

Relationship between leaf temperature and photosynthetic carbon in *Capsicum annuum* L. in controlled climates

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This study measures photosynthesis in *Capsicum annuum* L. by principles of gas analysis and expression of enzyme ribulose-1,5-biphosphate carboxylase/oxygenase (rubisco), and demonstrates that a relationship exists between leaf temperature and photosynthesis. Observed relationship between leaf temperature and photosynthesis may be applicable to other C₃ plants.

Keywords: Air temperature, Expression of rubisco, Gas analyzers Photosynthetic active radiation (PAR), RT-PCR

Introduction

Photosynthesis (PS) is conversion of carbon dioxide (CO₂) into organic compounds using energy from sun¹. Physiology, botany and biology are concerned with PS response of plant to climatic factors². PS, being a process, cannot be directly measured, but may only be inferred from the measurement of related variables³. Principal factors related to PS² are light radiation, CO₂ absorption, O₂ production, and leaf temperature. The C₃ PS is most common and ancient pathways, whereas C₄ PS and crassulacean acid metabolism (CAM) are derived from C₃¹. *Capsicum annuum* L. was chosen as experimental organism because it is a C₃ plant, small and portable produced round the year, and one of the most cultivated chili pepper species in the world. This study demonstrates relationship between temperature of leaves and its PS on *C. annuum* L. at different room temperatures. Although there have been earlier researches on changes in leaf temperature^{5,6}, this study is the first to correlate leaf temperature with PS. Such a relationship would ensure the inferring of PS through leaf temperature, and

would be a starting point for the possible designing of non-invasive PS measurement devices.

Experimental Section

PS uses spectrum of light, mostly the wavelengths of 400 and 700 nm, called Photosynthesis Active Radiation (PAR), which was used for all the experiments. There are many methods for measuring PS³, but only gas analysis and expression of enzyme [ribulose-1,5-biphosphate carboxylase/oxygenase (rubisco)] were selected as correlation between leaf temperature and PS. Expression of rubisco^{2,4} was chosen for being a biochemical indicator of PS process activity.

Experimental Organism

Experimental organism was cultivar Don Benito (Jalapeño chili pepper) of *C. annuum* L. Among five cultivated species of chili, *C. annuum* L. is the most widely known and the most economically important because it is distributed worldwide^{7,8}. It is an ingredient for food preparation and an important product utilized in pharmaceutical and cosmetic industries⁹. Its origin is Mesoamerica, and more specifically Mexico and Guatemala. Today, its main producers are China, Mexico, Turkey and the USA⁷. *C. annuum* is cultivated in

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different regions from sea level to 2500 m above sea level.

Cultivar Don Benito has plants with stems and vigorous branches with dichotomous upward branching. Its leaves are oval and, similar to branches, have a high degree of pubescence, which gives it a grayish green color to foliage. Plant height is up to 90 cm. Its fruits are dark emerald green, oblong, and medium sized (length, 6-8 cm; diam, 2.5-3.0 cm), have a thick pericarp of 7 mm and an evenly distributed placenta in inner cavity, which gives them strength and high specific gravity. Its yield potential is 40 tons per ha and it is best suited to areas of humid tropics and subtropics¹⁰. Selected plants were cultivated in sunshine substrate added with perlite. To maintain similarities among analytes, experiment used selected leaves of different chili plants with the same average age, size, height and nutrition.

Gas Analysis

Gas analysis, most used technique for research and commercial applications in bioprocesses^{3,11}, is based on that CO₂ molecule absorbs IR at the wavelengths of 2.66, 2.77, 4.26 and 14.99 µm³. Infrared gas analyzers (IRGAs) utilize light absorption characteristics of CO₂ molecules to measure the decrease in quantum flow density of infrared light as a quantitative measure of this gas. Production of this gas is an accurate measure for PS. Gas interchange chambers of IRGAs draw air from the chamber and from a reference atmosphere, comparing them in gas sensor. The time necessary for CO₂ concentration to decrease by a predetermined amount (30 ppm) is inversely proportional to PS rate of leaf³. Device used in these experiments was Li-6400XT¹². This gas interchange analyzer was chosen for its characteristics of accuracy of 1.5%, precision of ± 0.09% at CO₂ 350 ppm, rapid response, elimination of time delays, and portability. It is also the most referenced PS system in scientific publications^{13,14}. PS and leaf temperature were measured in plants at different room temperatures and different radiation levels.

Detection of Stabilization Period of Photosynthesis

Stabilization period is the time that plant needs to stabilize its PS at different radiation levels. Two room temperatures (RTs: 23 & 33°C) were used for this test. Under stabilization process, plants were placed in a completely darkened chamber with corresponding RT for 30 min. Plants were subjected to 6 levels of radiation (0 - 500 PAR in increments of 100 PAR) for 15 min

each. PS was monitored every minute to detect stabilization point.

Light Curves

Light curves were used to obtain leaf temperature tendencies at different radiation levels and, different PS levels. This part of experiment used three plans with three repetitions each, at 5 RT levels (11, 23, 33, 39 & 45°C), and 11 radiation levels (from 0 to 1000 PAR, with increments of 100 PAR). Under stabilization process, plants were subjected to each radiation level for a period equal to the obtained stabilization period (5 min). After radiation exposure, PS and leaf temperature were measured.

Reference Light Curves

To establish reference measurements, light curves procedure was repeated with two differences. First difference was to repeat one light curve without the plant, to measure device effect. Second difference was to repeat other light curve with a plant affected with a herbicide¹⁵ (SENCOR 480 SC), which prevents PS from occurring, to measure radiation effect on leaves without PS. SENCOR is based in Metribuzin, and inhibits PS at photosystem II. It is a selective systemic herbicide that controls broadleaf weeds through their leaves and roots. Herbicide mixture, SENCOR (126 µl) dissolved in 40 ml of distilled water, was applied to a plant at 24 h before the normal procedure of a light curve was followed.

Expression of Rubisco

All photosynthetic eukaryotes reduce CO₂ to carbohydrates via Calvin Cycle, which proceeds in three stages: 1) carboxylation, during which CO₂ is covalently linked to a carbon skeleton; 2) reduction, during which carbohydrates are formed from photochemically derived Adenosine TriPhosphate (ATP) and reducing equivalents in the form of Nicotinamide Adenine Dinucleotide Phosphate (NADPH); and 3) regeneration, during which CO₂ acceptor ribulose-1,5-bisphosphate re-forms². CO₂ fixation is central mechanism of primary production in almost all ecosystems, and it is the major biogeochemical process in Calvin cycle. Key enzyme of this cycle is rubisco, which is a bifunctional enzyme and controls reduction of CO₂ and oxygenolysis of ribulose-1, 5-bisphosphate. Rubisco exists in multiple forms, which differ in structure, catalytic property and O₂-sensitivity. However, the most distributed type of rubisco is the form I, which occurs in plants as well as in photo- and

chemoautotrophic bacteria. Form I rubisco is composed of 8 chloroplast-encoded large subunits and 8 nucleus-encoded small subunits (L8S8)⁴. Small subunits of rubisco are synthesized in cytoplasm and transported into chloroplast, where enzyme is assembled. Expression of rubisco was selected as a reference method because rubisco is key enzyme of Calvin cycle, and also is a biochemical indicator of photosynthetic process activity^{4,16}. Additionally, it is the most abundant enzyme in the world, representing up to 40% of total soluble protein of most leaves². Expression of rubisco was measured by Reverse Transcriptase of Polymerase Chain Reaction (RT-PCR).

Reverse Transcriptase of Polymerase Chain Reaction (RT-PCR)

RT-PCR is a useful variation of conventional Polymerase Chain Reaction (PCR). It allows amplification of specific transcripts of RNA from small samples of biological material. It does not require the same levels of rigorous extraction processes associated with purification of RNA for conventional purifying purposes. Buffer, Taq polymerase, primers, reverse transcriptase (RTase) and template of RNA are added together in reaction tube. Reaction is warmed up at 37°C. This allows RTase to produce a copy of DNA from RNA chains aligned with any of the primers of mixture. After synthesis of first chain, a normal PCR is realized for DNA amplification. This results on synthesis of a second chain. Subsequently, DNA of double chain is amplified¹⁷. RT-PCR procedure is as follows: i) Six treatments, designed for this part of experiment, consisted in two air temperatures (23 & 33°C) at three levels of PAR radiation (0, 191, and 520 $\mu\text{mol}/\text{m}^2\text{s}$) each, and one plant was used for each treatment; ii) Each plant was submitted to stabilization process. Then it was subjected to 5 min of the corresponding level of radiation. The sample was then cut and frozen at -75°C; iii) mRNA was extracted from sample (single leaf) with trizol technique. Leaf was frozen with liquid nitrogen, triturated, and put in an eppendorf tube. Triturated sample was mixed and homogenized with 1 ml of trizol. This mixture was centrifuged until it separated into two phases. Over-swimmer was separated and added to chloroform and isopropane. This last mixture was centrifuged once more until it separated into another two phases. Crystalline phase that contained RNA was added to ethanol to precipitate RNA. An RNA tablet was formed, dried and then suspended in sterile water with Diethylpyrocarbonate (DEPC); iv) cDNA was obtained

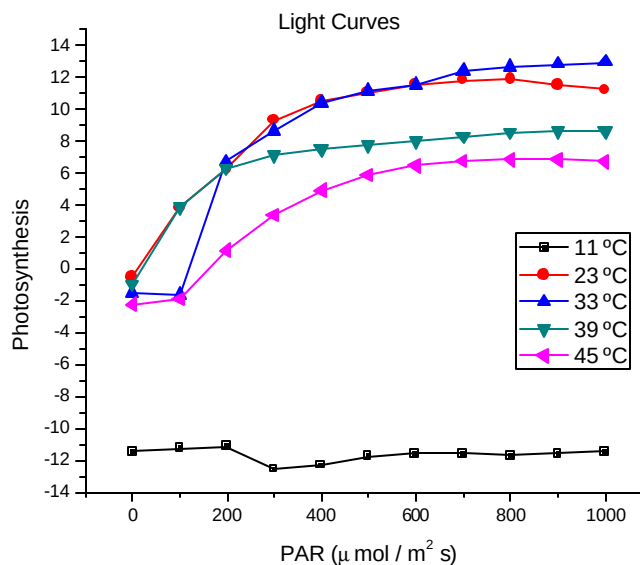


Fig. 1—Light curves at different room temperature levels

through the method of “Synthesis of first chain”. Quantity (300 ng) was calculated for each sample; v) Once first chain was obtained, a conventional PCR was realized for each sample, using chili oligos for quantifying rubisco enzyme; and vi) Obtained PCR products corresponded to transcribed rubisco. These products were charged in an agarose gel with ethidium bromide. The mass (ng) of transcribed was then calculated.

Results

Stabilization Period

Observations during this part of experiment indicate that 5 min were sufficient for a plant to stabilize its PS for applied radiation increments of 100 PAR. This period of time is equal for temperatures of 23 and 33°C. This stabilization period of 5 min was used for experiments in light curves.

Light Curves

At low temperatures (11°C) there is absolutely no PS (Fig. 1). On the contrary, effect is pure photorespiration, no matter the incidental radiation. Photorespiration and PS work in diametrically opposite directions. This explains why these plants die under long periods of low temperatures, for example during winter frosts. At RTs (23 & 33°C), *C. annuum* L. has the highest levels of PS, lowering it for higher RTs, as 45°C (Fig. 1). This behavior confirms that these plants are best suited to areas of humid tropics and subtropics¹⁰. Graphs of air and leaf temperatures at RT of 11°C were not plotted because there is no PS at this RT (Fig. 1). At 23

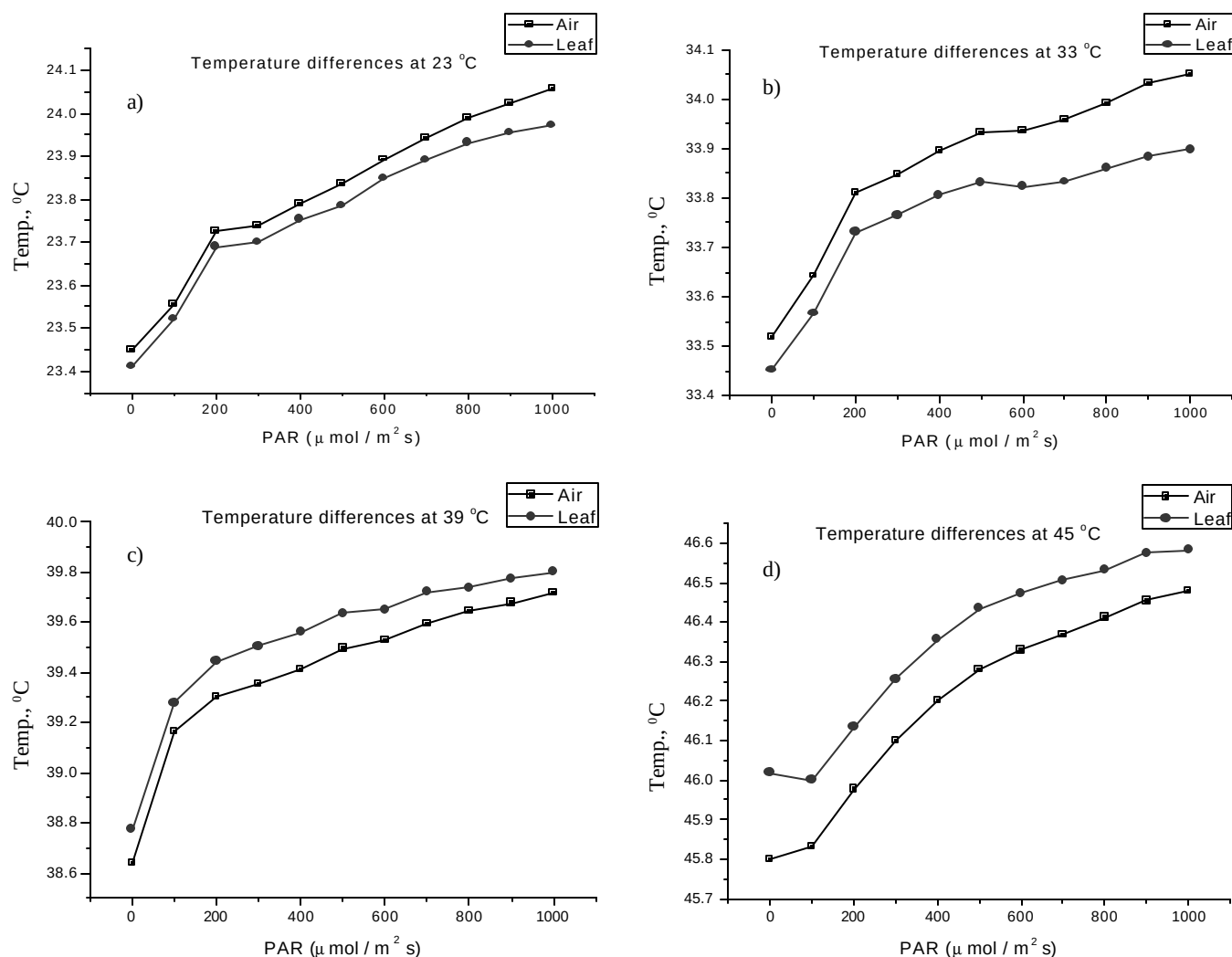


Fig. 2—Differences between air and leaf temperatures at different room temperatures : a) 23°C; b) 33°C; c) 39°C and d) 45°C

(Fig. 2a) and 33°C (Fig. 2b), air temperature is higher than leaf temperature, while at 39 (Fig. 2c) and 45°C (Fig. 2d) opposite behavior is observed.

Reference Light Curves

Highest values of PS are reached at RTs of 23 and 33°C (Fig. 1). Thus, temperature chosen for experiments of Reference light curves was 23°C. This part of experiment was conducted to study device effects and radiation on the sample. There is no PS in the absence of leaf (Fig. 3), as it was expected. Li-6400XT lamp does not have any effect on the sample (Fig. 4). Temperature variations at temperature measuring point are not related to the amount of radiation applied to that point. Thus there is no heating effect of Li-6400XT lamp on measuring point. Radiation itself had no effect on the sample (Fig. 5).

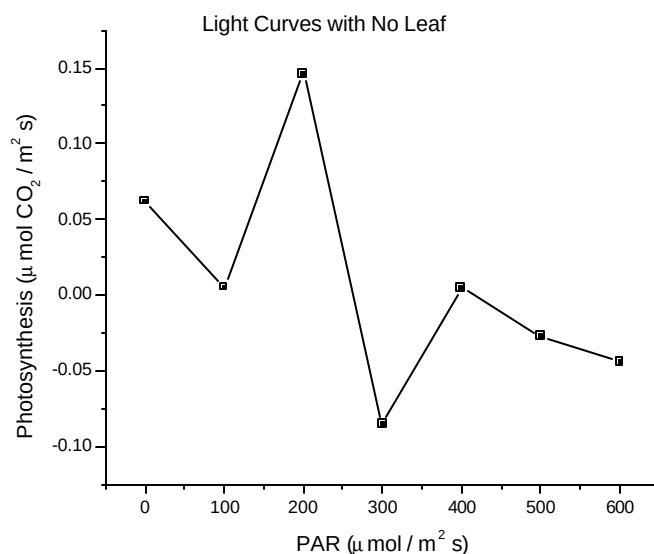


Fig. 3—Light curve without leaf

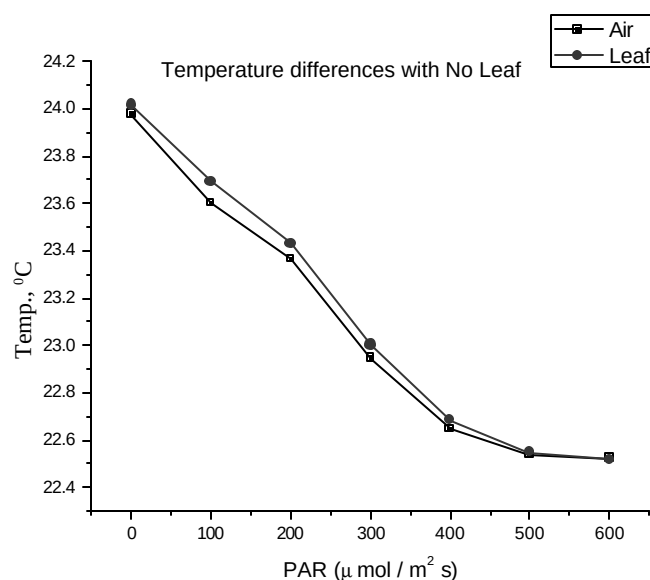


Fig. 4—Differences between air and leaf without leaf

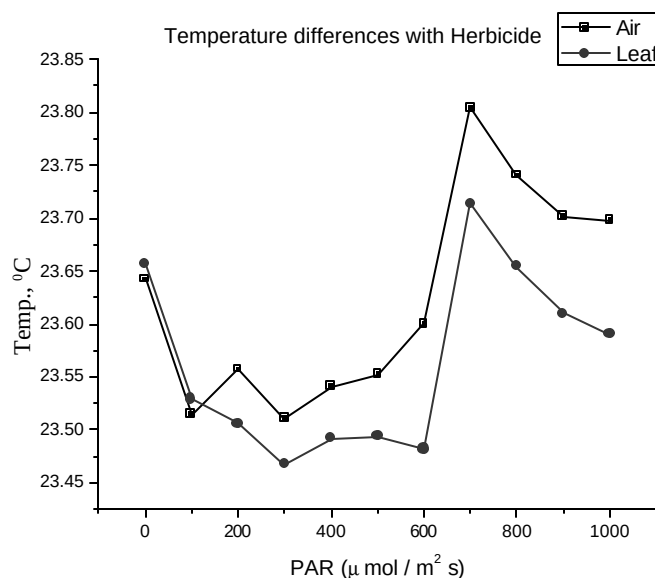


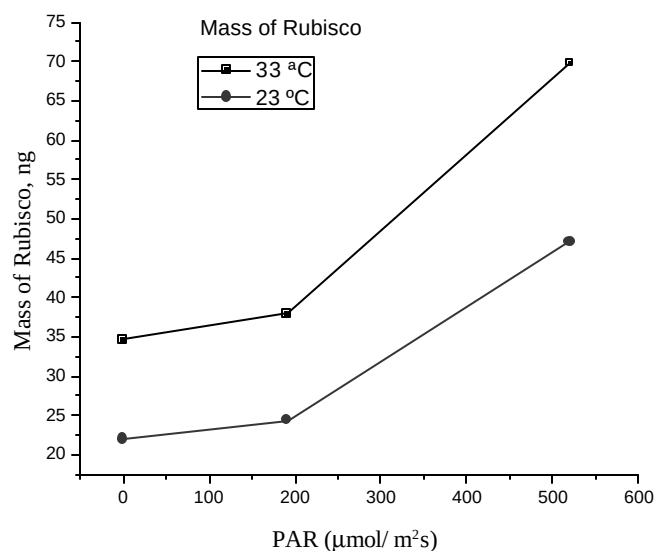
Fig. 5—Differences between air and leaf of the plant with herbicide

Expression of Rubisco by RT-PCR

Mass of rubisco expressed in *C. annuum* L. at two different air temperatures and three different levels of radiation (Fig. 6). Since highest PS values are reached at RTs of 23 and 33°C (Fig. 1), RT-PCR was run only at these two RTs.

Discussion

There is a direct relationship between RT and PS (Fig. 1), but only until an upper temperature limit. That limit is where the plant lowers its yield potential and hardly survives. This upper temperature limit seems to be 33°C. At higher temperatures (39 & 45°C) lower levels of PS were measured (Fig. 1). In addition, under 33°C, difference between air temperature and leaf temperature is positive (Figs 2a & 2b), but difference becomes negative for RTs above 33°C (Figs 2c & 2d). Such change might be because, at temperatures higher than 33°C, plant stops PS process and starts to dissipate heat in order to survive. With respect to radiation, for each RT, there is a direct relationship between PS and applied PAR until a photo-saturation point (Fig. 1). After such point, PS will likely remain constant, or it may even diminish. This photo-saturation point is around 500 to 600 PAR (Fig. 1). The radiation in present proofs only exceeded for few minutes the photo-saturation point, until 1000 PAR, in order to appreciate this saturation, but without damaging permanently the plant. There is a direct relationship between PS and leaf temperature from 0 PAR until photo-saturation point (between 500 and 600 PAR). Beyond

Fig. 6—Mass of rubisco expressed in *Capsicum annuum* L. at two different air temperatures

this photo-saturation point, PS remains constant or even diminishes (Fig. 1), as leaf temperature increases. At photo-saturation, incident extra energy causes the plant to become over heated. As a result, plant uses its energy to activate cooling processes, thus diminishing PS.

Expression of rubisco increases with increased radiation (Fig. 6). Rubisco proofs had a top level of radiation in 500 PAR because this is photo-saturation point (Fig. 1). This growing of rubisco is because it is one of the five light-regulated enzymes, which operate in Calvin cycle. Its activity increases in presence of light.

Since rubisco catalyzes both carboxylation and oxygenation, there is always a competition between these processes. However, rubisco fixes CO₂ about 80 times faster than it oxygenates². There is a CO₂ reduction due to PS process made by the plant during its exposure to these treatments of radiation and temperature (Fig. 1). The behavior of measured variable (Fig. 6) is related to the level of PS produced by the plant under each treatment. *C. annuum* L. has better PS rates under temperatures 33°C and with an incident radiation of 500 to 600 PAR (Figs 1, 2 & 6). Thus there exists a relationship between leaf temperature and PS. This relationship was proven by gas analysis^{3,11,13,14} and expression of rubisco^{2,4,16}. Behavior shown is due neither to the effect of Li-6400XT lamp nor to the warming effect of incident radiation in the plant (Figs 3-5).

Conclusions

PS does not occur in *C. annuum* L. variety “Don Benito” at 11°C, the best photosynthetic performance occurs between 23 and 33°C, and lower PS levels are present at RTs over 33°C. There is a relationship between leaf temperature and PS. This relationship represents not only a new physical method for inferring PS, but also an opportunity to develop non-invasive devices for measuring this important physiological process. Other variables should be studied in order to identify their relationship with PS beyond photo-saturation point. This would help in generation of new mathematical models for inferring PS. Although experiments were conducted only on *C. annuum* L. variety “Don Benito”, it is expected that experiments on other C₃ plants would produce similar results.

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