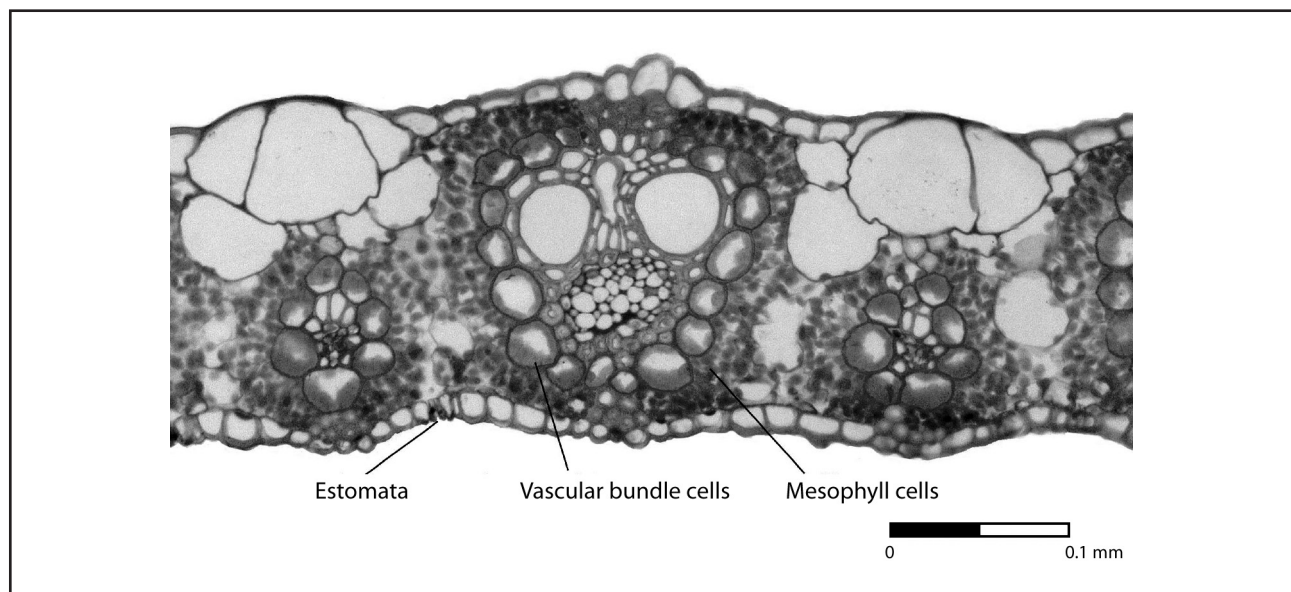


PHOTOSYNTHESIS IN SUGARCANE AND ITS STRATEGIC IMPORTANCE TO FACE THE GLOBAL CLIMATIC CHANGE

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The photosynthetic process consists of two couple reaction. The first, called photochemical phase, refers to the reactions related to light capture, electron transport and the formation of reducing power (i.e. NADPH and ATP). The second phase is the biochemical, where carbon dioxide (CO₂) is captured and transformed into compounds that link carbon atoms together and therefore retain the energy absorbed by the light reaction (BUCKERIDGE *et al.*, 2008). In the photochemical reactions, the light captured by leaves is capable to provoke changes in chlorophyll molecules present in the chloroplasts and these changes start the transportation of electrons

through the electron transport chain, formed by highly specialized proteins. At the end of the process one NADPH and one ATP molecule are formed. These compounds carry the energy and are used in the biochemical phase of photosynthesis. During this phase, CO₂ enters the mesophyll through the stomata (Figure 1), and it is assimilated by an enzyme called Rubisco (Ribulose-1,5-bisfosphate carboxylase/oxygenase). The process of carbon assimilation starts when the carbon of CO₂ is incorporated into a molecule of ribulose-1,5-bisphosphate (RuBP), what starts the so called Calvin Cycle. This cycle forms compounds with 3 carbon atoms, named 3-phosphooglyceric acid (3-



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FIGURE 1 Cross section of a sugarcane leaf showing a stomata, a vascular bundle cells and the mesophyll cells.

PGA). Every two 3-PGAs formed, one molecule of sugar with six carbons is synthesized. The sugars are then polymerized to starch stored in leaves or transported to the growing organs as sucrose.

The plants use this process to perform a type of photosynthesis that is called C_3 , since the enzyme that captures the CO_2 inside the cell forms primarily a compound containing 3 carbon atoms (3-PGA). However, certain plants (e.g. sugarcane, maize and sorghum) have been modified during evolution so that they contain complementary metabolic pathway where, instead of incorporating carbon from CO_2 in a 3-carbon acid, they incorporate into a 4-carbon acid and because of that this type of photosynthesis is called C_4 . We even refer to a given plant species as a C_4 or C_3 species depending on its metabolism.

The functioning of the C_4 photosynthetic system is directly related to the anatomical features of the leaves where it exists (FURBANK, 2000). Leaves of C_4 plants have two types of chloroplasts, the ones that occur in the mesophyll cells and the ones present at the vascular bundle cells (Figure 1). In this system, the differential feature is that the Calvin cycle (i.e. the cycle C_3) occurs only in the vascular bundle cells (TAIZ, 2004). This process permits the concentration of CO_2 in the vascular bundle to reach 10 times the atmospheric concentration within cell that contains Rubisco. This mechanism makes the C_4 photosynthesis system naturally more efficient than the C_3 one.

In all C_4 plants, the CO_2 is initially fixed in mesophyll cells by an enzyme named Phosphoenolpyruvate Carboxylase – PEPc, which forms oxaloacetate, an acid of four carbon atoms. This acid is then converted into malate or aspartate and one of these, depending on the species, diffuses to the cells of the vascular bundle, where it is decarboxylated and released as CO_2 . This CO_2 will be refixed by Rubisco, which feeds the Calvin cycle. A compound of three carbon atoms that comes from the decarboxylation reaction, returns to the mesophyll cells and is subsequently used as a precursor for regeneration of phosphoenolpyruvate (FURBANK, 2000). However, C_4 can present biochemical variations in relation to the type of decarboxylating enzyme presents in the cells of the

vascular bundle. These changes give rise to three different subtypes of C_4 photosynthesis: NADP-ME (NADP⁺ – *malic enzyme*), NAD-ME (NAD⁺ – *malic enzyme*) and PCK or PEPCK (*Phosphoenolpyruvate carboxykinase*) (SAGE, 2000).

Sugarcane, along with maize and sorghum, is considered a C_4 plant of the subtype NADP-ME (BOWYER, 1997). There is evidence that sugarcane can also work as a PEPCK type (CALSA JR. and FIGUEIRA, 2007), similarly to maize, that uses the PEPCK pathway to catalyse the regeneration of oxaloacetate in the cytosol (WINGLER *et al.*, 1999).

The rate of photosynthesis in a plant is dependent on the environmental changes, as well as to intrinsic features of plant, such as the demand of photoassimilates and the expression of certain genes.

In sugarcane plants, depending on the availability of water and nutrients, the rate of photosynthesis will vary according with light intensity. Under natural conditions, the photosynthetic rate in sugarcane is directly related with the variation of solar radiation during the day. This happens because enzymes that participate on the photosynthetic process (like the enzymes mentioned above) are regulated by light.

When measured during the day, it is observed that the period in which sugarcane plants photosynthesize from 8am to 4pm, the highest photosynthesis rates are observed at midday ($\sim 45 \mu\text{mol } CO_2 \cdot \text{m}^{-2} \cdot \text{s}^{-1}$), being therefore consistent with the local changes in the Photosynthetic Active Radiation – PAR. Although being slower, the stomatal conductance rates (that afford entrance of CO_2 into the mesophyll cells), also changes follow the variations in PAR. The changes in enzyme activity related to photosynthesis during one day are predominantly dependent on PEPc, which increases its activity to 170% during the period of higher carbon assimilation (DU *et al.*, 2000).

The C_4 plants are more efficient to use water. When we divide the amount of CO_2 that enters the leaf by the amount of water released by transpiration, it can be observed that C_4 plants are capable to absorb more carbon per water molecule when compared with the C_3 plants. However, although

very efficient in using water, sugarcane can drastically reduce its photosynthetic rate under conditions of drought. Water deficiency provokes a series of chemical and physiological alterations in plants, resulting in acclimation mechanisms that afford their survival. Among these alterations are the decrease in growth rate, leaf area, stomatal closure, senescence and leaf abscission and also the expression of stress-related genes (TAIZ, 2004).

In experiments with water deficit, it has been observed for sugarcane a decrease in leaf area, probably related to decreased leaf expansion and, a decrease in photosynthesis rates and biomass accumulation (INMAN-BAMBER *et al.*, 2005; SMIT, 2006). A reduction in photosynthesis rates under drought conditions is possibly correlated with stomatal closure, what prevents entrance of CO₂ in the leaves and latter on to the formation of reactive oxygen species – ROS as a consequence of severe stress. ROS tend to oxidise photosynthetic pigments, membrane lipids, proteins and nucleic acids, reducing the contents of chlorophyll and electron transport proteins.

C₄ plants present an optimal temperature for growth higher than the observed for C₃ species. This is because usually the former are plants that inhabit tropical and subtropical regions. Furthermore, elevated temperatures can cause problems. They lead to a rapid loss of water due to evapotranspiration and provoke dehydration (MACHADO, 2001). This dehydration leads to rupture of membranes, reducing plant metabolism and consequently the rates of photosynthesis and growth.

EBRAHIM e colleagues (1998) demonstrated that when sugarcane plants were grown at 45 °C, the internodes were shorter, the leaves dried earlier and there was an increase of tillering when compared to plants grown at 27 °C. Also, lower growth rate and sugar accumulation were observed.

Under lower temperatures, photosynthesis is also negatively affected. When cultivated in 10 °C, the photosynthetic rate of sugarcane was reduced in approximately 20% in relation to plants cultivated at 30 °C (DU, 1999). The reduction in the photosynthetic rate in this case is related with

the high demand of energy necessary to activate the enzymes and also due to the range of optimal temperature for photosynthesis in these plants, which is around 30 °C.

Another factor that interferes with the photosynthetic process that is becoming increasingly important during the last years is the increase in the concentration of CO₂ in the atmosphere. This greenhouse gas is increasing due to the intense utilization of fossil fuels that are used as sources of energy for humans, as well as due to the land use change (BUCKERIDGE and AIDAR, 2002). When cultivated under elevated CO₂ concentrations C₃ present an increase in photosynthesis rates and consequently an increase in the amount of biomass produced (WAND *et al.*, 1999; POORTER and NAVAS, 2003).

Although this is a quite common response for C₃, the C₄ plants presents varying results. In the case of sugarcane, de SOUZA *et al.* (2008) showed the effects of 720 ppm of CO₂ (concentration expected for around 2050) during 50 the first 50 weeks of growth. During this period, an increase of c.a. 30% in the photosynthetic rate was observed with the induction of expression of genes related with the electron transport system and with the enzyme PEPc. There was a reduction of stomatal conductance, which led to a better water use efficiency.

These results demonstrated that sugarcane photosynthesis under elevated CO₂ is possibly related with the increase due to the fact its light capture systems increase efficiency. This change in light capture pattern may be associated to a change in the capacity of consumption of photoassimilates, which is directly dependent on growth because, in general, the photosynthetic rate is regulated by the activity in sink organs (PAUL, 2001).

In sugarcane, McCORMICK, CRAMER and WATT (2006), observed a sink-source relationship that was dependent on a stronger sink and higher photosynthetic rate. These authors did not find significant changes in the contents of sucrose that could be associated with the observed increase in photosynthesis. However, they suggested the possibility of a regulation of the photosynthetic rate could be related with the concentration of glucose.



FIGURE 2 Culms of sugarcane after 75 days growing in open top chambers (OTCs) with elevated CO_2 . Left (four groups of culms) = control; right = grown in OTCs.

On the other hand, WU and BIRCH (2007) showed that the increase in sink strength in sugarcane lead an increase in the electron transport system which was followed by increase in the concentrations of sucrose in internodes under intermediate stage of development.

Under elevated CO_2 , it has been observed that after 75 days, sugarcane presents an increase of 177.98% in the fresh mass of culms (Figure 2), what is thought to increase sink due to the allocation of more biomass to this organ. These data indicate that the plant will be capable to accumulate more sugar at the end of the growth period. Indeed, de SOUZA *et al.* (2008) observed that after an average increase of 60% of biomass accumulation in culms, there was about 30% increase in sucrose after 50 weeks.

More recently, de SOUZA and BUCKERIDGE (unpublished) found that the electron transport rates are significantly higher in plants of sugarcane growing under elevated CO_2 , confirming previous observations in gene expression observed by de SOUZA *et al.* (2008).

The discoveries mentioned above posses the possibility that the changes in gene expression related to the electron transport system and higher CO_2 are possibly correlated with the increase in biomass production and sucrose concentration in culms of sugarcane. This knowledge opens opportunities for the selection of new varieties and/or genetic transformation of sugarcane plants in order to obtain higher photosynthesis rates and therefore higher productivity.

Although these results sound quite promising to be used for increase in productivity, it is important to remember that the increase in the concentration of atmospheric CO_2 used in our experiments are not natural and that this has consequences to the society that are negative in many ways.

On the other hand, for new technologies to be developed on the basis of our discoveries, deeper studies will be necessary in order to understand the possible consequences for the source-sink relationship in the plant. One of the ways is searching into the collections of varieties currently avail-

able in Brazil and other countries to try to find plants that would present even higher sensitivity to elevated CO₂. For genetic transformation, it will be necessary to develop a stable and secure methodology for transformation and latter on evaluate the general physiological responses of the plants to elevated CO₂, comparing them with non-transformed individuals.

Even though the way seems they have been paved to understand how plants of sugarcane will respond to the global climatic changes, many studies have yet to be carried out in order to understand what will be the metabolic and physiological responses in the future. For instance, experiments that combine elevated CO₂ with elevated tempera-

ture and water stress are necessary. Such studies were performed with sugarcane in Florida (USA) showed that an increase of up to 6 °C combined with drought did not cause loss of photosynthesis (VU, 2009a, b).

Another important issue will be to include physiological responses in modelling of sugarcane production so that the effects of elevated CO₂, temperature and water stress on growth in the field would be considered (DA SILVA *et al.*, 2008). The production of integrated models is important for decision making regarding sugarcane cropping in Brazil due to the strategic importance that this crop has to energy security in this country nowadays.

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