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ORIGINAL ARTICLE

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Participation of MIR775A in Post-transcriptional Regulation Glycerol-3-phosphatacyltransferases in Corn Leaves under Hypoxia

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Oxygen deficiency causes significant changes in plant metabolism related to the coordination of metabolic processes for stress adaptation. MicroRNAs provide regulation of target genes at the post-transcriptional level. The plant cell presents hypoxia-dependent microRNAs, including miR775A, whose quantity increases under these stress conditions. An electrophoretic study of total RNA samples from corn leaves under hypoxia has revealed the presence of two interfering complexes with miR775A at the 12th hour of the experiment. Sequencing and subsequent analysis of the nucleotide sequence of image 2 of the miR775A interfering complex with mRNA from corn leaves have showed a high degree of homology with the N-acyltransferase domain, and corresponded to a fragment of glycerol-3-phosphate acyltransferase mRNA. Inhibition of glycerol-3-phosphate acyltransferase in maize leaf cells under hypoxia can probably provide regulation of cellular fatty acid metabolism under the influence of a stress factor.

Key words: microRNA miR775A, glycerol-3-phosphate acyltransferase, hypoxia, RNA interference

Plants adapt to various environmental stresses through the regulation of a number of genes (Seki *et al.*, 2002, Zhu, 2002). Recent studies have shown that plant microRNAs and siRNAs participate in biotic and abiotic stress responses (Ruiz-Ferrer, Voinnet, 2009). Anaerobic or low-oxygen stress (hypoxia) causes changes in the transcriptome and a switch from aerobic respiration to anaerobic metabolism (Agarwal, Grover, 2006). It has been shown that during immersion of Arabidopsis seedlings in water, Zm-miR166, Zm-miR167, Zm-miR171, Os-miR396 and Zm-miR399 have been induced, but Zm-miR159, At-miR395, Pt-miR474 and Os-miR528 have been reduced (Zhang *et al.*, 2008). Nineteen hypoxia-dependent miRNA families have also been identified, the number of which is inversely correlated with the number of their specific target mRNAs. Levels of gene expression miR156g, miR157d, miR158a, miR159a, miR172a,b, miR391 and miR775 have been increased by hypoxia exposure (Khraiwesh *et al.*, 2012).

Inhibition of mitochondrial respiration pathways under hypoxia results in induction of miRNA775a, indicates its important role in the regulation of this process (Moldovan *et al.*, 2009). In oxygen-deficient conditions, fatty acids (FAs) play an important role. Obtained either through exogenous uptake or de novo synthesis, FAs are used as substrates for oxidation and energy production, membrane synthesis, energy storage in the form of triacylglycerols (TAGs) and production of signalling molecules (Khraiwesh *et al.*, 2012). Since the expression levels of a number of microRNAs including miR775 increased under hypoxia conditions (Moldovan *et al.*, 2009), the study of its role in post-transcriptional regulation of target genes of cell lipid metabolism is relevant.

MATERIAL AND METHODS

The action of low oxygen concentrations on plants was carried out by placing the plants with for 24 h in water. Plants under normal aeration conditions were used as a control group. To exclude the influence of the photosynthetic system, both groups of plants were pre-exposed in the dark for 24 h before the experiment.

RESULTS AND DISCUSSION

To identify targets of RNA interference involving miR775A, total cellular RNA have been isolated from maize leaves at different hours of hypoxia. Total RNA have been reverse transcribed and the resulting cDNA have been incubated with the specific fluorescent probe miR775AROX. Electrophoretic mobility shift assay of cDNA samples after incubation with the probe has showed the presence of hybridisation products, indicating the formation of interfering complexes with miR775A. It has been shown that up to 6 hours of exposure time, one target is detected for miR775A, but after 12 hours of exposure time, there are two targets (Fig.1). The results of hybridisation analysis indicate a change in the number and type of targets for miR775A in maize leaves during the development of hypoxic stress, as the fluorescent complexes with the miR775A-ROX probe have different sizes, as determined using DNA length markers.

Nucleotide sequence analysis of sample 2 of the miR775A interfering complex with mRNA from maize leaves has showed a high degree of homology with the N-acyltransferase domain of various enzymes that catalyse the transfer of the acyl group to the substrate (Fig.2).

Using a bioinformatics approach based on RPS-BLAST, a comparative analysis for domain element matching of annotated proteins based on the NAT_SF acyltransferase domain architecture has been performed (Jiyao *et al.*, 2023). In the analysed sequence, the characteristic elements of this domain have been identified, forming the corresponding active centre of the enzyme, as well as the appropriate surrounding by amino acid residues, ensuring its correct orientation in the protein globule.

The highest affinity of the resulting sequences have been shown with glycerol-3-phosphate acyltransferase mRNA (LOC100285746) (Fig 3). Members of this protein family include N-acetyltransferases (GNATs) such as aminoglycoside-N-acetyltransferases, histone N-acetyltransferase (HAT) enzymes and serotonin-N-acetyltransferases that catalyse the transfer of the acetyl group to the substrate, arginine/ornithine-N-

succinyltransferase, myristoyl-CoA: Protein-N-myristoyltransferase, etc (Jiyao *et al.*, 2023).

It has been shown that the attachment site of miR775A corresponds to the zone of the lyase domain, which confirms the possibility of forming an interfering complex with it (Fig. 4).

Also of note is the incomplete correspondence of miR775A with maize glycerol-3-phosphate acyltransferase mRNA. The 5'-end of miR775A shows the formation of a complementary element of 6 nucleotides (Fig. 4), confirming its participation in the formation of the interfering complex.

Glycerol-3-phosphate acyltransferase is involved in the metabolism of glycerophospholipids and phosphoglycerides, the alpha-glycerophosphate pathway (Shindou, Shimizu, 2009). Lipids are ubiquitously involved in the regulation of plant adaptive responses during hypoxia and post-hypoxic reoxygenation. In particular, polyunsaturation of long-chain acyl-CoA regulates hypoxia sensitivity in plants by modulating the dynamics of acyl-CoA-binding protein (Zhou *et al.*, 2020). Lipids also mediate signal transduction in plant response to biotic and abiotic stresses (Lung, Chye, 2019, Wang, 2004). The production of lipid signalling molecules is strictly regulated by a set of specialised lipid-modifying enzymes and acyl-lipid metabolic networks (Noack, Jaillais, 2020). In particular, abiotic stresses such as chilling, freezing, heating, drought and hypoxia activate lipid-mediated signaling cascades, increasing plant tolerance to these stresses (Hou *et al.*, 2016).

Long-chain acyl-CoAs act as central nodes of cellular metabolism and signal transduction, which are regulated by cis-CoA-binding protein in different compartments of plant cells (Xiao, Chye, 2011, Neess *et al.*, 2015). Two independent studies have shown that long-chain acyl-CoA may be signalling molecules that modulate membrane receptor association (Schmidt *et al.*, 2018, Zhou *et al.*, 2019). Schmidt *et al.* have showed that due to a hypoxia-induced decrease in cellular ATP levels, the activity of long-chain acyl-CoA synthetase is reduced, resulting in a shift in the composition of acyl-CoA pools (Schmidt *et al.*, 2018).

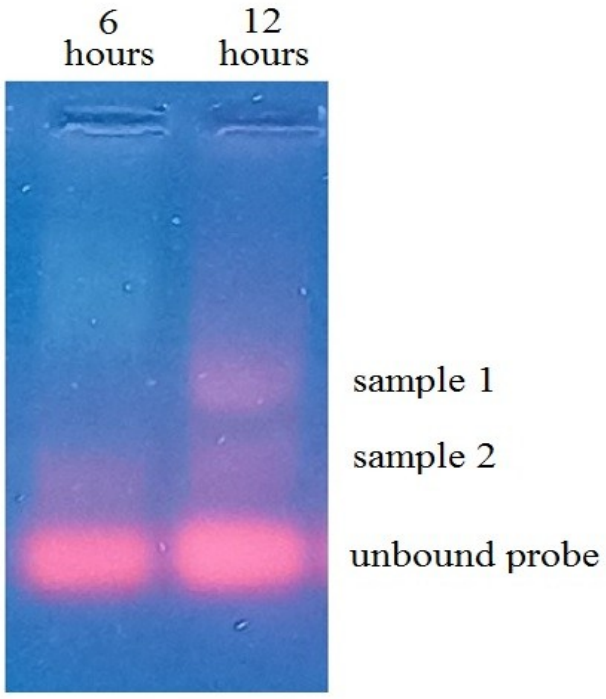

Figure 1: Electrophoretic study of the formation of fluorescent complexes of mRNA from maize leaves with the miR775A-ROX probe at different hours of hypoxic stress.

```
----fYEVCfLYIFd--q-----rtGIKLKRNFK------fni 94  sequence
--qrfAEVAfLAVTaneq---vrgyGTRLMNKFKDhmqk-qni 106 Tetrahymena thermophila
---dvTFLRdFQILpefq---gcgiGSKCLELVVRhald-rqs 130 Vibrio cholerae
elhvyGNAVgVGQEgddedhqhkgyGKKLLEKAEVlard-agf 519 Haloarcula marismortui
--nphHAVElVRMIavrp---glgiGKAMMEAVIDhafsglga 126 Rhodospirillum rubrum
-qgylLFIDeLYIVpdkr---slgyGRTCITLIEKtfh---di 117 Cytophaga hutchinsonii
yhkalREPFDLYMFgqny-------GIRPLVLCKPfsm---rg 180 Zea mays
          ***            *  **    *
```

Figure 2: Comparative analysis of the amino acid sequences of annotated proteins with a NAT_SF acyltransferase domain and a fragment of the amino acid sequence based on the sequence of the RNA interfering complex with maize microRNA775A. The sequences indicate the amino acids (highlighted underlining) that form this domain and the binding site for coenzyme A (highlighted in yellow).

```
GPAT    agagcagtatcatgaagcctgctctcctgagactgattccataactaaccttgccaatca   3660
seq     ----------------------------------------------------GCCCAGCC   8
                                                             *** * *

GPAT    gacaggcaatttattctgccaacaaagctgcagactgctgttcgaggaattgcagaatct   3720
seq     GACAGTCAATTTATTTTAGTGAATAAGCTGCATACAGCTTTGAAAGTAAATACAGGATAG   68
        ***** ********* *    *  ******** ** *** *   ** ** * *** **

GPAT    tcttgcagtcaagaaggatcttgaaccagaatattcatgcagagttgttcaacgcatcca   3780
seq     TGGTACAGTAACTACTGAGCTCGAACCTGAATATTCATGCAGAGTTGTTCAACGCATCCA   128
        *  * **** *  *  ** ** ***** ********************************

GPAT    tgaagatgtaccagaagaagtccttgctttggacaaaagagttgagtgtaactccaggat   3840
seq     TGAAGATTTATAAATAGAATTATTTGTTTTTGACAAAAGAGTTGAGTGTAACTCCAGTAT   188
        ******* ** **  **** *  *** *** ************************* **

GPAT    tgccgttgcactttcattaatggatgagtgcttccttccgattattgaccagagaactgg   3900
seq     TGCTTGTGTAAGATCTTTTTATGAGGTTTGCTTGATTTAGATATTTGACCAGAGAACTGG   248
        ***   ** *   ** **    ** *  *****  **  ***  ***************

GPAT    catcaacttgatacgaaatgttgtgtatagctgtggatcaaattttgcccggttggattt   3960
seq     CATCAAATTGAAACGAAATTTTTGATTTTGAATTGAGTATTTTTTTGTTTGGTTAGATTT   308
        ****** **** ******* **   * * *   **  *    *****   **** *****

GPAT    ccgtggattctatatatttattttagaacgtggagatgaaataatagctgcagcatctgt   4020
seq     CCGTGTAATCTATATTTTTTTTTTGAATATGAAGATAAAATTGTATCTCCCGCCCCCGC   368
        ***** * ******* *** **** ***  ** **** ****  ** ** * **  * *

GPAT    ccgaatacatggaaccaagttagctgagatgccgttcattggtacccggaatatgtatag   4080
seq     CCCCCCCCATGGCCCCCCGCTCGCTGAGCCGCCGATCATTGGTACCCGGAAAATGCGGGG   428
        **     *****  **  * * ******  **** *************** ***    *

GPAT    gcgacaagggatgtgccgccgacttgtagatggaatcgaaatgatcctcagctctctcaa   4140
seq     GCGACAAGGGATGTCCCGACGACACGTAGATGGAATCGAAATGATCCTCGACACCCCCTG   488
        ************** *** ****  ************************  * * * *

GPAT    tgttgagaagttgatcattcctgctatcacagaacttgtggacacctggacatcgaaatt   4200
seq     GGTTGAGGGGTTGCCCCTCCCGGCCGCCACCGAACTTGGGACCCGCAGGGCAC-------   541
         ******  ****  * * ** **   *** ******* *  *  *******
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Figure 3: Comparison of the nucleotide sequences of the obtained sequence (seq) of sample 2 and the maize glycerol-3-phosphate acyltransferase (GPAT) mRNA, indicating the homology of the conserved NAT_SF domain. * - nucleotide matching.

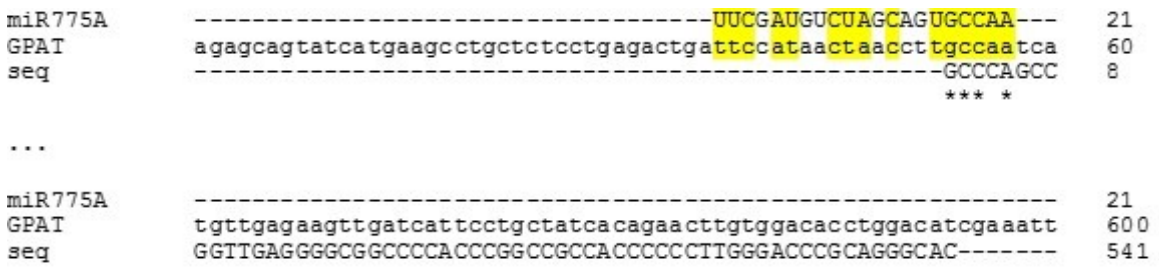

Figure 4: Nucleotide sequences of sequence (seq) of sample 2 and maize glycerol-3-phosphate acyltransferase (GPAT) mRNA, indicating the location of attachment of miR775A microRNA. * - nucleotide matching.

Glycerol-3-phosphatacyltransferase has different localisation in the cell: mitochondria, plasmolemma, endoplasmic reticulum. This enzyme catalyses the reaction of acyl-CoA and glycerol-3-phosphate (G3P) to form lysophosphatidic acid (LPA) and protect acyl-CoA from β-oxidation. In addition, by participating in the formation of triacylglycerides, this enzyme enables the synthesis of lipid droplets, which are responsible for intercellular signal transduction (Chen *et al.*, 2011).

Inhibition of glycerol-3-phosphatacyltransferase in maize leaf cells under hypoxia can probably provide changes in the rate of intra- and extracellular vesicle formation (Gallop *et al.*, 2005), which reflects the coordination of signalling pathways regulating cellular metabolism under stress factor action. The mechanism for this regulation of glycerol-3-phosphatacyltransferase is RNA interference involving miR775A.

CONCLUSION

One of the hypoxia-dependent microRNAs is miR775A, whose accumulation has been shown in plants under oxygen deprivation (Moldovan *et al.*, 2009). Application of a specific fluorescent probe to miR775A has allowed assessment of the formation of RNA interfering complexes in maize leaves under hypoxia. Electrophoresis results indicate that miR775A acts via the RNA interference pathway by binding to the RISC complex and interacts posttranscriptionally with its complementary mRNA. This is evidenced by the fluorescent structures formed - triplexes representing the mRNA-miR775A-probe complex. It has been shown that at 12 h of stress exposure miR775A forms two complexes with different electrophoretic mobility. The resulting sequences of the less mobile complex 2 have showed its correspondence to glycerol-3-phosphate acyltransferase mRNA. Evaluation of RNA-interfering complex formation using a specific ROX-containing probe has showed that miR775A acts through the RNA interference pathway by binding to the RISC complex and posttranscriptionally interacts with its complementary mRNA of glycerol-3-phosphate acyltransferase, reducing the formation of apoproteins of this enzyme in maize cells under hypoxia.

CONFLICTS OF INTEREST

The authors declare that they have no potential conflicts of interest.

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