Plant Omics Journal POJ

POJ 5(6): 547-552 (2012) ISSN:1836-3644

Transcription of mitochondrial and chloroplast related genes in rice plants under anoxic stress

Camila Pegoraro[1], Mariana Madruga Krüger[1], Railson Schreinert dos Santos[1,2], Liliane Marcia Mertz[3], Luciano Carlos da Maia[1] , Cesar Valmor Rombaldi[3], Antônio Costa de Oliveira[1]*

[1]Plant Genomics and Breeding Center, Crop Science Department, Eliseu Maciel school of Agronomy, Federal University of Pelotas, Campus Capão do Leão, s/n, 96010-900, P.O.Box 354, Pelotas - Rio Grande do Sul, Brazil
[2]Graduate Program in Biotechnology, Technological Development Center, Federal University of Pelotas, Campus Capão do Leão, s/n, 96010-900, P.O.Box 354, Pelotas - Rio Grande do Sul, Brazil
[3]Agroindustrial Science and Technology Department, Eliseu Maciel school of Agronomy, Federal University of Pelotas, Campus Capão do Leão, s/n, 96010-900, P.O.Box 354, Pelotas - Rio Grande do Sul, Brazil

***Corresponding author: acostol@terra.com.br**

Abstract

Most of mitochondrial and chloroplast proteins are encoded by nuclear genes and their import into organelles of destiny involve protein complexes located in the mitochondria (TIM and TOM) and chloroplast (TIC and TOC) membranes. Any damage in mitochondrial and chloroplast membranes impair the movement of important nuclear genome encoded proteins by these complexes, leading to plants death. The aim of this study was to evaluate the gene expression of TIM/TOM and TIC/TOC complexes in rice (*Oryza sativa* L.) plants under anoxic as well as other abiotic stresses, seeking to associate the expression of these genes with membrane integrity and transport of proteins into mitochondria and chloroplast under stress conditions. Expression data obtained from *Genevestigator* for 16 genes showed that the transcript levels of most TIM/TOM and TIC/TOC genes are increased when rice is under abiotic stress. The results from qRT-PCR analyses for the genes *TIM17/22-Os03g0305600, TIM17/22-Os04g0376100* and *TIM17/22-Os10g0519700* presented high levels of expression 24 h after anoxic conditions, suggesting a role in the primary response to anoxic stress adaptation. After 72 h under anoxia, most genes were inhibited, suggesting that an interruption in protein transport into mitochondria and chloroplast does occur after this period of stress.

Keywords: Cell damage; chloroplast; gene expression; mitochondria; *Oryza sativa*.
Abbreviations: ATP - adenosine triphosphate; GAPDH - *Glyceraldehyde-3-phosphate dehydrogenase*; ROS - reactive oxygen species; TIC - chloroplast inner envelope membrane translocon; TIM - translocase of the inner mitochondrial membrane; TOC - chloroplast outer envelope membrane translocon; TOM - translocase of the outer mitochondrial membrane.

Introduction

Mitochondria and chloroplasts contain ca. 2,000 and 4,000 proteins, respectively (Van Wijk, 2004; Millar et al., 2005). However, the majority (>95%) of mitochondrial and chloroplast proteins are nuclear encoded and translated in the cytosol, with target signal peptides in the N-terminal domains called transit peptides (Braun and Schmitz, 1999). The import of proteins into the mitochondria and chloroplast involves protein complexes located in the organelle membranes. The subunits of external and internal mitochondrial membrane machineries import nuclear encoded proteins by aiding their transport through the bipolar lipid layer. These subunits are called translocase of the inner mitochondrial membrane (TIM) and translocase of the outer mitochondrial membrane (TOM) (Neupert, 1997). In the chloroplasts, these complexes are called chloroplast inner envelope membrane translocon (TIC) and chloroplast outer envelope membrane translocon (TOC) (Kessler and Schnell, 2006). Anoxic stress is described by a decrease of environmental oxygen [O_2] concentration below 2% (Skutnik and Rychter, 2009), affecting plant growth and development. This condition usually occurs in situations such as plant complete submergence, which imposes stress due to O_2 and carbon dioxide diffusion reductions (Fukao and Bailey-Serres, 2008). O_2 deficiency dramatically reduces the efficiency of cellular adenosine triphosphate (ATP) production, affecting diverse paths in cellular metabolism and development (Fukao and Bailey-Serres, 2004). Previous reports have indicated that anoxic conditions cause changes in lipid content, protein synthesis, membrane fluidity and cytoplasmic acidosis. Such changes, accompanied by ATP depletion, favor the production of reactive oxygen species (ROS), lipid peroxidation and degradation of carbohydrates and nucleic acids (Blokhina et al., 2001; Blokhina et al., 2003).

Chloroplast membranes are oxygen-radical production sites especially affected by ROS intermediates. In addition, chloroplasts bear a particular risk of oxygen toxicity, since molecular O_2 can be reduced by light through Photosystem I electrons. On the other hand, oxidative damage can cause the inhibition of important enzymes in the mitochondria, such as Complex I and Aconitase (Mehler, 1951; Zhang et al., 1990). Damages in membranes and membrane-bound proteins involved in the production of energy can subsequently

influence whole plant metabolism (Holmberg and Bülow, 1998). Also, mitochondrial and chloroplast damages block the entry of nuclear encoded proteins, leading to plant death.
In rice (*Oryza sativa* L.), 24 loci coding for TIM/TOM and TIC/TOC complex subunits have been reported (Murcha et al., 2007). Under total or partial lack of O_2, as well as in other abiotic and biotic stress conditions, the excess ROS production causes the peroxidation of lipids on mitochondrial membranes. In chloroplasts, ROS production can be increased by many factors such as light, drought, cold injury and flooding, inducing membrane photo-oxidation (Elstner and Oswald, 1994). The by-products of lipid peroxidation can cause cell damage by reacting with other lipids, vital mitochondrial proteins and nucleic acids, causing mutations and gene expression changes (Sweetlove et al., 2002).
Transcription profiling of genes coding for TIM/TOM and TIC/TOC complexes could reflect the integrity/functionality of mitochondrial and chloroplast membranes, and consequently the import of proteins into these organelles. Therefore, the objective of this study was to evaluate the transcription profiles of sixteen genes coding for the protein complexes TIM/TOM and TIC/TOC in rice plants under different stresses in general and particularly under anoxic stress.

Results

Digital expression analysis (Fig. 1) showed that rice TIM/TOM and TIC/TOC complex transcript levels are affected by anoxia, as well as several other stresses (cold, drought and salt). In rice coleoptiles, anoxic stress caused the up-regulation of nine genes (*TOM7-Os01g0626300*, *TOM20-Os01g0921600*, *TIM10-Os03g0825400*, *TIM13-Os04g058-1300*, *TIM17/22-Os02g0672500*, *TIM17/22-Os03g0296300*, *TOC24-Os01g0969000*, *TIC21-Os06g0638100* and *TIC62-Os10g0100300*), down-regulation of two genes (*TIM10-Os07g0243100* and *TIM17/22-Os03g0305600*) and undetectable changes in five genes (*TIM17/22-Os02g0717300*, *TIM17/22-Os04g0376100*, *TIM17/22-Os10g0519700*, *TIM-17/22-Os12g0514900* and *TIM44-Os07g0409700*).

The results from qRT-PCR (Fig. 2) indicate that the majority of TIM/TOM and TIC/TOC related genes are inhibited in rice seedling leaves subjected to anoxic stress for 24 h. However, the genes *TIM17/22-Os03g0305600*, *TIM17/22-Os04g0376100* and *TIM17/22-Os10g0519700* were highly expressed after 24 h of stress. After 72 h of stress, the majority of the genes were either inhibited or presented basal activity, i.e., those seen in plants under normal conditions.

No gene was up-regulated by cold stress. Six genes did not change their expression level (*TOM20-Os01g0921600*, *TIM17/22-Os02g0717300*,*TIM17/22-Os12g0514900*, *TIM44-Os07g0409700*, *TIC21-Os02g0187600* and *TIC62-Os10g0100300*) and the other ten genes were down-regulated by this stress, suggesting loss of function in mitochondria and chloroplast membranes (Fig. 1).

Under drought stress, most genes were down-regulated, with the exception of two genes that were up-regulated (*TIM17/22-Os02g0672500* and *TIM17/22-Os03g0305600*) (Fig. 1).

Under salt stress, five genes were up-regulated (*TOM20-Os01g0921600*, *TIM17/22-Os02g0717300*, *TIM17/22-Os03g0305600*, *TIM17/22-Os04g0376100* and *TIM17/22-Os10g0519700*), two were down-regulated (*TOM7-Os01g0626300* and *TIM17/22-Os02g0672500*) and no changes in the transcriptional expression were detected for the remaining nine genes under this stress (Fig. 1).

In this work, the overlapping expression of TIM/TOM and TIC/TOC associated genes were investigated under different abiotic stresses (stored microarray data). The results indicate a lack of overlapping in gene expression.
Analyzing the expression data for plant anatomy (Fig. 3), it was observed that twelve genes (*TOM7-Os01g0626300*, *TOM20-Os01g0921600*, *TIM10-Os07g0243100*, *TIM10-Os03g0825400*, *TIM13-Os04g0581300*, *TIM17/22-Os02g077300*, *TIM17/22-Os03g0293600*, *TIM17/22-Os04g0376100*, *TIM17/22-Os10g0519700*, *TIM17/22-Os12g0514900*, *TIM44-Os07g0409700* and *TIC21-Os06g0638100*) were expressed in all plant organs, but exhibited different expression levels. The other genes exhibited variation in expression levels in different plant parts.

Expression data from different developmental stages of rice plants (Fig. 4) showed that eleven genes (*TOM7-Os01g0626300*, *TOM20-Os01g0921600*, *TIM10-Os07g0243100*, *TIM10-Os03g0825400*, *TIM13-Os04g0581300*, *TIM17/22-Os02g0717300*, *TIM17/22-Os03g0293600*, *TIM17/22-Os10g0519700*, *TIM17/22-Os12g0514900*, *TIM44-Os07g0409700* and *TIC21-Os06g0638100*) were expressed in all developmental stages, but at different levels. Four genes (*TOM7-Os01g0626300*, *TIM17/22-Os10g0519700*, *TIM17/22-Os12g0514900* and *TIM44-Os07g0409700*) exhibited higher levels of expression in all developmental stages.

Discussion

Up-regulation of nine TIM/TOM and TIC/TOC genes in rice coleoptiles under anoxic stress can be explained by the fact that mitochondria are the center of oxygen sensing. High protein import demand for ROS detoxification would be expected, thus requiring high activity of the TIM/TOM protein complex. Similarly, ROS can be also formed as by-products of anoxia stress in chloroplasts, also requiring a great import of antioxidant enzymes.

The ability of rice to germinate under anoxic conditions is similar to that in air, but in anoxic stress only the coleoptile elongates, whereas both root and primary leaf fail to grow, suggesting higher gene activity in the coleoptile (Lasanthi-Kudahettige et al., 2007). Thus, the high transcription activity of genes under anoxic stress can be associated with a higher metabolic activity of coleoptiles when subjected to low O_2 levels.

In rice seedling leaves subjected to anoxic stress for 24 h, the majority of TIM/TOM and TIC/TOC related genes are inhibited with exception of *TIM17/22-Os03g0305600*, *TIM17/22-Os04g0376100* and *TIM17/22-Os10g0519700*, that are highly expressed after 24 h of stress. This suggests that these genes are part of the plant primary responses to low O_2 conditions. Other studies have reported that rice plants subjected to anaerobic conditions (24 and 48 h) exhibited significant increases of proteins TIM17/22, TIM23, TIM44, TOM20 and TOM 40. The majority of the genes are inhibited or presented basal activity after 72 h of stress. The three genes that were up-regulated 24 h after the stress were completely inhibited after 72 h of stress.
The results obtained by qRT-PCR were not identical to the results obtained from stored microarray data using the *Genevestigator* software. These differences can be explained by the fact that for *Genevestigator* analysis the sample tissues were coleoptiles, while in the qRT-PCR analysis leaf tissue was used. As mentioned above, different tissues respond differently under anoxia condition. In this study leaf tissue was used in order to simulate submergence conditions that

Table 1. Genes evaluated in *Genevestigator* and qRT-PCR.

Gene	Forward	Reverse
TOM7-Os01g0626300	5'-GAAGCCGAAGCCCAAGGTCAA-3'	5'-TGGTCCACGTGGTCCACTCCTT-3'
TOM20-Os01g0921600	5'-GGACATGGGAGCGATGAGCG-3'	5'-ACCTTGGCGTTCTGGCATGC-3'
TOM10-Os07g0243100	5'-GAAAGGTCAGCCGGTGAATGTG-3'	5'-TGCCAATCCAAACATCTGCTCC-3'
TOM10- Os03g0825400	5'-TGGAGAAGGAGCAGATGTTCGG-3'	5'-CGACCCGGTACTCCATCTCCTT-3'
TOM13-Os04g0581300	5'-ATGGACTCGTTCTCGTCGCCGT-3'	5'-TGAGATGCTCCGTGGACGCAGT-3'
TIM17/22-Os02g0672500	5'-CCTCGCCTTCCCCACCTCGTAC-3'	5'-GCGAGGTGGGCAGGTCGTAGAG-3'
TIM17/22-Os02g0717300	5'-GGGCTCATCCGGACGCTCAA-3'	5'-ACGAGCTGCTCGACGCCGAT-3'
TIM17/22-Os03g0296300	5'-GGAGGAGATCAAGGGGCAGGAC-3'	5'-ATGACTCCACTGACGACGCTGC-3'
TIM17/22-Os03g0305600	5'-CGGCCTGGAGAAGAACTGGATG-3'	5'-ATGAAGTAAGACTCCCGCGCGA-3'
TIM17/22-Os04g0376100	5'-TGGAAGGAGCGGATCTTGCTGC-3'	5'-GAATCCAGCTCCGACCACACCG-3'
TIM17/22-Os10g0519700	5'-CTGTTCCCGTCGGGATCCAA-3'	5'-AGGGGTTGTACTTCCGGCGG-3'
TIM17/22-Os12g0514900	5'-CGCGAGGAGGAGGAAGGAGG-3'	5'-CTCAGCGTCGGTGGTGTCCC-3'
TIM44-Os07g0409700	5'-GGAGGGCAGGACACCATCCA-3'	5'-TGCTGGATCTCACGCAACCG-3'
TIC21-Os06g0638100	5'-GCCATGCTCGCCCAGGTTAG-3'	5'-CAGAATCCCAGGGTGCCCAA-3'
TIC62-Os10g0100300	5'-ATCGTGTCGGCCATTGGCAA-3'	5'-TGCAGCCTGCACGAGGTTGTT-3'
TOC24- Os01g0969000	5'-GGCCGCCGGCAATAATAAGG-3'	5'-GCACGTCCTCCTCCTCCAGCTT-3'
GAPDH- Os04g0486600	5'-AAGCCAGCATCCTATGATCAGATT-3'	5'-CGTAACCCAGAATACCCTTGAGTTT-3'

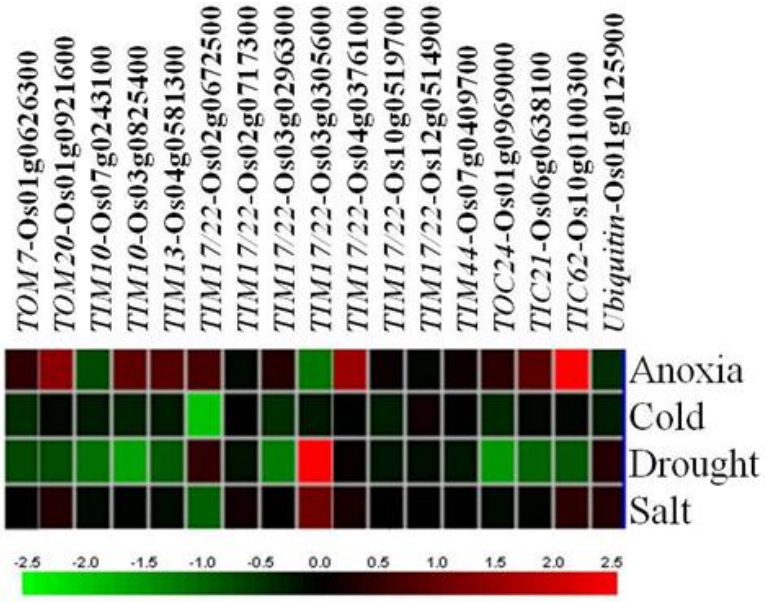

Fig 1. Expression pattern of rice (*Oryza sativa*) *TOM, TIM, TOC* and *TIC* genes under abiotic stresses in cultivar Nipponbare. The microarray data based expression profiles under stress conditions are presented as heat maps generated using meta-analysis tool at *Genevestigator* (http://www.genevestigator.ethz.ch) (Zimmermann et al., 2008). The transcript levels are depicted by color scale indicating log2 values. Ubiquitin expression is shown as control.

may occur during rice development later on and not during or soon after germination.

The reduction of transcript levels, observed for the majority of genes in rice seedling leaves subjected to anoxic conditions for 24 and 72 h, can be explained by damages in the mitochondrial and chloroplast membranes due to lipid peroxidation, mainly poly-unsaturated fatty acids (Rhoads et al., 2006). These results suggest that the lack of O_2 affects the electron transport chain, increasing redox state, inter-membrane pH acidification and; therefore, interfering in the protein trafficking to mitochondria. This imbalance in the preservation of importing complexes into the mitochondria (TIM/TOM) and chloroplast (TIC/TOC) contributes to increase negative physiological responses under anoxic stress.

The high expression levels recorded for the genes *TIM17/22-Os03g0305600*, *TIM17/22-Os04g0376100* and *TIM17/22-Os10g0519700* can allow the transport of essential proteins from cytoplasm to the mitochondria, at least during the first 24 h on anoxic stress. This result indicates a probable association of these genes with the development of a stress tolerance mechanism to low O_2 levels.

Interestingly, no gene was up-regulated by cold stress and ten genes were down-regulated in cold condition, suggesting loss of function in mitochondria and chloroplast membranes. These results support previous studies reporting the destruction of plasma and chloroplast membranes ultra-structures under heat (44ºC for 3h) or cold (-3ºC for 3h) stressed grape plants (Wang and Li, 2006).

Most genes were down-regulated under drought stress, indicating that some mechanisms provoked the arrest of gene transcription for most genes. Previous reports in other species had already demonstrated that drought stress causes damage in all cell membranes and induces chloroplast rupture (Carbonell et al., 1994).

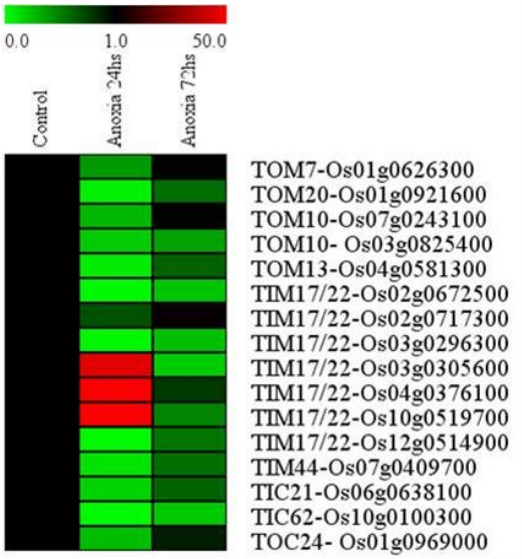

Fig 2. Relative mRNA abundance in Nipponbare rice (*Oryza sativa* L.) leaves under anoxia condition. mRNA abundance is represented at different scales, using the Multi Experiment Viewer (TIGR MeV) software. mRNA abundance of each gene from control times at 24 and 72 h served as the baseline for determining relative RNA levels.

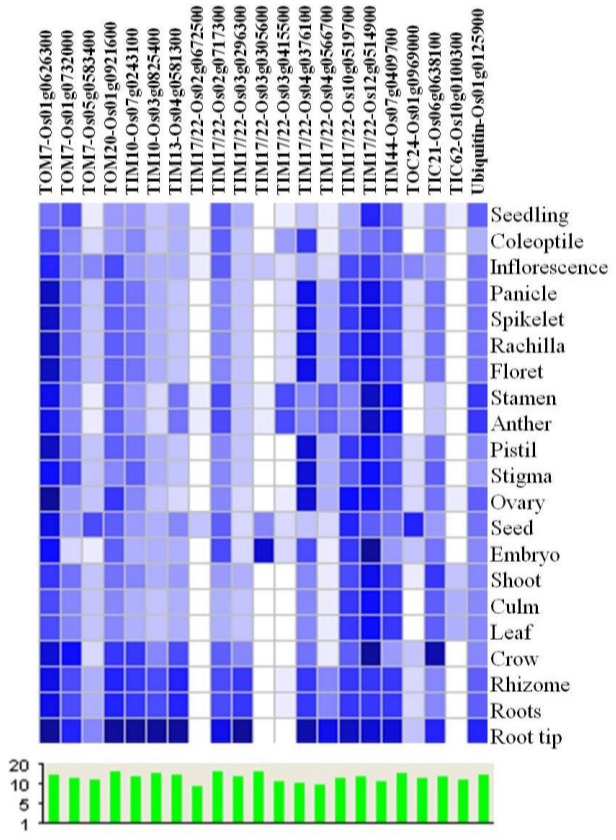

Fig 3. Microarray based expression profiles of rice (*Oryza sativa* L.) *TIM*, *TOM*, *TIC* and *TOC* genes in different rice organs. Expression profiles are presented as heat maps in blue/white colors generated using the meta-analysis tool of *Genevestigator* (http://www.genevestigator.ethz.ch) (Zimmermann et al., 2008). Darker colors correspond to stronger expression.

Under salt stress, only two genes were down-regulated indicating that there is no excessive damage to mitochondrial and chloroplast membranes, since the expression remained unchanged for the majority of the genes when compared to the control. However, in other studies rice plants subjected to intense salt stress presented chloroplast and mitochondrial breakages (Rahman et al., 2000).

In this study a lack of overlapping in gene expression was observed, which is in contrast to commonly seen in other stress related gene families such as *HSPs* (heat shock proteins), *HSFs* (heat shock factors) and *ERFs* (ethylene responsive factors).

In different plant organs it was observed that the majority of genes were expressed in all plant organs but presented different expression levels. Five genes could be highlighted by their overall high expression (*TOM7-Os01g0626300*, *TIM17/22-Os04g0376100*, *TIM17/22-Os10g0519700*, *TIM17/22-Os12g0514900* and *TIM44-Os07g0409700*). This observation indicates the constitutive action and the importance of these genes in rice plants organs.

Similarly, in different developmental stages of rice plants most of TIM/TOM and TIC/TOC genes were expressed in all developmental stages at different levels. Four genes presented higher levels of expression in all developmental stages, suggesting that these genes are constitutively expressed and; therefore, present vital function in these stages. Previous reports have demonstrated the rule of organelle protein importing apparatus in the regulation of physiological responses during plant development. The TIC/TOC complexes are important for the maintenance of cellular homeostasis when gene expression profile changes occur during chloroplast development (Inaba and Schnell, 2008).

Materials and methods

Plant materials

For the quantitative expression analysis of TIM/TOM and TIC/TOC genes, seeds of rice cv. Nipponbare were soaked for 15 min in 0.5% sodium hypochlorite, acidified to pH 5.5, followed by rinsing in sterile water (10 times) and then germinated on sterile filter paper at 25°C with 16 h photoperiod. Seedlings (15-d-old) were subjected to two treatments, the complete submergence (anoxia) for 24 and 72h as well as normal conditions (controls) for 24 and 72h. For both treatments the seedlings were placed in bottles with a volume of 580 mL. For the treatment of anoxia, the bottle was completely filled with Milli-Q water and the control treatment the plants were kept in the same bottles without water and with lids open. Three biological replicates were performed for each treatment, and each replicate consisted of 15 seedlings. After each period, rice leaves were collected, immediately frozen in liquid nitrogen and then stored at -80°C until total RNA extraction.

Bioinformatics analyzes

In order to verify gene transcriptional profiles of TIM/TOM and TIC/TOC complexes under different stress conditions, sixteen out of twenty four sequences were randomly chosen and obtained from RAP-DB (*The Rice Annotation Project data Base*) (http://rapdb.dna.affrc.go.jp/). The digital transcription profile was obtained using meta-analysis tool at *Genevestigator* (http://www.genevestigator.ethz.ch) for

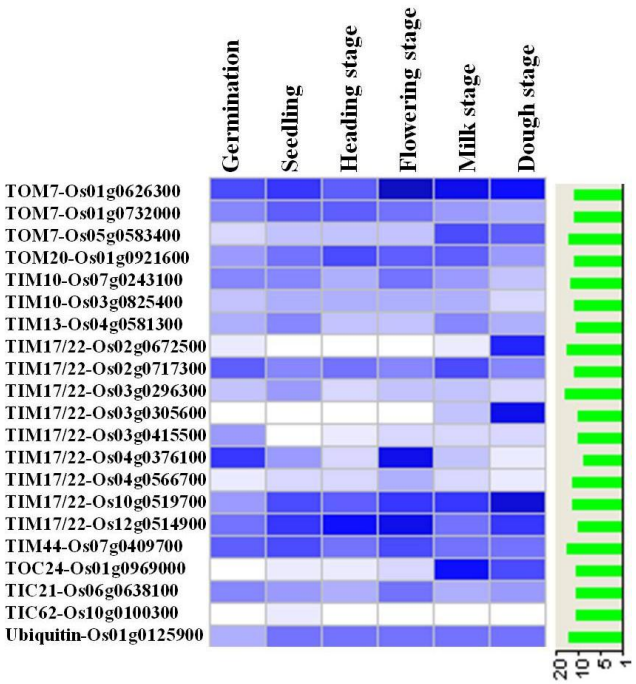

Fig 4. Microarray based expression profiles of rice *TIM, TOM, TIC* and *TOC* genes during different developmental stages of rice. Expression profiles are presented as heat maps in blue/white colors generated using the meta-analysis tool of *Genevestigator* (http://www.genevestigator.ethz.ch) (Zimmermann et al., 2008). Darker colors correspond to stronger expression.

different rice plant organs, developmental stages, anoxia and other abiotic stresses (Zimmermann et al., 2008).

Gene expression analysis by real-time PCR

The 16 genes evaluated in this study plus the endogenous control (*Glyceraldehyde 3-phosphate dehydrogenase - GAPDH*) are listed in Table 1. Primer design was performed using Vector™ (Invitrogen™, Carlsbad, CA, USA) from sequences obtained from RAP-DB. The criteria used for primer selection consisted of amplicon sizes between 50 and 150 bp, CG content between 40% and 60%, and melting temperature ranging from 60 to 65ºC according to recommendations (Applied Biosystems® - USA).

The qRT-PCR amplification was conducted using a 7500 Fast Real Time PCR System (Applied Biosystems® - USA). Reaction conditions were as follows: 50ºC for 2 min, 95ºC for 10 min, 40 cycles of three stages (95ºC for 30 s, 60ºC for 1 min and 72ºC for 1 min), and final extension at 72ºC for 5 min, following a standard dissociation curve, in which only one peak was observed. Only primers with amplification efficiency close to 100% were used.

Total RNA was extracted from 0.1 g of leaf tissue from a pool of 15 rice seedlings using Pure Link™ Plant RNA Reagent (Invitrogen™ - USA) followed by treatment with DNAse I™ Amplification Grade (Invitrogen™- USA) according to manufacturer's instructions. The RNA quality and quantity were assured by electrophoresis gel and spectrophotometry, respectively. cDNAs were obtained from 2µg of RNA through the use of SuperScript™ First-Strand System for RT-PCR (Invitrogen™ - USA) according to manufacturer's instructions. The qRT-PCR was performed using three physical replicates per individual biological replicate in a 7500 Fast Real-Time PCR System (Applied Biosystems® - USA). Gene expression data was analyzed using Multi Experiment Viewer (MeV), EASE Expression Analysis Systematic Explorer version 4.6 (Saeed et al., 2003) and presented in a color diagram in which the control times at 24 and 72 h were used as baseline.

Conclusions

The transcriptional expression quantification data obtained through qRT-PCR provided new precise information about modification in transcriptional expression of these genes in leaves of rice under anoxic conditions. The genes *TIM17/22-Os03g0305600*, *TIM17/22-Os04g0376100* and *TIM17/22-Os10g0519700* present high transcript levels 24 h after anoxic stress, suggesting a role in the early adaptation responses of rice plants. After 72 h of anoxia, there is inhibition of the expression of the majority of TIM/TOM and TIC/TOC complex coding genes, suggesting an interruption on protein transport to the mitochondria and chloroplast during the stress, due to organelle membrane damages. The analyses made with the *Genevestigator* tool indicate the participation of these genes in rice plant responses to different stresses. For of the analyzed stresses, there is no overlapping expression, indicating transcriptional expression specificity. The differences in transcriptional expression detected in *Genevestigator* tools for different organs and developmental stages of rice plants show the importance of their regulation during plant growth and development.

Acknowledgements

This work was supported by the Brazilian Ministry of Science and Technology - CNPq and the Brazilian Ministry of Education - Capes.

References

Blokhina OB, Chirkova TV, Fagersted KVV (2001) Anoxic stress leads to hydrogen peroxide formation in plant cells. J Exp Bot. 52:1179-1190

Blokhina OB, Virolainen E, Fagerstedt KVV (2003) Antioxidants, oxidative damage and oxygen deprivation stress: a review. Ann Bot. 91:179-94

Braun HP, Schmitz UK (1999) The protein-import apparatus of plant mitochondria. Planta. 209:267-274

Carbonell ML, Alegre L, Onckelen H (1994) Effects of water stress on cellular ultrastructure and on concentrations of endogenous abscisic acid and indole-3-acetic acid in *Fatsia japonica* leaves. Plant Growth Regul. 14:29-35

Elstner EF, Osswald W (1994) Mechanisms of activation during plant stress. Proc Roy Soc Edinb. 102:131-154

Fukao T, Bailey-Serres J (2004) Plant responses to hypoxia is survival a balancing act? Trends Plant Sci. 9:449-456

Fukao T, Bailey-Serres J (2008) Submergence tolerance conferred by *Sub1A* is mediated by SLR1 and SLRL1 restriction of gibberellin responses in rice. Proc Natl Acad Sci USA. 105:6814-16819

Holmberg N, Bülow L (1998) Improving stress tolerance in plants by gene transfer. Trends Plant Sci. 3:61-66

Inaba T, Schnell DJ (2008) Protein trafficking to plastids: one theme, many variations. Biochem J. 413:15-28

Kessler F, Schnell DJ (2006) The function and diversity of plastid protein import pathways: a multilane GTPase highway into plastids. Traffic. 7:248-257

Lasanthi-Kudahettige R, Magneschi L, Loreti E, Gonzali S, Licausi F, Novi G, Beretta O, Vitulli F, Alpi A, Perata P (2007) Transcript profiling of the anoxic rice coleoptile. Plant Physiol. 144:218-231

Mehler AH (1951) Studies on reactions of illuminated chloroplasts II. Stimulation and inhibition of the reaction with molecular oxygen. Arch Biochem Biophys 33:339-351

Millar AH, Heazlewood JL, Kristensen BK, Braun HP, Moller IM (2005) The plant mitochondrial proteome. Trends Plant Sci. 10:36-43

Murcha MW, Elhafez D, Lister R, Tonti-Filippini J, Baumgartner M, Philippar K, Carrie C, Mokranjac D, Soll J, Whelan J (2007) Characterization of the preprotein and amino acid transporter gene family in Arabidopsis. Plant Physiol. 143:199-212

Neupert W (1997) Protein import into mitochondria. Annu Rev Biochem. 66:863-917

Rahman MS, Matsuro T, Miyake H, Takeoka Y (2000) Salinity induced ultrastructural alterations in leaf cells of rice (*Oryza sativa* L.). Plant Prod Sci. 4:422-429

Rhoads DM, Umbach AL, Subbaiah CC, Siedow JN (2006) Mitochondrial reactive oxygen species. Contribution to oxidative stress and interorganellar signaling. Plant Physiol. 141:357-366

Saeed AI, Sharov V, White J, Li J, Liang W, Bhagabati N, Braisted J, Klapa M, Currier T, Thiagarajan M, Sturn A, Snuffin M, Rezantsev A, Popov D, Ryltsov A, Kostukovich E, Borisovsky I, Liu Z, Vinsavich A, Trush V, Quackenbush J (2003) TM4: a free, open-source system for microarray data management and analysis. Biotechniques. 34:374-378

Skutnik M, Rychter AM (2009) Differential response of antioxidant systems in leaves and roots of barley subjected to anoxia and post-anoxia. J Plant Physiol. 166:926-937

Sweetlove LJ, Heazlewood JL, Herald V, Holtzapffel R, Day DA, Leaver CJ, Millar AH (2002) The impact of oxidative stress on Arabidopsis mitochondria. Plant J. 32:891-904

Van Wijk KJ (2004) Plastid proteomics. Plant Physiol Biochem. 42:963-977

Wang JL, Li SH (2006) Salicylic acid-induced heat or cold tolerance in relation to Ca^{2+} homeostasis and antioxidant systems in young grape plants. Plant Sci. 170:685-694

Zhang Y, Marcillat O, Giulivi C, Ernster L, Davies KJ (1990) The oxidative inactivation of mitochondrial electron transport chain components and ATPase. J Biol Chem. 265:16330-16336

Zimmermann P, Laule O, Schmitz J, Hruz T, Bleuler S, Gruissem W (2008) Genevestigator Transcriptome Meta-Analysis and Biomarker Search Using Rice and Barley Gene Expression Databases. Mol Plant. 5:851-857