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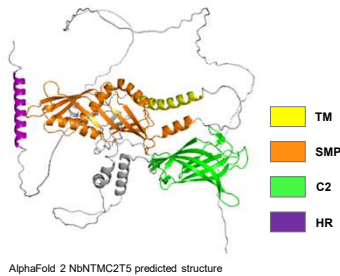
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**ABSTRACT:** Membrane contact sites (MCS) are specialized regions where the membranes of two different organelles come into close proximity, typically within 10–30 nm. These regions are enriched in specific lipids and proteins and play a crucial role in plant lipid metabolism by facilitating lipid transport between chloroplasts and the endoplasmic reticulum (ER) during the eukaryotic pathway of glycerolipid synthesis.

Our research focuses on proteins that localize to MCS and contain an SMP domain, which mediates lipid transfer between organelle membranes. Our recent studies have identified NTMC2T5 proteins, which are found in the outer envelope membrane of the chloroplast and associates the ER, although their specific functions remain unclear.

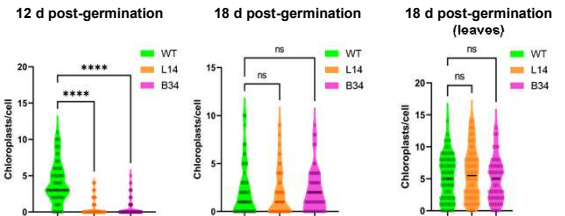
To explore their functional roles, we generated *nbntmc2t5* mutant lines —L14 and B34— of *Nicotiana benthamiana*. These mutants displayed a characteristic yellowing of cotyledons post-germination, prompting us to analyse plastid development under both light and etiolation conditions using confocal and transmission electron microscopy.

Our results indicate that the absence of NbNTMC2T5 protein significantly impacts plastid biogenesis, disrupting the transition from proplastids to mature chloroplasts in seedlings germinated in both light and darkness. These findings suggest that these alterations originate during early plastid development, specifically at the proplastid stage. Furthermore, the disruption of ER-chloroplast MCS in the *nbntmc2t5* mutants highlights the critical role of MCS-mediated lipid transfer in proper plastid differentiation and function.



NbNTMC2T5 protein is anchored to the chloroplast outer envelope through its transmembrane domain (TM), and is able to establish *in trans* interactions with the nuclear envelope via its C2 domain (C2). Additionally, the SMP domain (SMP) putatively performs a lipid transport function between the chloroplast and the nucleus and thus participates in retrograde signaling.

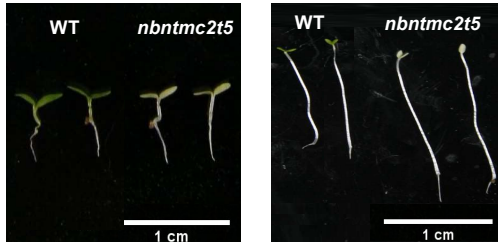
The C-terminal hydrophobic region (HR) of NbNTMC2T5 could have a relevant role in the establishment of chloroplast-ER MCS.



*Nbntmc2t5* mutants showed a reduced number of chloroplasts per cell in cotyledons compared to wild-type *N. benthamiana*, as observed through chlorophyll A fluorescence in confocal microscopy. However, this reduction was only evident until seedlings reached 18 days of age. In leaves, no significant differences were detected in chloroplast number per cell.

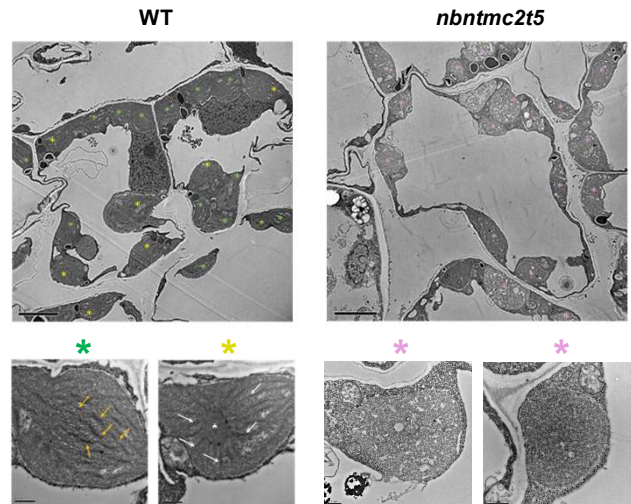
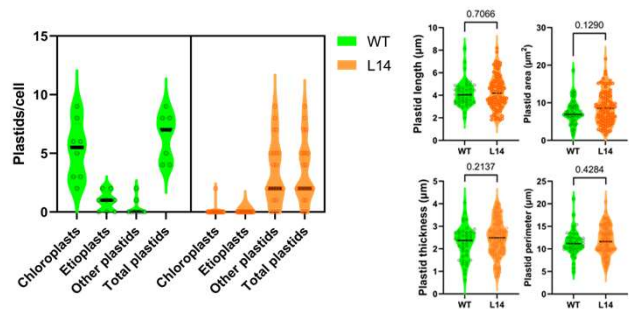
## 1. *Nbntmc2t5* mutants show a yellowing phenotype after germination under light and etiolation conditions

8 d post-germination under light conditions (16h/8h)      7 d etiolation + 1 d under light conditions (16h/8h)



*Nbntmc2t5* seedlings exhibited a yellowing phenotype in the cotyledons upon germination under both light and dark (etiolation) conditions. However, as the seedlings matured, this phenotype gradually faded, and the leaves developed normally. In the adult stage, *nbntmc2t5* mutants displayed no noticeable phenotypic differences compared to wild-type plants.

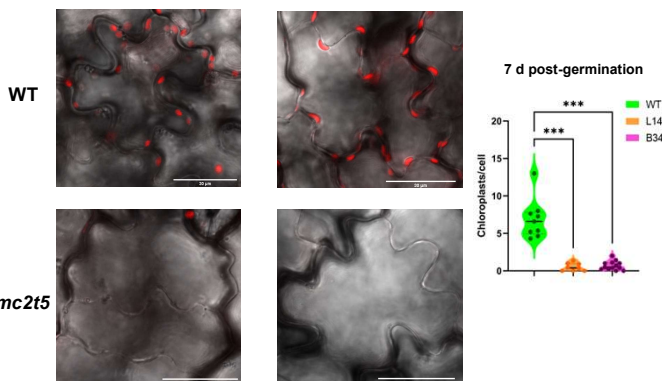
## 3. The lack of function of NbNTMC2T5 causes impaired plastid development under etiolation conditions



*Nbntmc2t5* mutants exhibited a depletion of mature chloroplasts seven days after germination under etiolation conditions. Moreover, no etio-plastids were observed in these mutants, suggesting that alterations were present during the proplastid stage.

## 2. *Nbntmc2t5* mutants show a reduction in the number of chloroplasts per cell during early development stages

Suc+      Suc-



*nbntmc2t5*