudes of the O-J, J-I, and I-P phases (*i.e.*, A_{OJ}, A_{JI}, and A_{IP}) in each sample. Further, we used the graphs of W_{OJ}, W_{JI}, and W_{IP} to determine the half-time ($t_{1/2}$) of the fluorescence rise in each phase (*see* Fig. 1S, *supplement*); then, the rate constants of PSII closure during each phase (k_{OJ}, k_{JI}, and k_{IP}) were calculated as the inverse of the respective values of $t_{1/2}$.

JIP-parameters calculated from Chl fluorescence data: A different set of fluorescence parameters were calculated from the OJIP transients by using the method of Strasser and Strasser (1995) and Tsimilli-Michael and Strasser (2008), which is based on the general concepts of energy fluxes in the photosynthetic apparatus (Strasser 1978). The so-called JIP-parameters, obtained from the analysis of all data, are proxies of different characteristics of PSII and of the photosynthetic electron transport.

For definitions of the set of JIP-parameters used in the analysis of OJIP Chl fluorescence transients, *see* Strasser *et al.* (2004), Stirbet and Govindjee (2011). The symbols and the meaning of most of the JIP-parameters, used in this paper, are described below in the following text table. O is for origin (the minimum fluorescence, F_O), J and I for two intermediate levels at 2 ms and 30 ms (F_J and F_I), and P for peak (F_P, or F_M when the fluorescence is maximal); RC is for the number of active PSII reaction centers in the measured area; and Q_A is for the first plastoquinone electron acceptor of PSII.

Chlorophyll fluorescence parameters	Meaning
$F_v = F_t - F_O$	Variable fluorescence
$F_V = F_M - F_O$	Maximum variable fluorescence
$V_{OP} = F_v/F_V$	Relative variable fluorescence
$M_0 = (\Delta V/\Delta t)_0 \approx 4(F_{0.3ms} - F_{0.05ms})/F_V$	Initial slope (in ms^{-1}) of the O to J fluorescence rise
$S_m = Area/F_V$	Normalized area between the OJIP curve and the line $F = F_M$
Energy fluxes	
ABS	Photon flux absorbed by PSII antenna
TR	Part of ABS trapped by the active PSII, leading to Q_A reduction
DI = ABS – TR	Part of ABS dissipated in PSII antenna in processes other than trapping
ET	Energy flux associated with the electron transport from Q_A^- to intersystem electron acceptors

RE	Energy flux associated with the electron transport from Q_A^- to final electron acceptors of PSI
Specific energy fluxes	
$ABS/RC = (M_0/V_J)/(F_V/F_M)$	Absorbed photon flux per active PSII
$TR_0/RC = M_0/V_J$	Trapped energy flux per active PSII
$DI_0/RC = ABS/RC - TR_0/RC$	Dissipated energy (as heat and fluorescence) flux per active PSII
$ET_0/RC = (M_0/V_J)(1 - V_J)$	Electron flux from Q_A^- to the PQ pool per active PSII
$RE_0/RC = (M_0/V_J)(1 - V_I)$	Electron flux from Q_A^- to the final electron acceptors of PSI per active PSII
Quantum yields and efficiencies	
$TR_0/ABS = F_V/F_M$	Maximum quantum yield of PSII photochemistry
$DI_0/ABS = 1 - TR_0/ABS$	Quantum yield of energy dissipation (as heat and fluorescence) in PSII antenna
$ET_0/ABS = (F_V/F_M)(1 - V_J)$	Quantum yield of electron transport from Q_A^- to the PQ pool
$RE_0/ABS = (F_V/F_M)(1 - V_I)$	Quantum yield of electron transport from Q_A^- to final electron acceptors of PSI
$ET_0/TR_0 = 1 - V_J$	Efficiency with which a PSII trapped electron is transferred from Q_A^- to the PQ pool
$RE_0/TR_0 = 1 - V_I$	Efficiency with which a PSII trapped electron is transferred from Q_A^- to final electron acceptors of PSI
$RE_0/ET_0 = (1 - V_I)/(1 - V_J)$	Efficiency with which electrons from the PQ pool are transferred to final electron acceptors of PSI
Performance index	
$PI_{abs} = (RC/ABS)\cdot[(TR_0/ABS)/(1 - TR_0/ABS)]\cdot[(ET_0/TR_0)/(1 - ET_0/TR_0)]$	Performance index on absorption basis

Statistical analysis: Significant differences of the mean values of different fluorescence parameters were evaluated between control (cells grown in photoautotrophic conditions) and cells treated with sucrose, or the leaf, with the *Kruskal-Wallis* or the *K-sample T*-test, by using the '*Location Equivalence Test*' package of the software *Mathematica*. These calculations were done separately for samples measured in suspension and those deposited on the filter paper; we note that for *p* values lower than 0.05 ($p<0.05$), differences between the means were considered to be statistically significant.

Results and discussion

Chl fluorescence induction data

The OJIP curves measured in blue grama grass leaves and cells grown in 0, 0.15, 0.3, and 3% sucrose are presented in Fig. 1, both for cells measured in suspension in the culture medium (Fig. 1*A*), and those deposited on the filter paper (Fig. 1*B*). Here, we have assumed that differences in the initial Chl fluorescence (F_O) (measured with direct light) from the blue grama grass cells are mainly due to differences in Chl concentration between the samples (*see e.g.*, Strasser *et al*. 2004). This is a reasonable assumption since the PQ pool is expected to be all in the oxidized state (*see* discussion in Stirbet *et al*. 2019; *cf.* Feild *et al*. 1998 for information on chlororespiration that could, in principle, affect it). Data in Fig. 1*A* suggest that the isolated cells grown in 3% sucrose had a significantly higher Chl concentration than the cells grown photoautotrophically, or in lower sucrose concentration. This is in agreement with the observation of García-Valenzuela *et al.* (2005), who found that the Chl content of isolated green cells increases with the osmotic stress. Further, the results shown in Fig. 1*B* suggest that the samples deposited on filter paper had a higher Chl concentration than those measured in suspension, including the sample with 3% sucrose. Since, unlike the other cells, the sample with 3% sucrose was grown without added CO_2, the F_O increase in these cells cannot be attributed to a higher Chl content induced by added CO_2, but rather to a higher density of the cells deposited on filter paper than of those measured in suspension.

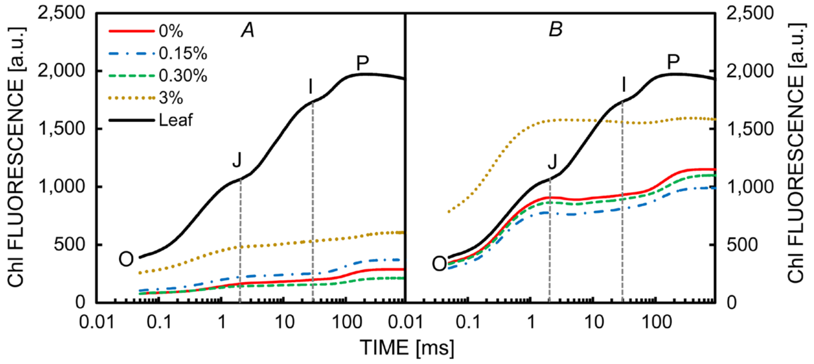

Fig. 1. Chlorophyll *a* fluorescence induction curves measured in dark-adapted blue grama grass (leaf), as well as in cell suspensions in culture medium with 0, 0.15, 0.3, and 3% sucrose concentrations (*A*), or deposited on filter paper (*B*). Fluorescence data were obtained with a *PEA* instrument (*Hansatech*) during one second illumination with saturating [3,000 µmol(photons) m^{-2} s^{-1}] red light (λ_{excit} = 650 nm). Data represent average of five independent measurements.

OJIP induction curves normalized at F_O

ChlFI curves normalized at F_O emphasize differences between the relative maximum variable fluorescence ($F_V/F_O = (F_M - F_O)/F_O$); this is consistent with the trend of F_V/F_M variation in different samples, observed by Essemine *et al.* (2017). The curves in Fig. 2 show that all isolated blue grama grass cells had a significantly lower F_V/F_O ratio compared with that in blue grama grass leaves; however, the photoautotrophic cells measured in suspension had a higher F_V/F_O compared to cells grown in sucrose, for which F_V/F_O decreased at increasing sucrose concentrations (Fig. 2*A*). On the other hand, all the cells deposited on filter paper had similar F_V/F_O values, with the exception of those grown in 3% sucrose, which had the lowest F_V/F_O, both in cells deposited on the filter paper or those in suspension in culture medium (Fig. 2). The similarity between the ChlFI curves and their F_V/F_O values in samples grown in culture medium with 0, 0.15, and 0.3% sucrose, and deposited on filter paper, may be due to the additional CO_2 provided during the growing period, which was not the case for any of the cells measured in suspension, or for the cells grown in 3% sucrose, which were grown under atmospheric CO_2.

OJIP induction curves double normalized at F_O and at F_M

Fig. 3 shows ChlFI curves normalized between F_O and F_M (*i.e.*, the relative variable fluorescence, $V_{OP} = (F_t - F_O)/F_V$) representing the kinetics of PSII closure from all PSIIs being open ($V_O = 0$) to all PSIIs closed ($V_P = 1$).

The O-J fluorescence rise, observed in ChlFI curves plotted on a logarithmic time scale, is the 'photochemical' phase of the OJIP transient, when mainly Q_A is reduced; this step is dependent on light intensity, but much less on temperature. The subsequent rise in fluorescence, *i.e.*, the J-I-P phase, is the 'thermal' phase, as it is sensitive to temperature. It involves the reduction of the PQ pool *via* the double reduction of Q_B and exchange with a new PQ from the pool (*see* Introduction). Further, during the IP phase, there is a bottle-neck in electron flow beyond PSI, due to a transient inactivation of the Calvin-Benson cycle, which limits the consumption of NADPH (Munday and Govindjee 1969). Thus, at the F_M level, all the components of the photosynthetic electron transport chain are reduced.

The normalized area between the OJIP curve and the line $V_{OP} = 1$ (*i.e.*, S_m; *see* the text table in Materials and methods) is shown in Fig. 3*B*,*D*, where Chl fluorescence was plotted on a linear time scale. S_m is proportional to the number of electrons transferred from Q_A^- into the electron transport chain during the OJIP rise, and thus, also with EC/RC, the number of electron carriers per active PSII reaction centre (Malkin and Kok 1966, *cf.* Lavergne and Trissl 1995). These data show that, with the exception of the cells in 3% sucrose deposited on filter paper, the S_m (and thus EC/RC) in all isolated cells was larger than in leaves. The increased S_m in isolated cells may be due to an unusually large PQ pool (or to the presence of unknown exogeneous electron carriers similar to PQ), since the oxidation/reduction kinetics of the plastoquinone pool has been shown to control the appearance of the I-peak (Joly and Carpentier 2007, 2009). Indeed, the OJIP transients measured by Joly and Carpentier (2009), on isolated intact chloroplasts treated with decyl-plastoquinone (dPQ) (*see* their figure 6), are comparable with those measured here on isolated blue grama grass cells: in the presence of dPQ, both F_O and F_M were lowered and the apparent I level was considerably decreased, while the time to reach the P level was retarded. A larger electron acceptor pool size thus explains the significant increase in t_{Fm} (*i.e.*, the time to reach F_M) in isolated cells compared to that in leaves; the t_{Fm} ranged from ~ 200 ms to > 600 ms (Fig. 3*B*,*D*). However, the cells deposited on filter paper had a smaller S_m than those in suspension in the culture medium (Fig. 3*B*)

Analysis of the K-band

Stress induced by high temperature or drought is known to lead to the appearance of a new inflection (or a maximum) at 0.3 ms in the ChlFI transient, labeled as the K-step (F_K; Guissé *et al.* 1995, Lazár *et al.* 1997, De Ronde *et al.* 2004, Oukarroum *et al.* 2013), which was attributed to the inactivation of the oxygen-evolving complex (OEC) of PSII resulting from its partial functional disconnection from the electron transfer chain (Strasser 1997). Further, Strasser *et al.* (2004) found that low to moderate inactivation of the OEC (when the K-step is not yet visible) can be detected by using the difference between the double normalized ChlFI curves of a stressed and nonstressed plant samples (*i.e.*, $\Delta W_{OJ} = \Delta[(F_t - F_O)/(F_J - F_O)]$); in this difference curve, a positive K-band ~ 0.3 ms is observed.

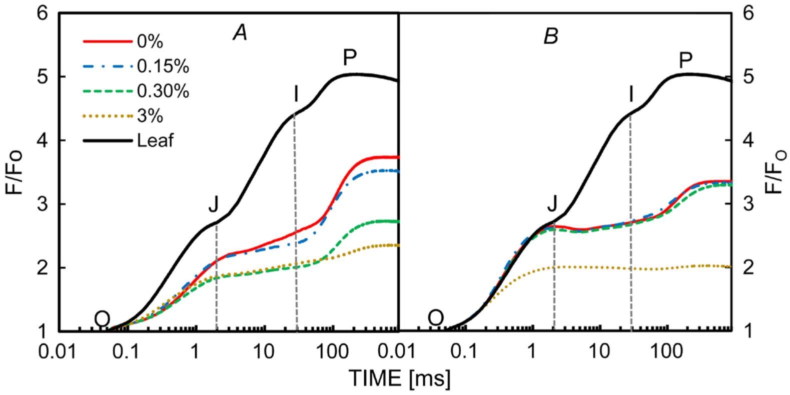

Fig. 2. Chlorophyll *a* fluorescence induction curves normalized to the initial fluorescence (F_O), calculated for dark-adapted blue grama grass (leaf), as well as for isolated cells in suspension in culture medium with 0, 0.15, 0.3, and 3% sucrose concentrations (*A*), or deposited on filter paper (*B*).

In Fig. 4, we show the ΔW_{OJ} graphs for the isolated cells of blue grama grass measured in suspension (Fig. 4*A*), and those measured deposited on the filter paper (Fig. 4*B*), where the control was the leaf, which was assumed to have a normal OEC. As seen in Fig. 4*A*, the K-bands for the cells in suspension are negative, not positive, with the exception of the cells with 3% sucrose; thus, we may assume that OEC is slightly inactivated by the osmotic stress only in the presence of 3% sucrose. Since negative K-bands are the result of a steeper fluorescence increase in the control (leaf), they may be related to differences in PSII antenna size, as suggested by Yusuf *et al.* (2010); *see* also discussion in Stirbet *et al*. (2014). On the other hand, the K-bands for the cells deposited on filter paper are all positive (Fig. 4*B*), indicating low to moderate inactivation of OEC by a possible dehydration of the samples when measured deposited on filter paper. These data suggest that the OEC activity in photoautotrophic cells deposited on filter paper was less affected compared to that in the cells grown with added sucrose: 3% sucrose gave the highest OEC inactivation.

Analysis of the O-J, J-I and I-P phases of the OJIP transient

An excellent method to analyze the ChlFI data, is to fit the OJIP curve with a sum of three exponential functions (Pospíšil and Dau 2000):

$$F_t = F_O + A_{OJ} \cdot (1 - e^{-k_{OJ} t}) + A_{JI} \cdot (1 - e^{-k_{JI} t}) + A_{IP} \cdot (1 - e^{-k_{IP} t})$$

where F_t is fluorescence at time t, F_O is initial fluorescence, while A_{OJ}, A_{JI}, and A_{IP} are the amplitudes, and k_{OJ}, k_{JI}, and k_{IP} are the rate constants of the O-J, J-I and I-P phases. This method has been used by many others (Pospíšil and Dau

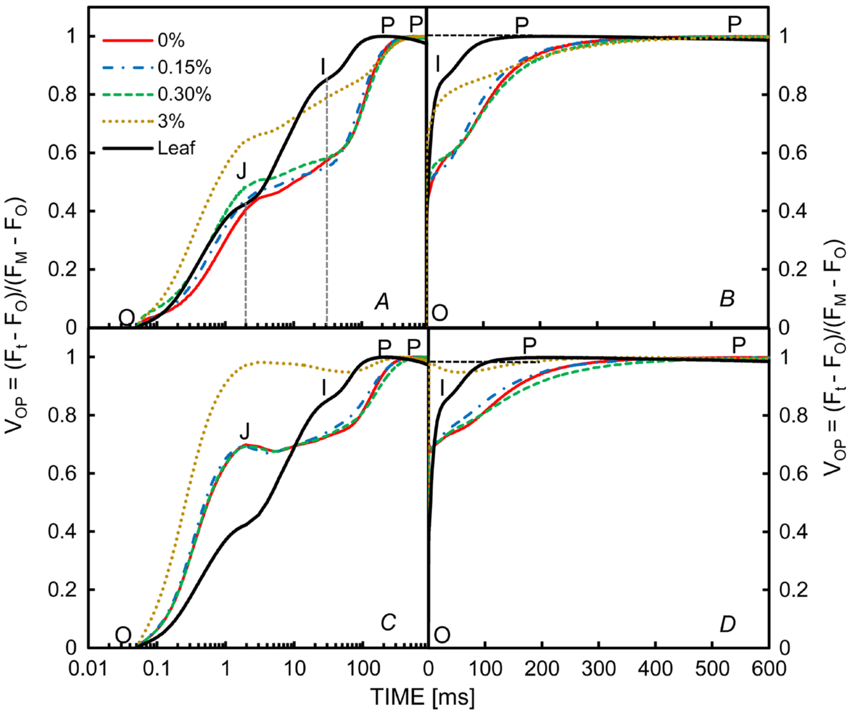

Fig. 3. Chlorophyll *a* fluorescence induction curves double normalized to the initial (F_O) and maximum (F_M) fluorescence, calculated for dark-adapted blue grama grass (leaf), as well as for isolated cells in suspension in culture medium with 0, 0.15, 0.3, and 3% sucrose concentrations (*A*,*B*), or deposited on filter paper (*C*,*D*). In (*A*) and (*C*) the curves are shown on a logarithmic time scale, and in (*B*) and (*D*) on a linear time scale.

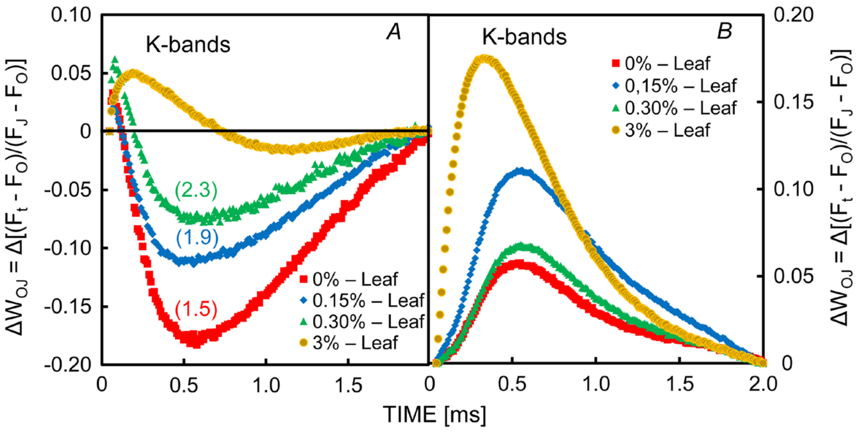

Fig. 4. Plots of difference (ΔW_{OJ}) between the double normalized O-J fluorescence rise for isolated cells of blue grama grass in suspension in culture medium with 0, 0.15, 0.3, and 3% sucrose concentrations (*A*), and of cells deposited on filter paper (*B*), and that of dark-adapted blue grama grass leaves as control. The K-band(s) emphasized in these graphs may be related either to a partial inactivation of the oxygen-evolving complex (when positive; Strasser *et al.* 2004) or to differences in the apparent PSII antenna (Yusuf *et al.* 2010).

2002, Boisvert *et al.* 2006, Antal and Rubin 2008, Joly and Carpentier 2009, Hamdani *et al.* 2015) to characterize photosynthetic electron transport under different treatments. As an alternative to this approach, we have used here other graphical methods (*see* Materials and methods and Fig. 1S) to evaluate the A_{OJ}, A_{JI}, and A_{IP} and their respective rate constants k_{OJ}, k_{JI}, and k_{IP} (calculated as the reverse of the half-time of fluorescence rise, $k = 1/t_{½}$, during each phase), by assuming that the J- and I-steps take place at 2 ms and 30 ms (Strasser and Strasser 1995). The above parameters for isolated cells measured in suspension or deposited on filter paper, as well as for the leaf, are shown comparatively in Fig. 5. Note that for isolated cells deposited on filter paper in 3% sucrose, only the parameters related to the O-J phase are shown, since the ChlFI curve in this case was mostly an OJ rise (*see* Fig. 3*C*), similar to that in samples treated with diuron [DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea]. The numerical values of these parameters are shown in Table 1.

Measurements on blue grama grass cells in suspension

The amplitudes of the O-J, J-I, and I-P phases for blue grama grass leaves, and for cells measured in suspension, are shown in Fig. 5*A*. The A_{OJ} for the leaf was comparable with that of the photoautotrophic cells. However, for the cells grown with added sucrose, the A_{OJ} increased with sucrose concentration, its relative value (calculated as $[A_{OJ}]_{sample}/[A_{OJ}]_{0\%}$) reaching the value of 1.6 for cells grown in 3% sucrose. The amplitude of the J-I phase for isolated cells was much smaller than for the leaf (which had a relative A_{JI} of 2.6), the cells grown in 0.15 and 0.3% sucrose having the smallest relative A_{JI}, of ~ 0.67. On the other hand, the amplitudes of the I-P phase in isolated cells, grown in 0, 0.15, and 0.3% sucrose, were relatively similar, but much higher than in the leaf and in cells grown in 3% sucrose, which had relative A_{IP} of 0.35 and 0.5, respectively.

The rate constants of the O-J, J-I, and I-P phases (Table 1, Fig. 5*B–D*) decreased in the order $k_{OJ} > k_{JI} > k_{IP}$ in all the samples. For the cells in suspension, the k_{OJ} had the smallest value in photoautotrophic cells, but increased with sucrose concentration, so that the relative k_{OJ} (*i.e.*, $[k_{OJ}]_{sample}/[k_{OJ}]_{0\%}$) of the cells in 3% sucrose was even higher than in leaf (1.8 compared to 1.6). Further, the relative k_{JI} for cells, grown in 0, 0.3 and 3% sucrose, were more or less similar, but lower than in the leaf (which had a value of 1.2), while the cells grown in 0.15% sucrose had the highest value (*i.e.*, 1.5). And finally, in all the isolated cells, the relative k_{IP} had values of 1.3, 1, and 0.75 for 0.15, 0.3 and 3% sucrose, but were significantly lower than in the leaf, which had a relative k_{IP} of 2.5.

The above results support the idea, presented earlier, of an enlarged size of the acceptor pool of PSII in isolated cells measured in suspension than in the leaf. Indeed, the PSII acceptor pool is mainly reduced during the I-P phase, and not only the values of A_{IP} were significantly higher than in the leaf in all the samples (Fig. 5*A*), but all the k_{IP} values in isolated cells were significantly smaller than in the leaf (Fig. 5*D*). On the other hand, since the A_{OJ} and the rate constant k_{OJ} had higher values in cells in suspension at higher sucrose concentrations, indicating a slower rate of oxidation of the reduced Q_A, we suggest that, besides the photoautotrophic cells, all the cells in suspension in culture medium containing sucrose had an increased number of Q_B-nonreducing PSII centers, compared to those in the leaf. It is well known that PSII *in vivo* is heterogeneous, especially in antenna size, energetic connectivity, and types of inactivation. Further, Chl fluorescence induction has been used as a tool to study this heterogeneity (Melis 1985, Hsu and Lee 1991, Strasser and Stirbet 1998, Lazár *et al.* 2001). The nature and extent of PSII heterogeneity

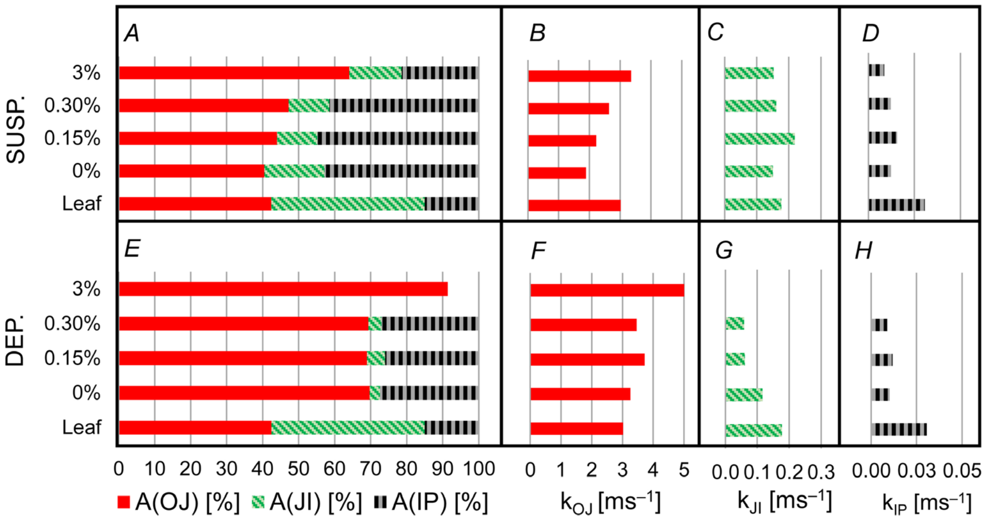

Fig. 5. The amplitudes A_{OJ}, A_{JI}, and A_{IP} (expressed as percentage of the maximum variable fluorescence $F_V = F_M - F_O$), the half-time fluorescence rise $t_{½}$, and the rate constants k_{OJ}, k_{JI}, and k_{IP} of the O-J, J-I, and I-P phases of the chlorophyll fluorescence transients OJIP: for cells measured in suspension (Susp.) in culture medium with 0, 0.15, 0.3, and 3% sucrose concentrations (panels *A–D*), and cells measured deposited (Dep.) on filter paper (panels *E–H*), as well as for blue grama grass (leaf). The parameters are the mean of five replicates.

Table 1. The amplitudes (A_{OJ}, A_{JI}, and A_{IP}, expressed as percentage of the maximum variable fluorescence $F_V = F_M - F_O$), the half-time fluorescence rise $t_{½}$, and the rate constants k_{OJ}, k_{JI}, and k_{IP} of the O-J, J-I, and I-P phases of the relative variable chlorophyll fluorescence transients for blue grama grass leaves (leaf), as well as for cells in suspension in culture medium with 0, 0.15, 0.3, and 3% sucrose (Susp.), and deposited on filter paper (Dep.). The parameters are the mean of five replicates ± SE. nd – not determined. *, $^\#$ – statistically significant result, $p<0.05$, for suspended and deposited samples, respectively.

Parameter	Leaf		0% sucrose	0.15% sucrose	0.3% sucrose	3% sucrose
A_{OJ} [%]	42.40 ± 1.10$^{\#}$	Susp.	40.50 ± 1.10	44.00 ± 1.20	48.20 ± 1.10*	64.00 ± 0.73*
		Dep.	70.00 ± 3.70	69.00 ± 2.40	69.00 ± 0.80	97.00 ± 4.00$^{\#}$
$t_{½}$ [ms]	0.33 ± 0.01*	Susp.	0.53 ± 0.01	0.45 ± 0.01*	0.40 ± 0.01*	0.30 ± 0.01*
		Dep.	0.31 ± 0.01	0.27 ± 0.01$^{\#}$	0.30 ± 0.01	0.20 ± 0.01$^{\#}$
k_{OJ} [ms^{-1}]	3.00 ± 0.10*	Susp.	1.90 ± 0.03	2.20 ± 0.05*	2.59 ± 0.05*	3.35 ± 0.10*
		Dep.	3.26 ± 0.10	3.70 ± 0.10$^{\#}$	3.46 ± 0.10	5.00 ± 0.37$^{\#}$
A_{JI} [%]	42.60 ± 1.00$^{*\#}$	Susp.	16.70 ± 0.04	11.20 ± 0.50*	9.70 ± 1.60*	14.60 ± 0.40*
		Dep.	3.00 ± 1.80	5.00 ± 1.60	3.60 ± 0.60	nd
$t_{½}$ [ms]	5.65 ± 0.10$^{*\#}$	Susp.	6.80 ± 0.06	4.64 ± 0.23*	6.22 ± 0.02	7.00 ± 0.50
		Dep.	15.40 ± 3.30	17.00 ± 1.60	17.30 ± 1.00	nd
k_{JI} [ms^{-1}]	0.180 ± 0.003$^{*\#}$	Susp.	0.150 ± 0.001	0.220 ± 0.010*	0.160 ± 0.005	0.140 ± 0.010
		Dep.	0.120 ± 0.060	0.060 ± 0.005	0.060 ± 0.003	nd
A_{IP} [%]	15.0 ± 0.40$^{*\#}$	Susp.	42.8 ± 1.0	45.0 ± 1.0	42.0 ± 1.0	21.4 ± 1.0*
		Dep.	27.4 ± 2.0	25.9 ± 1.0	27.0 ± 0.6	nd
$t_{½}$ [ms]	33.0 ± 2.0$^{*\#}$	Susp.	82.0 ± 2.0	66.0 ± 5.0*	88.0 ± 4.6	118.0 ± 0.6*
		Dep.	103.4 ± 10.7	91.4 ± 11.0	116.4 ± 7.6	nd
k_{IP} [ms^{-1}]	0.030 ± 0.002$^{*\#}$	Susp.	0.012 ± 0.001	0.015 ± 0.001*	0.011 ± 0.001	0.008 ± 0.001*
		Dep.	0.010 ± 0.001	0.012 ± 0.001	0.009 ± 0.001	nd

varies under different physiological conditions (Cao and Govindjee 1990, Lavergne and Briantais 1996), such as temperature, salinity, and pH stress (Singh-Tomar *et al.* 2012). The Q_B-nonreducing PSII centers, besides their inability to reduce Q_B, are characterized by a smaller antenna, absence of energetic connectivity, and their localization in stromal thylakoid membranes (Andrée *et al.* 1998).

In addition to what we have presented above, we also analyzed the O-J, J-I, and I-P phases of the V_{OP} curves of isolated cells in suspension in culture medium by fitting their data separately with the first order kinetic function $A\ (1 - e^{-kt})$, where A and k are the amplitude and rate constant of the respective phase. The fitted rate constants (data not shown) were somewhat lower than those shown in Fig. 5 (and Table 1) in all samples, but their values followed the same trend. We speculate that the higher rate constants of the fluorescence rise, estimated using the experimental ChlFI curves, may be due to the effect of PSII excitonic connectivity on Q_A reduction rate (Strasser and Stirbet 2001, Stirbet 2013), which is neglected when a first order kinetics is used as fitting function for the O-J, J-I, and I-P phases.

Measurements on blue grama grass cells deposited on filter paper

We note that for the cells grown in 3% sucrose and deposited on filter paper, the ChlFI curves showed mainly an O-J phase, similar to that in samples treated with DCMU. The amplitudes of the O-J, J-I, and I-P phases for cells measured deposited on filter paper, as well as for the blue grama grass leaf, are shown in Fig. 5*H* (*cf.* Table 1): (*1*) the values of A_{OJ} in cells grown in 0, 0.15, and 0.3% sucrose were very similar, but higher than in isolated cells measured in suspension (Fig. 5*A*), or in the leaf (which had a relative A_{OJ} of ~ 0.6, calculated considering the deposited photoautotrophic cells as control), while in cells grown in 3% sucrose, the relative A_{OJ} was ~ 1.3; (*2*) the relative A_{JI} values in cells grown in 0, 0.15 and 0.3% sucrose were much lower than in isolated cells measured in suspension (Fig. 5*A*), or in the leaf (which had a relative A_{JI} of 14.7); and (*3*) the A_{IP} in cells grown in 0, 0.15 and 0.3% sucrose had closer values, but clearly lower than those in isolated cells measured in suspension with 0, 0.15, and 0.3% sucrose (Fig. 5*A*), and higher than in the leaf (which had a relative A_{IP} of ~ 1.8).

The rate constants of the O-J, J-I, and I-P phases for the cells, deposited on filter paper, are shown in Fig. 5*F–H*. The rate constants of the O-J phase in these samples were higher than in cells in suspension (Fig. 5*B*), and in the leaf. The relative k_{OJ} (*i.e.*, $[k_{OJ}]_{sample}/[k_{OJ}]_{0\%}$) were 1.14, 1.06, and 1.5, for cells grown in 0.15, 0.3, and 3% sucrose, respectively. Further, the k_{JI} and k_{IP} for all the cells deposited on filter paper were clearly lower than in cells in suspension and in the leaf (Fig. 5*C*,*D*).

From the above data, we emphasize that, for cells deposited on filter paper, A_{OJ} and k_{OJ} were higher, while A_{JI} and the k_{JI} were much lower than in cells in suspension. These changes suggest an increase in Q_B-nonreducing PSII centers in these samples. Also, the cells deposited on filter paper had smaller A_{IP} than in cells in suspension; we assume that this may be due to a smaller PSII acceptor pool here than in cells in suspension.

JIP-test analysis of the OJIP transients

The OJIP curves have been widely used to estimate the maximum quantum yield of PSII by using the F_V/F_M ratio (Kitajima and Butler 1975), where F_V (= $F_M - F_O$) is the (maximum) variable Chl *a* fluorescence. Furthermore, other fluorescence parameters, defined in the so-called JIP-test (Strasser and Strasser 1995, Strasser *et al.* 1999, 2000, 2004; Tsimilli-Michael and Strasser 2008, also *see* Stirbet and Govindjee 2011), and calculated by using the F_K, F_J, and F_I, in addition to F_O and F_M, are often used to characterize the PSII activity. In order to further analyze the OJIP transients of our samples, we calculated a selected set of JIP-parameters characterizing electron transfer from PSII to the end acceptors of PSI (*see* the text table for definitions and Table 2 for results; *cf.* Fig. 2S, *supplement*).

Measurements on blue grama grass cells in suspension

The JIP-parameters calculated for cells in suspension had significantly different values from those for the leaf. We remind the readers that the maximum quantum yield of PSII photochemistry, as inferred from the ratio F_V/F_M, is equivalent to TR_0/ABS in the JIP-test; we found it to decrease with increased sucrose concentration in isolated cells, while the leaf and the photoautotrophic cells had the highest values, *i.e.*, ~ 0.8 and ~ 0.73 (Table 2). These results are consistent with the observed decrease of F_M/F_O in cultured cells shown in Fig. 2. The lower TR_0/ABS ratio in cells grown with added sucrose suggests an increase in Q_A-nonreducing PSII reaction centers in these samples. Further, DI_0/ABS (= $1 - TR_0/ABS$), which is the quantum yield of excitation energy dissipation in PSII antenna through both fluorescence and heat in dark-adapted samples, increased with sucrose concentration in isolated cells, having the smallest value in the leaf.

On the other hand, the specific trapping flux TR_0/RC, which is the fraction of ABS/RC used for Q_A reduction (with $ABS/RC = TR_0/RC + DI_0/RC$), had the largest value in cells grown in 3% sucrose (~ 1.8) compared to the leaf (~ 1.6), while in all other isolated cells, ABS/RC was smaller than in the leaf, with the smallest value in photoautotrophic cells (~ 1.1). Since DI_0/ABS had the lowest value in photoautotrophic cells, the low TR_0/RC in these cells is most probably due to a greater number of active PSII RCs (*i.e.*, Q_A-reducing PSII centers) than in cells grown in medium with sucrose, with the cells grown in 3% sucrose having the lowest number of active PSII. Indeed, ABS/RC in cells grown in 3% sucrose was ~ 1.53 times higher than in the leaf, while TR_0/RC was only 1.1 times higher. This type of result is often assumed to be due to the inhibition of a fraction of PSII units, which still participate in light absorption, but dissipate totally or partially the absorbed energy since they cannot reduce Q_A (for PSII inhibition, *see* Hendrickson *et al.* 2005, Sarvikas *et al.* 2010, Kou *et al.* 2012). The above results indicate that the fraction of Q_A-nonreducing PSII units increases in the presence of sucrose, and PSII antenna size in photoautotrophic cells, as well as in those cells grown in lower sucrose concentrations, is lower compared to that in the leaf. Indeed, the specific absorption flux ABS/RC (where ABS is the absorbed photon flux and RC is the number of active PSII units), which is a measure of the apparent PSII antenna size, was higher in cells grown in 3% (~ 3.1) and 0.3% (~ 2.3) sucrose, compared to the leaf (~ 2.0), but it was lower (~ 1.5) in photoautotrophic cells or those grown in 0.15% sucrose (~ 1.8); *see* Fig. 4*A*.

Further, the electron transport from reduced Q_A to Q_B is characterized by ET_0/ABS, ET_0/TR_0, and ET_0/RC (*see* the text table). These parameters for the cells in suspension with added sucrose had lower values than in the leaf, with the cells grown in 3% sucrose having the lowest values of these JIP-parameters; this is often due to the presence of Q_B-nonreducing PSII, and manifested by an increase in V_J (Fig. 3*B*). On the other hand, ET_0/ABS and ET_0/TR_0 in photoautotrophic cells were very similar to those in the leaf, with the exception of ET_0/RC, which had the smallest value in all the samples. However, this can be explained by a lower ABS/RC in the photoautotrophic cells, *i.e.*, due to a smaller antenna size. These results indicate, again, a higher number of Q_A-active PSII centers in the photoautotrophic cells than in those grown with added sucrose. Therefore, the impediment in electron transfer from reduced Q_A to Q_B, which leads to a slower PQ pool reduction, is probably caused by the presence of sucrose.

Several parameters, labeled as RE_0/ABS, RE_0/RC, and RE_0/ET_0, are known to characterize electron transport to the end electron acceptors of PSI (*see* the text table). As shown in Table 2, these parameters are higher than in leaf for all the cells in suspension, due to their low $V_I = (F_I - F_O)/F_V$ (Fig. 3*A*). Beside the presence of a larger acceptor pool of PSII, this can also suggest that these samples may be in the so-called State 2, when part of PSII antenna attaches to PSI antenna, and the fluorescence intensity is low (Papageorgiou and Govindjee 2011).

The performance index on absorption basis, PI_{abs}, is often used in studies related to plant stress (Strasser *et al.* 1999, Stirbet *et al.* 2018), and has been defined as performance index for energy conservation from photons absorbed by PSII until the reduction of intersystem electron acceptors; its value for the photoautotrophic cells in suspension and the leaf were found to be essentially the same (*i.e.*, ~ 27), but it decreased gradually in cells in suspension with sucrose (~ 18.5, ~ 8, and ~ 2.5 for cells grown in 0.15, 0.3 and 3% sucrose). Compared with the F_V/F_M ratios, which is also widely used in stress studies, the descending trend of PI_{abs} in samples with different sucrose concentrations was steeper, which shows that PI_{abs} is a much more sensitive parameter than F_V/F_M (= TR_0/ABS) to identify stressed samples.

Table 2. JIP-parameters for blue grama grass (leaf), as well as for isolated cells in suspension (Susp.) in culture medium with 0, 0.15, 0.3 and 3% sucrose, and cells deposited on filter paper (Dep.) (*see* the text table for definitions). The parameters are the mean of five replicates ± SE. nd – not determined. *, $^\#$ – statistically significant result, $p<0.05$, for suspended and deposited samples, respectively.

Parameter	Leaf		0% sucrose	0.15% sucrose	0.3% sucrose	3% sucrose
ABS/RC	2.02 ± 0.05$^{*\#}$	Susp.	1.50 ± 0.03	1.77 ± 0.03*	2.28 ± 0.08*	3.10 ± 0.11*
		Dep.	2.50 ± 0.08	2.70 ± 0.08	2.53 ± 0.07	4.60 ± 0.30$^\#$
RC/ABS	0.49 ± 0.01$^{*\#}$	Susp.	0.67 ± 0.01	0.56 ± 0.01*	0.44 ± 0.01*	0.30 ± 0.01*
		Dep.	0.40 ± 0.01	0.37 ± 0.01	0.39 ± 0.01	0.22 ± 0.02$^\#$
TR_0/ABS	0.800 ± 0.001$^{*\#}$	Susp.	0.730 ± 0.003	0.720 ± 0.004*	0.640 ± 0.006*	0.580 ± 0.010*
		Dep.	0.700 ± 0.010	0.700 ± 0.005	0.700 ± 0.001	0.510 ± 0.015$^\#$
DI_0/ABS	0.200 ± 0.001$^{*\#}$	Susp.	0.270 ± 0.003	0.280 ± 0.004*	0.360 ± 0.006*	0.420 ± 0.010*
		Dep.	0.300 ± 0.010	0.300 ± 0.005	0.300 ± 0.001	0.490 ± 0.015$^\#$
ET_0/ABS	0.460 ± 0.010$^\#$	Susp.	0.440 ± 0.009	0.400 ± 0.010*	0.330 ± 0.009*	0.200 ± 0.002*
		Dep.	0.210 ± 0.020	0.220 ± 0.020	0.210 ± 0.005	0.014 ± 0.025$^\#$
RE_0/ABS	0.120 ± 0.035$^{*\#}$	Susp.	0.310 ± 0.008	0.320 ± 0.010	0.270 ± 0.008*	0.120 ± 0.003*
		Dep.	0.190 ± 0.015	0.180 ± 0.010	0.190 ± 0.006	nd
TR_0/RC	1.62 ± 0.04$^{*\#}$	Susp.	1.10 ± 0.02	1.27 ± 0.02*	1.45 ± 0.04*	1.79 ± 0.05*
		Dep.	1.75 ± 0.04	1.89 ± 0.05	1.76 ± 0.05	2.30 ± 0.10$^\#$
ET_0/RC	0.93 ± 0.01$^{*\#}$	Susp.	0.66 ± 0.01	0.71 ± 0.02*	0.75 ± 0.03*	0.64 ± 0.03
		Dep.	0.53 ± 0.06	0.58 ± 0.06	0.54 ± 0.20	0.06 ± 0.09$^\#$
RE_0/RC	0.240 ± 0.010$^{*\#}$	Susp.	0.470 ± 0.003	0.570 ± 0.015*	0.610 ± 0.006*	0.380 ± 0.023*
		Dep.	0.480 ± 0.030	0.490 ± 0.025	0.470 ± 0.020	nd
ET_0/TR_0	0.58 ± 0.01$^\#$	Susp.	0.60 ± 0.01	0.56 ± 0.01	0.52 ± 0.01*	0.36 ± 0.01*
		Dep.	0.30 ± 0.04	0.30 ± 0.02	0.30 ± 0.01	0.03 ± 0.04$^\#$
RE_0/ET_0	0.26 ± 0.01$^{*\#}$	Susp.	0.72 ± 0.01	0.80 ± 0.01*	0.81 ± 0.03*	0.60 ± 0.01*
		Dep.	0.90 ± 0.05	0.84 ± 0.04	0.88 ± 0.02	nd
PI_{abs}	27.00 ± 2.00$^\#$	Susp.	27.14 ± 1.80	18.44 ± 1.37*	8.27 ± 0.62*	2.46 ± 0.07*
		Dep.	4.00 ± 1.00	3.80 ± 0.50	4.00 ± 0.20	0.06 ± 0.20$^\#$

Measurements on blue grama grass cells deposited on filter paper

As shown in Table 2 (*cf.* Fig. 2S), the JIP parameters for cells grown with added CO_2 in the culture medium containing 0, 0.15, and 0.3% sucrose, and deposited on filter paper during fluorescence measurements, were statistically similar. The TR_0/ABS for the deposited cells with 3% sucrose was lower than in similar cells measured in suspension (*i.e.*, ~ 0.51 *vs*. 0.58); further, cells grown in 0, 0.15, and 0.3% sucrose, had values of ~ 0.7, compared with 0.73, 0.72, and 0.64, respectively, in cells measured in suspension (but grown under atmospheric CO_2). Further, both the specific absorption (ABS/RC) and trapping (TR_0/RC) fluxes were higher in all the deposited cells, compared to the cells measured in suspension, but especially in the cells grown in 0, 0.15, and 0.3% sucrose (Table 2). Since the increase of ABS/RC was higher than the increase in TR_0/RC, we can assume (as discussed earlier) the presence of an increased fraction of Q_A-nonreducing PSII centers in these cells.

The JIP parameters characterizing the electron transport from reduced Q_A to the PQ pool (*i.e.*, ET_0/ABS, ET_0/TR_0, and ET_0/RC) had lower values for all cells deposited on filter paper than for the cells in suspension in culture medium, with the lowest values for cells grown in 3% sucrose (Table 2). These changes may be the consequence of an increased fraction of Q_B-nonreducing PSII centers in all cells deposited on filter paper, which led to a high V_J level (Fig 3*C*).

We were unable to calculate the RE_0/ABS, RE_0/RC, and RE_0/ET_0 in cells grown in 3% sucrose and deposited on filter paper, for which the fluorescence transient had mainly an O-J phase (Fig. 3*C*). For the cells grown in 0, 0.15, and 0.3% sucrose, all these three JIP-parameters had statistically similar values, which were higher than in the leaf (Table 2). We note: (*1*) RE_0/ABS values were clearly lower here than that in photoautotrophic cells measured in suspension, probably because ABS/RC was higher in the cells deposited on the filter paper; (*2*) RE_0/RC values were similar here to that from the photoautotrophic cells in suspension; but (*3*) RE_0/ET_0 values were higher than that in all the cells measured in suspension, which is understandable, because the electron transport from the reduced Q_A to the PQ pool was much more inhibited in the cells deposited on filter paper. These results suggest that the measurements on the cells deposited on filter paper may be 'distorted' due to possible 'contamination' with exogenous quinone molecules, but this may be to a lesser extent than in the cells measured in suspension.

Finally, the performance index on the absorption basis (PI_{abs}) had very small values in the cells deposited on filter paper, *i.e.*, ~ 4 for 0, 0.15, and 0.3% sucrose, and ~ 0.06 for 3% sucrose, compared to ~ 27 in photoautotrophic cells in suspension and the leaf. These low PI_{abs} values indicate increased fraction of both Q_B- and Q_A-nonreducing centers in the cells deposited on filter paper, while TR_0/ABS is affected only by the Q_A-nonreducing centers; however, note that an eventual change from State 1-to-State 2 will also lower both PI_{abs} and TR_0/ABS.

Conclusions

Given the economic advantages of the blue grama grass, we have studied here the activity of the photosynthetic apparatus in its isolated cells based on the analysis of the fast (< 1 s) Chl fluorescence transient (*i.e.*, the OJIP curve). We conclude that, despite differences between their OJIP transients, the photoautotrophic blue grama grass cells suspended in culture medium had an optimal PSII activity, as they showed closer F_V/F_M and PI_{abs} values to those in blue grama grass leaves. On the other hand, all samples grown in culture medium containg sucrose had a reduced PSII activity, due to an increase in the fractions of Q_A- and Q_B-nonreducing PSII centers, which seemed to be in proportion to the sucrose concentration in the culture medium. Therefore, our work clearly showed the deleterious effect of sucrose on the photosynthetic activity of cultured cells, despite the observed increase in Chl concentration in cells grown in higher sucrose concentrations (*e.g.*, 3%). Further, results on isolated blue grama grass cells grown with added CO_2, and measured on filter paper, showed somewhat increased F_V/F_M ratio in cells grown with 0.3% sucrose, compared to similar cells grown under atmospheric CO_2 and measured in suspension (*i.e.*, 0.7 *vs.* 0.64); however, all the cells had lowered PI_{abs} values, due to an even slower oxidation of the reduced Q_A. These results seem to indicate that the added CO_2 may be advantageous, but the deposition of the isolated cells on filter paper is probably not a good alternative method to measure the ChlFI kinetics on these samples. Further research is needed to fully understand all these novel observations, and especially those obtained for blue grama grass cells when they were deposited on filter paper.

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