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Comprehensive analysis of the regulatory network of carbon and nitrogen metabolism in rapeseed under waterlogging stress

Prof. Ni Ma

Presented by: Xiaohua Bian

Oil Crops Research Institute, Chinese Academy of Agricultural Sciences

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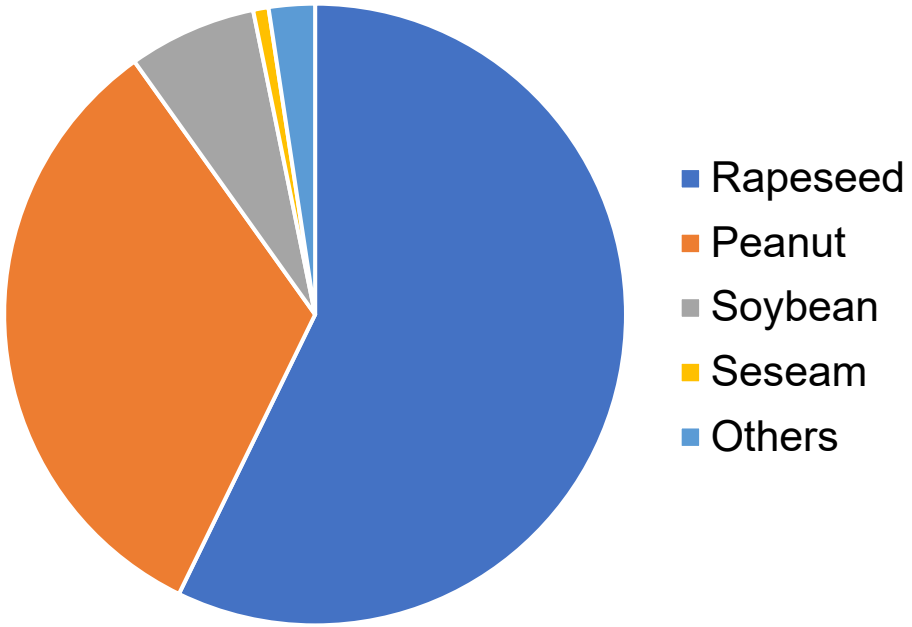
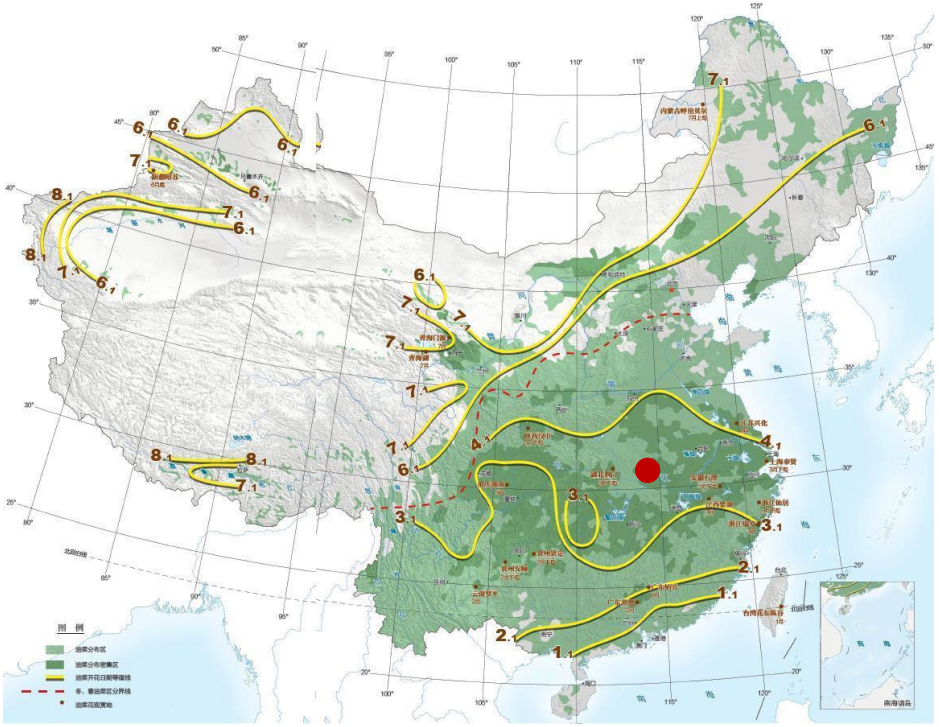
Introduction

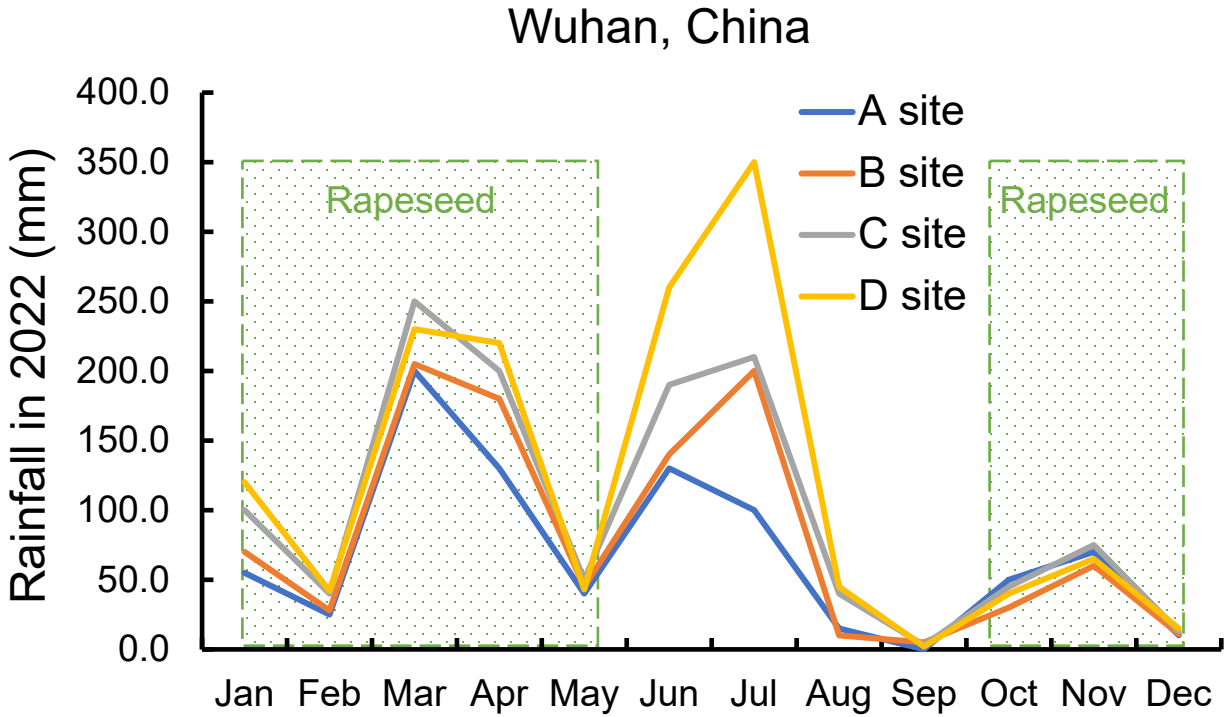
- Rapeseed is an important oil crop, with the largest planting area as well as total oil production in China.



Rapeseed oil accounts for 55% of domestic edible oil in China.

- Rapeseed is mainly distributed in the Yangtze River basin in China.
- Heavy rainfall and poor soil drainage combined with a humid climate make rape susceptible to waterlogging.

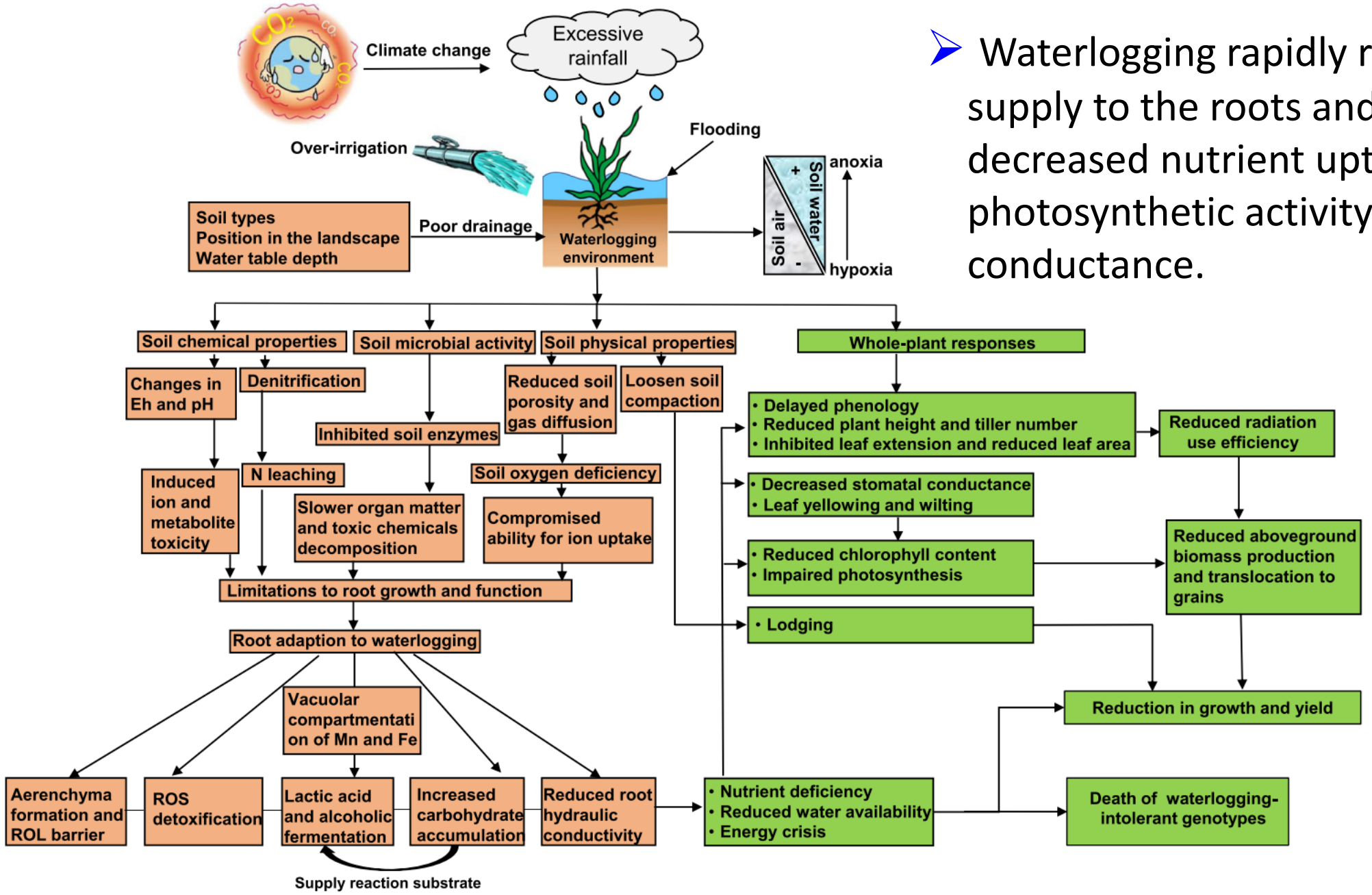




- Plant height, number of effective branches, number of siliques, and number of grains per silique decreased under waterlogging stress.
- The yield of rapeseed could decrease by 17%-42% due to waterlogging stress in China.



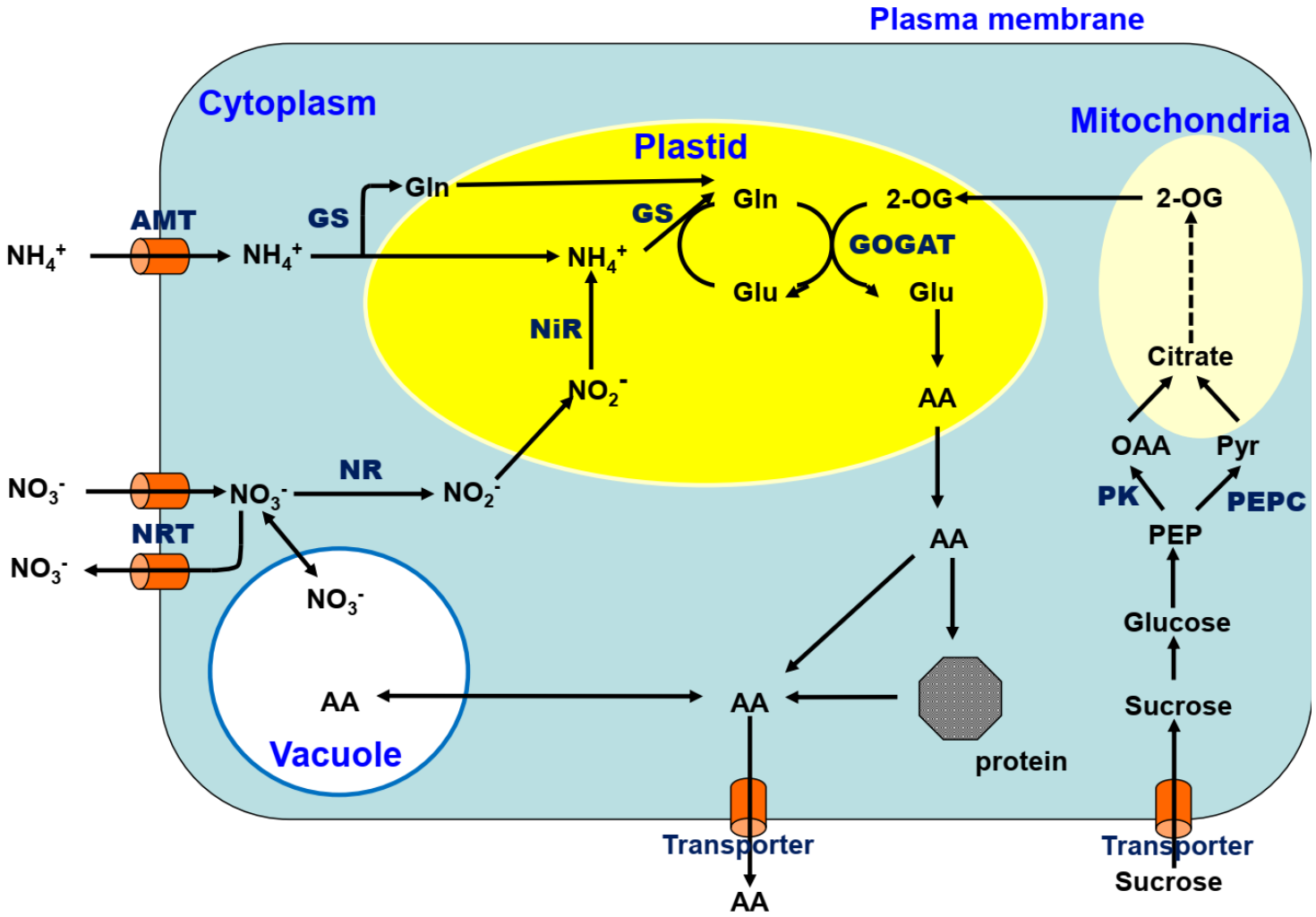
Hu et al., 2017; Wang et al., 2022



- Waterlogging rapidly reduced oxygen supply to the roots and it resulted in decreased nutrient uptake, growth, photosynthetic activity and stomatal conductance.

Ploschuk et al., 2018;
Liu et al., 2020;
Langan et al., 2022

- Nitrate is absorbed through the nitrate transporters located in the plasma membranes of the cells and is reduced to nitrite by the enzyme nitrate reductase (NR) in the cytoplasm.
- The ammonium ion is initially combined with glutamic acid (Glu) and assimilated into glutamine (Gln) by the enzyme glutamine synthetase (GS).
- The amide group of Gln is then transferred to an organic acid, 2-oxoglutarate (2-OG), by glutamate synthase (GOGAT) in plastids.

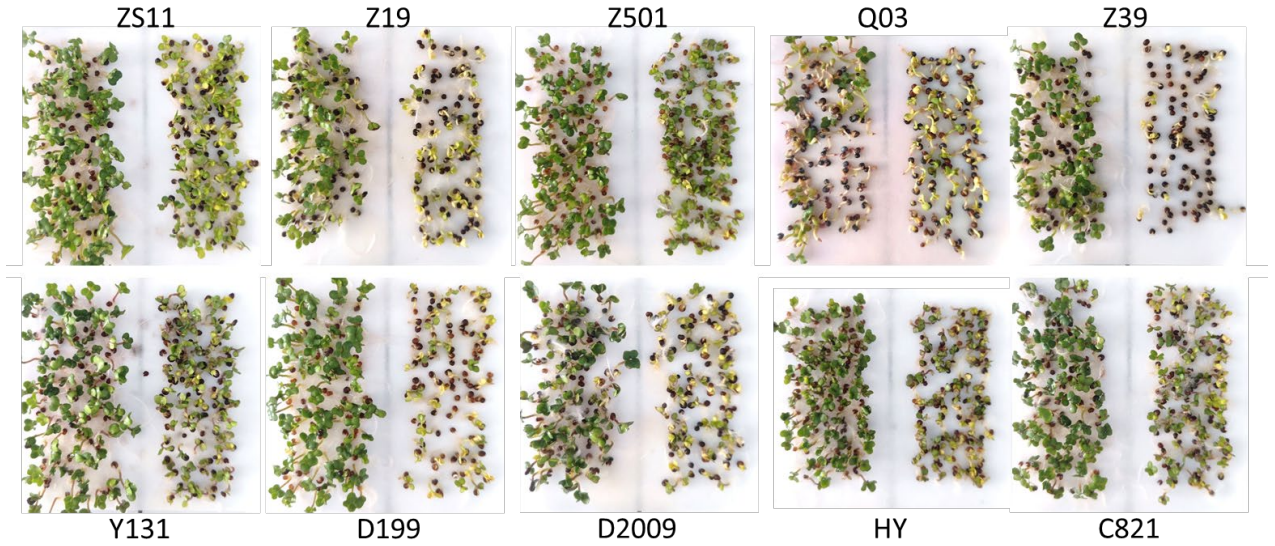


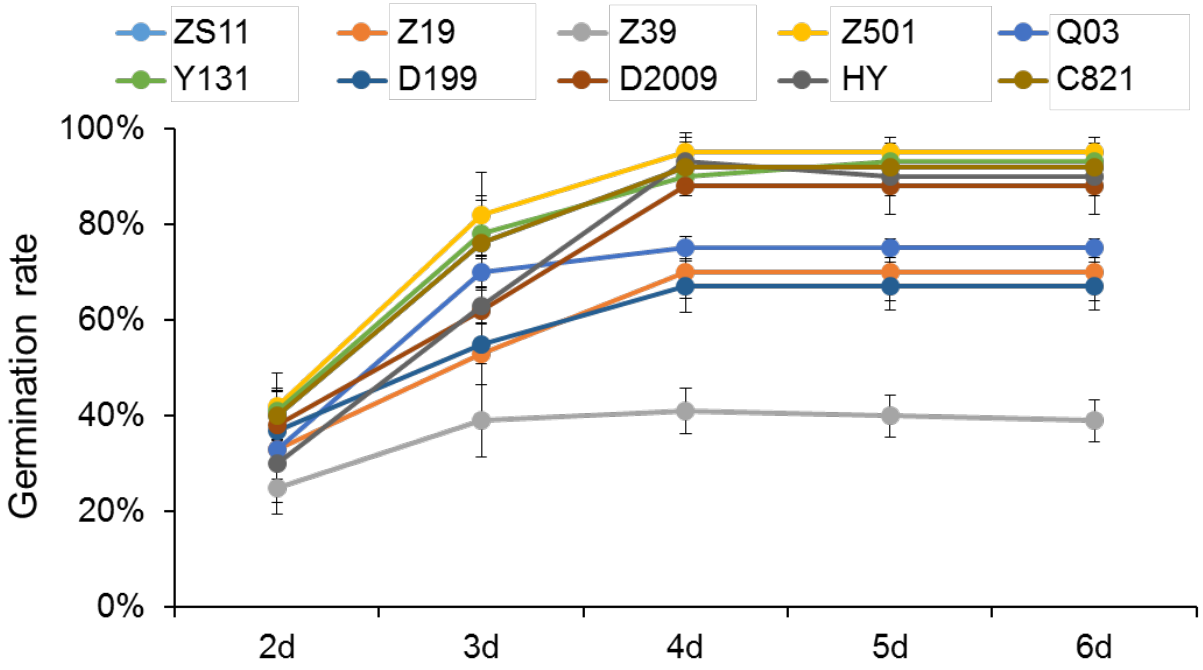
Kuai et al., 2014; Ren et al., 2017; Men et al., 2020; Alves et al., 2021; Baslam et al., 2021; Wei et al., 2022

How does carbon and nitrogen metabolism participate in the regulation of waterlogging stress?

Screening of waterlogging tolerance at germination stage

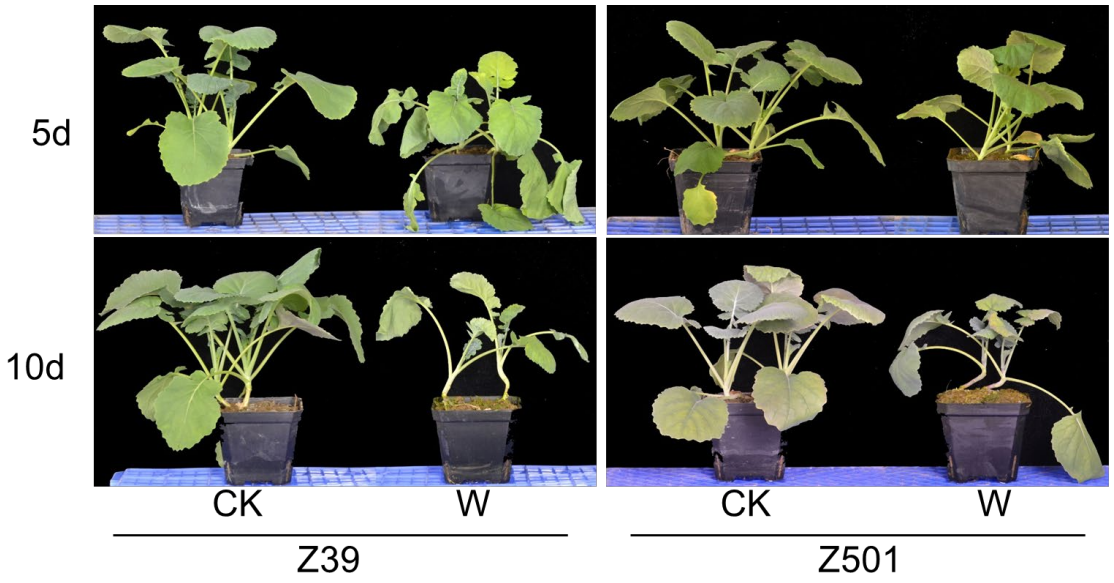
- After waterlogging treatment, the germination rate of Z501 was more than 90%, while the germination rate of Z39 was only about 40%.
- Z501 showed strong resistant to waterlogging stress according to the gemination rate and germination vigor.

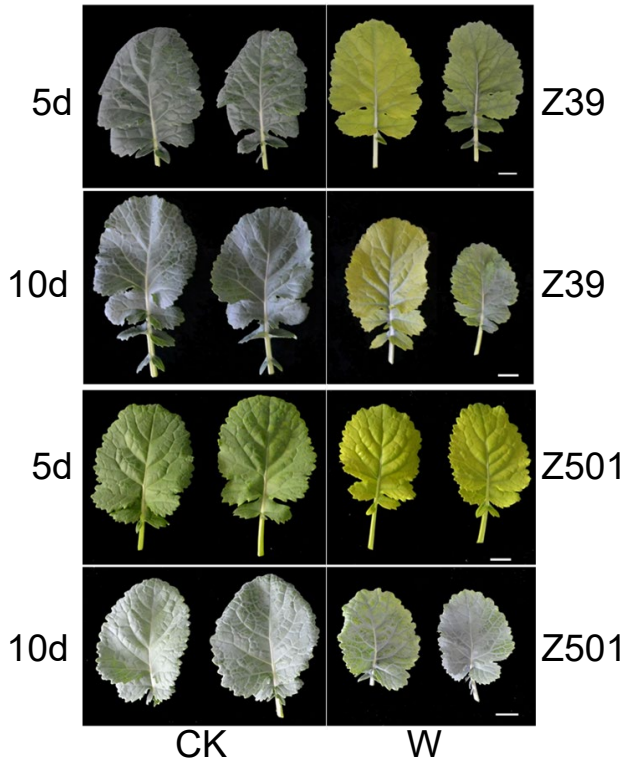


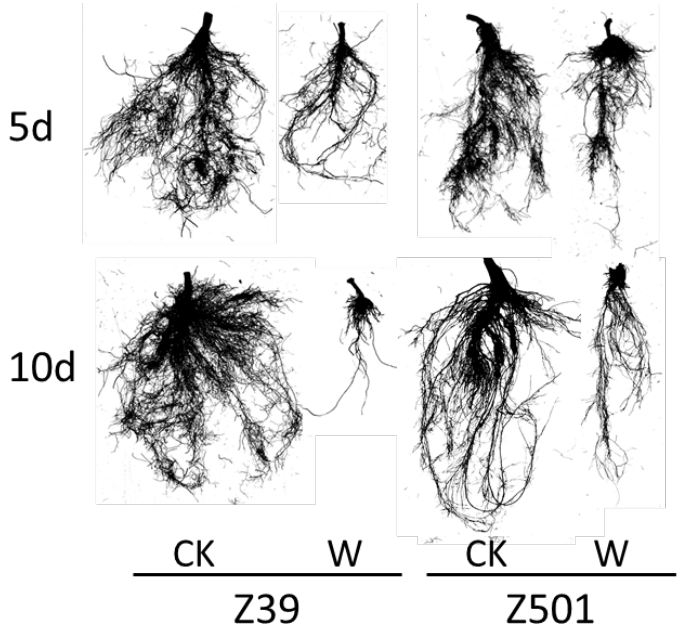


Phenotypic analysis of Z39 and Z501 with waterlogging treatment

- Waterlogging treatment was performed at the 5-leaf stage and phenotypic analysis was conducted on the 5th and 10th day of treatment.
- Z501 showed strong resistant to waterlogging stress at seedling stage, compared with Z39.

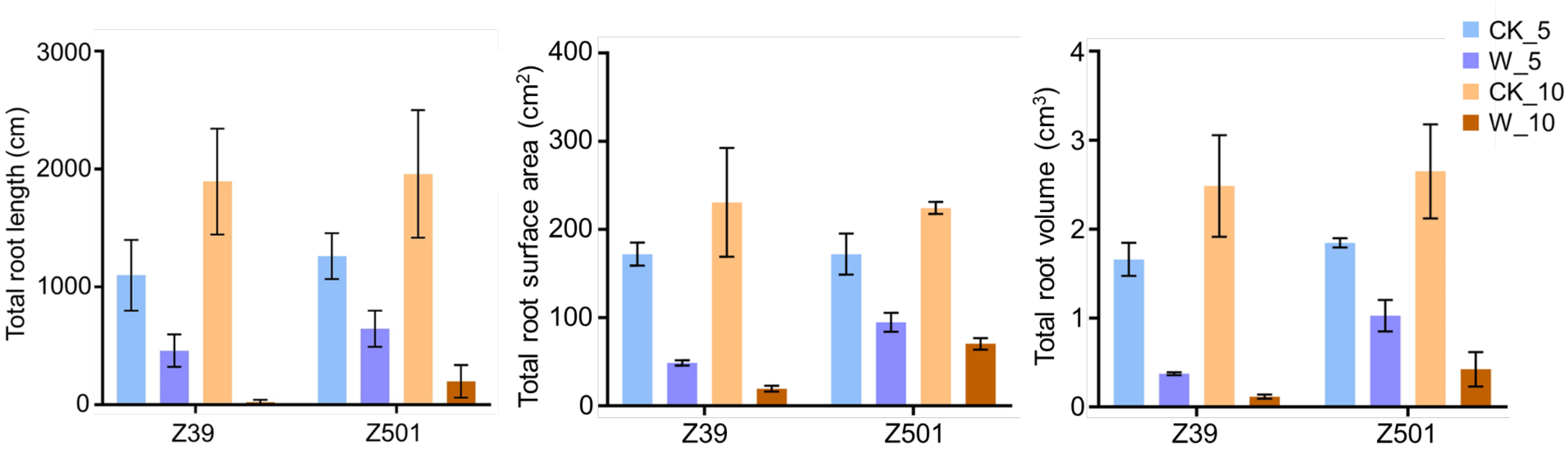






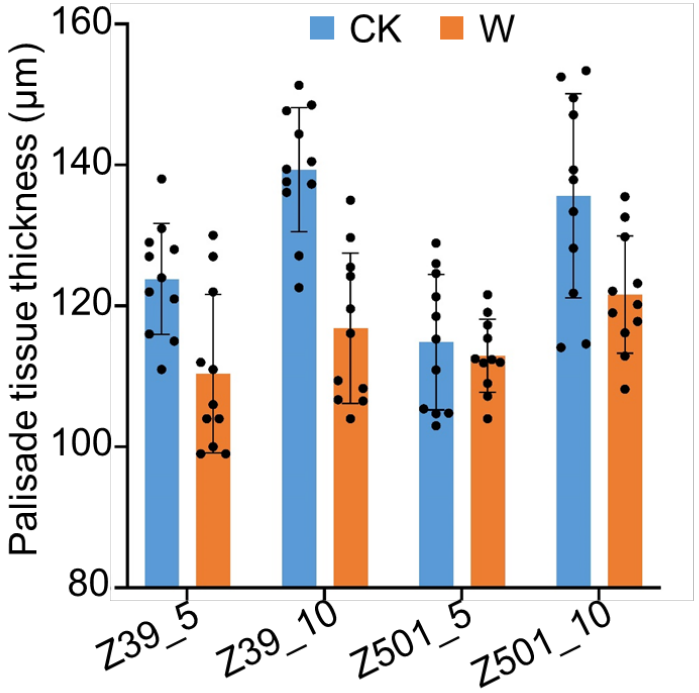
Root architecture analysis after waterlogging treatment

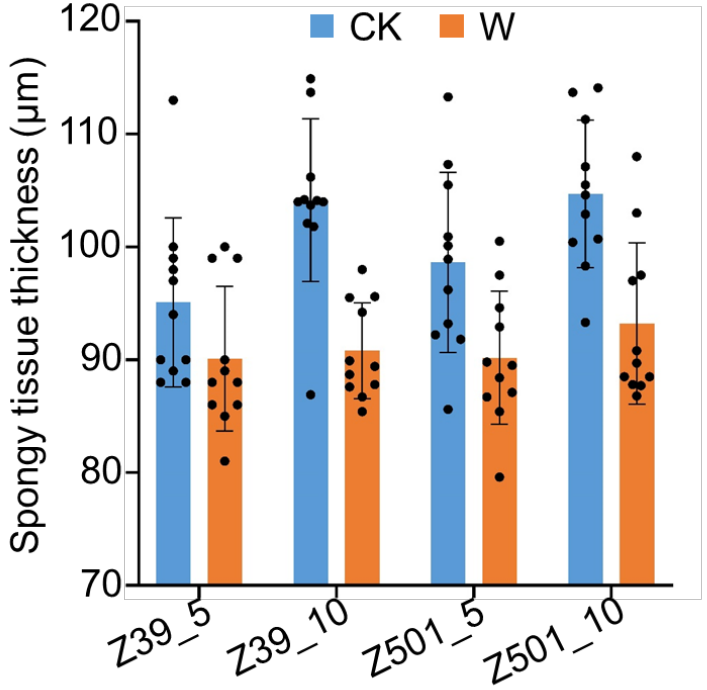
- The total root length, root surface area and root volume of Z39 and Z501 decreased after 5 and 10 days of waterlogging treatment, but the reduction of root length of Z501 was lower than that of Z39.

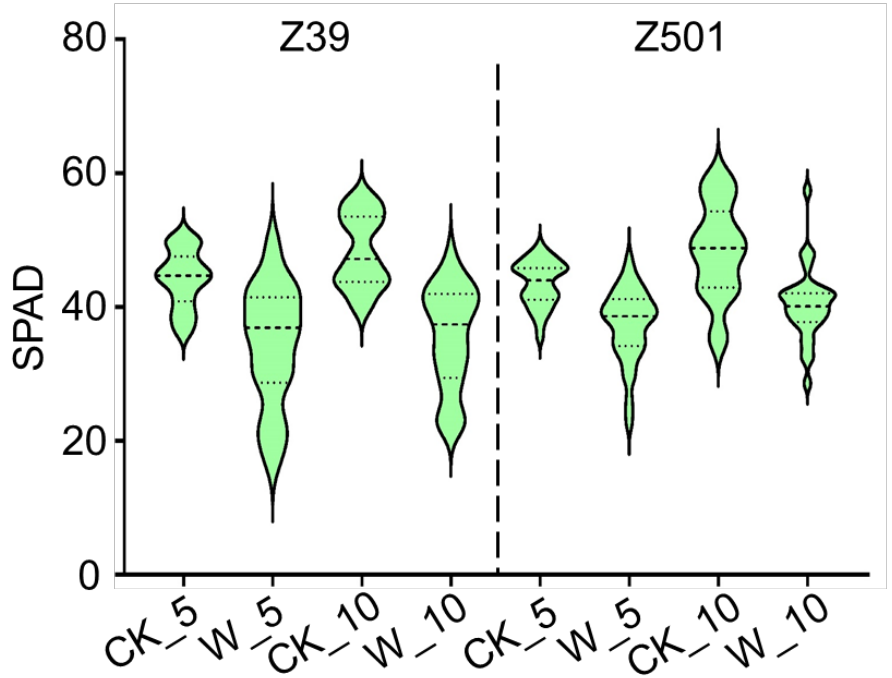


Leaf analysis after waterlogging treatment

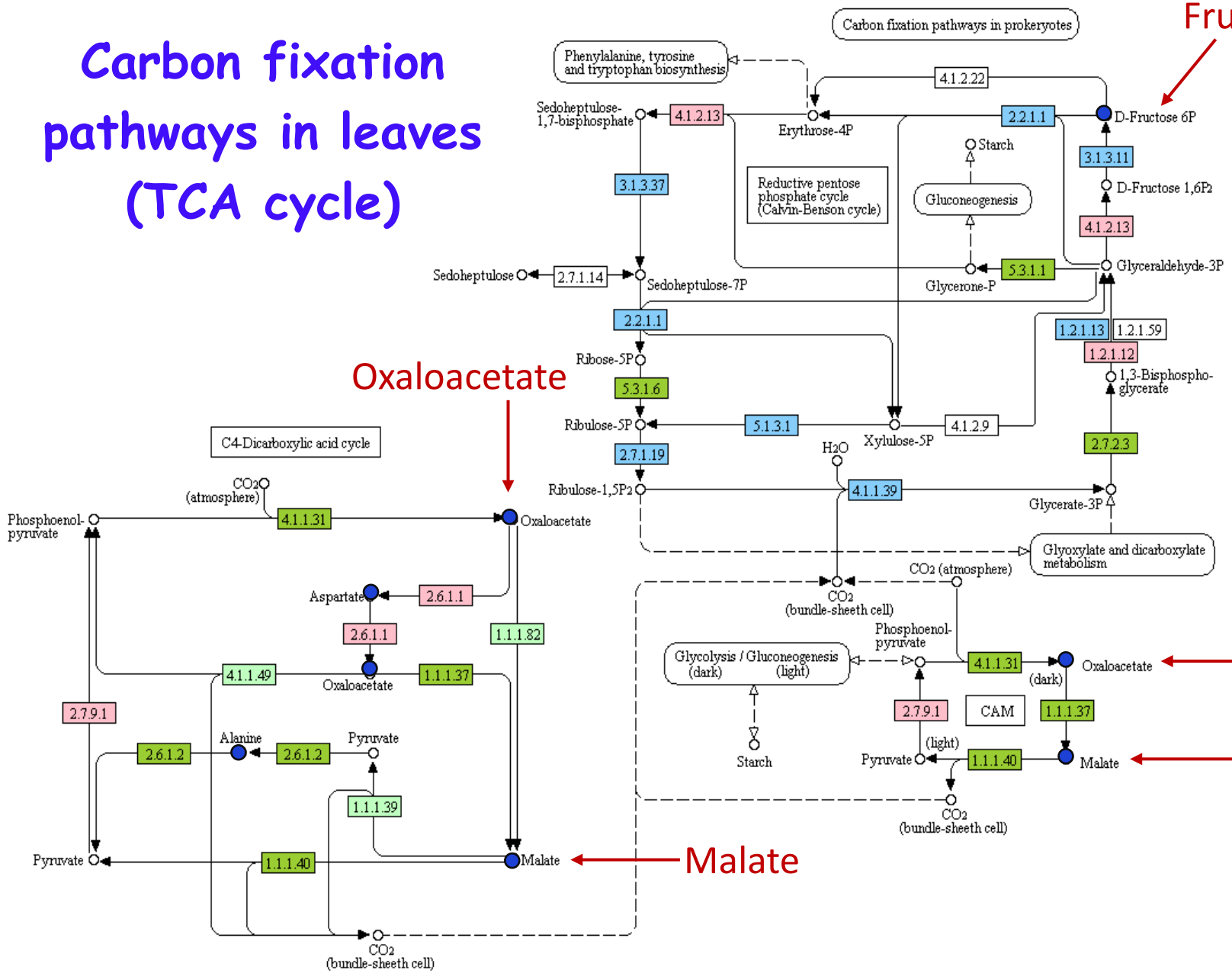
- The thickness of palisade tissue and sponge tissue, the content of chlorophyll in leaves of Z39 and Z501 decreased after 5 days and 10 days of waterlogging.







Carbon fixation pathways in leaves (TCA cycle)



Fructose-6-phosphate

Oxaloacetate

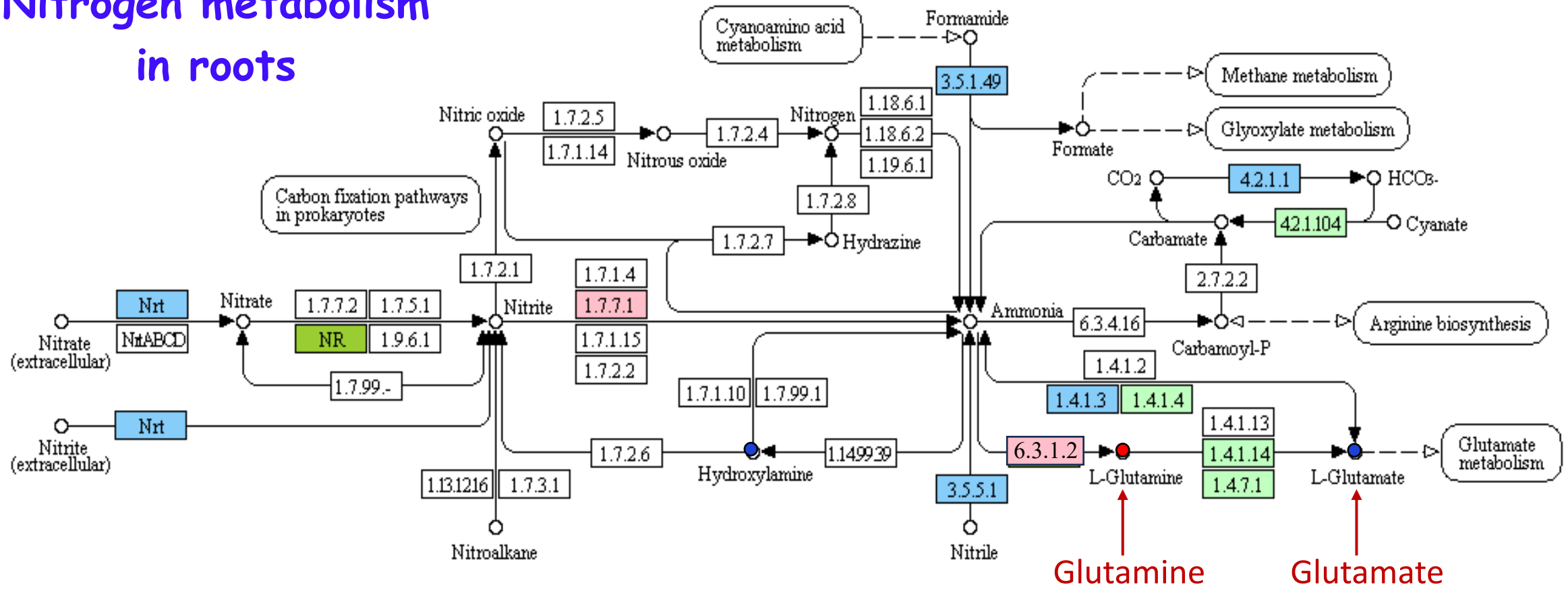
Oxaloacetate

Malate

Malate

- With metabolism analysis, fructose-6-phosphate, oxaloacetate and malate were decreased after waterlogging stress treatment, resulting in a decrease in photosynthetic carbon fixation products.

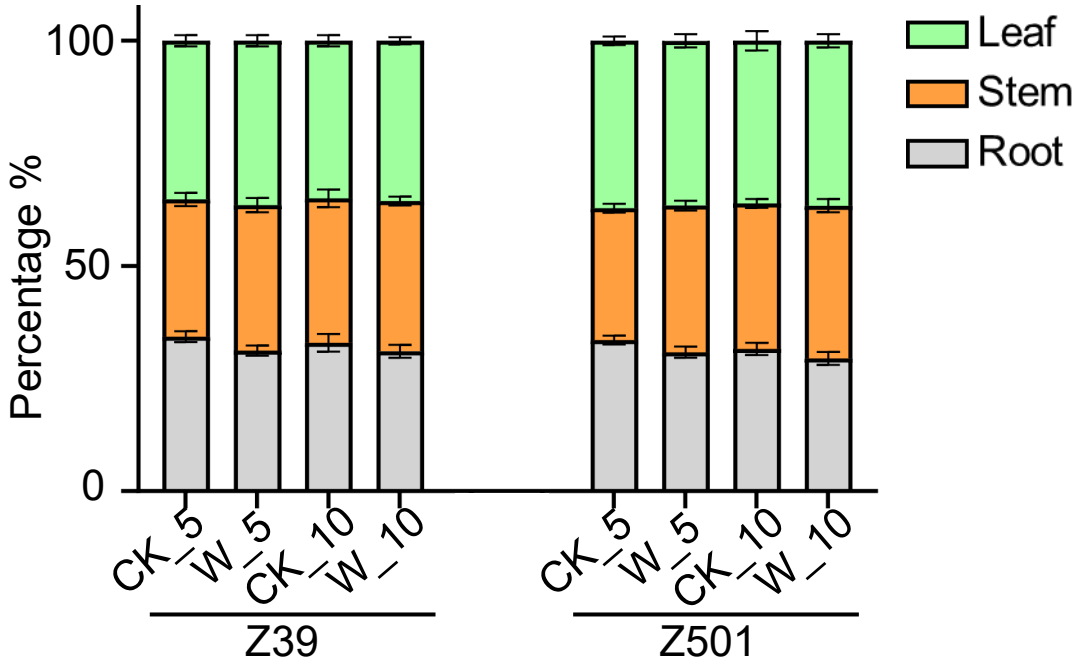
Nitrogen metabolism in roots



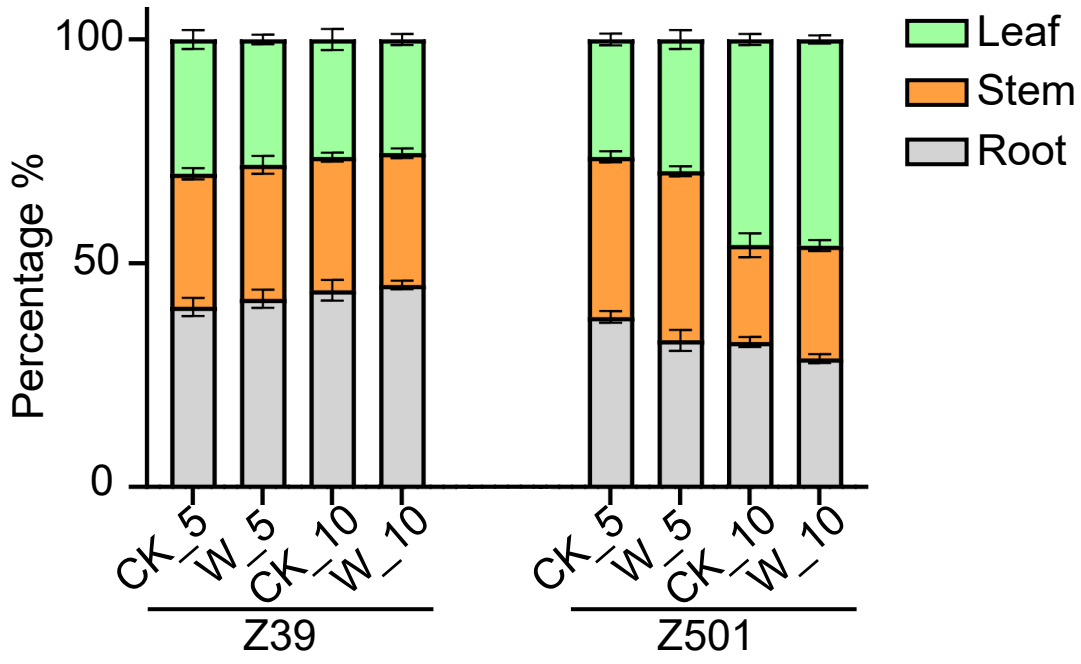
- Metabolism data showed that glutamine was increased and glutamate was decreased after waterlogging treatment.

C/N content analysis after waterlogging treatment

- There was no significant change in plant carbon content before and after waterlogging treatment.
- Nitrogen accumulated more in Z501 leaves, but more in Z39 roots under both normal and waterlogging conditions.



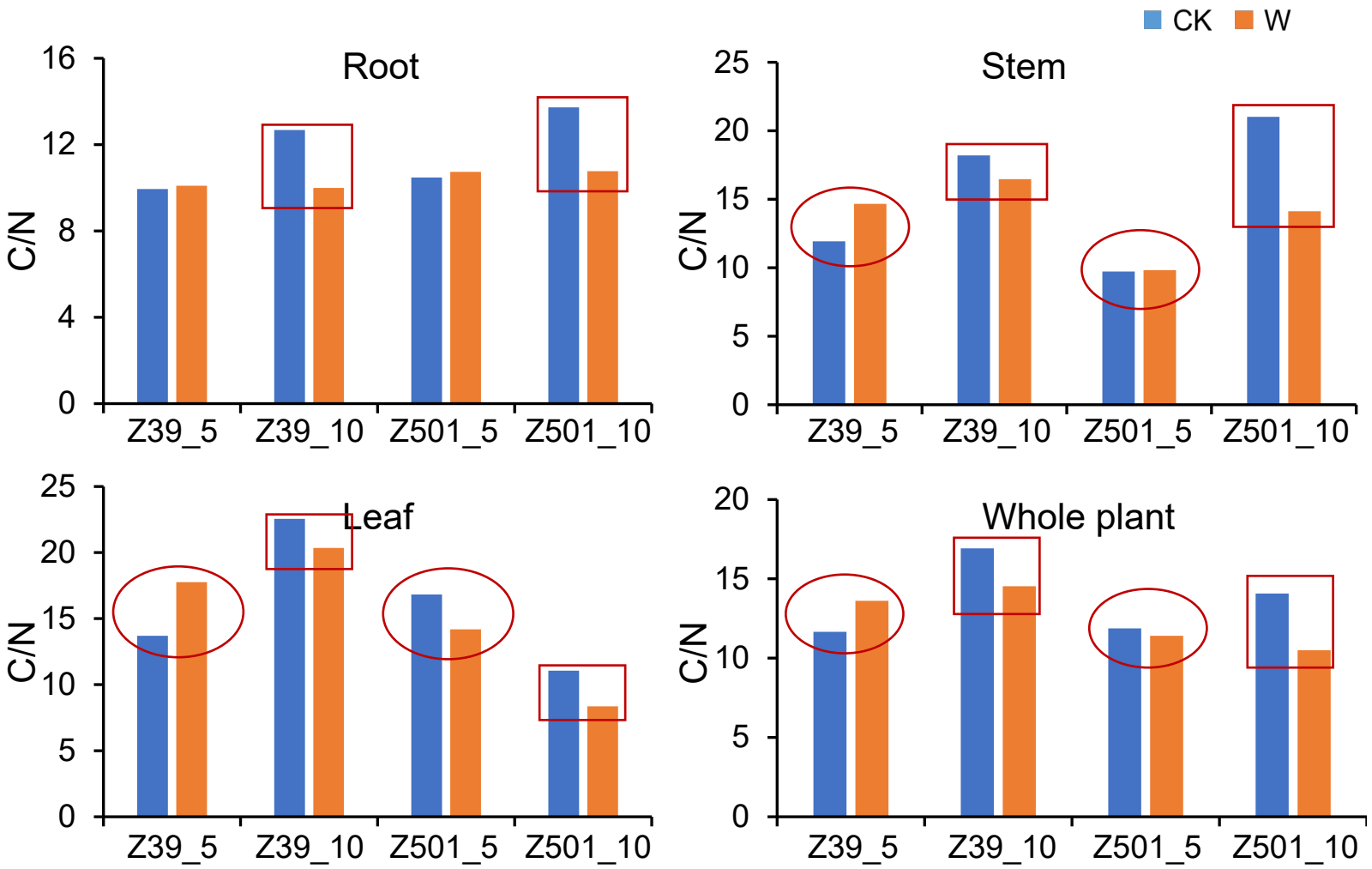
The proportion of carbon content in various tissues



The proportion of nitrogen content in various tissues

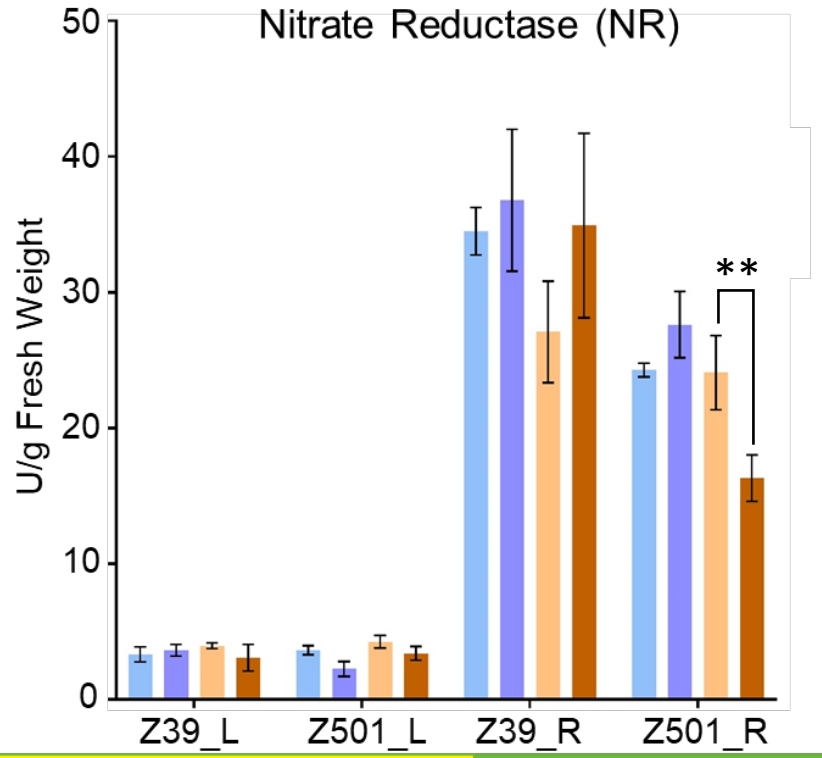
C/N ratio analysis after waterlogging treatment

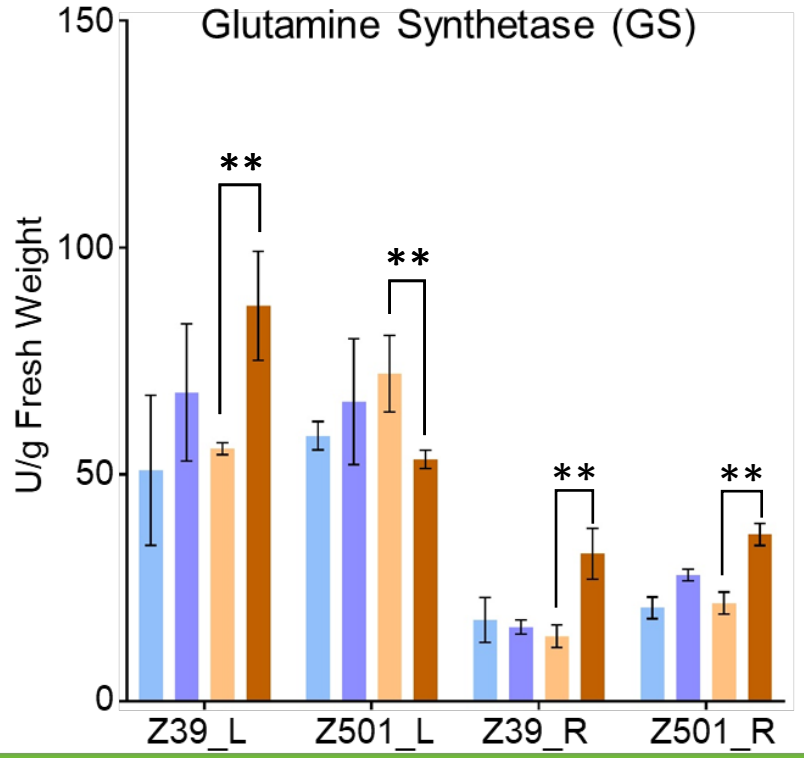
- After 5 days of waterlogging treatment, the C/N in the stems and leaves of Z39 increased, while the C/N in the stems and leaves of Z501 remained unchanged or decreased.
- While, after 10 days of waterlogging treatment, the C/N ratio decreased in both varieties.

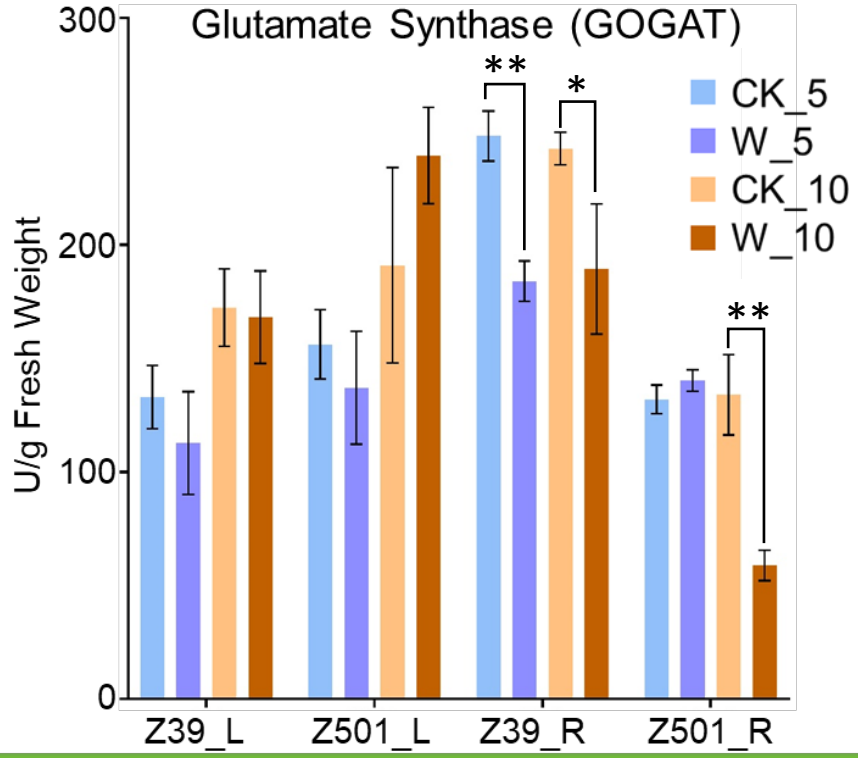


C/N enzymatic activity analysis after waterlogging treatment

- After 10 days of waterlogging treatment, NR activity was lower in Z501 roots, while NR activity did not change significantly in Z39 roots.
- The changes of GS enzyme activity in Z39 and Z501 leaves were opposite.
- The same change in GOGAT enzyme activity was observed in Z39 and Z501.







Summary

- Compared with Z39, Z501 showed stronger resistance to waterlogging at both germination and seedling stages.
- TCA cycle (fructose-6-phosphate, oxaloacetate and malate) and nitrogen metabolism (glutamine and glutamate) were regulated by waterlogging stress.
- The results indicated that regulating the activities of key enzymes of C/N metabolism, such as NR and GOGAT, was a way to improve waterlogging stress tolerance.
- The nitrogen utilization efficiency after waterlogging treatment will be further studied with ^{15}N tracer method, as well as the gene expression of C/N metabolism.

Acknowledgment

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Thank you for your attention!