AP52 PIF1, a phytochrome-interacting factor negatively regulates drought tolerance and carotenoids biosynthesis in tobacco

- Presenter: Shaohua Liu
- Authors: Shaohua Liu; Yinchao Zhang; Xuhao Pan; Bin Li; Qing Yang; Changqing Yang; Jianhui Zhang; Fengyan Wu; Aiguo Yang; Yiting Li
- *Key laboratory of Tobacco Genetic Improvement and Biotechnology, Tobacco Research Institute, Chinese Academy of Agricultural Sciences, Qingdao 266100, China*
- *Shenzhen Yupeng Technology Co., Ltd., Shenzhen, Guangdong 518000, China*

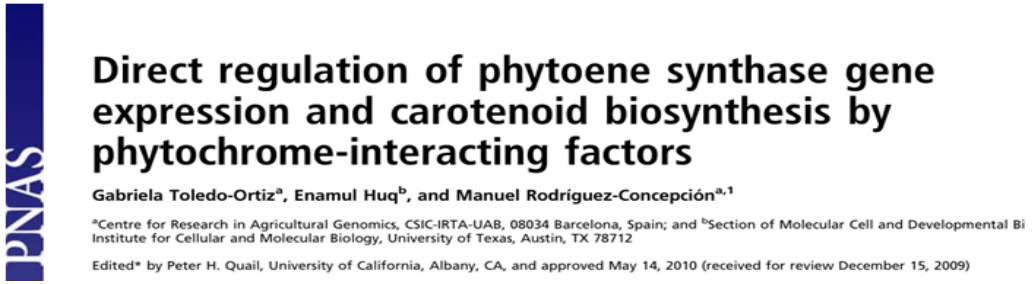
Contents

Background-drought stress

- Drought is one of the most serious environmental stresses and has been widely concerned for a long time.
- The crop yield reduction caused by water scarcity, especially in arid and semi-arid regions, poses a serious threat to food security worldwide.
- Thus, understanding the molecular regulation mechanism of plants in response to drought has great significance for improving the drought resistance of crops and maintaining sustainable agricultural production.
- Transcription factors (TFs) play critical role in the defense mechanism of plant water deficiency by controlling downstream stress-related genes expression. Such as the MYB/AP2/WRKY/bHLH/bZIP.

Background-phytochrome interacting factors

- PIFs have been demonstrated to regulate multiple developmental and physiological processes.



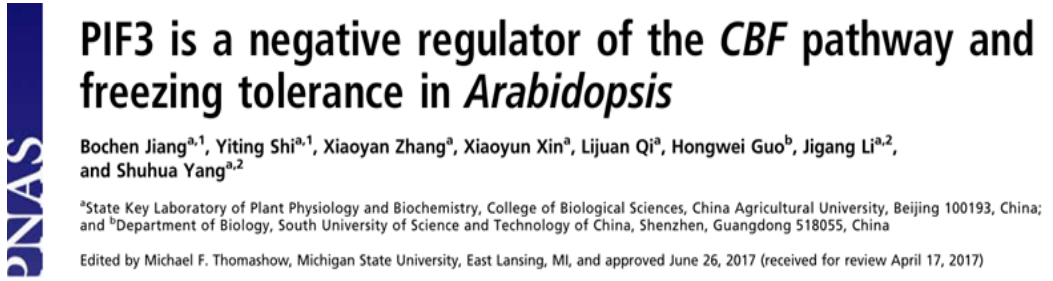
PNAS

Direct regulation of phytoene synthase gene expression and carotenoid biosynthesis by phytochrome-interacting factors

Gabriela Toledo-Ortiz[a], Enamul Huq[b], and Manuel Rodríguez-Concepción[a,1]

[a]Centre for Research in Agricultural Genomics, CSIC-IRTA-UAB, 08034 Barcelona, Spain; and [b]Section of Molecular Cell and Developmental Bi Institute for Cellular and Molecular Biology, University of Texas, Austin, TX 78712

Edited* by Peter H. Quail, University of California, Albany, CA, and approved May 14, 2010 (received for review December 15, 2009)



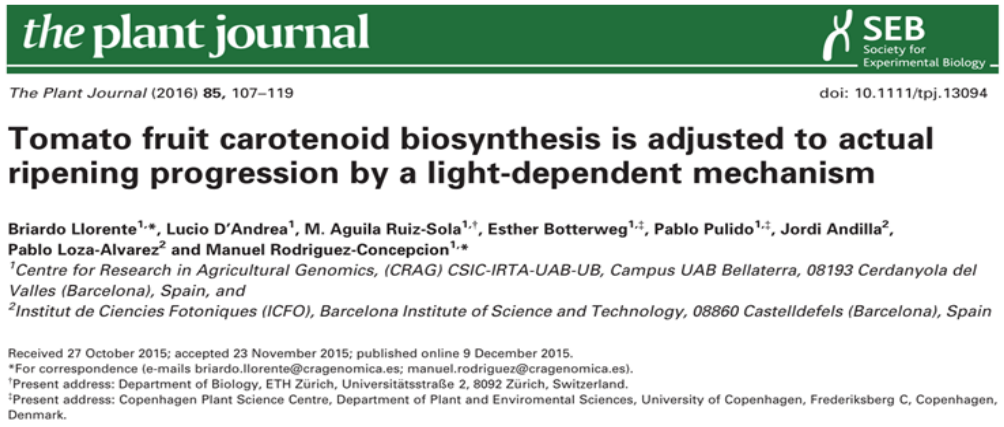
PNAS

PIF3 is a negative regulator of the *CBF* pathway and freezing tolerance in *Arabidopsis*

Bochen Jiang[a,1], Yiting Shi[a,1], Xiaoyan Zhang[a], Xiaoyun Xin[a], Lijuan Qi[a], Hongwei Guo[b], Jigang Li[a,2], and Shuhua Yang[a,2]

[a]State Key Laboratory of Plant Physiology and Biochemistry, College of Biological Sciences, China Agricultural University, Beijing 100193, China; and [b]Department of Biology, South University of Science and Technology of China, Shenzhen, Guangdong 518055, China

Edited by Michael F. Thomashow, Michigan State University, East Lansing, MI, and approved June 26, 2017 (received for review April 17, 2017)



the plant journal — SEB Society for Experimental Biology

The Plant Journal (2016) **85**, 107–119 doi: 10.1111/tpj.13094

Tomato fruit carotenoid biosynthesis is adjusted to actual ripening progression by a light-dependent mechanism

Briardo Llorente[1,*], Lucio D'Andrea[1], M. Aguila Ruiz-Sola[1,†], Esther Botterweg[1,‡], Pablo Pulido[1,‡], Jordi Andilla[2], Pablo Loza-Alvarez[2] and Manuel Rodriguez-Concepcion[1,*]

[1]*Centre for Research in Agricultural Genomics, (CRAG) CSIC-IRTA-UAB-UB, Campus UAB Bellaterra, 08193 Cerdanyola del Valles (Barcelona), Spain, and*

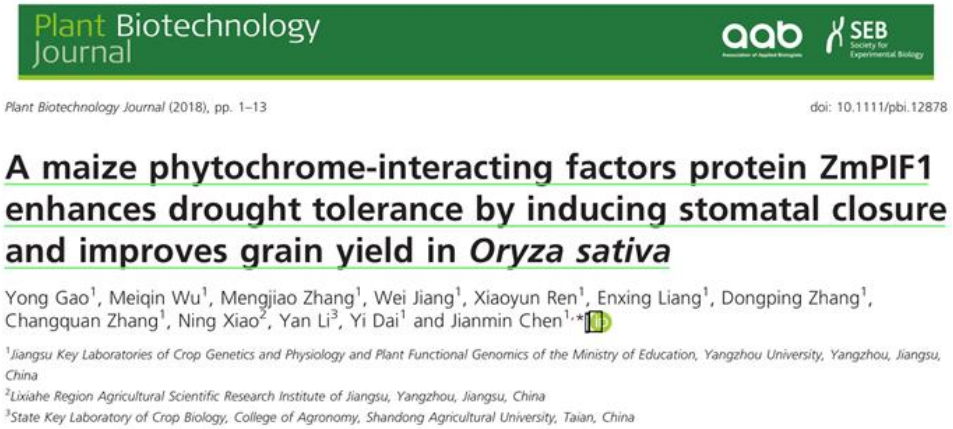
[2]*Institut de Ciencies Fotoniques (ICFO), Barcelona Institute of Science and Technology, 08860 Castelldefels (Barcelona), Spain*

Received 27 October 2015; accepted 23 November 2015; published online 9 December 2015.

*For correspondence (e-mails briardo.llorente@cragenomica.es; manuel.rodriguez@cragenomica.es).

†Present address: Department of Biology, ETH Zürich, Universitätsstraße 2, 8092 Zürich, Switzerland.

‡Present address: Copenhagen Plant Science Centre, Department of Plant and Enviromental Sciences, University of Copenhagen, Frederiksberg C, Copenhagen, Denmark.



Plant Biotechnology Journal — aab — SEB Society for Experimental Biology

Plant Biotechnology Journal (2018), pp. 1–13 doi: 10.1111/pbi.12878

A maize phytochrome-interacting factors protein ZmPIF1 enhances drought tolerance by inducing stomatal closure and improves grain yield in *Oryza sativa*

Yong Gao[1], Meiqin Wu[1], Mengjiao Zhang[1], Wei Jiang[1], Xiaoyun Ren[1], Enxing Liang[1], Dongping Zhang[1], Changquan Zhang[1], Ning Xiao[2], Yan Li[3], Yi Dai[1] and Jianmin Chen[1,*]

[1]*Jiangsu Key Laboratories of Crop Genetics and Physiology and Plant Functional Genomics of the Ministry of Education, Yangzhou University, Yangzhou, Jiangsu, China*

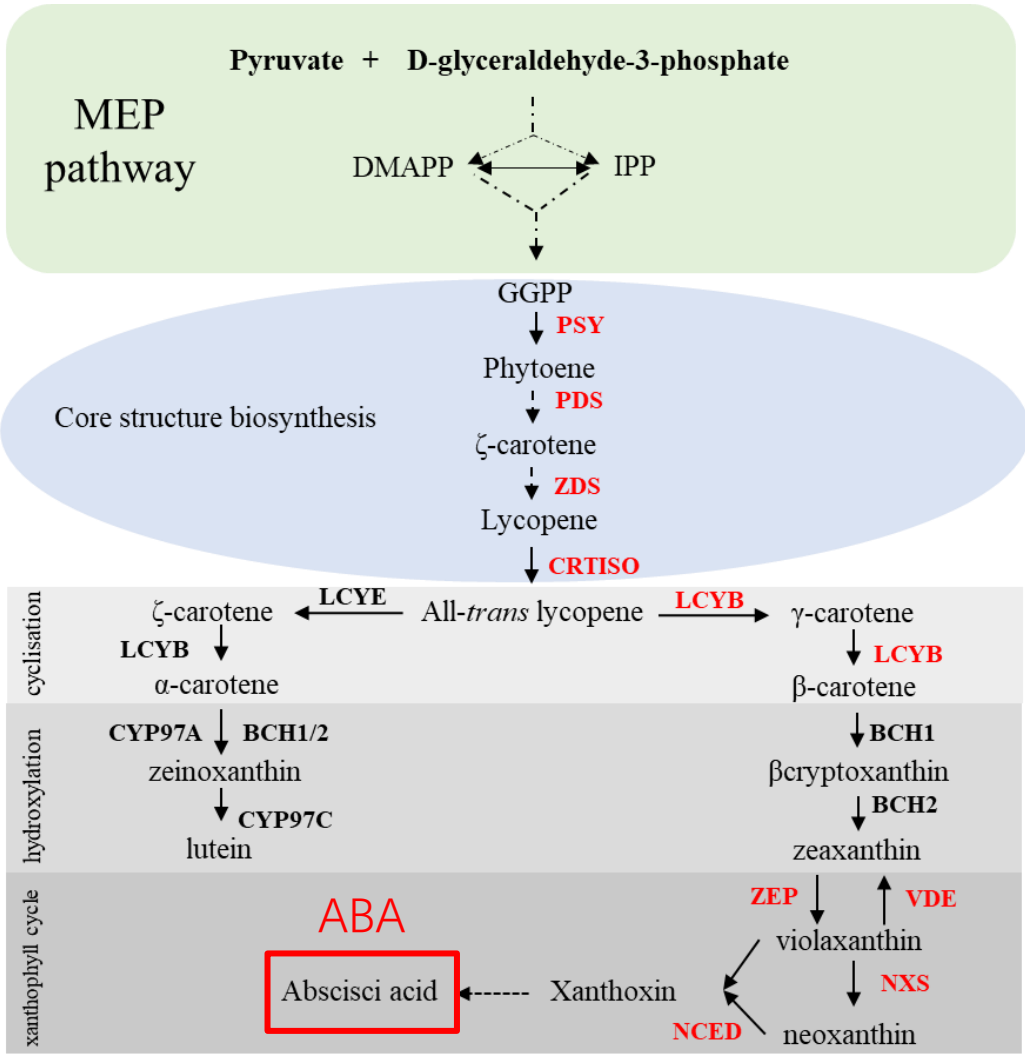
[2]*Lixiahe Region Agricultural Scientific Research Institute of Jiangsu, Yangzhou, Jiangsu, China*

[3]*State Key Laboratory of Crop Biology, College of Agronomy, Shandong Agricultural University, Taian, China*

- Previous studies have shown that PIF not only regulates the biosynthesis of carotenoids in plants, but also widely participates in plant resistance to stress.

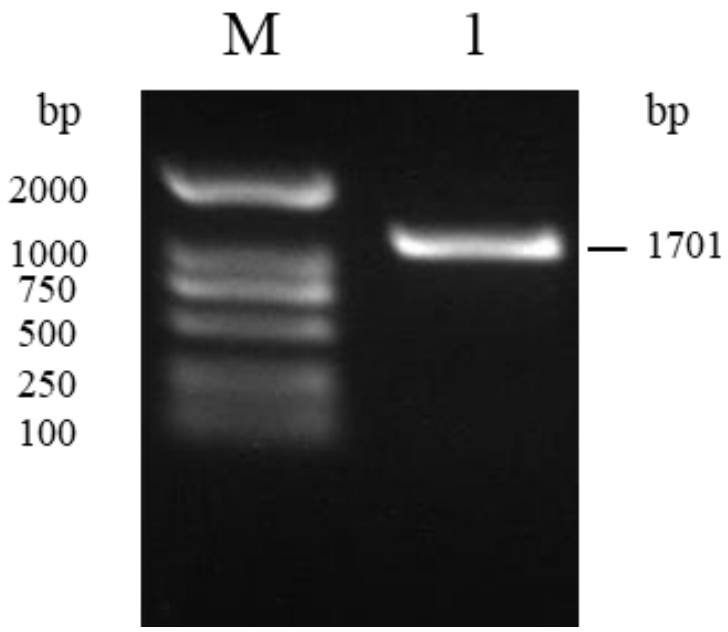
Background-carotenoids

- Carotenoids are a diverse group of natural polyunsaturated isoprenoids distributed widely in higher plants, algae, fungi, and bacteria.
- They color flowers and fruits, serve as precursors for phytohormones, such as ABA and strigolactones, regulating plant growth and development.
- The most critical function of carotenoids is to harvest light to provide energy for photosynthesis and transfer excess energy away from chlorophylls to protect photosynthetic apparatus against photo-oxidative damage.
- So far, the biosynthetic pathway of carotenoids has been well established and a series of key enzymes in this pathway have been identified.

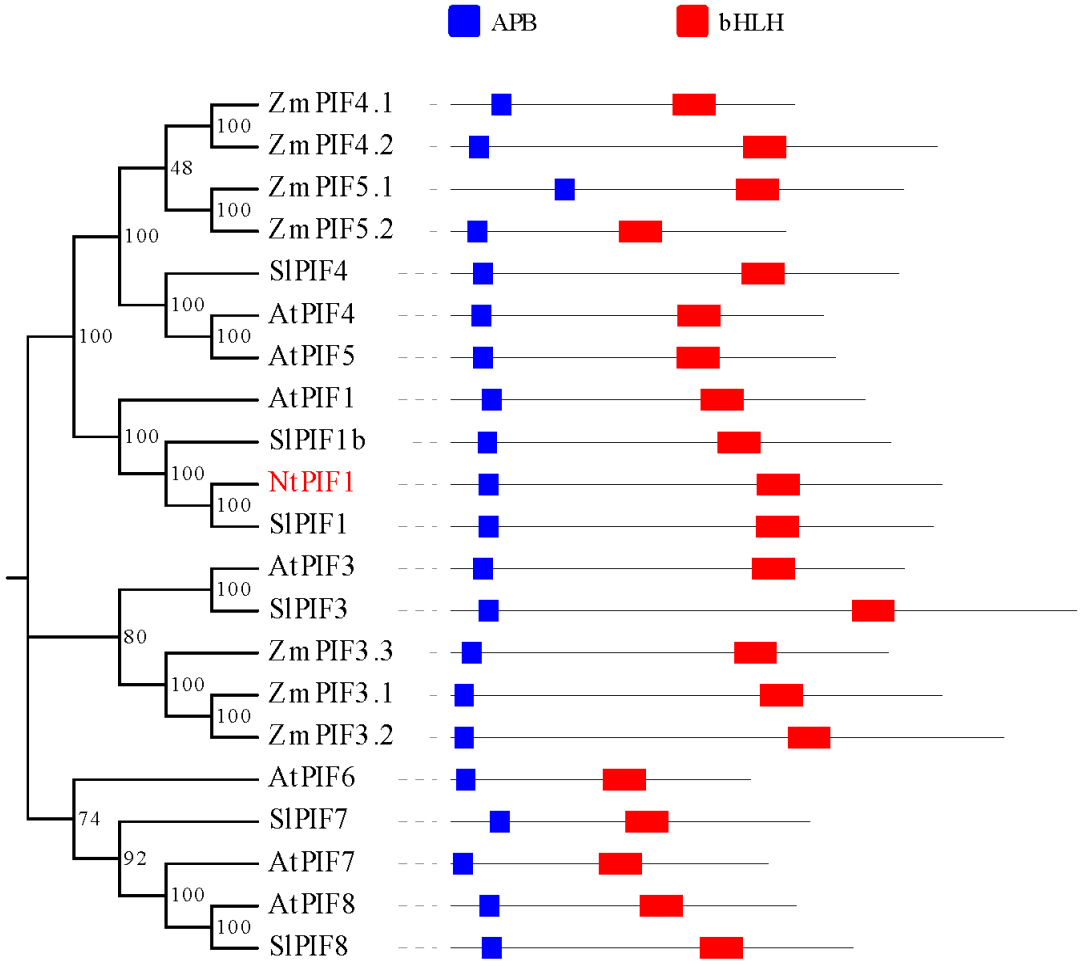


Carotenoid biosynthesis pathway

Background-previous studies



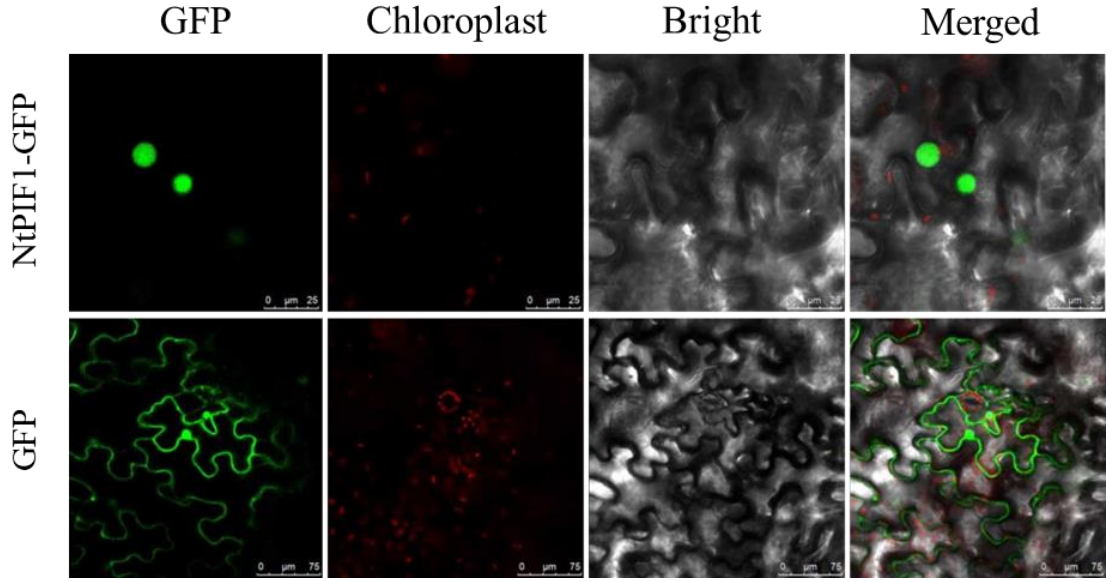
characterization of *NtPIF1*



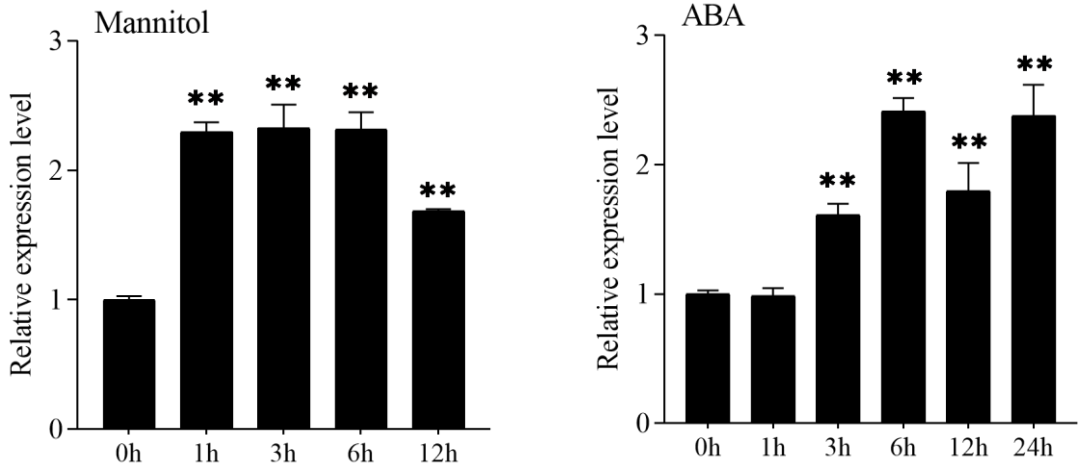
- NtPIF1 has APB and bHLH domain, and has the closest sequence similarity with SlPIF1.

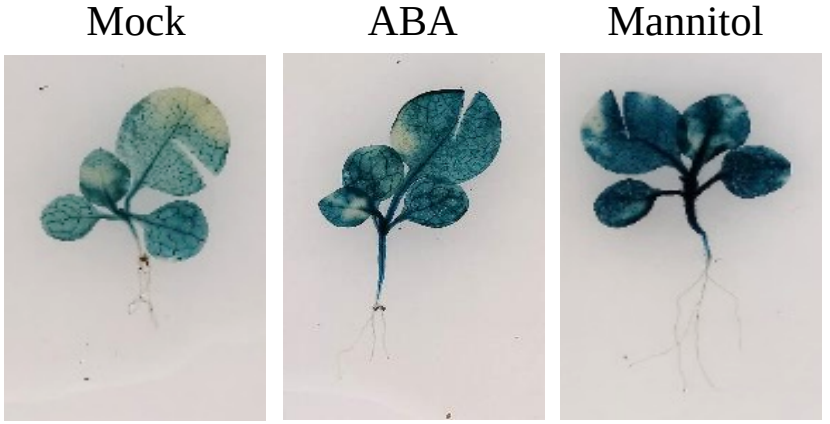
Background-previous studies

- NtPIF1 is a nuclear localized transcription factor.



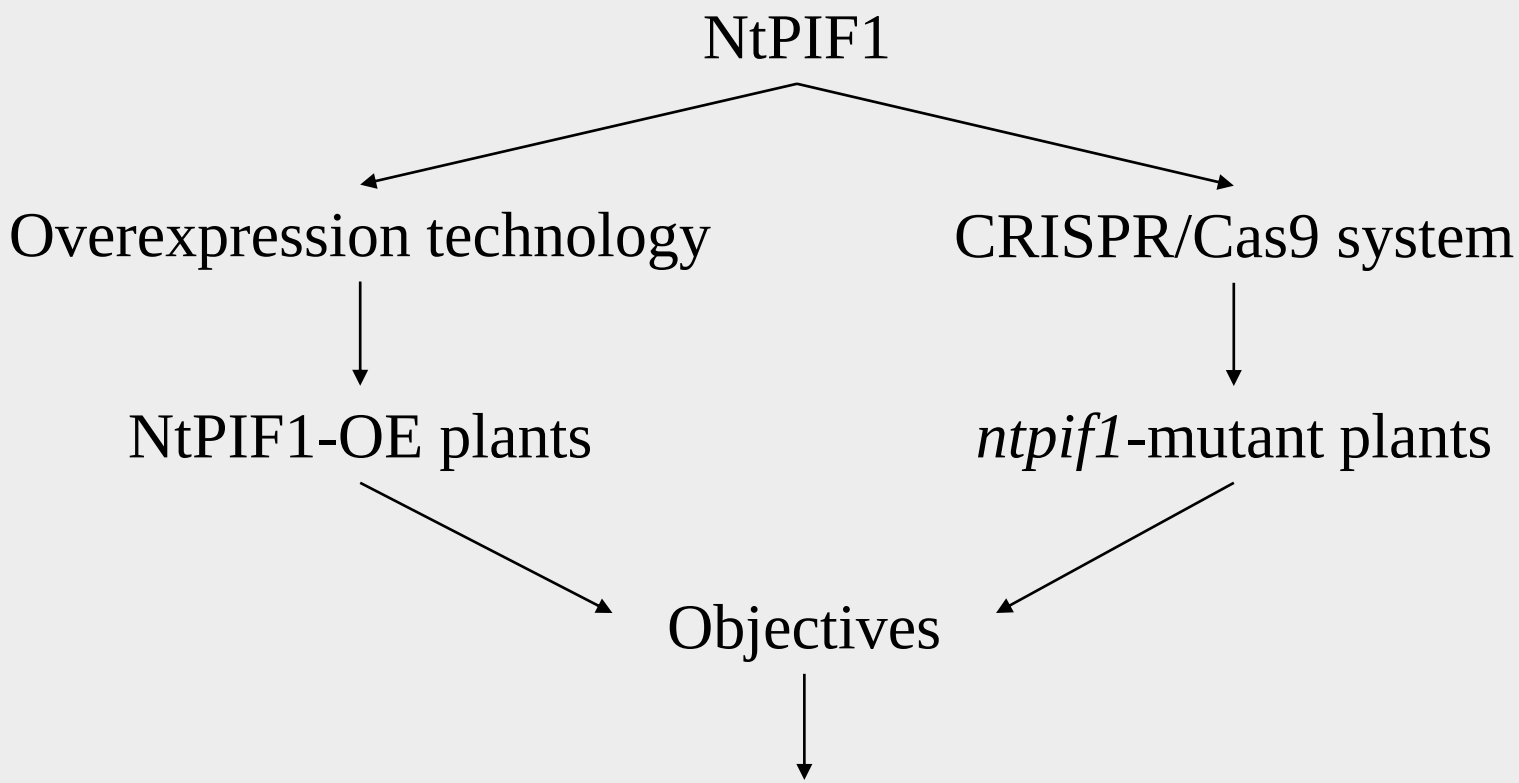
Subcellular localization analysis of NtPIF1





- NtPIF1 can be induced by mannitol and ABA for expression.

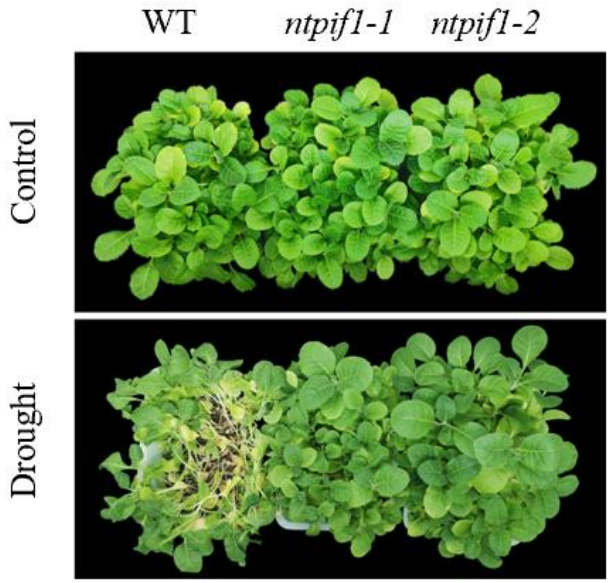
Study Roadmap

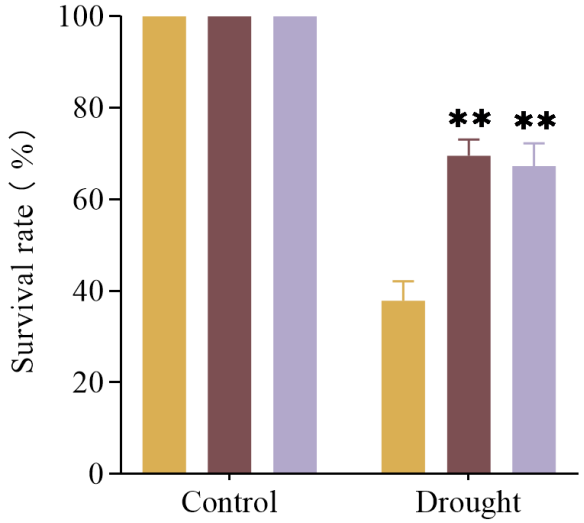


1. Explore the effect of *NtPIF1* on Tobacco drought resistance
2. Explore the effect of *NtPIF1* on Tobacco carotenoid biosynthesis
3. Is there any relationship between carotenoid accumulation and drought tolerance

Result 1. *NtPIF1* negatively regulates plants drought tolerance

- For drought tolerance test, the *ntpif1* mutant lines were grown in pots containing mixed soid under normal conditions for 4 weeks. And then, these plants were deprived of water for 21 days.

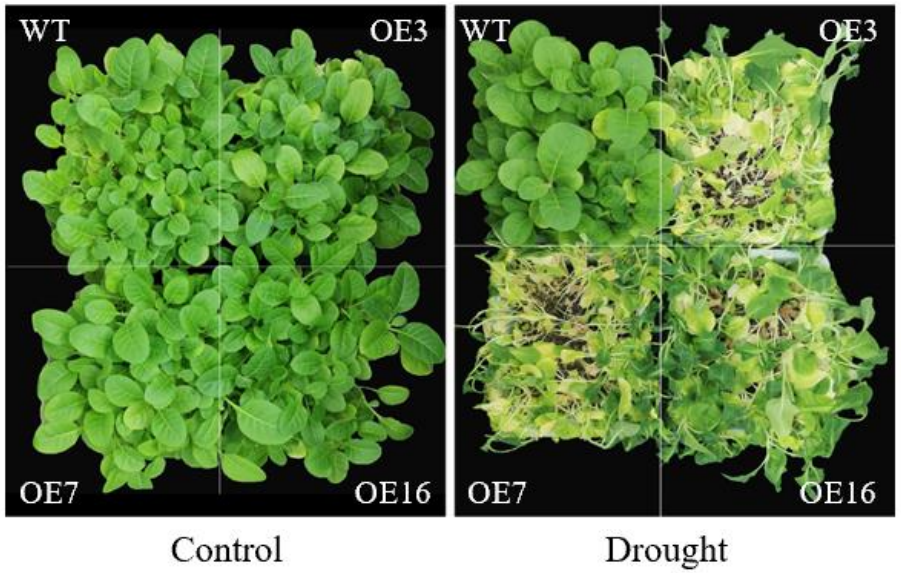


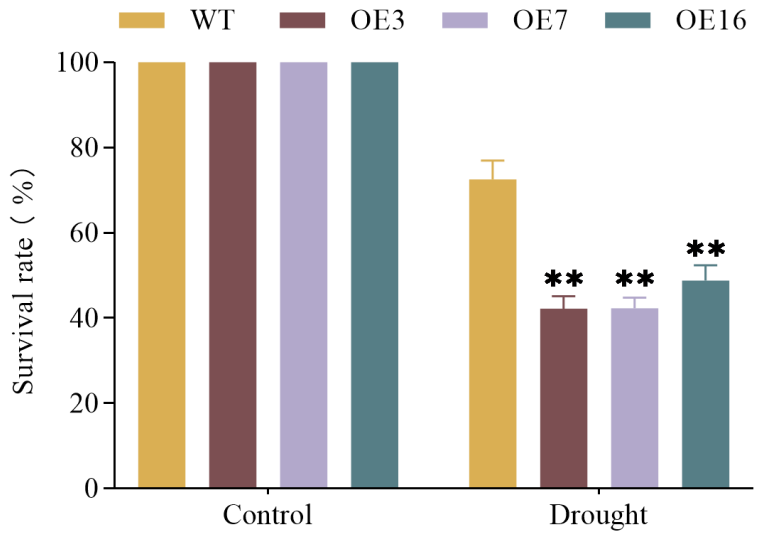


- Under control conditions, no phenotypic and physiological differences were observed between *ntpif1* mutants and wild-type (WT) plants.
- Upon drought stress, the *ntpif1* mutants showed lighter leaves wilting and higher survival rates than that of WT plants.

Result 1. *NtPIF1* negatively regulates plants drought tolerance

➢ The *NtPIF1-OE* lines showed the opposite results.

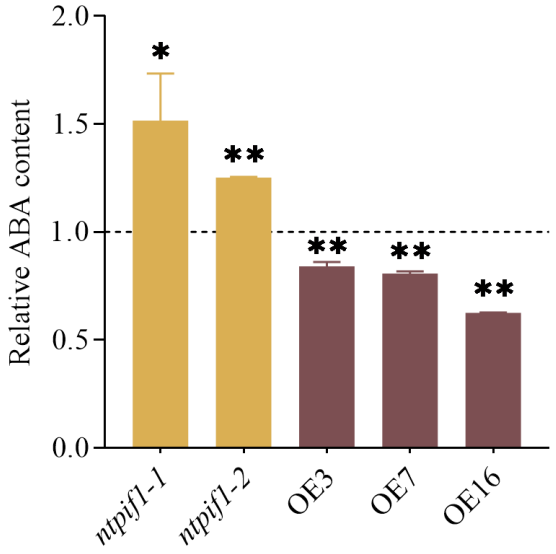




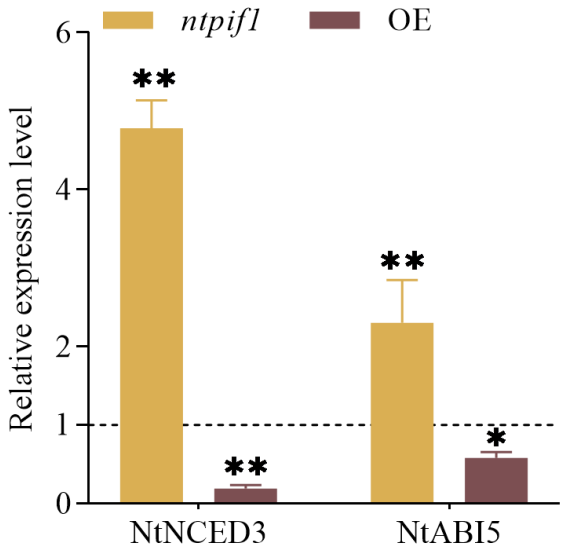
- The phenotypic and physiological traits of *NtPIF1-OE* lines and WT were similar under normal conditions.
- When exposed to drought stress, the *NtPIF1-OE* plants displayed heavier leaves wilting phenotypes and lower survival rates than WT plants.

Result 2. *NtPIF1* decrease ABA sensitivity and accumulation in response to drought stress

- ABA plays an important role in plant drought response. In order to verify whether NtPIF1 participates in the tobacco drought resistance defense by regulating ABA-dependent pathway, we first analyzed the ABA contents of *ntpif1* mutants, *NtPIF1-OE*, and WT plants under drought stress.

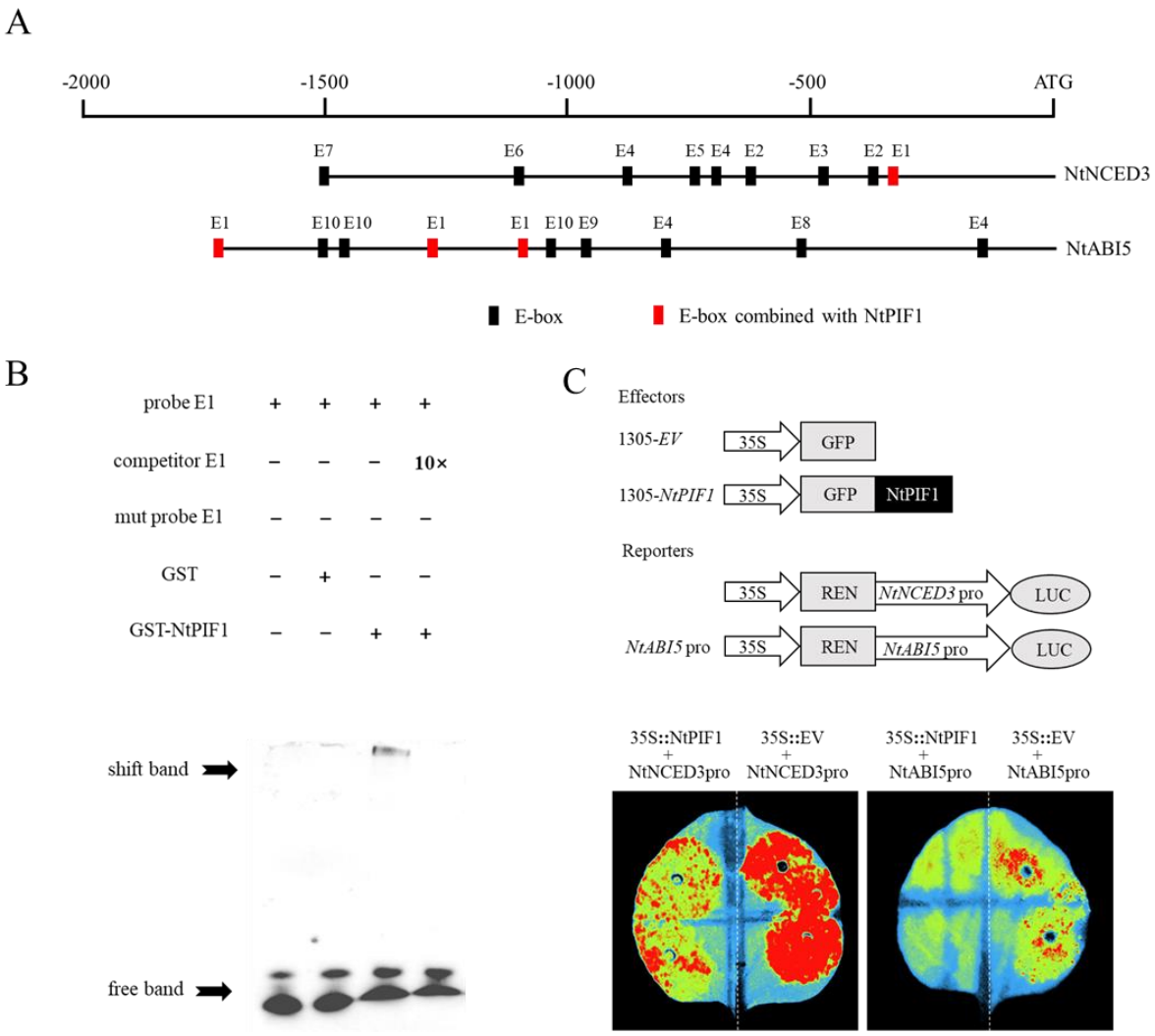


NtPIF1 may regulate ABA-dependent manner by targeting the gene *NtNCED3* and *NtABI5*.



- Compared with WT plants (set to 1), *ntpif1* mutants accumulated higher ABA levels, whereas *NtPIF1-OE* plants exhibited lower ABA contents , suggesting that *NtPIF1* could be involved in the regulation of ABA biosynthesis.
- Notably, qRT-PCR results indicated that only *NtNCED3* and *NtABI5* were strongly upregulated in *ntpif1* mutants and downregulated in *NtPIF1-OE* plants

Result 2. *NtPIF1* decrease ABA sensitivity and accumulation in response to drought stress



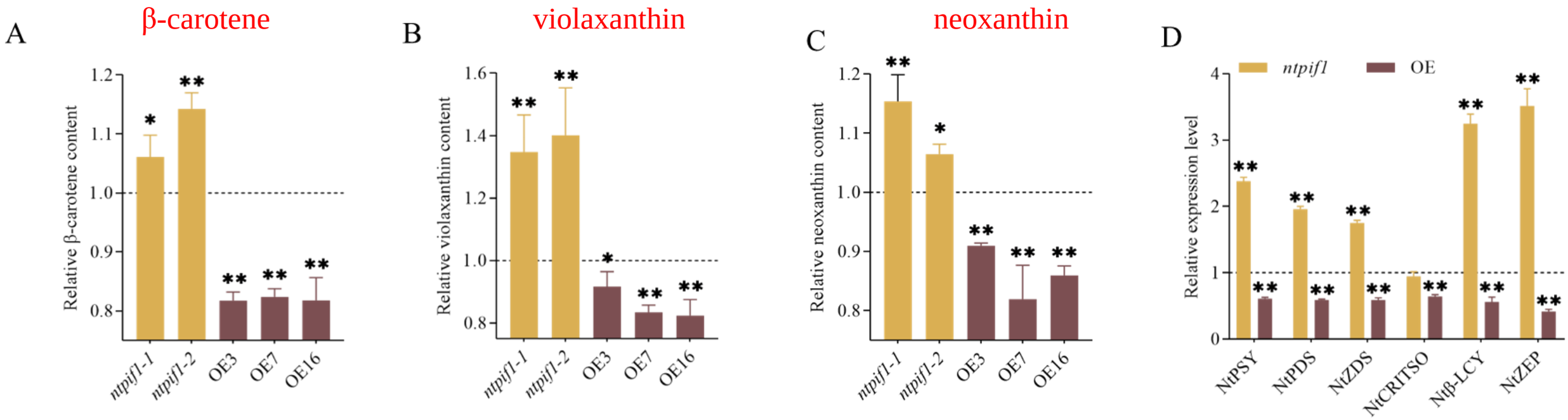
- Further molecular experiments proved NtPIF1 could directly combine and regulate the genes (NtNCED3 and NtABI5) involved the biosynthesis of ABA.

A. E-box analysis of promotor： CANNTG

B. EMSA assay

C. Luc assay

Result 3. *NtPIF1* reduces the biosynthesis of carotenoids under drought stress



- Compared with the WT plants (set to 1), the results showed that *ntpif1* mutants accumulated larger carotenoids contents, while the contents of carotenoid compounds contents in *NtPIF1-OE* tobacco seedlings are lower (A-C).

- The expression levels of *NtPSY*, *NtPDS*, *NtZDS*, *Ntβ-LCY*, and *NtZEP* were significantly higher in the *ntpif1* mutants than in the WT seedlings (set to 1) under drought stress, while *NtPIF1-OE* tobacco seedlings showed lower expression level of these genes.

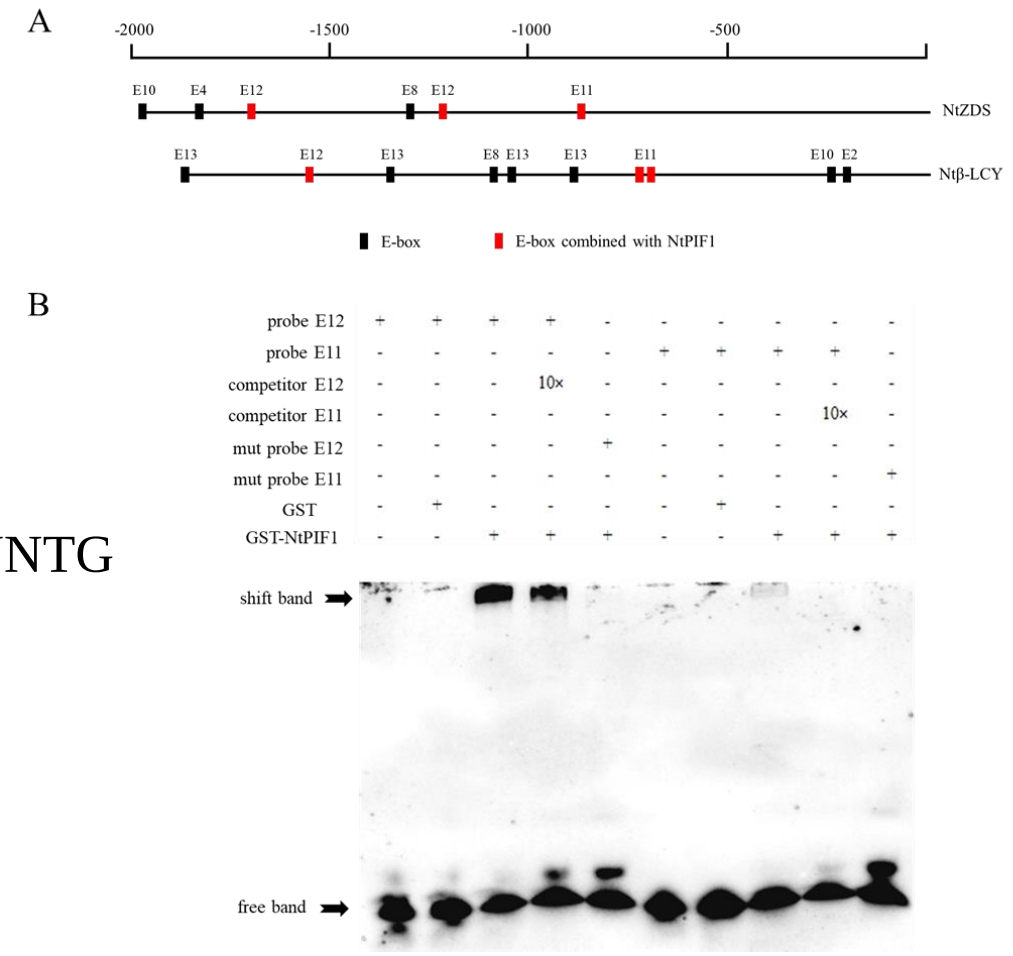
Result 3. *NtPIF1* reduces the biosynthesis of carotenoids under drought stress

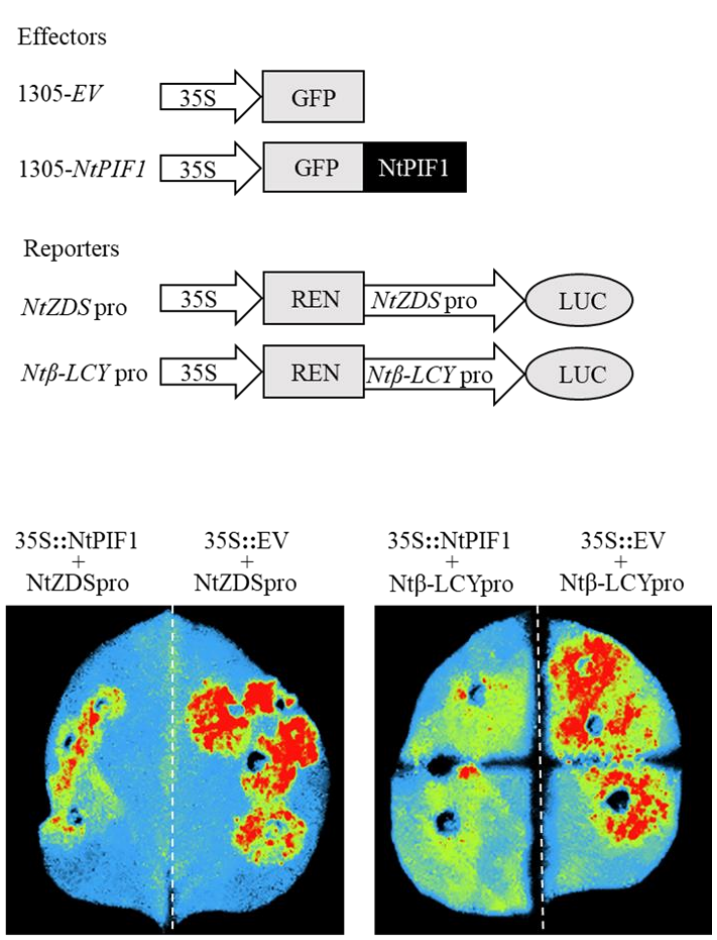
- Further molecular experiments also identified NtPIF1 could directly combine and regulate the genes (NtZDS and Ntβ-LCY) involved in the biosynthesis of Carotenoids.

A. E-box of promotor： CANNTG

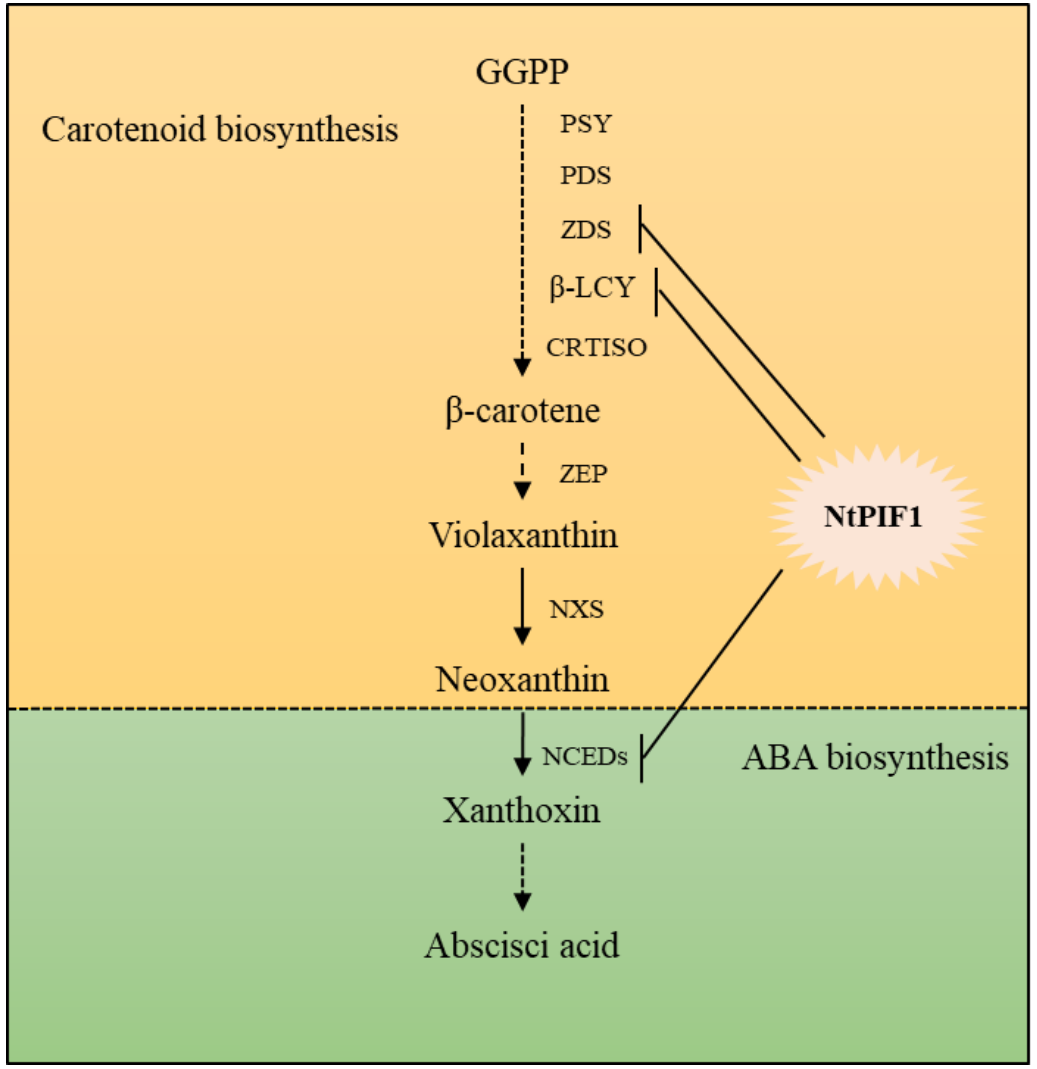
B. EMSA assay

C. Luc assay

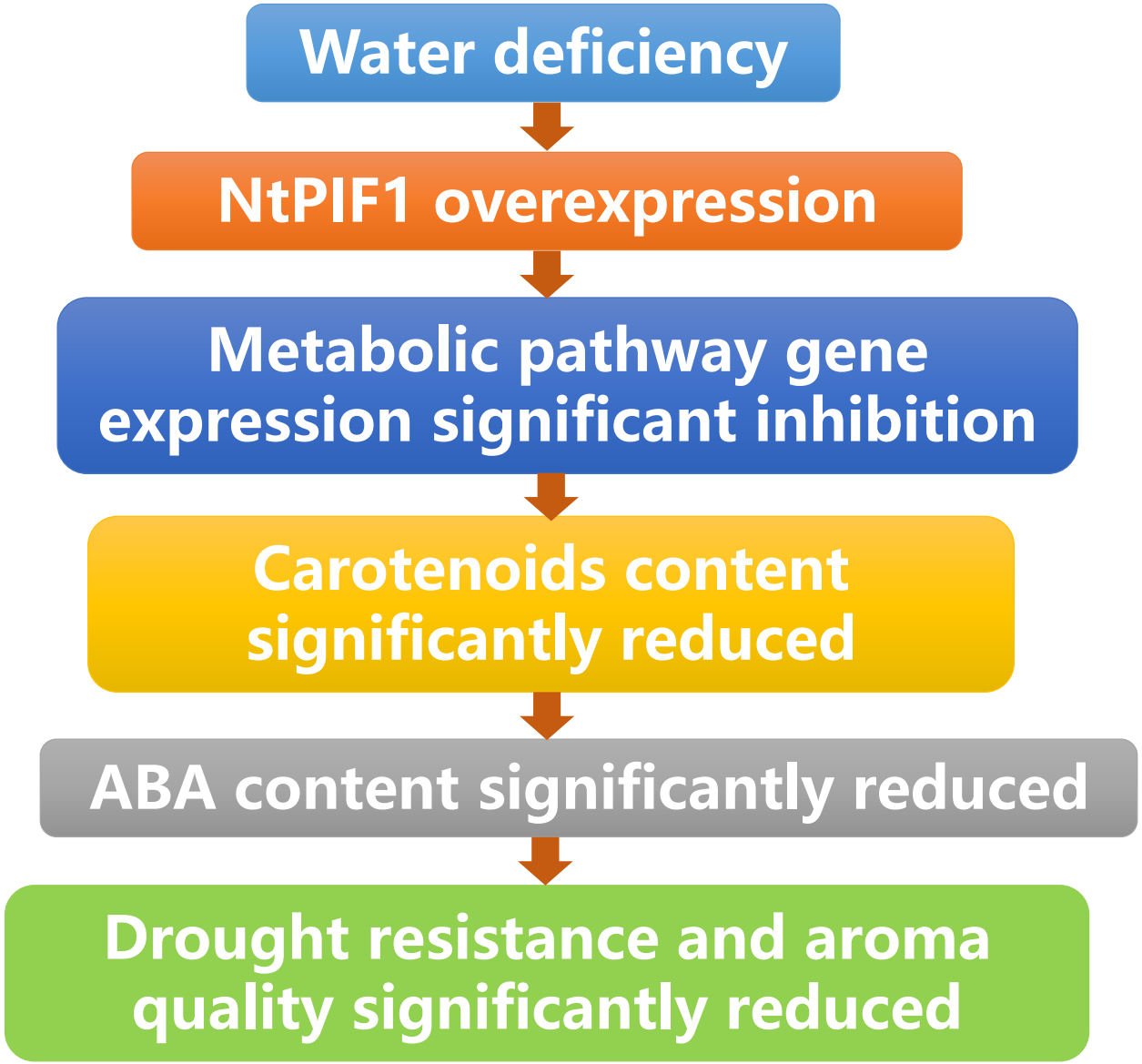




Discussion



A schematic diagram of carotenoids and ABA biosynthesis pathways, including functional roles of *NtPIF1* under drought stress in tobacco.



Conclusions

Objectives

1. The effect of NtPIF1 on Tobacco drought resistance ?
2. The effect of NtPIF1 on Tobacco carotenoid biosynthesis?
3. Any relationship between carotenoid accumulation and drought tolerance ?

- NtPIF1 was identified as a negative regulator in tobacco adaptation to drought stress and carotenoids biosynthesis.
- Furthermore, NtPIF1 not only directly repressed the expression of ABA biosynthesis and signaling related genes *NtNCED3* and *NtABI5*, but also inhibited the carotenogenic genes *Ntβ-LCY* and *NtZDS* to decrease tobacco drought tolerance.
- This discovery complements the studies on the involvement of carotenoids metabolic pathways in drought stress and provides new ideas for the resolution of drought resistance mechanisms.

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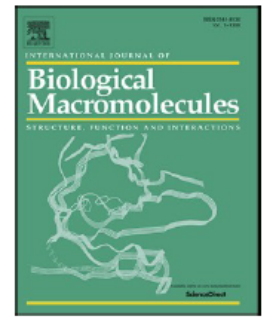

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Shaohua Liu [a,b,1], Yinchao Zhang [a,1], Xuhao Pan [a], Bin Li [c], Qing Yang [a], Changqing Yang [a], Jianhui Zhang [c], Fengyan Wu [a], Aiguo Yang [a,*], Yiting Li [a,*]

[a] *Key Laboratory of Tobacco Genetic Improvement and Biotechnology, Tobacco Research Institute, Chinese Academy of Agricultural Sciences, Qingdao 266100, China*
[b] *Shenzhen Yupeng Technology Co., Ltd, Shenzhen 518110, China*
[c] *Sichuan Tobacco Corporation, Chengdu 610014, China*

Thanks!

lsh@szupon.com

liyiting@caas.com