Revista Ciência Agronômica, v. 47, n. 1, p. 178-186, jan-mar, 2016
Centro de Ciências Agrárias - Universidade Federal do Ceará, Fortaleza, CE
www.ccarevista.ufc.br

Artigo Científico
ISSN 1806-6690

Chlorophyll fluorescence in the evaluation of photosynthetic electron transport chain inhibitors in the pea[1]

Fluorescência da clorofila na avaliação de inibidores da cadeia de transporte de elétrons da fotossíntese em ervilha

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ABSTRACT - The study aimed to evaluate the behavior of the chair of photosynthetic electron transport in the presence of DCMU and atrazine in detached leaves of pea through simultaneous measurements of the kinetics of fluorescence transient, fluorescence delayed and modulated reflection at 820 nm. The petioles of the leaves were immersed for two hours in solution of inhibitors at concentrations of 0 (control), 25, 50, 100, 250 and 500 µM. Measurements of the kinetics of fluorescence transient and fluorescence delayed and modulated reflection at 820 nm were recorded simultaneously by the M-PEA fluorometer. Simultaneous measurement of fluorescence transient, fluorescence delayed and modulated 820 nm reflection is important for assessments of the photosynthetic electron transport chain activity tool. The use of specific inhibitors of the electron transport chain allows you to collect and correlate a lot of information about the effect of different inhibitors at specific points in the photosynthetic electron transport chain. DCMU and atrazine are inhibitors of photosystem II and the concentration of 500 mM affects more strongly the flow of photosynthetic electrons.

Key words: *Pisum sativum* L.. Fluorescence transient. Delayed fluorescence. DCMU. Atrazine.

RESUMO - O trabalho teve como objetivo avaliar o comportamento da cadeia de transporte de elétrons fotossintética na presença de DCMU e atrazina, em folhas destacadas de ervilha, através de medidas simultâneas das cinéticas da fluorescência transiente, decaída da fluorescência da clorofila *a* e reflexão modulada a 820 nm. O pecíolo das folhas foi imerso por duas horas em solução de inibidores nas concentrações de 0 (controle), 25, 50, 100, 250 e 500 µM. Medidas da cinética da fluorescência transiente, da decaída da fluorescência e reflexão modulada a 820 nm foram gravadas, simultaneamente, pelo fluorômetro M-PEA. A medida simultânea da fluorescência transiente, decaída da fluorescência e reflexão modulada a 820 nm é ferramenta importante para avaliações da atividade da cadeia de transporte de elétrons fotossintética. O uso de inibidores específicos da cadeia de transporte de elétrons permite coletar e correlacionar uma série de informações sobre o efeito de diferentes inibidores em pontos específicos na cadeia de transporte de elétrons fotossintético. DCMU e atrazina são inibidores do fotossistema II e a concentração de 500 µM afeta de maneira mais acentuada o fluxo de elétrons fotossintético.

Palavras-chave: *Pisum sativum* L.. Fluorescência transiente. Decaída da fluorescência. DCMU. Atrazina.

DOI: 10.5935/1806-6690.20160021
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[1]Recebido para publicação em 28/08/2014; aprovado em 29/10/2015
Parte da Dissertação de Mestrado do primeiro autor apresentada ao Programa de Pós-Graduação em Fisiologia Vegetal da Universidade Federal de Pelotas, pesquisa financiada pelo CNPq, CAPES e FAPERGS
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INTRODUCTION

The electron transport chain (ETC) is made up of protein complexes embedded in the thylakoid membrane. The main components are photosystem II (PSII) and photosystem I (PSI), connected in series via the cyt_{b6f} complex and plastocyanin (PC). The interconnection between the complexes involved in electron flow is mediated by plastoquinones, mobile carrier molecules, which are located in the hydrophobic region of the thylakoid membrane (PRIBIL; LABS; LEISTER, 2014).

Chlorophyll (Chl) molecules of the antenna system of the photosystems, upon capturing luminous energy, pass from ground state to an excited state. This excitation energy can be utilised by means of a photochemical effect. However, not all the absorbed energy is directed to this end; part may be dissipated as heat or re-emitted as fluorescence. In this way, the analysis of Chl fluorescence can provide information about the amount of luminous energy used by the photochemistry and dissipated as heat (STIRBET; GOVINDJEE, 2012).

Analysis of Chl *a* fluorescence emission, makes it possible to evaluate this process in terms of energy flow (STRASSER; TSIMILLI-MICHAEL; SRIVASTAVA, 2004), in which the fluorescence transient (FT) is important for obtaining information about the pigment complexes, the organisation and transfer of excitation energy between them, and about various transfer reactions of specific PSII electrons (STIRBET; GOVINDJEE, 2012). However, just as the chlorophylls of the PSII emit light as FT, they also emit another type of light, known as delayed fluorescence (DF), as a result of the reversal of charge separation followed by the rapid transfer of P_{680}* excitation energy to the chlorophylls of the antenna system (GOLTSEV *et al*., 2009; OUKARROUM; GOLTSEV; STRASSER, 2013; STRASSER *et al*., 2010).

To distinguish the two types of light emission, FT is measured at the same time as illumination, while DF is measured after the actinic light is turned off, in alternating cycles of light and dark (GOLTSEV *et al*., 2009; OUKARROUM; GOLTSEV; STRASSER, 2013). According to Grabolle and Dau (2005), the similarity between the spectral emissions of FT and DF shows that the emitted photon is the result of the radioactive deactivation of the excited state of the PSII Chl.

It has been proposed that the kinetics of modulated reflection at 820 nm (MR_{820}) may reflect PSI activity (STRASSER *et al*., 2010; YAN *et al*., 2012), indicating alterations in the redox state of the PC and of the PSI (P_{700}) reaction centre (RC) (OUKARROUM; GOLTSEV; STRASSER, 2013; YAN *et al*., 2013). Strasser *et al*. (2010) emphasize that simultaneous measurements of the FT, DF and MR_{820} are essential for complete correlation of the information on the different dominions of the ETC. By virtue of this correlation, research is being conducted to demonstrate the importance of such simultaneous analysis, mainly using artificial chemical compounds that can donate or accept electrons at specific locations in the electron transport chain (YAN *et al*., 2012). For this, DCMU (3-(3',4'-dichlorophenyl)-1,1-dimethylurea)) and atrazine (1-chloro-3-ethylamino-5-isopropylamino-2,4,6-triazine) are used, since they are considered to be PSII inhibitors; however, no data can be found in the literature on the simultaneous analysis of FT, DF and MR_{820} where these inhibitors are used.

The aim of this study was to evaluate changes in the kinetics of Chl *a* fluorescence transient emission, delayed fluorescence and modulated reflection at 820 nm as tools in the study of electron flow in the electron transport chain of detached pea leaves.

MATERIALS AND METHODS

Seeds of the pea (*Pisum sativum* L.) were left to germinate in two-litre polyethylene pots, containing soil as substrate; these were kept in a greenhouse and irrigated by micro sprinkler system. One week after emergence, the weekly application of Hoagland and Arnon nutrient solution (1950) was started in order to prevent possible mineral deficiencies.

When the plants presented young and fully expanded leaves, they were taken to the laboratory, where the leaves were detached with the aid of scissors, and the petiole immediately immersed in a 10 ml solution of each inhibitor [DCMU (3-(3',4'-dichlorophenyl)-1,1-dimethylurea) and atrazine (2-chloro-4-ethylamino-6-isoproprilano s-triazine)].

The immersion time for the petioles, and the concentrations of the inhibitors were defined in preliminary trials, by observing the behaviour of the OJIP curve in readings carried out over 24 h to determine the soaking time which would give the greatest effect from the inhibitors; this immersion time was determined as two hours, at concentrations of 0 (control), 25, 50, 100, 250 and 500 µM. During soaking, the leaves were maintained under low-light conditions (5 to 10 µmol photons m^{-2} s^{-1}) to induce photosynthesis, at a temperature of 22 ± 2 °C. After the leaves had been exposed to the treatments, the evaluations were carried out, using eight replications per treatment.

In the final 30 minutes of the immersion period, the leaves were adapted to the dark using clips supplied by the manufacturer. Measurements of transient fluorescence

kinetics, delayed fluorescence and modulated reflection at 820 nm were recorded simultaneously using the Multifunctional Plant Efficiency Analyser - M-PEA fluorometer (Hansatech Instrument Ltd. Kings Lynn, Norfolk, PE30 40NE, UK).

In the M-PEA analyser, there are emitters with wavelengths in the 627 ± 10 nm band for actinic light, 820 ± 25nm band for modulated light and 735 ± 15nm band for far-red light, the latter using a filter (RG9) to remove some of the components of visible light. High quality optical filters were used to protect the FT and DF detectors (730 ± 15 nm) and the modulated reflection detector (820 ± 20 nm) (STRASSER *et al*., 2010). The saturating light pulse, with an intensity of 5,000 µmol photons m^{-2} s^{-1}, was emitted by the machine for 60 s.

Data acquisition for the three signals, FT and MR_{820} in the light and DF in the dark, was carried out every 0.01 ms for range 1 (0.01 to 0.30 ms), every 0.1 ms for range 2 (0.3-3.0 ms), and every 1 ms for range 3 (3.0 to 30 ms). Simultaneous measurement of FT and DF require a cycle with periods of light and dark, so that during each cycle, the FT is measured when the actinic light is turned on, and the DF recorded when the light is off.

The FT was analysed as per the JIP test (Strasser, Strasser, 1995), using original data: minimum fluorescence (F_0), recorded 20 µs after actinic illumination; a fluorescence intensity of 2 ms (F_{2ms}: J-point) and 30 ms (F_{30ms}: I-point); and maximum fluorescence (F_M). From the modulated reflection signal at 820 nm (MR_{820}), the MR/MR_0 ratio was calculated, where MR_0 is the value at the start of actinic illumination (at 0.7 ms). This ratio reflects changes in the redox state of the PSI reaction centre (P_{700}+) and of the plastocyanin (PC^+). A reduction in amplitude of the intensity of the modulated reflection at 820 nm therefore represents an accumulation of these cofactors in the oxidised state, whereas an increase in this amplitude indicates a reduction in $P700^+$ and PC^+ (STRASSER *et al*., 2010). The DF induction curve shows a rapid increase up to a peak, I_1 (7 ms), a subsequent decline to a peak, I_2 (around 100 ms) and a long period (between 0.5 and 10 s), where level I_3 is located.

RESULTS AND DISCUSSION

Analysis of OJIP fluorescence transient kinetics and JIP test

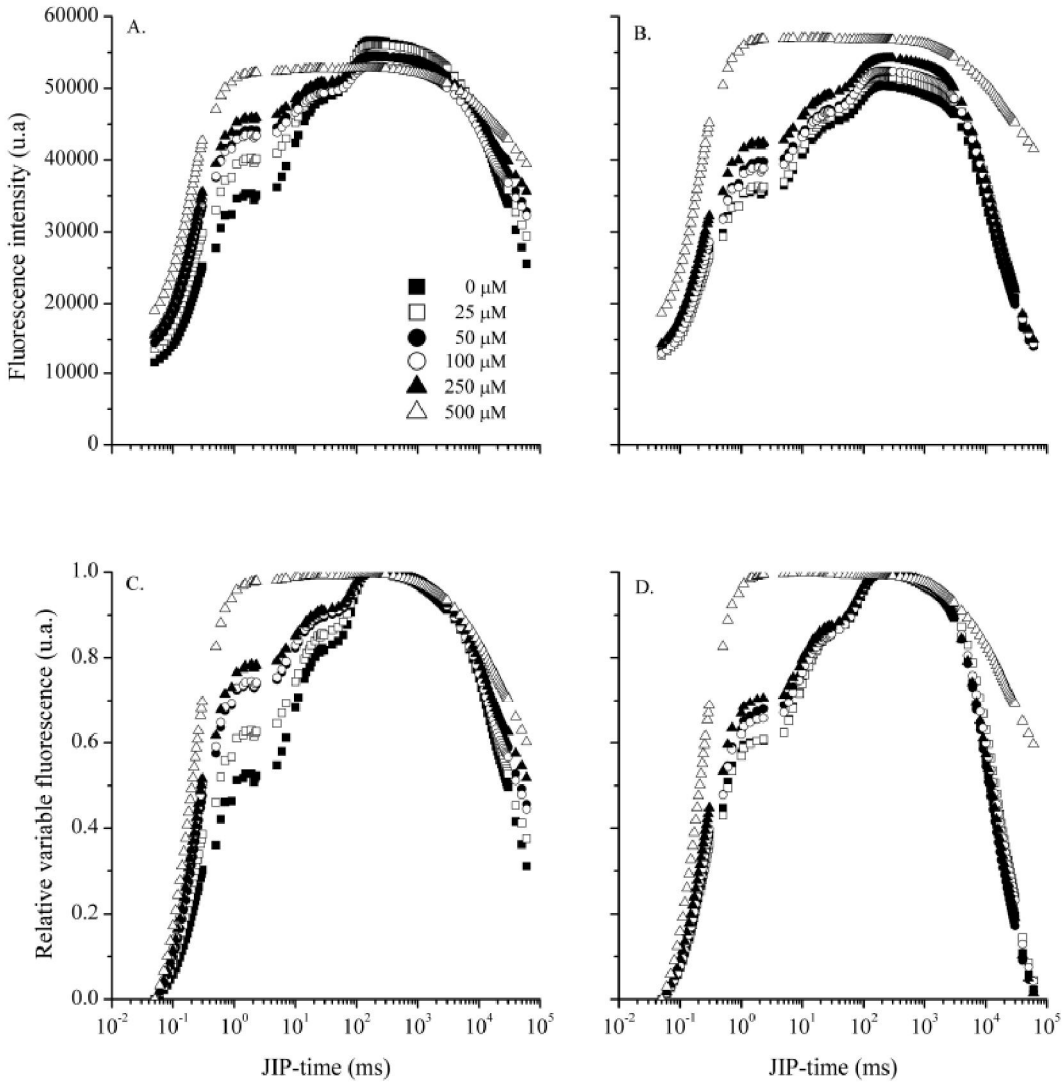
The FT of detached pea leaves treated with different concentrations of the inhibitors DCMU and atrazine is shown in Figure 1. The Chl *a* fluorescence transient, represented on a logarithmic time scale starting at 50 µS, shows an increase in the level of J as the concentrations of DCMU increase (Figures 1A and 1C). Similar behaviour was seen for values of F_0, being more significant at a concentration of 500 µM when compared to the other concentrations and the control; none of the treatments however showed changes in the values of F_M.

At a DCMU concentration of 500 µM, the induction curve lost its sigmoid characteristic between steps J and I, since the fluorescence intensity in phase J was similar to F_M (Figures 1A and 1C), suggesting an accumulation of Q_A^- by the complete inhibition of the transfer of electrons from Q_A to Q_B (LAZAR, 2009). DCMU blocks the transfer of electrons from Q_A to QB (VELTHUYS, 1981), as it competes with the Q_B binding site on the D1 protein of PSII (TREBST; DRABER, 1986). When the petioles of the leaves were immersed in 250 µM of DCMU for 14 hours in the dark (TÓTH; SCHANSKER; STRASSER, 2005), the behaviour was similar to that observed in the present study. The increase in fluorescence is related to the redox state of Q_A, and blocking the reoxidation leads to a simplification of the kinetics of Chl fluorescence induction (TÓTH; SCHANSKER; STRASSER, 2005) due to a complete inhibition of the transport of electrons on the reductor side of PSII preventing the reduction of $NADP^+$, required for CO_2 fixation (OETTMEIER, 1992). At the remaining DCMU concentrations, the curves show the J-I and I-P phases as being defined, but with fluorescence intensities higher than the control.

When the leaves were treated with different concentrations of atrazine, the fluorescence induction curves showed the same behaviour as the curves of leaves treated with DCMU, starting with a concentration of 50 µM (Figures 1B and 1D). Atrazine blocks the transport of electrons between Q_A and Q_B, since it prevents the binding of Q_B in the D1 protein, and with this the flow of electrons to the PSI stops (HESS, 2000), resulting in a reduced accumulation of Q_A. The results indicate that high concentrations of atrazine on the leaves directly interfere with the plant's ability to convert the luminous energy absorbed into a biologically available form of energy. In studies of phytoplankton, Deblois, Dufresne and Juneau (2013) found the same loss in energy conversion caused by atrazine, with a significant effect on the cyanobacteria and algae.

Parameters for the JIP-Test in treatments up to a concentration of 250 µM of each inhibitor, normalised to the respective controls, are shown in Figure 2. Treatment with DCMU (Figure 2A) caused a slight reduction in RC/ABS (active reaction centre), and in a more pronounced manner, a reduction in the values for V_F/F_0, as concentrations of the inhibitor increased. The opposite behaviour was seen in the values of ABS/RC, a parameter

Figure 1 - Fluorescence transient in detached pea leaves adapted to the dark for 30min, treated with different concentrations of DCMU (A and C) and atrazine (B and D). (A and B) fluorescence intensity and (C and D) relative variable fluorescence

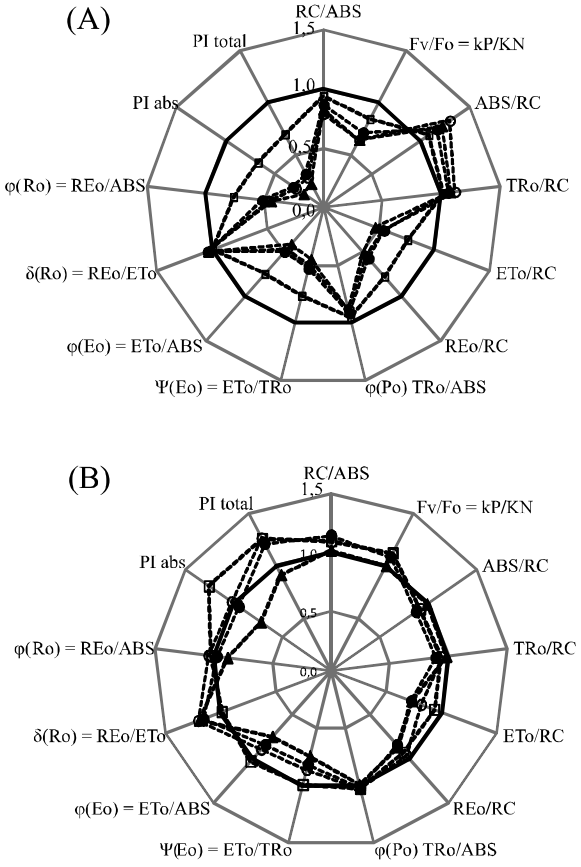


related to the absorption flow of the RC, which also relates to the apparent size of the antenna system. Similarly, the same behaviour occurs with the energy flow captured by the reaction centre (TR_0/RC).

Flows at the reaction centre, which represent the transport (ET_0/RC) and the reduction flow of the terminal acceptors on the electron acceptor side of PSI (RE_0/RC), showed similar behaviour, characterised by a reduction in value as the tested concentrations increased. The same occurs when the fluorescence values are treated as: (1) the probability of a captured exciton moving an electron in the electron transport chain after Q_A^- (ψ_{Eo}); (2) the quantum yield of electron transport from Q_A^- to the intersystem of electron acceptors (φ_{Eo}); and (3) the quantum yield related to the reduction of the terminal electron acceptors of PSI by absorbed photons (φ_{Ro}). This shows that there was blocking of the electron transport from Q_A^-.

The increase in the ABS/RC ratio indicates an apparent increase in the antenna complex of the photosystem to compensate for the high loss of energy as heat (CHRISTEN *et al*., 2007). The consequence of this loss of energy is a reduction in the rate of electron transport (ET_0/RC) and a reduction in the terminal acceptors of PSI (RE_0/RC), since the absorbed energy was not used for photochemistry. In addition, the parameters related to yields, efficiencies and probabilities (φ_{Eo}, Ψ_{Eo} and φ_{R0}) were also reduced with high doses of DCMU; it can be said that these concentrations of the inhibitor

Figure 2 - Quantitative parameters of the photosynthetic machinery, derived by JIP test from the OJIP polyphasic fluorescence in detached pea leaves treated with different concentrations of DCMU (A) and atrazine (B). All parameters were normalised for a concentration of 0µM. (----- 0 µM; --- --- 25 µM; ---●--- 50 µM; ---○--- 100 µM and ---▲--- 250 µM)



demonstrate an efficient mechanism for inhibiting electron transport between Q_A and Q_B. To reinforce this action, the reduction in the quantum yield of the photochemical and non-photochemical processes (F_V/F_0) demonstrates that this parameter is related to the energy captured and the energy dissipated.

The indices of photosynthetic performance (PI_{abs} and PI_{total}) were affected negatively by the increases in concentration, showing a reduction when compared to the control. The performance index (PI_{total}) is the most sensitive parameter to the JIP-test, as it incorporates several parameters which are evaluated from OJIP fluorescence transients (YUSUF *et al*., 2010). Such results indicate a negative effect on the photosynthetic machinery, since these parameters are related to energy flow throughout the ETC.

When in a solution of atrazine, pea leaves undergo minor changes to the parameters RC/ABS and F_V/F_0, with a tendency for values to increase, and to the parameters ABS/RC and TR_0/RC, with a tendency to decrease (Figure 2B). The values of ET_0/RC decrease as the concentration of the inhibitor increases. The same can be seen for RE_0/RC, however to a lesser extent.

For the parameters related to quantum yield, only φ_{Eo} decreases with increasing concentrations of atrazine from 50 µM; a concentration of 250 µM hardly changed φRo. The values of the parameter ψ_{Eo} were reduced at the lowest concentration used, and an increase in the values of δ_{Ro} can be seen at the three highest concentrations.

PI_{abs} showed an increase in relation to the control at a concentration of 25 µM atrazine, and a drop at 250 µM atrazine. PI_{total} showed an increase at concentrations of 25, 50 and 100 µM, the opposite being seen at a concentration of 250 µM. The reduction in these parameters indicates a loss in the capacity of the plant to carry out photochemical reactions, i.e. in the efficiency of using the energy absorbed by the antenna for converting energy into the form of ATP and NADPH. The performance indices demonstrate more sensitive behaviour than the parameter related to the maximum photochemical quantum yield of PSII (φ_{Po}) in plants under conditions of stress (OUKARROUM *et al*., 2007). This can be confirmed by the results of this work, since the parameter F_V/F_M suffered no variation in relation to the control for any of the inhibitors being used.

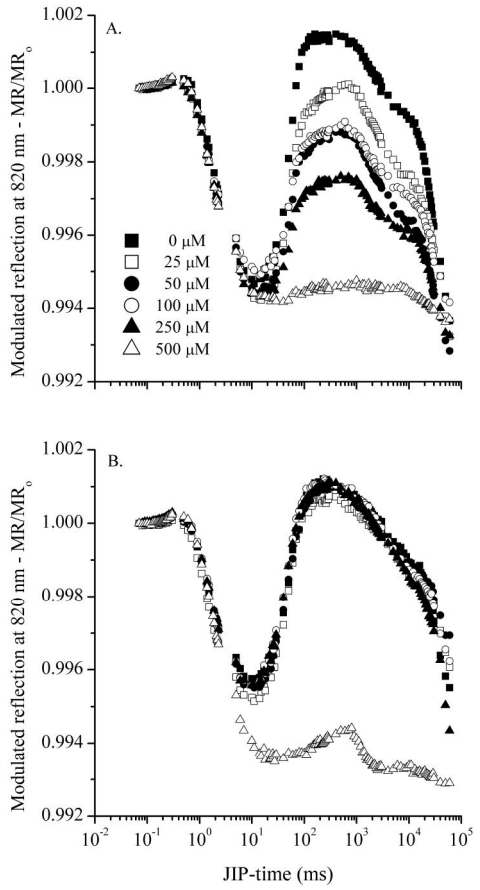
Analysis of the kinetics of modulated reflection at 820 nm (MR/MR_0)

Modulated reflection at 820 nm has recently been studied to evaluate the photosynthetic capacity of PSI, by analysing the redox state of the P_{700} reaction centre and the PC (YAN *et al*., 2013). The signals of the modulated reflection at 820 nm are shown in Figure 3. At the beginning of actinic illumination (5,000 µmol m^{-2} s^{-1}), detached pea leaves, treated with inhibitors and adapted to the dark for 30 min, showed completely reduced P_{700} and plastocyanins at all concentrations of both inhibitors. With the passage of illumination time, there is a drop in amplitude of the MR/MR_0 curve, indicating an increase in the oxidised state of the P_{700} and plastocyanins to the point at which re-reduction takes place, illustrated by a further increase in curve amplitude.

The accumulation of oxidised P_{700} and plastocyanin increases the absorbance at 820 nm, resulting in a reduction in MR/MR_0 (rapid phase). There is a subsequent re-reduction of PC and P_{700} by the intersystem electron carriers, increasing the MR/MR_0 ratio (slow phase) (SCHANSKER *et al*., 2003). Generally, the length of time for which MR/MR_0 is minimal (a state of transition where

the rates of oxidation and re-reduction are equal) is in the same range as the J-I phase of fluorescence transient, while the slow phase appears mainly in the I-P phase (SCHANSKER *et al*., 2003; STRASSER *et al*., 2010).

Figure 3 - Kinetics of modulated reflection at 820 nm in detached pea leaves adapted to the dark for 30min, treated with different concentrations of DCMU (A) and atrazine (B). The signals of modulated reflection at 820 nm are represented by the MR/MR_0 ratio, where MR_0 is the value at which actinic illumination begins (at 0,7ms)



In the pea leaves treated with DCMU, a decrease can be seen in the amplitude of the curve, a phase which corresponds to the re-reduction of P_{700} and plastocyanin as inhibitor concentrations increase, where a concentration of 500 µM causes the extinction of this re-reduction (Figure 3A) due to the complete inhibition of the electron transport chain beyond Q_A^-. The same behaviour of the transient MR/MR_0 is reported by Tóth, Schansker and Strasser (2005), showing that P_{700} and plastocyanin were oxidised and remained oxidised during a sequence of red and far-red light, since the blocking of electrons in the transport chain did not allow a reduction in the plastoquinone pool, which remained as oxidised PQ during the different light pulses (SCHANSKER; TÓTH; STRASSER, 2005).

When the inhibitor used is atrazine, the concentrations under study do not modify the behaviour of the curve when compared to the control, except for the sharp fall in the re-reduction phase of P_{700} and plastocyanin for a concentration of 500 µM (Figure 3B).

Analysis of the kinetics of delayed fluorescence (DF)

Just as the chlorophylls of the PSII antenna emit a fluorescence transient signal, they also emit an DF signal, this being a result of the reversal of charge separation followed by the transfer of energy from the rapid excitation of the excited P_{680} to the antenna chlorophylls (GOLTSEV *et al*., 2009). According to Goltsev *et al. (*2009), DF kinetics comprises various components, each with a different duration and amplitude, being emitted due to the return of the electron transfer and to the charge recombination of various redox states of PSII, such as $P_{680}^+Pheo^-$, $P_{680}^+Q_A^-$, $Z^+Q_A^-$, $S_3Z^+Q_A^-Q_B$, $S_3Z^+Q_A^-Q_B^{2-}$. Two phases can be seen on the FD induction curve, a phase that occurs for up to around 300 ms, including the peaks at I_1 (7 ms) and I_2 (50 ms), and a slow phase that can last for several minutes (GOLTSEV *et al.*, 2009; OUKARROUN; GOLTSEV; STRASSER, 2013; STRASSER *et al*., 2010).

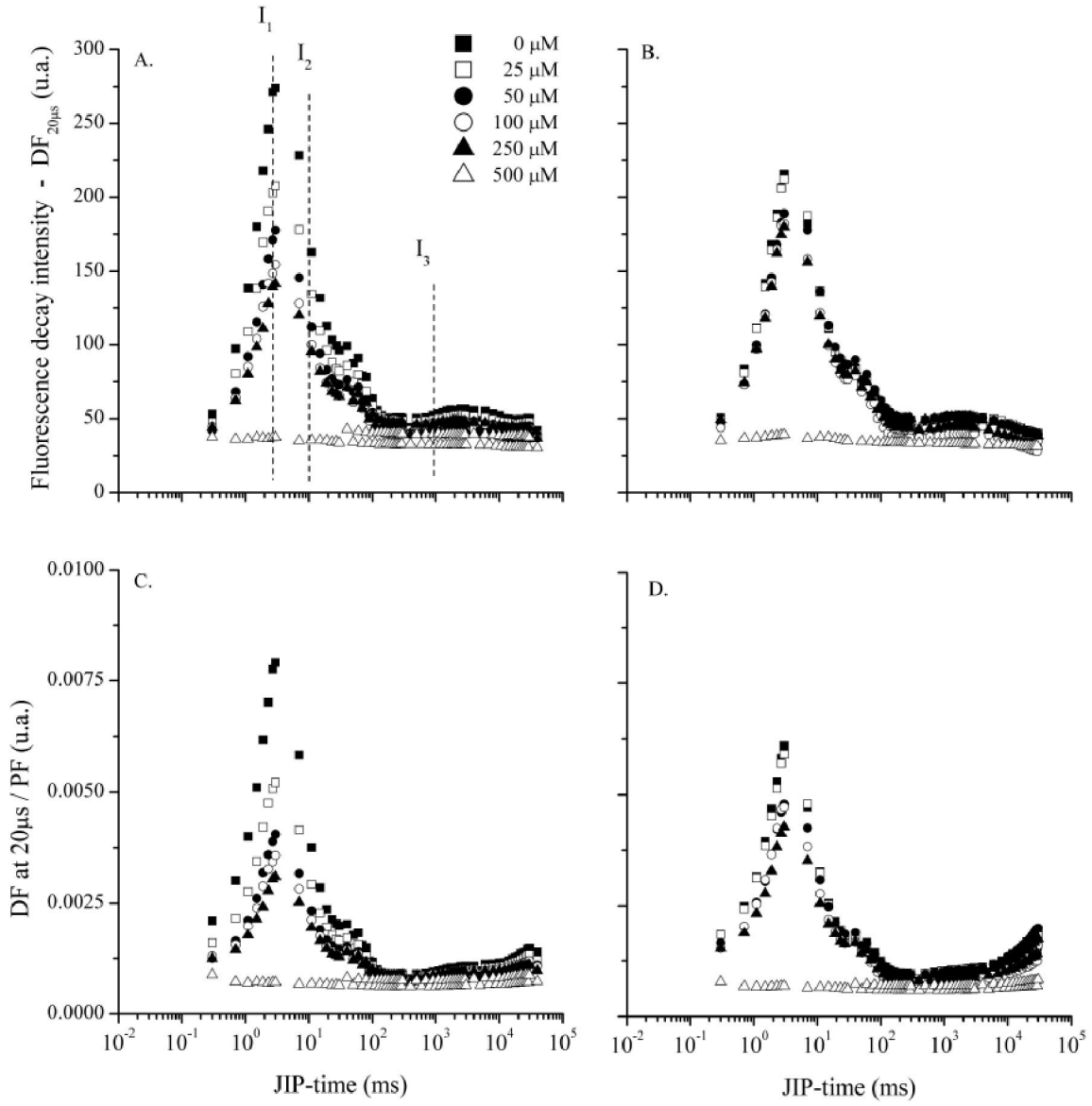
The FD induction curves (DF vs. JIP-time) proposed by Strasser *et al. (*2010), were constructed as DF kinetics in relation to JIP-time in the periods of delayd at 0.02, 0.03, 0.05, 0.09, 0.15, 0.25, 1, 3, 30 and 230 ms (data not shown). The characteristic points of the curves for DF vs. JIP-time, i.e. the I_1 peak (at 3 ms), the I_2 shoulder (at 100 ms) and I_3 (at 1000 ms, plateau) were named as per terminology proposed by Goltsev (GOLTSEV *et al*., 2009).

The amplitude of the curves at different OJIP times gradually reduced with the increase in DCMU concentration, especially in relation to the I_1 peak. At the greatest concentration (500 µM), the DF induction curve disappears. The same was found in leaves treated with atrazine, albeit with a smaller intensity of reduction. At a concentration of 500 µM of atrazine, the induction curve again loses its characteristics. According to Goltsev *et al. (*2009), the I_1 peak is a result of an increase in the transmembrane electrical gradient, formed by the PSI when P_{700} is oxidised, in addition to the accumulation of reaction centres with semi-reduced Q_B ($Z^+P_{680}Q_AQ_B^-$). The I_2 shoulder is related to an increase in the $Z^+P_{680}Q_A^-Q_B^-$ state during reduction of the PQ pool. These states display a relatively high yield in the emission of DF. In addition, the I_2 peak of the DF induction curve is

probably associated with the prolonged reopening of P_{680} through the transfer of electrons accelerated by the quinone Q_B, while PQH_2 is being reoxidised by the PSI before the complete reduction of the plastoquinone pool (GOLTSEV *et al.*, 2009; STRASSER *et al*., 2010).

For the sake of simplicity, it was decided only to present the DF intensity measured at 20 µs after interruption of the actinic light ($DF_{20\mu s}$). Accordingly, a rapid increase can be seen in the I_1 peak (at 3 ms JIP-time), with a subsequent polyphasic decline represented by the I_2 shoulder (at 100 ms JIP-time), followed by a long plateau (between 0.5 and 10 s JIP-time), where I_3 is located at 1s (Figure 4). For the treatments with DCMU, the intensity of $DF_{20\mu s}$ decreases with the increase in concentration of the inhibitor, there is a consequent reduction of the I_1 peak, with the $DF_{20\mu s}$ kinetics disappearing at a concentration of 500 µM, compared to the control (Figure 4A). Treatment of the leaves with atrazine showed a similar behaviour on the $DF_{20\mu s}$ induction curve at a concentration of 500 µM (Figure 4A).

Figure 4 - Delayed fluorescence intensity measured at 20 µs after interruption of the actinic light ($DF_{20\mu s}$) (A and B) and the ratio between the delayed and the intensity of fluorescence transient, measured at the same JIP-time (B and D), in detached pea leaves treated with DCMU (A and C) and atrazine (B and D)



DCMU and atrazine display as an action mechanism the blocking of the transfer of electrons from Q_A to Q_B, i.e. at a concentration of 500 µM for each inhibitor, the loss of $DF_{20\mu s}$ kinetics was noted, when the two peaks, I_1 and I_2, also disappeared, since there was no formation of the $Z^+P_{680}Q_AQ_B^-$ (I_1) and $Z^+P_{680}Q_A^-Q_B^-$ (I_2) states, which are the charge recombinations responsible for DF induction.

When the relationship between delayed fluorescence at 20µs and the intensity of Chl fluorescence ($DF_{20\mu s}$/PF) is shown (Figure 4C and 4D), it is possible to express the repopulation rate of the Chl excited by absorption (STRASSER *et al*., 2010). For leaves treated with DCMU, evaluations of $DF_{20\mu s}$/PF showed a reduction in the intensity of this relationship as the concentration of the inhibitor increased. At the greatest concentration (500 µM), the curve lost its characteristics (Figure 4C). The same was found in the treatments with atrazine (Figure 4D).

The $DF_{20\mu s}$/PF ratio is determined by a concentration of the light-emitting states (the result of charge recombination) at the time the actinic light is turned off, which in turn is dependent on the rate of closed reaction centres (reduced Q_A), i.e. a decrease in $DF_{20\mu s}$/PF is expected as the open reaction centres decrease (STRASSER *et al*., 2010). As in the $DF_{20\mu s}$ curve for the leaves treated with DCMU and atrazine, the $DF_{20\mu s}$/PF induction curve also decreased with the increase in concentration, disappearing at a dose of 500 µM and indicating a low repopulation rate of excited chlorophylls; consequently, a decrease in charge recombination for those states that emit DF also occurs.

CONCLUSIONS

1. The simultaneous measurement of chlorophyll fluorescence transient, delayed fluorescence and the modulated reflection at 820 nm is an important method for evaluating the activity of the photosynthetic electron transport chain in pea leaves;
2. The use of the specific inhibitors of the electron transport chain, DCMU and atrazine, makes it possible to collect and correlate a variety of information on the effect of different inhibitors for specific points of the entire photosynthetic transport chain of electrons;
3. For pea leaves, DCMU and atrazine are inhibitors of photosystem II, affecting the flow of electrons in the photosynthetic electron transport chain; a concentration of 500 µM more markedly affects the flow of photosynthetic electrons.

ACKNOWLEDGEMENTS

The authors wish to thank the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and the Fundação de Amparo à Pesquisa do Estado do Rio Grande do Sul (FAPERGS) for the financial aid and scholarships.

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