NTMC2T5.1 is involved in chloroplast clustering around nucleus in *Nicotiana benthamiana*

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Plants have developed mechanisms to protect themselves from pathogens and resist their attacks. Among these, clustering of chloroplasts around the nuclear envelope is a phenomenon that has been proposed as a general response to the perception of pathogens, such as viruses and bacteria [1]. Additionally, it has been hypothesized that chloroplast clustering could serve as an efficient way of transferring signals — such as lipids and reactive oxygen species— between these two organelles in order to induce changes in the expression of pathogen defence-related genes.

Synaptotagmin-like mitochondrial-lipid-binding (SMP) domain proteins are evolutionarily conserved in eukaryotes and participate in the formation of membrane contact sites between organelles. In particular, their SMP domains allow the lipid transport between the membranes of different organelles [2]. Our group is focus on studying NTMC2T5 proteins, a group of SMP proteins, with two homologs in *Arabidopsis thaliana.* By transiently overexpressing *AtNTMC2T5.1* and some truncated versions in *Nicotiana benthamiana* leaf cells, and using confocal microscopy, we have determined the subcellular localization of the encoded protein. This protein is anchored to the chloroplast outer envelope and interacts with the membranes of other organelles, like the ER and the nuclear envelope membrane [3]. Additionally, we have detected that overexpression of NTMC2T5 proteins causes a significant chloroplast clustering around nucleus, that it was not observed when overexpressing other proteins. Thus, we have estimated the contribution of its functional domains in the clustering. Our results suggest that the C-terminal hydrophobic region (HR) of AtNTMC2T5.1 is essential for this process. Moreover, our *Arabidopsis ntmc2t5.1/t5.2* knock-down plants showed slower growth when treated with flagelin22 and reduced chloroplast clustering when treated with H_2O_2. Altogether, our results suggest that NTMC2T5 proteins have a role in pathogen defence, although the molecular mechanisms are still to be elucidated.

References

[1] Ding, X., Jiménez-Góngora, T., Krenz, B., & Lozano-Durán, R. (2019). Chloroplast clustering around the nucleus is a general response to pathogen perception in *Nicotiana benthamiana*. *Molecular Plant Pathology*, *20*(9), 1298–1306. https://doi.org/10.1111/mpp.12840

[2] Prinz, W. A., Toulmay, A., & Balla, T. (2020). The functional universe of membrane contact sites. In *Nature Reviews Molecular Cell Biology* (Vol. 21, Issue 1, pp. 7–24). https://doi.org/10.1038/s41580-019-0180-9

[3] Huércano, C., Percio, F., Sánchez-Vera, V., Morello-López, J., Botella, M. Á., & Ruiz-López, N. (2022). Identification of plant exclusive lipid transfer SMP proteins at membrane contact sites in *Arabidopsis* and Tomato. *BioRxiv*. https://doi.org/https://doi.org/10.1101/2022.12.14.520452