PEROXISOMAL METABOLISM ALTERATIONS ASSOCIATED TO *Z. AETHIOPICA* SPATHE SENESCENCE AND REGREENING

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The development of C3 monocot calla lilly (*Zantedeschia aethiopica* (L.) Spreng.) floral spathe has been used as a natural model for studying physiological and biochemical features of senescence. Soon after its formation, this photosynthetic leaf-like organ undergoes senescence, which ultimately leads to the organ death if pollination does not occur (Figure 1-A). Fruiting inhibits the ongoing spathe senescence and the regreening of its abaxial parenchyma cells is observed. Previous studies have shown that during the time course of spathe development dramatic changes occurs, namely in the ultrastructure of plastids (Figure 1-B). Moreover, the decline in photorespiratory activity during spathe whitening and its recovery during spathe regreening was also observed.

In this work, changes in peroxisomal metabolism namely in what concerns leaf-type peroxisome-glyoxysome transition were evaluated during *Z. aethiopica* spathe development.

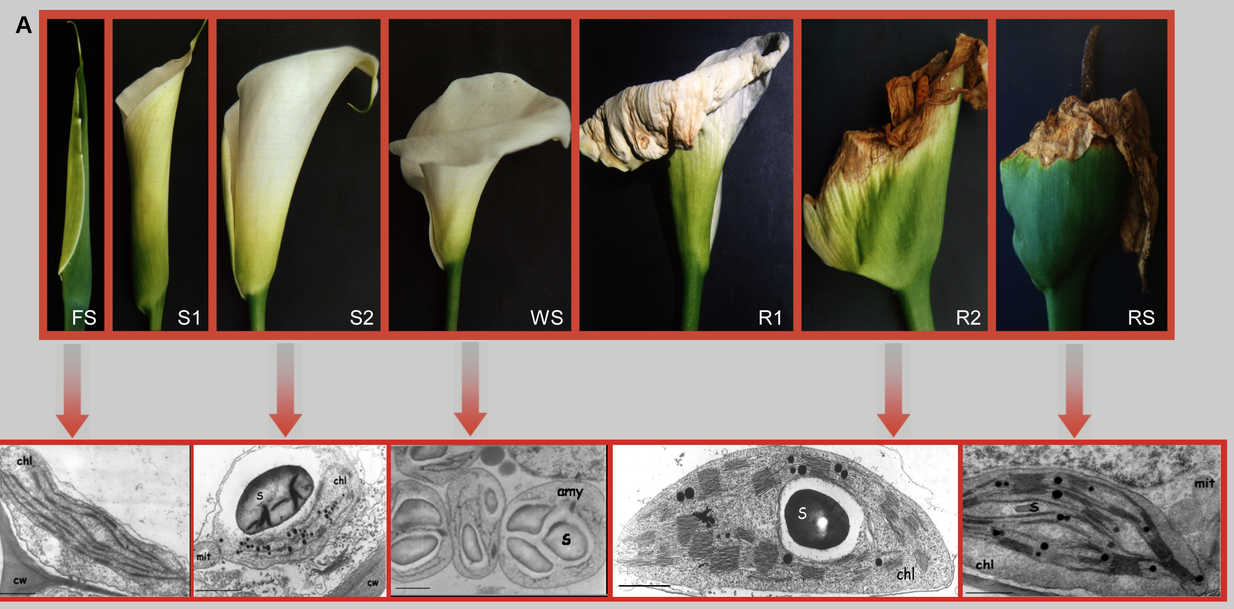

Figure 1 – A - Macroscopic aspects of *Z. aethiopica* spathe whitening and regreening. FS-Floral bud spathe; S1 and S2-First and second intermediate stages of spathe whitening; WS-White spathe; R1 and R2-First and second intermediate stages of spathe regreening; RS-Regreened spathe. B - Ultrastructural aspects of *Z, aethiopica* plastids during spathe whitening and regreening. 1 bar = 1 μm. S -Starch ; Chl -Chloroplast; Mit -Mitochondria; Amy -Amyloplast; CW -Cell Wall.

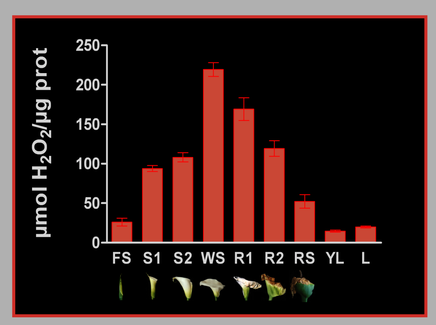

Figure 2 - Evaluation of the H_2O_2 content during *Z. aethiopica* spathe and leaf development, expressed per mg of total soluble protein. Hydrogen peroxide content was determined according to Velikova *et al*. (2000) based in the oxidation of KI.

The levels of H_2O_2 (Figure 2) during the initial stages of spathe whitening seem to parallel catalase activity (Figure 3).

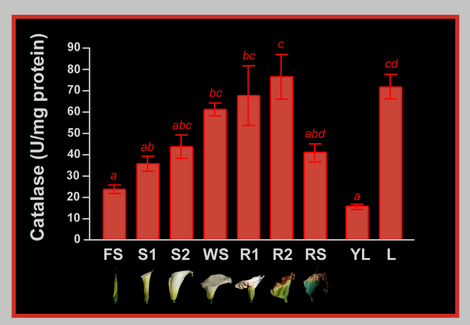

Figure 3 - Evaluation of catalase activity during *Z. aethiopica* spathe and leaf development. Catalase activity was determined on extracts obtained from three independent pools of leaves or spathes in different developmental stages by following the decomposition of H_2O_2 at 240 nm (Aebi, 1983). Bars represent SE of three to five independent experiments. The letters above bars indicate significant differences at $P < 0.05$.

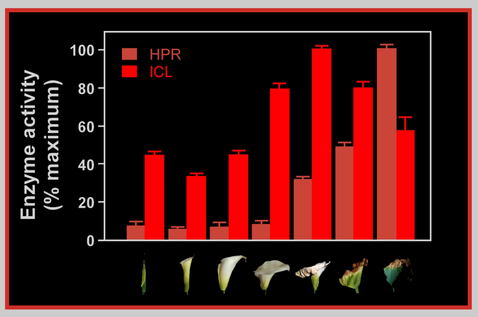

Figure 6 - Evaluation of isocitrate lyase (ICL) and hydroxypyruvate reductase (HPR) activity during the time course of *Z. aethiopica* spathe and leaf development. The activity of the glyoxysomal key enzyme ICL and the leaf-type peroxisomal key enzyme HPR were determined in extracts obtained from three independent pools of spathes in different developmental stages according to Zelitch (1988) and Liang *et al*. (1984), respectively. Bars represent SE of three independent experiments.

Increase in ICL activity during *Z. aethiopica* spathe whitening could reflect an induction of glyoxysomal metabolism suggesting that the conversion of peroxisomes into glyoxysomes occurs during spathe senescence.

Increase in HPR activity during spathe regreening, accompanied by the glyoxylate key-enzyme ICL, points to an induction of leaf-type peroxisomal metabolism suggesting the conversion of glyoxysomes back into peroxisomes, during spathe regreening. This hypothesis was further supported by the expression analysis of genes encoding enzymes from photorespiratory pathways.

Two catalase genes were identified in *Z . aethiopica*

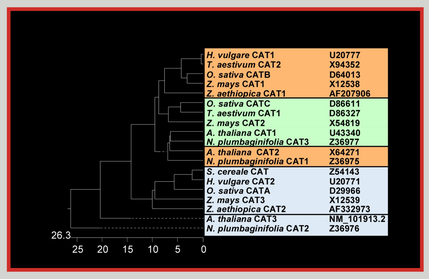

Figure 4 – Phylogenetic tree representing the relationship between *Z. aethiopica* CAT1 and CAT2 and other higher plant catalases. Amino acid sequences were aligned with the MegAlign program (DNASTAR) using clustal method with PAM250 residue weight table. The length of each pair of branches represents the distance between sequence pairs. The scale beneath the tree indicates the distance between sequences. Catalase types based on phylogenetic relationships are indicated. Catalase classes grouped by their putative function are shadowed as follows: class I in green, class II in blue and class III in orange.

Phylogenetic analysis (Figure 4) of *Z. aethiopica* catalse genes has revealed:

CAT1 (AF207906) is more related to those catalases reported as playing a role on glyoxysomal metabolism (Class I).

CAT2 (AF332973) is more related to those catalases with

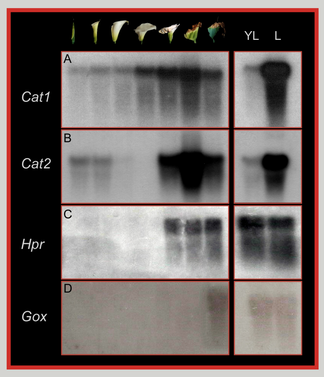

Figure 5 - Expression analysis of *Z. aethiopica Cat1* and *Cat2* genes and photorespiratory *Hpr* and *Gox* genes during spathe and leaf development. Total RNA samples (20 μg per lane) were separated on formaldehyde agarose, blotted and hybridized with corresponding ^{32}P-labelled homologous probes. A- Expression analysis of *Z. aethiopica Cat1* gene. B- Expression analysis of *Z. aethiopica Cat2* gene. C- Expression of *Z. aethiopica* hydroxypyruvate reductase (*Hpr*) gene. D- Expression analysis of *Z. aethiopica* glycolate oxidase (*Gox*) gene.

Conclusions:

During *Z. aethiopica* spathe whitening, a transition from leaf-type peroxisomes into glyoxysomes occurs.

During spathe regreening, spathe senescence is inhibited, and the conversion of glyoxysomes back into leaf-type peroxisomes takes place.

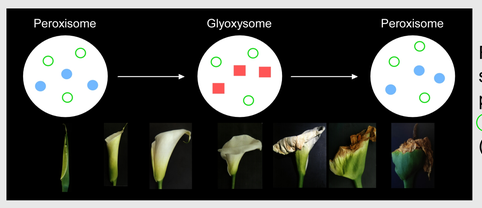

Figure 7 – Peroxisomal transition during *Z.aethiopica* spathe senescence and regreening. ● - Leaf-type peroxisomal enzymes; ■ - Glyoxysomal enzymes; ○- Enzymes common to both types of peroxisomes (adaptation of Nishimura *et al*., 1993).

$Cat2$ transcript levels show identical variation during *Z. aethiopica* spathe development as photorespiratory genes (*Hpr and Gox*), suggesting that *Cat2* is specifically associated to photorespiratory H_2O_2 decomposition.

The transcript levels of *Cat1* increase until the late stages of spathe whitening.

However, as *Cat1* expression is also strongly induced during regreening, a dual role of *Cat1* in scavenging leaf-type peroxisomal and glyoxysomal H_2O_2 could also be considered.

References:
Aebi H. (1983). Catalase, in: HU Bergmeyer, (Ed.), Enzymes 1: Oxidoreductases, Transferases, Verlag Chemie, NY, 273-286. **Liang Z., Yu C., Huang AHC. (1984).** Conversion of glycerate to serine in intact spinach leaf peroxisomes. Arch. Biochem. Biophys. 233: 393-401. **Loreto F., Velikova V. (2001).** Isoprene produced by leaves protects the photosynthetic apparatus against ozone damage, quenches ozone products, and reduces lipid peroxidation of cellular membranes. Plant Physiol. 127: 1781-1787. **Nishimura, M., Takeuchi, Y., De Bellis, L., Hara-Nishimura, I. (1993)** Leaf peroxisomes are directly transformed to glyoxysomes during senescence of pumpkin cotyledons. Protoplasma 175: 131-137. **Willekens H., Inzé D., Van Montagu M., Van Camp W. (1995)** Catalases in plants. Molecul. Breed. 1: 207-228. **Zelitch I. (1988).** Synthesis of glycolate from pyruvate via isocitrate lyase by tobacco leaves in light. Plant Physiol. 86: 463-468.

Acknowledgments >>>Cátia Barbeta is supported by Fundação para a Ciência e Tecnologia, grant ref. SFRH/BD/12081/2003.