In vivo regulation of electron and proton transport under different environmental conditions
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ABSTRACT

Nowadays, it is considered that improvements in the photosynthetic rates of individual leaves within the canopy may be the solution to increase crop yields for the growing population in the coming decades. In that logic, it is of great importance to understand the operation, regulation, and limitations of photosynthesis under different environments. To contribute with this objective, the regulation of electron and proton fluxes produced in the thylakoid discs under increasing irradiance, decreasing CO₂ concentrations and decreasing temperatures was studied in the Solanacea species *Juanulloa aurantiaca* through the use of non-invasive biophysical techniques (chlorophyll fluorescence and absorbance changes at 520 and 820 nm) *in vivo*. The results showed that the mechanisms that regulate the flux of electrons and protons when the metabolism in the stroma is reduced by decreasing CO₂ concentrations or decreasing temperatures works in the same way. The flux of electrons generated a proton motive force (*pmf*) that decreased the pH in the lumen, thereby decreasing the rate of oxidation of plastoquinol (*k*e) and increasing the non-photochemical quenching (NPQ). A decrease of *k*e was accompanied by a decrease of the conductivity of ATP synthase to protons (*g*<sub>H⁺</sub>). Leaves exposed to increasing irradiance showed lower *pmf values* and a relatively non-altered *k*e and *g*<sub>H⁺</sub>.

**Keywords.** Photosynthesis, decreasing temperature, decreasing CO₂ concentrations, metabolism, flux of protons, flux of electrons.
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ACKNOWLEDGEMENTS

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SUMMARY

Nowadays, it is considered that improvements in the photosynthetic rates of individual leaves within the canopy may be the solution to increase crop yields for the growing population in the coming decades. In that logic, it is of great importance to understand the operation, regulation, and limitations of photosynthesis under different environments. In this context, few studies have been realized in leaves of the tropical plant species *Juanulloa aurantiaca*. By the use of biophysical techniques (i.e., leaf gas exchange, chlorophyll fluorescence and absorbance changes at 820 nm), such studies showed the responses of the thylakoid electron transport of this C₃ plant under different environmental conditions in which the photosynthetic photon flux densities (PPFD), CO₂ and O₂ concentrations were modulated (Harbinson, 1994; Genty and Harbinson, 1996). With these antecedents, the scope of this research is to generate more information regarding to the regulation of the photosynthetic apparatus of this tropical plant species under different environmental conditions through the use of a broad combination of non-invasive techniques.

Cuttings of the tropical C₃ plant species *Juanulloa aurantiaca* were planted and placed in a climate chamber with relative humidity of 70%, a day/night temperature of 25/23 °C, ambient CO₂ concentration and 12 hours of photoperiod with 600 μmolm⁻²s⁻¹ of fluorescent light. The photosynthetic performance of each plant was evaluated in a customised equipment designed by Wageningen University. This equipment offered the opportunity to measure *in vivo* the following photosynthesis parameters: leaf CO₂ exchange, chlorophyll fluorescence and absorbance changes at 520 and 820 nm in leaves exposed to different light intensities, temperatures and CO₂ concentrations. Photorespiration was avoided in this study fixing the O₂ concentration in the gas inflow at 2%.

The results showed that a reduction of the metabolism in the stroma by decreasing CO₂ concentrations or temperatures lessened the rate constant supply of reductant to the reaction centre P700⁺ (kₑ). The values for kₑ when the CO₂ concentrations were decreased ranged from 15 to 68.37 s⁻¹ while for decreasing temperatures kₑ ranged from 22.15 to 67.68 s⁻¹. This control of electron transport was caused by a decrease of the intra-thylakoid pH that could be identified by a relative increase of the proton motive force (pmf) with respect to the pmf generated when the light intensities were increased. The pmf for leaves under decreasing CO₂ concentrations and decreasing temperatures ranged from 0.019 to 0.0543 and from 0.019 - 0.0589 respectively. The pmf generated during the increase of light intensities (0.0069 - 0.0376) did not altered the kₑ.
The efficiency of photosystem II (PSII) decreased during all environmental scenarios. Nevertheless, under decreasing CO\textsubscript{2} concentrations and decreasing temperatures the inefficiency of PSII was caused mainly by losses of photochemical quenching (q\textsubscript{p}) than a reduction of the maximum quantum efficiency of PSII photochemistry (Fv'/Fm'). On the other hand, the inefficiency of PSII under increasing light intensities was due to a simultaneous loss of q\textsubscript{p} and Fv'/Fm'. The effect of pmf over the non-photochemical quenching (NPQ) was observed when plotting these parameters together. Both parameters increased when subjecting the leaves to increasing light intensities and decreasing CO\textsubscript{2} concentrations. There was not a clear trend for the relationship of NPQ and pmf under decreasing temperatures.

The ratio H'/e', represented by a linear relationship between ETR through PSII and the flux of protons across the thylakoid membrane (v\textsubscript{H+}), was kept relatively constant when leaves were exposed to increasing light intensities. In the same way, k\textsubscript{e} and the conductivity of the thylakoid membrane to protons (g\textsubscript{H+}) were also maintained unaltered when increasing light intensity. In contrast, the relationship between ETR through PSII and v\textsubscript{H+} for leaves exposed to decreasing CO\textsubscript{2} concentrations was found to have an exponential or linear trend (it changed among the different studied leaves). It means that the stoichiometries of ATP and NADPH were altered by decreasing CO\textsubscript{2} concentrations. Contrarily to the effect of light intensities over g\textsubscript{H+}, decreasing CO\textsubscript{2} concentrations lowered g\textsubscript{H+} in a range between 4.6 to 6.6 fold. Finally, the relationship between ETR through PSII and v\textsubscript{H+} for leaves exposed to decreasing temperatures did not followed the expected pattern as observed for CO\textsubscript{2} concentrations. However, the relationship between k\textsubscript{e} and g\textsubscript{H+} was comparable to the one observed when the leaves were subjected to decreasing CO\textsubscript{2} concentrations. Although, in this case the values for g\textsubscript{H+} decreased in less proportion (from 1.9 to 3.8 fold) when the temperatures were diminished.

The results suggests that the mechanisms by which the electron fluxes are regulated, when the metabolism in the stroma is reduced by decreasing CO\textsubscript{2} concentrations or temperatures, work identically. Similarly, when taking in consideration the relationships between k\textsubscript{e} Vs. g\textsubscript{H+} for both environments, it seems that the mechanisms regulating the proton fluxes across the thylakoid membrane work in the same manner too. However, the later cannot be stated with certainty due to possible inaccuracy in the data as described below. On the other hand, the regulation of proton fluxes across the thylakoid membrane under increasing light conditions differed from the observed at decreasing CO\textsubscript{2} concentrations by having an non-altered g\textsubscript{H+} and lower pmf.

The relationships between NPQ Vs. pmf and ETR through PSII Vs. v\textsubscript{H+} under decreasing temperatures were very peculiar and totally out of the expectations. It may be that the plants were totally stressed, taking into account that each leaf was subjected to extreme environments during three consecutive days, thereby affecting the expected results. On the other hand, it is very possible that the equipment to measure absorbance changes at 520 nm still needs to be fine-tuned to reduce noisy data.
1. INTRODUCTION

1.1 THEORETICAL BACKGROUND

Components involved in electron and proton fluxes in leaves

The major role of the photosynthetic apparatus of higher plant thylakoids is to transduce light energy into ATP and reductants (NADPH) to meet the metabolic requirements for carbon assimilation and other energy requiring processes (Baker et al. 2007; Raines 2011; Yamori et al. 2011; Foyer et al. 2012). Light-harvesting complexes capture and absorb light to transfer excitation energy to the reaction centres of photosystem II (PSII) and photosystem I (PSI) to drive the primary photochemical reactions and create a separation of electrical charge (Figure 1). These light-driven charge separations at PSI and PSII drive electron flux from water to terminal electron acceptors (Baker et al. 2007). The linear electron flux (LEF) from water through the PSII and PSI reaction centres is coupled with the release of protons inside the lumen. The accumulation of protons produce an electrochemical potential difference, or proton motive force (pmf), across the thylakoid membrane (Takizawa et al. 2007; Baker et al. 2007). A cyclic electron flux around PSI (CEF1) can increase the accumulation of protons in the lumen and contribute to the pmf. The activation of CEF1 has been considered to be a mechanism to trigger non-photochemical quenching (NPQ) to protect plant from photodamage (Foyer, et al. 2012) or correct imbalances in the ratio ATP/NADPH (Avenson et al. 2005). The pmf drives ATP synthesis by the transport of protons through the ATP synthase back into the stroma (Takizawa et al. 2007; Baker et al. 2007). The pmf is comprised by two components: the electric field (Δψ) and the (ΔpH) gradient. The ΔpH specifically modulates non-photochemical quenching (NPQ) by activating the enzyme violaxanthing de-epoxidase and protonation of the antenna PsbS protein. Besides, ΔpH controls the oxidation of QH₂ at the cytochrome b₆f, thereby, regulating the electron transport rate (Bakker, et al. 2007; Foyer, et al. 2012).

The overall rate of leaf photosynthesis can fluctuate according to environmental factors such as variations in irradiance and temperature. In conditions where irradiance is limiting, energy transfer to the reaction centres needs to be maximized. If irradiance is not limiting, it is possible to have an over-excitation of the reaction centres due to an increased production of ATP and NADPH caused by higher LEF. The later could lead to photodamage. On the other hand, plants use ATP and reductants not only for carbon assimilation but also for other metabolic process with different stoichiometric requirements. Thus, besides having to regulate the rate of excitation of the reaction centres when CO₂ assimilation is restricted (light limitation/surplus), leaves also have to regulate the ratio of ATP to NADPH being produced by the thylakoid photosynthetic apparatus. (Baker et al. 2007).
Figure 1. Schematic representation of the electron (orange arrows) and proton transfers (blue arrows), and associated processes that can occur as a result of light absorption by the thylakoid photosystems. Plastoquinone (PQ), plastiquinol (PQH$_2$), ferredoxin (Fd), plastocyanin (Pc), violaxanthin de-epoxidase (VDE), violaxanthin (V), antheraxanthin (A) and zeaxanthin (Z). The brown arrow represents loss of heat regulated by energy-dependent quenching (qE) (Baker et al. 2007).

**PSII photosystem**

Photochemistry of PSII competes with the processes of fluorescence and heat loss for excitation energy in the pigment antenna of PSII. PSII fluorescence emission is used to monitor changes in photochemistry, provided that the rate constants for fluorescence and heat loss do not change. The PSII operating efficiency, measured through Chlorophyll a fluorescence, is related linearly to linear electron flux. It allows estimating the Linear Electron Flux (LEF) through PSII (Baker et al. 2007; Baker 2008). In addition, a linear relationship is observed between PSII operating efficiency and CO$_2$ assimilation when photorespiration is inhibited. In this case, the CO$_2$ assimilation is the only major sink for ATP and NADPH. Hence, PSII operating efficiency can be used to monitor LEF and $^4$CO$_2$ changes (Baker 2008).

When the quantum yield of PSI photochemistry and PSII operating efficiency are measured simultaneously at different light intensities, linear relationships between them can be observed (Harbinson et al. 1989; Baker 2008). The operation of linear electron flux (LEF) from water through PSII and PSI to electron acceptors requires similar electron fluxes through the reaction centres of both PSII and PSI. (Baker 2008).
In many circumstances, the rate at which NADPH and ATP are consumed determines PSII. The rate of carbohydrate transport out of the cell, CO₂ supply from the atmosphere through the stomata, rates of regeneration of ribulose 1,5-bisphosphate are the main factors that controls affect NADPH and ATP consumption (Baker 2008).

**PSI photosystem**

Measurements of PSI electron transport are often focused on analysing limitations of PSI electron transport on its donor and acceptor side; and, to measure relative contributions of linear and cyclic fluxes to the regeneration of P700 from P700⁺ (Baker et al. 2007).

In order to have a continuous electron flux through PSI, the following requirements must be met: (1) there must be molecules of P700 which can be photochemically oxidized; (2) an electron transport chain that is capable of transferring the electron from P700 to ferredoxin; (3) an electron donor system receiving electrons via either the linear or cyclic pathways that can re-reduce P700⁺; and (4) metabolism (or a non-metabolic electron acceptor activity, such as O₂ reduction) that will reoxidize reduced ferredoxin. A limitation of any of these requirements will decrease the light-use efficiency of PSI (Baker et al. 2007).

**Cyt-b6/f and ATP synthase complexes**

The Cyt b₆/f complex acts in both linear electron transport (production of ATP and NADPH) and cyclic electron transport (ATP generation only). In the linear electron transport system, Cyt b₆/f mediates the electron transfer between PSII and PSI by oxidizing QH₂ and reducing Pc. The oxidation of QH₂ occurs in the Cyt b₆/f. The rate of oxidation of QH₂ is the limiting step in the electron transport chain between PSII and PSI. On the other hand, the APT synthase is a macromolecule which couples the energy-donating processes of proton transfer from the lumen to stroma to form ATP. It is activated by the pmf induced by the light (Tikhonov, 2013).

There is a strong linear relationship between chloroplast electron transport rate (ETR) and cytochrome b₆/f and ATP synthase complexes content (Yamori et al. 2011) when leaves are exposed to high irradiance. At low irradiance, the ETR (on an absorbed light basis) is similar for all non-stressed C3 leaves (von Caemmerer et al. 2010). However, the manner in which each complex does this and their relative contributions are distinctly different. The Cyt b₆/f complex exhibits much tighter control of electron transport capacity and photosynthesis than that of the ATP synthase complex content (Yamori et al. 2011). Moreover, the electron transport capacity is strongly related to the cytochrome f content per unit of leaf area across a diverse range of C3 species (von Caemmerer et al. 2010).
1.2 PROBLEM DEFINITION

To support the forecasted growth in the human population of 10 billion before reaching a plateau in the later part of this century, large increases in crop yields will be required (Zhu et al. 2008; Evans 2013). Increasing the yield potential is viewed as an important strategy for achieving this required increase in yield. Nevertheless, retrospective comparisons of cultivars released over time, but grown concurrently under favourable conditions with weed, pest, and disease control and physical support to prevent lodging, reveal that while modern cultivars yield more grain, they have similar total aboveground biomass (Evans 2013).

Rice and wheat yields have more than doubled over the past half century since modern semi-dwarf varieties with high harvest indices were released in the mid-1960s. However, as harvest indices are close to the theoretical maximum and further large increases in harvest index are unlikely, comparatively little of this increase can be attributed to increased biomass (Parry et al. 2011). Moreover, massive increases in biomass production as a result of improvements in canopy architecture are questionable because canopy architecture is now also close to optimal (von Caemmerer et al. 2010). In addition, an increase in leaf area index may not result in a higher crop biomass since this is already high in most crops (Horton 2000). Improvements in agronomic practices are also largely responsible for the increased yield. However, improvements in agronomic practice alone probably would not satisfy the increasing food demand in the coming decades (Parry et al. 2011).

In the absence of any constraints, an increase of photosynthesis will increase crop yields as shown by the effects of CO₂ enrichment experiments. Due to the fact that carbon is accumulated during all the growing season by crops, a small increase in the rate of net leaf photosynthesis will represent an increase of biomass and therefore yield. In addition, several factors contributing to an increase in total photosynthesis such as canopy architecture, light interception, and photosynthetic duration have already been optimized, as mentioned before. Hence, an increase in photosynthesis will only be achieved by an increase in the photosynthetic rate per unit leaf area (Parry et al. 2011). Moreover, the theoretical photosynthetic energy conversion efficiency of C₃ plants is about 4.6% taking into account 1000 kJ of incident solar radiation at 30 °C with a CO₂ atmosphere concentration of 380 ppm, while the recorded energy conversion efficiency in the field is usually less than one-third of this value. It means that there is possibility to improve photosynthetic energy conversion efficiency (Zhu et al. 2008). Thus, improvements in rates of photosynthesis of individual leaves within the canopy have become the focus of current efforts to increase crop yields (von Caemmerer et al. 2010; Adachi et al. 2013; Evans 2013). In that logic, it is of great importance to understand the operation, regulation, and limitations of photosynthesis (Harbinson, 2012).
According to Bakker et al., (2007), knowledge regarding to the structure, composition and function of the components of photosynthetic apparatus is nowadays well understood; nevertheless, few is known about the interaction of this components at different environmental conditions in vivo. The application of biophysical techniques such as gas exchange, chlorophyll fluorescence and absorbance changes at 820 and 520 nm permits to understand the regulation and limitations of the photosynthetic apparatus in a non-destructive way. In this context, few studies have been realized in leaves of the Solanaceae species *Juanullao aurantiaca*. By the use of some of the biophysical techniques mentioned above (gas exchange, chlorophyll fluorescence and absorbance changes at 820 nm), such studies showed the responses of the thylakoid electron transport of this C₃ plant under different environmental conditions in which the photosynthetic photon flux densities (PPFD), CO₂ and O₂ concentrations were modulated (Harbinson, 1994; Genty and Harbinson, 1996). Nevertheless, the regulation of the proton circuit in the thylakoid discs of this plant species is still unknown due to the fact that spectroscopic tools, able to measure absorbance changes at 520 nm, were not used in these experiments.

In addition, the majority of the research, in which a broad combination of non-invasive techniques has been applied, has only considered variations in light intensity and CO₂ and O₂ concentrations. Not much information has been reported about the regulation of the photosynthetic apparatus in vivo under environmental conditions different than the mentioned above (i.e., temperature changes). With these antecedents, the scope of this research is to generate more information regarding to the regulation of the photosynthetic apparatus of this Solanaceae species (*J. aurantiaca*) through the use of a broad combination of non-invasive techniques. Knowledge related to the photosynthetic physiology of this plant family might be used by breeding programs in order to improve the biomass production of some crop species that have horticultural and economical potential such as potato, egg plant, tomato, tobacco, among others.

### 1.3 AIM AND RESEARCH QUESTIONS

The aim of this research is to examine the regulation of the electron and proton fluxes produced in the thylakoid discs on leaves of the Solanaceae species *J. aurantiaca* under different environmental conditions (i.e., increasing irradiance, decreasing CO₂ concentration and decreasing temperature) through the use of non-invasive biophysical techniques in vivo.

Do the mechanisms involved in the regulation of electron flux work in the same manner when leaves of *J. aurantiaca* are exposed to decreasing CO₂ concentrations or decreasing temperatures?

Do the mechanisms involved in the regulation of proton flux work in the same manner when leaves of *J. aurantiaca* are exposed to increasing light intensity, decreasing CO₂ concentrations or decreasing temperatures?
1.4 HYPOTHESIS

The mechanisms involved in the regulation of electron fluxes when the metabolism in the stroma is reduced by decreasing CO₂ concentrations or decreasing temperatures will work in a similar way. A decrease of the metabolism in the stroma will reduce the consumption of ATP and NADPH, producing acidification in the lumen, thereby stimulating NPQ and also reducing the rate of oxidation of QH₂.

The mechanisms involved in the regulation of proton fluxes when the metabolism in the stroma is reduced by decreasing CO₂ concentrations or decreasing temperatures will work in a similar way. A decrease of the metabolism in the stroma will reduce the consumption of ATP, thereby producing acidification in the lumen (high $pmf$). Nevertheless, the conductance to protons of the ATP synthase will be reduced to satisfy a low demand of ATP in the stroma. This will increase the NPQ.

The mechanisms involved in the regulation of proton fluxes will work differently under circumstances in which the metabolism in the stroma is not suppressed. The conductance to protons of the ATP synthase will be maintained relatively non-altered to satisfy the demand of ATP by the metabolism in the stroma. The $pmf$ will be lower when compared to the abovementioned situation.
2. MATERIALS AND METHODS

2.1 Plant material and grow conditions

Four cuttings of *J. aurantiaca* were planted in pots (2.85 litres capacity) containing potting soil and fertilized with 13.47g of Osmocote Pro (17-11-10). The plants were placed in a climate chamber with relative humidity of 70%, a day/night temperature of 25/23 °C, ambient CO₂ concentration and 12 hours of photoperiod with 600 μmol·m⁻²·s⁻¹ of fluorescent light. All plants were watered to conserve the potting soil at field capacity throughout experimentation.

2.2 Measuring photosynthetic performance

To evaluate the photosynthetic performance of each plant, a customised equipment designed by Wageningen University was used. This equipment offered the opportunity to measure *in vivo* the following photosynthesis parameters, described below: leaf CO₂ exchange, chlorophyll fluorescence with red and green beams, and absorbance changes at 520 and 820 nm in leaves exposed to different light intensities, temperatures and CO₂ concentrations. The use of a 560 nm excitation beam to measure chlorophyll fluorescence is more suitable to establish relationships between chlorophyll fluorescence parameters and the parameters obtained by measuring absorbance changes produced with 820 and 520 nm beams, as these wavelengths (520, 560, and 820 nm) are less absorbed by the leaf, which is not the case for red. On the other hand, the red excitation beam for chlorophyll fluorescence was used to establish relationships between the quantum efficiency of CO₂ and PSII in the different environments (Annexes 6-8) because the actinic light had the same wavelength as the red excitation beam (660 nm), making the data more suitable to relate. All the measurements were realized when the gross assimilation of CO₂ reached a steady state. Photorespiration was avoided in this study fixing the O₂ concentration in the gas inflow at 2% to evaluate only the effect of the Calvin Cycle as ATP and NADPH sink.

To evaluate the photosynthetic performance under different light intensities, the leaves were exposed to the following increasing photosynthetic photon flux densities (PPFD): 25, 50, 100, 200, 300, 500, 600, 800, 900, 1200 and 1300 mol·m⁻²·s⁻¹. This values were chosen to generate a more accurate resolution, especially at lower PPFD’s. The saturating light intensity was different for almost all the four replicates used, i.e. the saturating light intensity for two replicates was 800 μmol·m⁻²·s⁻¹; meanwhile, for the other two replicates, the saturating light intensities were 600 and 1200 μmol·m⁻²·s⁻¹ (Appendix 6).
To evaluate the photosynthetic performance under different CO₂ concentrations and temperatures, the saturating light intensity for each leaf was used. To assess the photosynthetic performance in leaves exposed to decreasing CO₂ concentrations, the next decreasing scale was used: 500, 400, 350, 300, 250, 200, 150, 100, 50 and 35 ppm. In the same way, leaves were exposed to a decreasing temperatures to evaluate the photosynthetic performance: 25, 21, 19, 17, 15, 13, 11, 9 and 6 °C.

**CO₂ exchange**

Infrared gas analysers “ADC” were used to measure leaf CO₂ exchange by difference in concentration of ingoing and outgoing flows in the leaf chamber system. This allowed to assess the net assimilation rate of leaves at different light intensities, temperatures and CO₂ concentrations. Measurements of CO₂ exchange were recorder with the help of chart recorders and software developed in Wageningen University.

**Chlorophyll fluorescence**

Red and green modulated beams were used to measure the next chlorophyll fluorescence parameters described in Table 1. The electron transport rate (ETR) through PSII was calculated using the following equation:

$$ETR_{PSII} = I \times A_{leaf} \times fraction_{PSII} \times \left( \frac{F_q'}{F_m'} \right)$$

Where,

- $I$: incident PPFD on the leaf
- $A_{leaf}$: spectral absorbance of the leaf
- $fraction_{PSII}$: fraction of incident photons that are absorbed by PSII
- $F_q'/F_m'$: operating efficiency of PSII

The absorptivity of the leaf was obtained with a spectrophotometer enclosure with a light covering a wave length spectrum from 400 to 700 nm, all developed in Wageningen University.

**Absorbance changes at 820 nm**

To measure the relative efficiency of PSI and the rate constant for electron transport between PQH₂ and P-700⁺ ($k_e$) absorbance changes at 820 nm were measured in the leaf. To know the relative efficiency of PSI, it is necessary to know the size of the P700 pools, non-oxidized [P700₀] and oxidized [P700⁺]. Later, the following equation was applied:
\[ \Phi_{PSI} = \frac{P700^0}{(P700^0 + P700^+)} \]

For this, it was necessary to know the absorbance at 820 nm during the following steps described in chronological order (Figure 2):

1) actinic light turned on (P700 partially oxidized),
2) actinic light turned off (P700 reduced)
3) far-red light turned on (P700 almost fully oxidized)
4) fast flash of broad band irradiance (P700 fully oxidized)
5) total darkness after a quick flash of far red light (P700 fully reduced)

The rate constant for electron transport between PQH₂ and P-700⁺ (k₄) was measured during the change in absorbance from step 1) to 2).

![Figure 2](image-url). Raw data of absorbance changes at 820 nm. Y axis indicates the amplitude of the signal measured in voltage. X axis indicates the time in ms.
Table 1. Chlorophyll fluorescence parameters evaluated in this study. Table modified from Baker et al. (2007).

<table>
<thead>
<tr>
<th>Fluorescence parameter</th>
<th>Definition</th>
<th>Physiological relevance</th>
</tr>
</thead>
<tbody>
<tr>
<td>$F, F'$</td>
<td>Fluorescence emission from dark or light adapted leaf respectively.</td>
<td>Provides little information on the photosynthetic performance as they are influenced by many factors.</td>
</tr>
<tr>
<td>$F_o, F_o'$</td>
<td>Minimal fluorescence from dark and light adapted leaf respectively.</td>
<td>Level of fluorescence when PSII primary quinone electron acceptors are maximally oxidized (PSII centres ‘open’).</td>
</tr>
<tr>
<td>$F_m, F_m'$</td>
<td>Maximal fluorescence from dark and light adapted leaf respectively.</td>
<td>Level of fluorescence when $Q_a$ is maximally reduced (PSII centres ‘closed’).</td>
</tr>
<tr>
<td>$F_v, F_v'$</td>
<td>Variable fluorescence from dark and light adapted leaf respectively.</td>
<td>Demonstrates ability of PSII to perform primary photochemistry ($Q_a$ reduction).</td>
</tr>
<tr>
<td>$F_q'$</td>
<td>Difference in fluorescence between $F_m'$ and $F'$.</td>
<td>Photochemical quenching of fluorescence caused by (PSII centres ‘open’).</td>
</tr>
<tr>
<td>$F_v/F_m$</td>
<td>Maximum quantum efficiency of PSII photochemistry</td>
<td>Maximum efficiency at which light absorbed by PSII is converted to chemical energy ($Q_a$ reduction).</td>
</tr>
<tr>
<td>$F_q'/F_m'$</td>
<td>PSII operating efficiency</td>
<td>Estimates the efficiency at which light absorbed by PSII is used for photochemistry ($Q_a$ reduction); at a given light intensity, it provides an estimate of the quantum efficiency of linear 4-electron transport through PSII; has previously been termed $\Delta F/F_m$ and $q^*_\text{PSII}$ in the literature.</td>
</tr>
<tr>
<td>$F_q'/F_v'$</td>
<td>PSII efficiency factor</td>
<td>Relates the PSII maximum efficiency to the PSII operating efficiency. Nonlinearly related to the proportion of PSII centres that are ‘open’ ($Q_a$ oxidized). Mathematically identical to the coefficient of photochemical quenching, $q_o$.</td>
</tr>
<tr>
<td>NPQ</td>
<td>Non – photochemical quenching</td>
<td>Estimates the non-photochemical quenching from $F_m$ to $F_m'$; monitors the apparent rate constant for non-radiative decay (heat loss) from PSII and its antennae.</td>
</tr>
</tbody>
</table>

Electrochromic shift (ECS) measurements.

To assess ECS parameters (Table 2), absorbance changes at 520 nm were produced with an spectrophotometer incorporated in the leaf chamber.
Table 2. Electrochromic shift parameters evaluated in this study. Table modified from Baker et al. (2007).

<table>
<thead>
<tr>
<th>Electrochromic shift parameter</th>
<th>Definition</th>
<th>Physiological relevance</th>
</tr>
</thead>
<tbody>
<tr>
<td>$E_{CS}$</td>
<td>The total rapid (&lt;1 s) change in ECS signal upon rapidly switching off actinic light from steady state.</td>
<td>Proportional to the total light-dark difference in transthylakoid pmf.</td>
</tr>
<tr>
<td>$g_{H^+}$</td>
<td>The inverse of the lifetime of the rapid decay of ECS, upon rapidly switching off the actinic light from steady state</td>
<td>Proportional to the aggregate conductivity (or permeability) of the thylakoid membrane to protons, predominantly determined by ATP synthase activity.</td>
</tr>
<tr>
<td>$V_{H^+}$</td>
<td>The initial rate of decay of the ECS signal to a quasi-stable state, tens to hundreds of ms after rapidly switching off actinic light from steady state</td>
<td>Proportional to the proton flux through the photosynthetic apparatus and thus ATP synthesis; can be used to estimate changes in proton translocation by circular electron flux (CEF1).</td>
</tr>
</tbody>
</table>
3. RESULTS

3.1 Photosynthesis regulation under light intensity changes

It can be observed in Figure 3-A, that \( k_e \) had the trend to be non-altered while the \( ^*\text{PSII} \) decreased in leaves exposed to an increasing irradiance. The relationship between Fv'/Fm' and \( q_p \) (Figure 3-B), shows that decreasing values of Fv'/Fm' were accompanied by reductions in \( q_p \) when increasing gradually the light intensity. The relationship between NPQ and Fv'/Fm' (Figure 3-C) shows that both parameters were inversely related. While NPQ increased, Fv'/Fm' decreased with an increasing light intensity. It can be observed in Figure 3-D that the \( ^*\text{PSI} \) decreased while \( k_e \) was maintained constant with an increasing light intensity.

Figure 3. Relationships between: (A) \( ^*\text{PSII} \) Vs. \( k_e \), (B) Fv'/Fm' Vs. \( q_p \), (C) NPQ Vs. Fv'/Fm', and (D) \( ^*\text{PSIV} \) Vs. \( k_e \), when increasing gradually the light intensity in Juanulloa aurantiaca grown under 600 \( \mu \text{mol m}^{-2} \text{s}^{-1} \) of irradiance. Different symbols represent individual leaves measured from different plants.
In Figure 4-A, it can be observed that the pmf had the trend to increase while $k_e$ was maintained almost constant when increasing gradually the light intensity. Figure 4-B shows that an increase of the ETR through PSII was tightly coupled to an increase of $V_{H+}$ when the light intensity was increased gradually. The relationship between $k_e$ and $g_{H+}$ shows that both parameters were not altered when increasing gradually the light intensity (Figure 4-C). In Figure 4-D it can be observed that NPQ and pmf increased when the light intensity was increased. Measurements for R2 were too noisy; thereby, they were excluded.

**Figure 4.** Relationships between: (A) $k_e$ Vs. pmf, (B) ETR of PSII Vs. $v_{H+}$, (C) $k_e$ Vs. $g_{H+}$, and (D) NPQ Vs. pmf when increasing gradually the light intensity in *Juanulloa aurantiaca* grown under 600 $\mu$mol m$^{-2}$ s$^{-1}$ of irradiance. Different symbols represent individual leaves measured from different plants.
3.2 Photosynthesis regulation under CO\(_2\) concentration changes

It can be observed in Figure 5-A, that \(k_e\) and \(^4\)PSII decreased when the leaves were exposed to a decreasing CO\(_2\) concentration. The relationship between \(Fv'/Fm'\) and \(q_p\) (Figure 5-B), shows that decreasing values of \(Fv'/Fm'\) were accompanied by a reduction of \(q_p\) especially during the first decreasing steps of CO\(_2\) concentration, i.e. from 500 to approximately 200 ppm. At lower CO\(_2\) concentrations, it can be observed that \(q_p\) diminished in greater proportions than \(Fv'/Fm'\). The relationship between NPQ and \(Fv'/Fm'\) (Figure 5-C) shows that both parameters were inversely related. While NPQ increased, \(Fv'/Fm'\) decreased with a decreasing CO\(_2\) concentration. It can be observed in Figure 5-D that \(^4\)PSI and \(k_e\) declined simultaneously when the CO\(_2\) concentration was reduced. However, this relationship was not observed when the CO\(_2\) concentration reached 35 ppm. At this CO\(_2\) concentration, \(^4\)PSI increased while \(k_e\) decreased.

**Figure 5.** Relationships between: (A) \(^4\)PSII Vs. \(K_e\), (B) \(Fv'/Fm'\) Vs. \(q_p\), (C) NPQ Vs. \(Fv'/Fm'\), and (D) \(^4\)PSI Vs. \(K_e\) when decreasing gradually the air CO\(_2\) concentration from 500 to 35 ppm. in *Juanulloa aurantiaca* grown under 600 μmol m\(^{-2}\) s\(^{-1}\) of irradiance. Different symbols represent individual leaves measured from different plants.
The relationship between $k_e$ and $pmf$, shows that both parameters were inversely related if excluding the measurements at 35 ppm CO$_2$ concentration (Figure 6-A). When decreasing the CO$_2$ concentration $k_e$ decreased while $pmf$ increased. Observations for R3 did not follow this trend during the first decreasing CO$_2$ steps. Figure 6-B shows that ETR through PSII and $v_{hi}$ decreased with a decreasing CO$_2$ concentration. The relationship between $k_e$ and $g_{hi}$ (Figure 6-C), shows that both parameters decreased when leaves were exposed to decreasing CO$_2$ concentrations. Figure 6-D shows the relationship between NPQ and $pmf$. Both parameters increased when the CO$_2$ concentration was decreased.

Figure 6. Relationships between: (A) $k_e$ vs. $pmf$, (B) ETR of PSII vs. $v_{hi}$, (C) $k_e$ vs. $g_{hi}$, and (D) NPQ vs. $pmf$ when decreasing gradually the air CO$_2$ concentration from 500 to 35 ppm in _Juania llao aurantiaca_ grown under 600 μmol m$^{-2}$ s$^{-1}$ of irradiance. Different symbols represent individual leaves measured from different plants.
3.3 Photosynthesis regulation under temperature changes

It can be observed in Figure 7-A, that $k_e$ and $F_P$ decreased when the leaves were exposed to decreasing temperatures. With an exemption for R2 which did not have the same trend at 25 and 21 °C. The relationship between $F_V/F_m$ and $q_p$ (Figure 7-B), shows that both parameters decreased when temperature was decreased gradually. Nonetheless, it can be seen that $F_V/F_m$ was relatively lowered in less proportion than $q_p$. The relationship between NPQ and $F_V/F_m$ (Figure 7-C) shows that both parameters were inversely related. While NPQ increased, $F_V/F_m$ decreased with declining temperatures. It can be observed in Figure 7-D that $F_P$ and $k_e$ were reduced with declining temperatures. It can be observed that data from R2 continued being noisy as can be observed in Figures 7-A and B.

Figure 7. Relationships between: (A) $F_P$ Vs. $k_e$, (B) $F_V/F_m$ Vs. $q_p$, (C) NPQ Vs. $F_V/F_m$, and (D) $F_P$ Vs. $k_e$, when decreasing gradually the temperature from 25 to 6 °C in *J. aurantiaca* grown under 600 μmol m$^{-2}$ s$^{-1}$ of irradiance. Different symbols represent individual leaves measured from different plants.
Figure 8-A shows that the relationship between $k_e$ and $pmf$ did not follow a determined pattern as observed previously in the different environmental scenarios. Nevertheless, it can be seen that during the first decreasing temperature steps both parameters were inversely related. While $k_e$ decreased, $pmf$ increased. At lower temperatures $pmf$ had the trend to decrease in the cases of R3 and R4. For R1, it is not possible to describe the trend because the data is scattered. Figure 8 (C) shows that decreases of $k_e$ were accompanied by decreases of $g_{H+}$ when the temperature was decreased. The data obtained for the relationships between ETR through PSII Vs. $V_{m\nu}$ (Figures 8-B) and NPQ Vs. $pmf$ (Figure 8-D) was very scattered, making impossible to describe trends as previously, when the leaves were exposed to increasing light intensities or decreasing CO$_2$ concentrations.

Figure 8. Relationships between: (A) $k_e$ Vs. $pmf$, (B) ETR of PSII Vs. $V_{m\nu}$, (C) $k_e$ Vs. $g_{H+}$ and (D) NPQ Vs. $pmf$ when decreasing gradually the temperature from 25 to 6 °C in *J. aurantica* grown under 600 μmol m$^{-2}$ s$^{-1}$ of irradiance. Data for R2 was not taken into account due to electric interferences occurred during the measurement day.
4. DISCUSSION

As expected, when comparing $k_e$ behaviour at changing environments (i.e. increasing light intensities, decreasing CO$_2$ concentrations and decreasing temperatures) it could be seen that at different light intensities $k_e$ had the trend to be unaltered while at declining the CO$_2$ concentrations and temperatures, it had the trend to decrease (Appendix 1). Genty and Harbinson (1996), found that $k_e$ was almost constant, with values around 55 s$^{-1}$, when leaves of *J. aurantiaca* were exposed to increasing light intensities, which corresponds to the results obtained in this research (Appendix 1-A). According to Harbinson (1994), a non-altered $k_e$ accompanied with a decreasing ρPSII, ρPSI, and $Fv'/Fm'$ is commonly found with increasing light intensities, which corresponds to the results shown in Figure 3. Laisk and Oja (1994), stated that an unaltered $k_e$ is due to an absent or constant proton counter pressure at the site of plastoquinol oxidation. However, results obtained for pmf, which according to Kanazawa and Kramer (2002) could be taken as an indicator of ΔpH, showed that there was a reduction in the intra-thylakoid pH that did not altered $k_e$(Figure 4-A). It is explained by a low phosphate potential represented by the ratio [ATP]/([ADP][Pi]) (Tikhonov, 2013), which implies that there was enough amount of ADP and P, caused by ATP metabolism in the Calvin cycle. In other words, the efflux of protons from the lumen to the stroma to produce ATP was not limited (enough to maintain a constant H$^+$/e$^-$ ratio) so there was not a high acidification in the lumen that could avoid the oxidation of QH$_2$ and diminish $k_e$. On the other hand, a reduction of metabolism in the stroma by decreasing CO$_2$ concentrations or temperatures reduces the demand for NADPH and ATP by the Calvin Cycle, increasing the control of electron transport (drop of $k_e$) through a decrease of the intra-thylakoid pH (Harbinson, 1994; and, Genty and Harbinson, 1996). According to Tikhonov, A. (2013), this reduction in ATP consumption increases the phosphate potential, meaning with it less amount of ADP and P, for the formation of ATP, (i.e. less efflux of protons from the lumen to the stroma) which causes a decrease of the intra-thylakoid pH that avoids the oxidation of QH$_2$ and diminishes the $k_e$. In agreement to these staments, the results shown in Figures 6-A and 8-A, for declining CO$_2$ concentrations and declining temperatures respectively, represent how a decrease in the intra-thylakoid pH, identified through an increase in pmf lessened the $k_e$. When comparing the ranges for pmf changes obtained during increasing light intensities (0.0069 - 0.0376) with the ranges for pmf changes obtained when decreasing CO$_2$ concentrations (0.019 - 0.0543) and decreasing temperatures (0.019 - 0.0589), it could be observed that even when the differences between the highest and lowest values of the ranges were similar for all the environmental scenarios, the pmf changes when increasing light intensities were produced in a lower scale than for declining CO$_2$ concentrations and declining temperatures. Considering that pmf is an indicator of ΔpH (Kanazawa and Kramer, 2002), it explains a non-altered $k_e$ when increasing light intensities and a diminishing $k_e$ when decreasing the CO$_2$ concentrations and the temperatures.
As expected, \(^{\circ}\)PSII decreased while exposing the leaves to increasing light intensities, decreasing CO\(_2\) concentrations and decreasing temperatures as can be observed in Figures 3-A, 5-A and 7-A respectively. A linear relationship between \(^{\circ}\)PSII and \(k_e\) can be observed when decreasing CO\(_2\) concentrations and decreasing temperatures. This can be explained by the fact that a reduction of the intra-thylakoid pH simultaneously stimulated NPQ (Iliola et al. 2011), thereby reducing \(^{\circ}\)PSII; and also controlled the oxidation of QH\(_2\), thereby diminishing \(k_e\). On the other hand, a decrease of \(^{\circ}\)PSII is not only dependent of Fv'/Fm', but also of \(q_p\). In that way, a decrease of \(^{\circ}\)PSI due to increasing light intensities was caused by a simultaneous decrease of Fv'/Fm' and \(q_p\) (Figure 3-B). Both parameters decreased almost in the same proportion. While Fv'/Fm' had a decrease of 26.9%, \(q_p\) had a decrease of 23.71%. This results does not correspond with the results obtained by Harbinson (1994), in which the registered decreases for Fv'/Fm' and \(q_p\) were approximately 20% and 42% respectively, when leaves from the same plant species were exposed to increasing light intensities in the range of: 0 to 450 \(\mu\)mol m\(^{-2}\) s\(^{-1}\). It could be that the plants used by Harbinson (1994) were grown at a lower light intensity than the used in this experiment (600 \(\mu\)mol m\(^{-2}\) s\(^{-1}\)). The plants used in this experiment were acclimated for a relative high light intensity showing less accumulation of a reduced pool of Q\(_A\), represented by less reduction of \(q_p\) at high light intensities. On the other hand, the decrease of \(^{\circ}\)PSII due to decreasing CO\(_2\) concentrations was caused principally by a reduction of \(q_p\) (accumulation of reduced Q\(_A\)) than by a diminishing Fv'/Fm' as can be observed in Figure 5-B. In this case, Fv'/Fm' had a reduction of 40.9%, while \(q_p\) suffered a reduction of 65.4%. The same trend was observed by Harbinson (1994), although the decrease for Fv'/Fm' and \(q_p\) were approximately 18.5 and 58% respectively. As mentioned above, it could be that the plants used by Harbinson (1994) were grown under low light intensities. According to Genty and Harbinson (1996), plants that are adapted to low light intensities have low capacity to dissipate excess of energy (low NPQ) and the inefficiency of PSII is caused in greater proportions by accumulation of reduced Q\(_A\) pool. This phenomena can be noticed when comparing the relative decrease of Fv'/Fm' and \(q_p\) obtained by Harbinson (1994) with increasing light intensities (20% Vs 42%) and decreasing CO\(_2\) concentrations (18.5% Vs 58%). The decline of \(^{\circ}\)PSII when the leaves were exposed to decreasing temperatures was due to a relatively higher decline of \(q_p\) than a decline of Fv'/Fm', as observed in Figure 7-B. The values of Fv'/Fm' decreased 20.8% while the values of \(q_p\) decreased 45.1%. It is in agreement with results found for barley and spinach leaves, in which the rate of Q\(_A\) reduction overtook the rate of non-photochemical dissipation when lowering the temperature (Falks et al. 1996). The high relative effect of decreasing CO\(_2\) concentrations and temperatures on \(q_p\) was due to accumulation of reduced Q\(_A\), caused by a down-regulation of the electron transport through cytb6f (Genty and Harbinson 1996 and Foyer et al. 2012).

A decrease of \(^{\circ}\)PSI was observed while exposing the leaves to increasing light intensities, decreasing CO\(_2\) concentrations and decreasing temperatures as can be observed in Figures 3-D, 5-D and 7-D respectively. A decrease of \(^{\circ}\)PSI did not have a linear relationship with \(k_e\) (Figures 3-D) due to the fact the later parameter was non-altered when the light intensity were increased, as explained above. In contrast, a linear relationship between \(^{\circ}\)PSI and \(k_e\) can be observed when decreasing CO\(_2\) concentrations and decreasing temperatures. According to Genty and Harbinson (1996), a decrease of \(^{\circ}\)PSI is caused by the accumulation of photo-oxidized P700, i.e. during the light response curve. Nevertheless, when the metabolism in the stroma is reduced by
decreasing CO₂ concentrations or temperatures, the oxidation of P700 can be limited by a lack of electron acceptors (Genty and Harbinson, 1996), thereby, diminishing the light use efficiency of PSI (Bakker et al. 2007). It is important to mention that at low CO₂ concentrations the linearity between ᶲPSI and kᵣ was broken and an apparent increase in ᶲPSI was registered. According to Harbinson (1994), it is caused by an accumulation of reducing equivalents at the donor side of PSI, creating a P700 pool unable to be oxidized (inactive pool of reduced P700) which is not taken into account in the calculations that determine ᶲPSI. An inactive pool of reduced P700 produces an overestimation of ᶲPSI while kᵣ continues with a decreasing trend (Figure 5-D). According to Genty and Harbinson (1996); the inflection point in which the ᶲPSI apparently improves, determines the lower value of a range of CO₂ concentrations at which an effective control of electron transport can be exerted, i.e. in this experiment an effective control of electron transport was observed at CO₂ concentrations above 50 ppm, limit that does not match with the value of 35 ppm obtained by Genty and Harbinson (1996) in the same plant species. However, the values of kᵣ obtained at a CO₂ concentration of 35 ppm were in the range of 15.53 to 18.65 s⁻¹, values that are similar to the kᵣ (16 s⁻¹) obtained by Genty and Harbinson (1996), meaning that could be possible that the IRGA equipment that was measuring the absolute CO₂ concentration of air flowing into the chamber lost sensitivity at this low CO₂ concentrations.

In agreement to Bakker (2008), an increasing NPQ was accompanied by a decreasing Fv'/Fm' showing an inverse linear relationship with increasing light intensities as shown in Figure 3-C. The same phenomena was observed when the CO₂ concentrations and temperatures were decreased as shown in Figures 5-C and 7-C, respectively. This can be explained by the fact that the mechanisms that activate NPQ are considered to be the same in all the environmental sceneries. However, it can be observed that reducing the CO₂ concentration from 500 to 35 ppm triggered more the formation of NPQ than reducing temperatures from 25 to 6 °C.

The relationship between NPQ and pmf was exponential when the leaves were exposed to increasing light intensities and decreasing CO₂ concentrations as can be observed in Figures 4-D and 6-D, respectively; and Appendix 8. These results are in agreement with the results obtained by Avenson et al. (2004) in which the same trend can be observed when leaves of wild-type Nicotiana tabacum xanthi (tobacco) were subjected to increasing light intensities ranging from 32 to 820 µmol m⁻² s⁻¹ and different gas compositions (370 ppm CO₂/21% O₂, 50 ppm CO₂/21% O₂ and 50 ppm CO₂/1% O₂). However, in previous research realized by Kanazawa and Kramer (2002), another trend was found when leaves of the same plant species (tobacco) were exposed to increasing light intensities (45 to 2000 µmol m⁻² s⁻¹) with different gas compositions (2000, 350, 50 and 0 ppm of CO₂ with 21% O₂). This difference can be observed even when comparing results obtained for tobacco plants exposed to similar environments i.e., 370 ppm CO₂/21% O₂ and 350 ppm CO₂/21% O₂. In contrast, when leaves were exposed to decreasing temperatures, there was not a clear trend for the relationship of NPQ and pmf as can be observe in Figure 8-D. It is possible that the leaves suffered a lot of stress during the experimental period, taking into account that each leaf was subjected to extreme environments during three consecutive days, thereby affecting the expected results. On the other hand, it is very possible that the equipment to measure absorbance changes at 520 nm still needs to be fine-tuned to reduce noisy data. Indeed, when reviewing literature related to absorbance changes at 520 nm (Kramer, 1999; Sacksteder et al. 2000; Kanazawa
and Kramer, 2002; Avenson et al. 2004 and, Avenson et al. 2005) it can be noticed that there is a lot of variation on the produced data, especially for pmf values.

The ratio $H^+/e^-$, represented by a linear relationship between ETR through PSII and $v_{int}$, was kept relatively constant when leaves were exposed to increasing light intensities (Figure 4-B). This results reconcile with the results obtained by Sacksteder et al. (2000) and Avenson et al. (2005) in which the same relationship was obtained for Nicotiana tabacum and Arabidopsis thaliana plants, respectively. In the same way as the ratio between $H^+/e^-$, it can be observed in Figure 4-C that $k_e$ and $g_{int}$ were also maintained unaltered when increasing light intensity. Similar trend for $g_{int}$ was observed by Kanazawa and Kramer (2002) in Nicotiana tabacum plants, concluding that pmf was always above the needed to activate the ATP synthase during light changes. In contrast and within the noise, the relationship between ETR through PSII and $v_{int}$ for leaves exposed to decreasing CO$_2$ concentrations was found to have an exponential trend for R1, R2 and R4, while for R3 the trend was more linear (Figure 6-B). It means that the stoichiometries of ATP and NADPH were altered by decreasing CO$_2$ concentrations.

The lack of electron acceptors in the stroma at low CO$_2$ concentrations (Figure 5-D) was also reflected in the relationship between $k_e$ and pmf (Figure 6-A). It can be seen that a decrease of $k_e$ was accompanied by an increase of pmf until reaching CO$_2$ concentration levels ranging from 100 to 50 ppm. Below these CO$_2$ concentrations the pmf decreased too, showing that besides a down regulation of $g_{int}$ (explained below), there was an attempt to control the ratio $H^+/e^-$ by decreasing the pmf. Contrarily to the effect of light intensities over $g_{int}$, decreasing CO$_2$ concentrations lowered $g_{int}$ in a range between 4.6 to 6.6 fold (Figure 6-C) which is congruent with the results found by Kanazawa and Kramer (2002) for Nicotiana tabacum when CO$_2$ concentrations were reduced from 2000 to 0 ppm. Although the relationship between $k_e$ and $g_{int}$ was not very clear, being exponential for R1 and R2 and linear for R3 and R4, it can be observed (Figure 6-C) that both parameters were lessened with decreasing CO$_2$ concentrations. Interestingly, the relationship between ETR through PSII and $v_{int}$ for leaves exposed to decreasing temperatures (Figure 8-B) did not followed the expected pattern as observed for CO$_2$ concentrations. As mentioned above, it may be possible that the leaves were under high level of stress due to experimental conditions or it could be that measurements for pmf were too noisy, thereby affecting the values calculated for $v_{int}$. On the other hand, the relationship between $k_e$ and $g_{int}$ was exponential (Figure 8-C), which is more comparable to the data obtained when leaves were subjected to decreasing CO$_2$ concentrations, although in this case the values for $g_{int}$ decreased in less proportion (from 1.9 to 3.8 fold) when the temperatures were diminished.

The results suggests that the mechanisms by which the electron fluxes are regulated, when the metabolism in the stroma is reduced by decreasing CO$_2$ concentrations or decreasing temperatures, work in the same way. Similarly, when taking in consideration the relationships between $k_e$ Vs. $g_{int}$ for both environments, it seems that the mechanisms regulating the proton fluxes across the thylakoid membrane work in the same manner too. However, the later cannot be stated with certainty due to possible inaccuracy in the data generated by ECS measurements. On the other hand, the regulation of proton fluxes across the thylakoid
membrane under increasing light conditions differed from the observed at decreasing CO$_2$ concentrations and decreasing temperatures by having an non-altered $g_{in}$, and lower $pmf$. 
5. REFERENCES


6. APPENDIXES

Appendix 1. Rate constant supply of reductant to $P700^+$, $k_e$, at different changing environments. (A) When increasing gradually the light intensity, (B) when decreasing gradually the CO$_2$ concentration and (C), when decreasing gradually the temperature. Different symbols represent individual leaves measured from different plants.
Appendix 2. Parameters determined from electrochromic shift (ECS) signals when decreasing gradually the light intensity from 100 to 1300 μmol m$^{-2}$ s$^{-1}$ in *J. aurantiaca* grown under 600 μmol m$^{-2}$ s$^{-1}$ of irradiance. Different symbols represent individual leaves measured from different plants. (A) flux of protons, $V_{\text{H}^+}$, (B) total rapid change in ECS signal, pmf, (C) conductivity of the ATP synthase to protons, $g_{\text{H}^+}$. Measurements for R2 were too noisy; thereby, they were excluded.
Appendix 3. Parameters determined from electrochromic shift (ECS) signals when decreasing gradually CO$_2$ concentration in *Juanulloa aurantiaca* grown under 600 μmol m$^{-2}$ s$^{-1}$ of irradiance. Different symbols represent individual leaves measured from different plants. (A) flux of protons, $V_{H^+}$, (B) total rapid change in ECS signal, $pmf$, (C) conductivity of the ATP synthase to protons, $g_{H^+}$. 

**A**  

![Graph A](image1)

**B**  

![Graph B](image2)

**C**  

![Graph C](image3)
Appendix 4. Parameters determined from electrochromic shift (ECS) signals when decreasing gradually temperature in *Juanulloa aurantiaca* grown under 600 μmol m⁻² s⁻¹ of irradiance. Different symbols represent individual leaves measured from different plants. (A) Flux of protons, $V_{H^+}$, (B) total rapid change in ECS signal, $pmf$, (C) conductivity of the ATP synthase to protons, $g_{H^+}$. 

![Graph A](image1.png)

![Graph B](image2.png)

![Graph C](image3.png)
Appendix 5. Relationship between NPQ and pmf when plotting the same parameters using the data obtained during light and CO₂ changes together. It can be seen that the relationship had the trend to be exponential. Different symbols represent individual leaves measured from different plants.

Appendix 6. (A) Gross CO₂ assimilation and (B) Relationship between quantum efficiency for CO₂ assimilation and φPSII when increasing gradually light intensity.
Appendix 7. (A) Gross CO$_2$ assimilation and (B) Relationship between quantum efficiency for CO$_2$ assimilation and $\Phi_{PSII}$ when decreasing gradually the CO$_2$ concentration. Measurements of gross CO$_2$ assimilation for R4 were done in two consecutive days. In the first day, the first four measurements were taken (500 to 300 ppm). Measurements for the remaining CO$_2$ concentrations were done the next day.

Appendix 8. (A) Gross CO$_2$ assimilation and (B) Relationship between quantum efficiency for CO$_2$ assimilation and $\Phi_{PSII}$ when decreasing gradually the temperature.