

HIGHLIGHTS

Open Access



TT3.1: a journey to protect chloroplasts upon heat stress

Jin-Yu Li and Jian-Xiang Liu*

Abstract

Rice (*Oryza sativa* L.) is a staple crop that feeds over half the world's population. High temperature stress is a great threaten to sustainable agriculture and leads to yield loss and impaired grain quality in major crops. Rice is sensitive to heat stress at almost all the growth stages and the molecular mechanisms underlying responses to heat stress in rice is emerging. Through quantitative trait locus (QTL) mapping, a recent study conducted by Zhang et al. shows that one genetic locus Thermo-tolerance 3 (TT3) contains two genes that are required for thermotolerance in rice. The TT3.1–TT3.2 genetic module in rice links the plasma membrane to chloroplasts to protect chloroplasts from heat stress damage and increases grain yield under heat stress conditions. This breakthrough provides a promising strategy for future breeding of high temperature resilient crops.

Keywords: Chloroplast, ER, Heat stress, NTL3, Plasma membrane, TT3.1, TT3.2

Global warming threatens harvests and has negative impacts on agricultural production worldwide. Chloroplasts are essential for photosynthesis, and their biogenesis and function are tightly regulated by environmental temperatures (Li et al. 2022). Although much has been learned on understanding heat stress response in plants (Ding et al. 2020), the interactions and communications between different subcellular organelles, especially chloroplasts under heat stress conditions are still less understood. A recent study reports a novel quantitative trait locus (QTL) named *Thermo-tolerance 3* (TT3) which encodes an E3 ubiquitin ligase TT3.1 and a chloroplast-localized membrane protein TT3.2 to enhance thermotolerance in rice (Zhang et al. 2022). These exciting findings reveal a novel communication mechanism between plasma membrane (PM) and chloroplasts upon heat stress, and provide an efficient strategy for breeding thermotolerant crops.

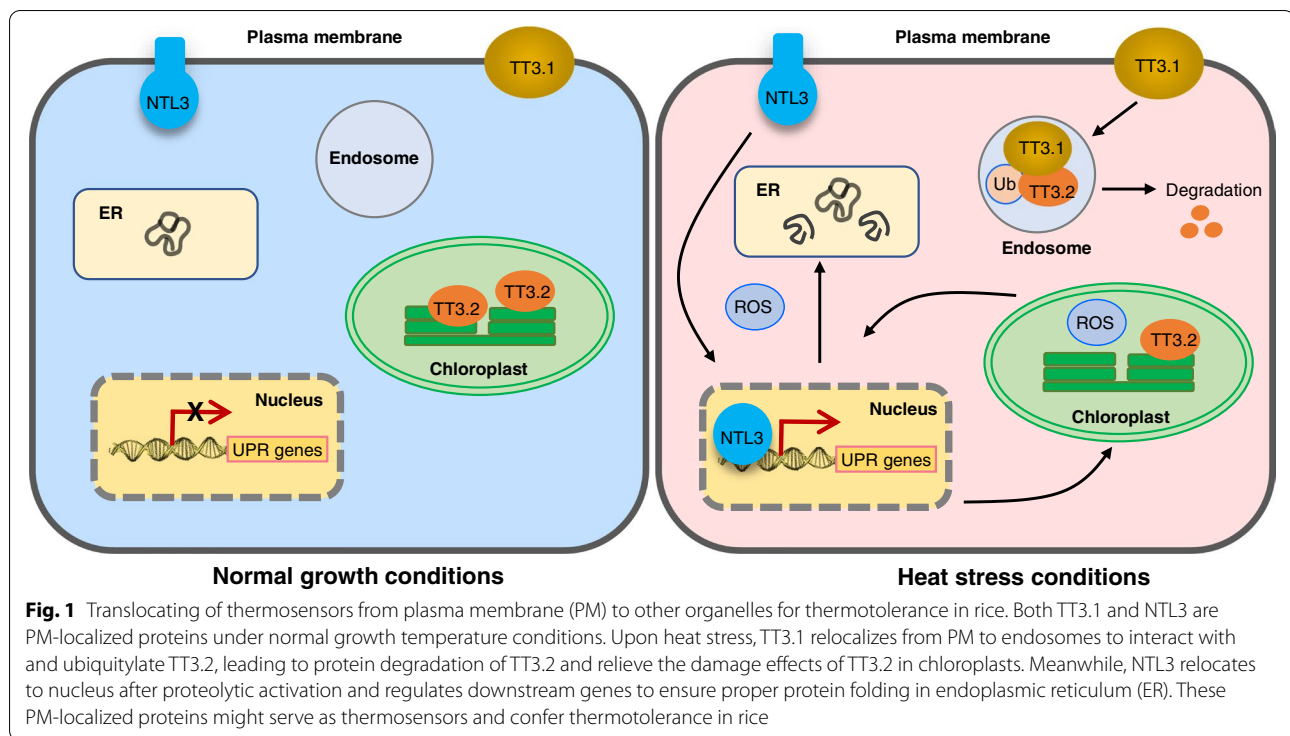
QTL mapping with chromosome segment substitution lines developed with the African rice variety CG14

as the donor parent and the Asian rice variety Wuyunjing (WYJ) as the recurrent parent leads to the identification of TT3 (Zhang et al. 2022) and TT1, encoding an $\alpha 2$ subunit of the 26S proteasome (Li et al. 2015), both of which are responsible for thermotolerance in rice. The nearly isogenic line (NIL) NIL-TT3^{CG14} shows more tolerant than the NIL-TT3^{WYJ} plants under heat stress conditions, and the loss-of-function mutant of TT3.1 is more sensitive to heat stress while mutation of TT3.2 confers thermotolerance comparing to the wild-type control WYJ. Overexpression of both TT3^{WYJ} and TT3^{CG14} in WYJ background increase thermotolerance. In greenhouse and field trials, either TT3.1^{CG14} overexpression plants or tt3.2 mutant plants exhibit a significant increase in seed setting rate, 1000-grain weight and ultimate grain yield per plant, indicating that TT3 also enhances thermotolerance at reproductive stages. Together, these results indicate that TT3 locus contain two casual genes that acts in an opposite way for thermotolerance in rice.

TT3.1 is a RING-type E3 ligase with predicted trans-membrane domains and it translocates from PM to endosomes in response to heat stress. Coincidentally, TT3.2 shows more endosome localization and less chloroplast localization under heat stress conditions

*Correspondence: jianxiangliu@zju.edu.cn

State Key Laboratory of Plant Physiology and Biochemistry, College of Life Sciences, Zhejiang University, Hangzhou 310027, China



comparing to that under normal temperature conditions. Intriguingly, Zhang et al. (2022) demonstrate that TT3.1 and TT3.2 interacts in yeast two-hybrid and split-luciferase assays. They later found that TT3.1 is a functional E3 ligase that polyubiquitinates TT3.2^{WYJ} and TT3.2^{CG14} *in vitro*. They also found that TT3.1^{CG14} has a stronger activity than TT3.1^{WYJ} *in vivo* in terms of TT3.2 degradation, and this degradation process is mediated by the endosome-to-vacuole pathway.

Since the degradation of TT3.2 upon heat stress is essential for heat tolerance in rice, it prompted the authors to study the molecular function of TT3.2. The results showed that the thylakoid organization of chloroplasts in NIL-TT3^{WYJ} and *tt3.1* mutant plants is impaired. They also found the abundance of photosynthetic protein complexes and the core subunits of photosystem II (PSII), D1 and D2, are decreased in NIL-TT3^{WYJ} and *tt3.1* mutant plants under heat stress conditions. These results suggest that the accumulation of chloroplast-localized TT3.2 plays a negative role in the maintenance of thylakoid organization, photosynthetic protein complexes and core subunits of PSII under heat stress conditions. How TT3.2 damages thylakoids and the PSII complex under heat stress conditions is not known. Nevertheless, the current report uncovers a novel mechanism in which TT3.1 travels from PM to endosomes to degrade TT3.2 for protecting

chloroplasts upon heat stress, which is essential for thermotolerance in rice.

Heat stress denatures proteins and disrupts protein homeostasis in the cytosol, endoplasmic reticulum (ER), chloroplasts, mitochondria, and even nucleus (Sun et al. 2021). Previously, we reported that a membrane-associated NAC transcription factor NTL3 relocates from PM to nucleus under heat stress conditions to regulated the expression of genes involved in reactive oxidative species (ROS) detoxification and protein folding in ER (Liu et al. 2020). These PM-associated proteins TT3.1 and NTL3 might serve as thermosensors in rice plants (Fig. 1).

In summary, Zhang et al. (2022) reported a novel QLT consists of TT3.1 and TT3.2 in rice. TT3.1 and TT3.2 forms a regulatory module to communicate among PM, endosomes and chloroplasts under heat stress conditions. In future studies, it would be exciting to find out how TT3.1 receives high temperature signals. TT3.1 and TT3.2 are conserved in other major crops such as maize and wheat, it would be interesting to know whether these orthologous genes could be used for breeding heat tolerant crops.

Acknowledgements

Not applicable.

Authors' contributions

J.Y.L. and J.X.L. prepared the figure and wrote the paper. The author(s) read and approved the final manuscript.

Funding

The projects in Liu Lab was financially supported by grants from the State Key Project of Research and Development Plan (2021YFF1000400), Natural Science Foundation of Zhejiang, China (LD21C020001), and Zhejiang Provincial Talent Program (2019R52005).

Availability of data and materials

Not applicable.

Declarations

Competing interests

J.X.L. is a member of the editorial board but was not involved in the journal's review of, or any decisions related to, this submission.

Received: 22 June 2022 Accepted: 6 July 2022

Published online: 12 July 2022

References

- Ding Y, Shi Y, Yang S (2020) Molecular regulation of plant responses to environmental temperatures. *Mol Plant* 13:544–564. <https://doi.org/10.1016/j.molp.2020.02.004>
- Li XM, Chao DY, Wu Y, Huang X, Chen K, Cui LG, Su L, Ye WW, Chen H, Chen HC, Dong NQ, Guo T, Shi M, Feng Q, Zhang P, Han B, Shan JX, Gao JP, Lin HX (2015) Natural alleles of a proteasome $\alpha 2$ subunit gene contribute to thermotolerance and adaptation of African rice. *Nat Genet* 47:827–833. <https://doi.org/10.1038/ng.3305>
- Li JY, Yang C, Tian YY, Liu JX (2022) Regulation of chloroplast development and function at adverse temperatures in plants. *Plant Cell Physiol* 63:580–591. <https://doi.org/10.1093/pcp/pcac022>
- Liu XH, Lyu YS, Yang W, Yang ZT, Lu SJ, Liu JX (2020) A membrane-associated NAC transcription factor OsNTL3 is involved in thermotolerance in rice. *Plant Biotechnol J* 18:1317–1329. <https://doi.org/10.1111/pbi.13297>
- Sun JL, Li JY, Wang MJ, Song ZT, Liu JX (2021) Protein quality control in plant organelles: current progress and future perspectives. *Mol Plant* 14:95–114. <https://doi.org/10.1016/j.molp.2020.10.011>
- Zhang H, Zhou JF, Kan Y, Shan JX, Ye WW, Dong NQ, Guo T, Xiang YH, Yang YB, Li YC, Zhao HY, Yu HX, Lu ZQ, Guo SQ, Lei JJ, Liao B, Mu XR, Cao YJ, Yu JJ, Lin Y, Lin HX (2022) A genetic module at one locus in rice protects chloroplasts to enhance thermotolerance. *Science* 376:1293–1300. <https://doi.org/10.1126/science.abo5721>

Publisher's Note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.