Changes in peroxisomal metabolism during Zantedeschia aethiopica spathe senescence and regreening: differential expression of two catalase genes

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The development of the C3 monocot Zantedeschia aethiopica floral spathe has been used as a natural model for studying the 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). 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 some dramatic changes occurs, namely in the ultra-structure of chloroplasts and peroxisomes. Some evidences suggest that, during the spathe whitening, peroxisomes differentiate into glyoxysomes and chloroplasts into amyloplasts. The regreening process is followed by the restructuration of chloroplasts and peroxisomes, resulting in the re-acquisition of photosynthetic and photorespiratory capacities. This work was undertaken to support this hypothesis with molecular and biochemical data concerning peroxisomal enzymes.

Figure 1 - 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; S-Senesced spathe.

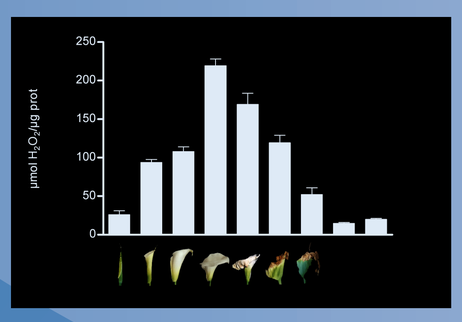

Figure 2 - Evaluation of the H_2O_2 content during Z. aethiopica spathe and leaf development, expressed per mg of total soluble protein. Hydrogen peroxide content was determined according to Velikova et al. (2000) based on the oxidation of KI.

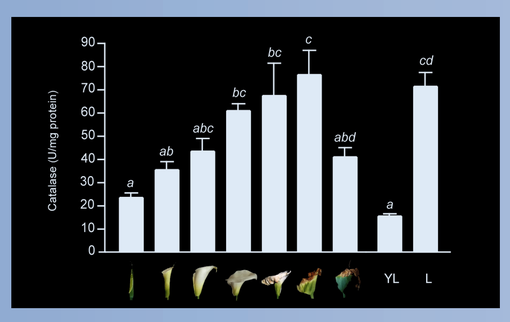

Figure 3 - Evaluation of catalase activity during Z. aethiopica spathe and leaf development. Catalase activity was determined on extracts obtained from three independent pools of leaves or spathes in different developmental stages by following the decomposition of H_2O_2 at 240 nm (Aebi, 1983). Bars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

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

§ The increase on catalase activity during spathe regreening could be associated to the increased photosynthetic and photorespiratory rates that occur during this developmental phase.

§ The increase on catalase activity observed during spathe senescence could be explained by the expression of a catalase form suitable for destroying H_2O_2 produced during •-oxidation of fatty acids .

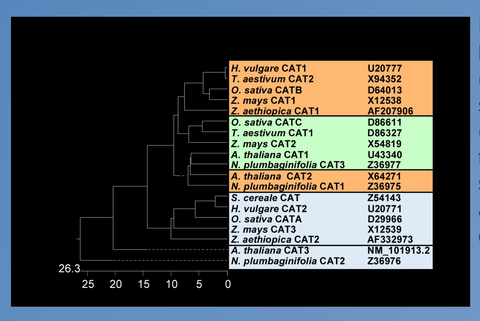

Figure 4 – Phylogenetic tree representing the relationship between Z. aethiopica CAT1 (AF207906) and CAT2 (AF332973) and other higher plant catalases. Amino acid sequences were aligned with the MegAlign program (DNASTAR) using clustal method with PAM250 residue weight table.The scale beneath the tree indicates the distance between sequences. Catalase classes grouped by their putative function are shadowed as follows: class I in green, class II in blue and class III in orange.

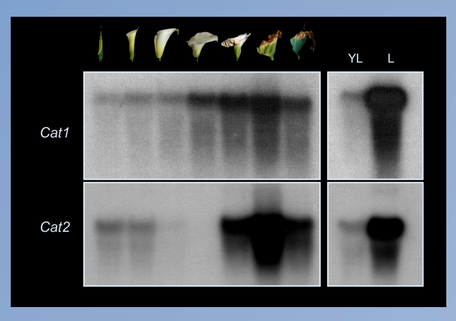

Figure 5 - Expression analysis of Z. aethiopica Cat1 (AF207906) and Cat2 (AF332973) genes during spathe and leaf development. Total RNA samples (20 µg per lane) were separated on formaldehyde agarose, blotted and hybridized with corresponding ^{32}P-labelled homologous probes.

Two catalase genes were previously identified in Z. aethiopica. Sequence analysis of these two genes (Figure 4) has revealed that:

i) CAT1 (AF207906) is more phylogenetically related to those catalases reported as playing a role on glyoxysomal metabolism (Class I catalases, according to Willekens et al.,1995);

ii) CAT2 (AF332973) is more phylogenetically related to those catalases with unspecified cellular function (Class II catalases, according to Willekens et al., 1995).

The expression analysis of these two catalase genes from Z. aethiopica was performed (Figure 5).

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

§ Cat1 is probably associated to scavenging of glyoxysomal H_2O_2 (Class I catalase), which is in accordance with its phylogenetic analysis.

§ 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.

Cat2 transcript levels are higher during spathe regreening

§ Cat2 is specifically associated to photorespiratory H_2O_2 decomposition, which suggests that CAT2 is a Class I catalase, although it shares higher phylogenetic proximity with Class II catalases.

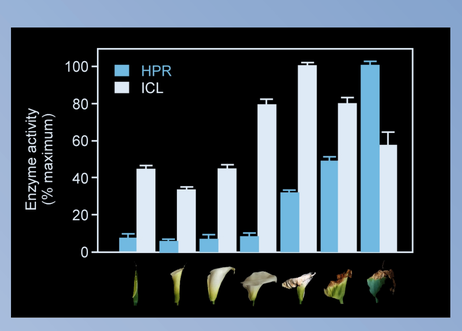

Figure 6 - Evaluation of isocitrate lyase (ICL) and hydroxypyruvate reductase (HPR) activity during the time course of Z. aethiopica spathe and leaf development. The activity of the glyoxysomal key enzyme ICL and the leaf-type peroxisomal key enzyme HPR were determined in extracts obtained from three 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 spathe whitening could reflect the induction of glyoxysomal metabolism.

§ Increase on HPR during spathe regreening could reflect the induction of leaf-type peroxisomal metabolism.

Conclusions

In this work, the hydrogen peroxide content during the time course of Z. aethiopica spathe development was determined. The results show a significant increase in the levels of H_2O_2 during the whitening process, which seems to parallel catalase activity. The results obtained from the expression analysis of catalase genes suggest that CAT2 functions on scavenging photorespiratory H_2O_2, while CAT1 appears to have a dual role in scavenging glyoxysomal and peroxisomal H_2O_2. Changes in peroxisomal metabolism were studied in what concerns key enzymes of glyoxylate and glycolate pathways. During spathe whitening, it seems likely that a transition from leaf-type peroxisomes into glyoxysomes occurs. During the regreening, this process is reversed with the conversion of glyoxysomes back into leaf-type peroxisomes.

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References:
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