

Arabidopsis thaliana NIP2;1 , a lactic acid transporter, is essential for plant survival during hypoxic stress

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Abstract

Nodulin-Intrinsic Proteins (NIPs) are a plant specific aquaporin subfamily known for transporting water, glycerol, metalloids and other uncharged solutes at different parts of the plants. AtNIP2;1 is one of the NIP I subfamily members expressed when plants are exposed to flooding stress or hypoxia. Hypoxia is a condition in which most of the oxygen has been removed from the environment. In hypoxic environments, plants construct a specific mechanism to cooperate, such as increasing glycolytic flux to provide ATP, increase of fermentation metabolism, and induction of morphological and developmental changes. Due to the significant increase in AtNIP2;1 expression during hypoxia, it is therefore considered to be one of the core hypoxia response genes and hypothesized to be a critical component of plant survival during prolonged hypoxia.

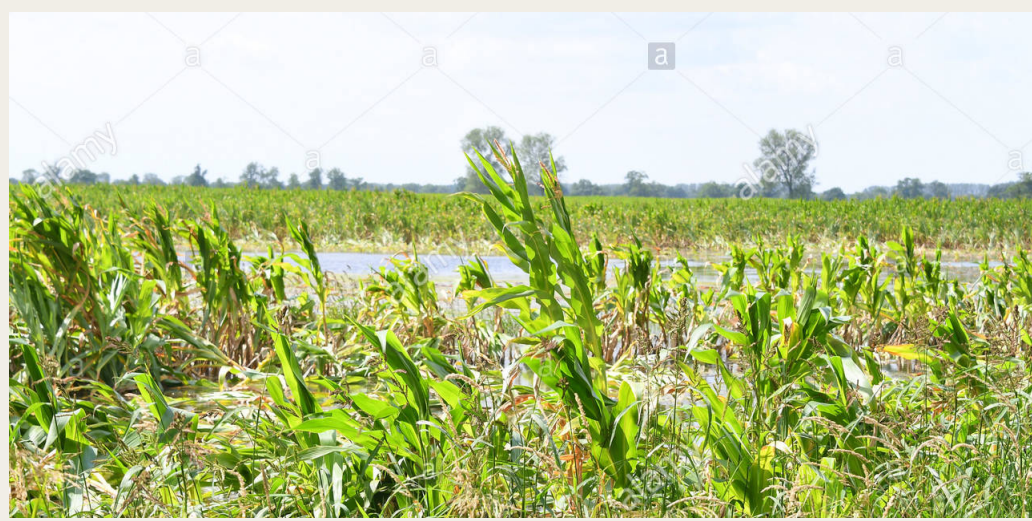
A functional analysis of AtNIP2;1 has shown it transports lactic acid and may play a role in the adaptation to fermentation of lactic acid under anaerobic stress.

In this study, the biological significance of the AtNIP2;1 is investigated using T-DNA insertion loss-of-function mutant. Under hypoxia, or flooding stress, loss-of-function mutant of *AtNIP2;1* failed to survive as wildtype, suggesting its essential role in plant survival under hypoxia stress. Furthermore, loss of *AtNIP2;1* increased the expression of alcohol fermentation pathway enzymes indicating plants preference for alcohol fermentation pathway in absence of *AtNIP2;1*.

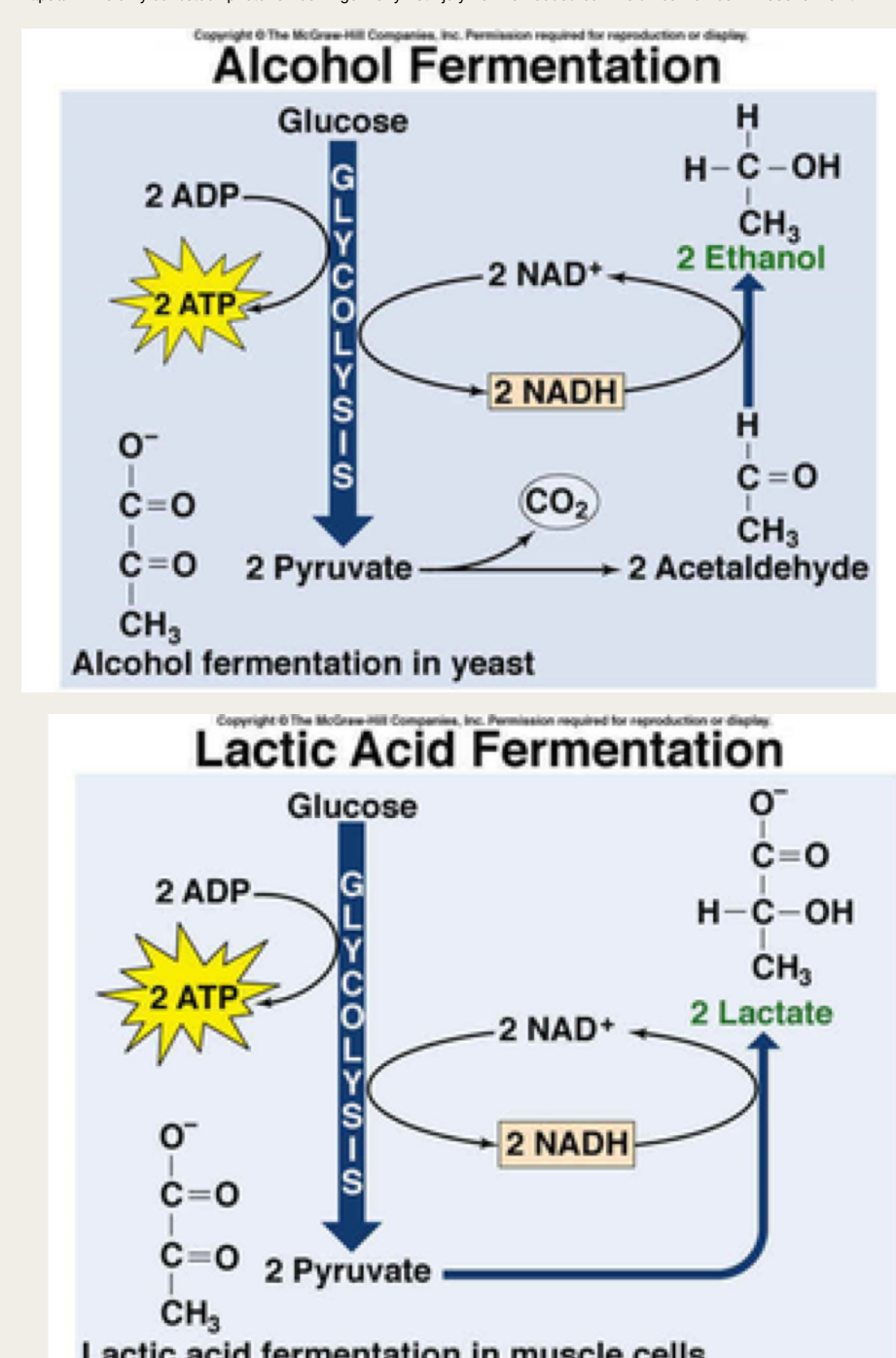
Introduction

A) Flooding and Hypoxia stress

- Flooding caused by nature or manmade is one of the major abiotic stresses for plant growth and development
- It restricts the gas exchange and oxygen availability for the plants.
- As a consequence, the cellular O₂ level reduced to the level to restrict aerobic respiration.
- Furthermore, the flooding also restricts the light availability to submerged plants for photosynthesis.



A flooded corn field.



B) Anaerobic respiration

- O₂ is the final electron acceptor in the mitochondrial electron transport chain
- During the low oxygen condition, termed as hypoxia or lack of oxygen called anoxia the cells generally rely on glycolysis and fermentation process to generate ATP.
- With in minutes of O₂ depleted, cells switched to the anaerobic method of ATP generation.
- And shuts down the high energy consumptive metabolic processes.
- Pyruvates that is produced by glycolysis is either directed to alcohol fermentation or lactic acid fermentation.
- The lactic acid fermentation process, pyruvate is converted to lactic acid which higher accumulation lowers the cytosolic pH, hence toxic for the cell.
- To overcome, the lactic acid toxification, plants are equipped with Lactic acid transporter or other different mechanisms yet to be known.

C) *Arabidopsis* NIP2;1 is a core hypoxia response gene

- Apart from shutting down most of the energy consuming metabolic pathways, plants induced certain set of genes in response to low oxygen condition.
- The genes are known as core hypoxia-responsive genes.
- In *Arabidopsis*, there are 49 core hypoxia genes, most of them are glycolytic and fermentative metabolic enzymes, heat shock proteins, signal proteins and transporters
- Arabidopsis* NIP2;1 is one of the core hypoxia gene.
- The expression of *AtNIP2;1* is most predominant in roots. The expression induced further under hypoxic condition.

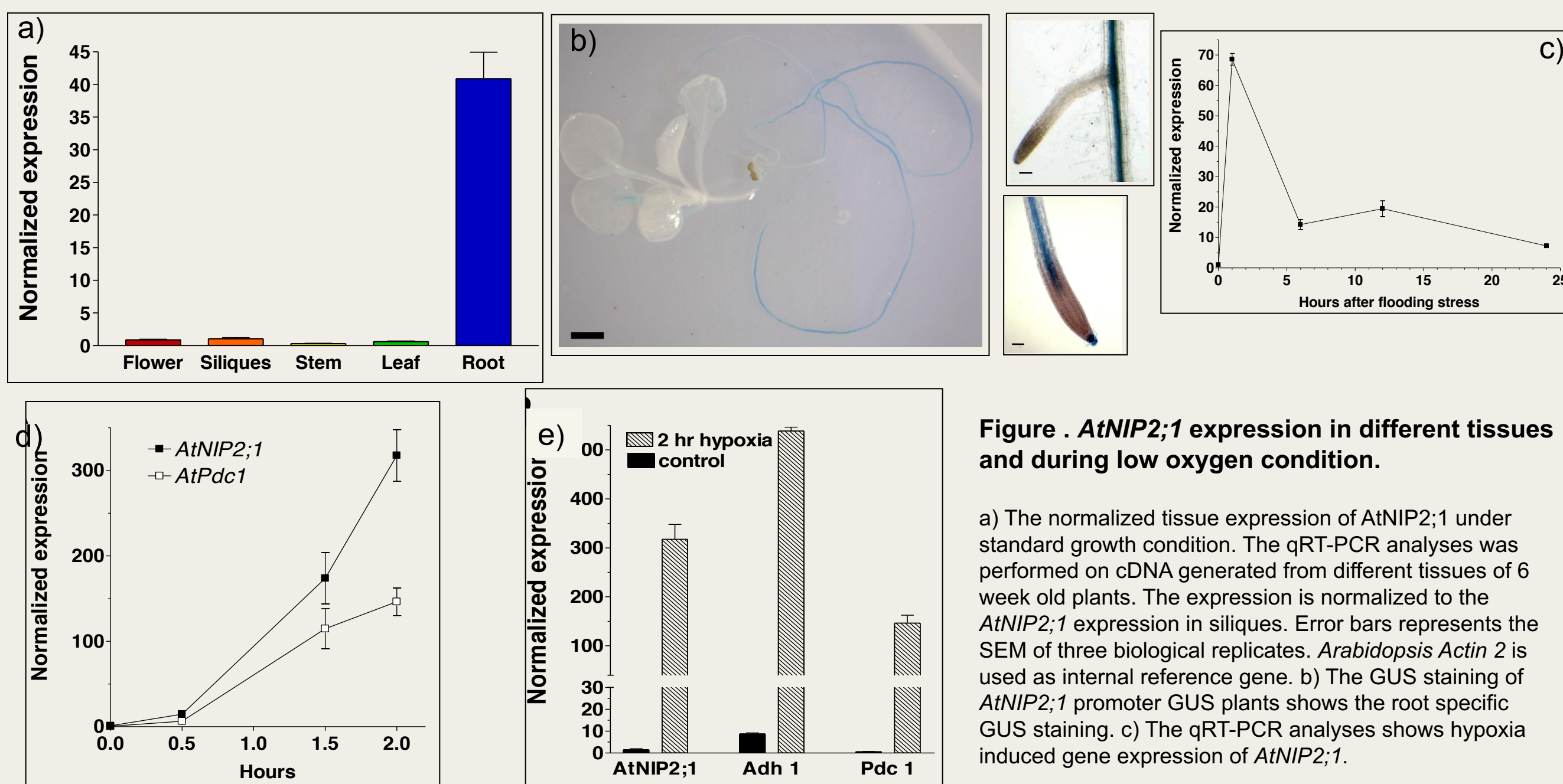


Figure. *AtNIP2;1* expression in different tissues and during low oxygen condition.

a) The normalized tissue expression of AtNIP2;1 under standard growth condition. The qRT-PCR analyses was performed on cDNA generated from different tissues of 6 week old plants. The expression is normalized to the *AtNIP2;1* expression in siliques. Error bars represents the SEM of three biological replicates. *Arabidopsis* *Actin 2* is used as internal reference gene. b) The GUS staining of *AtNIP2;1* promoter GUS plants shows the root specific GUS staining. c) The qRT-PCR analyses shows hypoxia induced gene expression of *AtNIP2;1*.

D) AtNIP2;1 is a member of plant specific aquaporin subfamily

- Aquaporins (AQP) ancient family of membrane protein channels present in all organisms.
- Helps in bidirectional transport of water and uncharged solutes.
- All members have a conserved Hourglass fold structure that assemble into tetramers
- Each monomer has a single transport pore with a selectivity filter guided by aromatic/arginine region-four residues (Ar/R).
- Unlike animal, plants AQP family is diverse and widespread.
- Nodulin Intrinsic Proteins (NIPs) is a plant specific aquaporin subfamily.
- Based on composition of the Ar/R region, NIPs are broadly divided into three subgroups.
- AtNIP2;1 belongs to the NIP I family members of the *Arabidopsis*

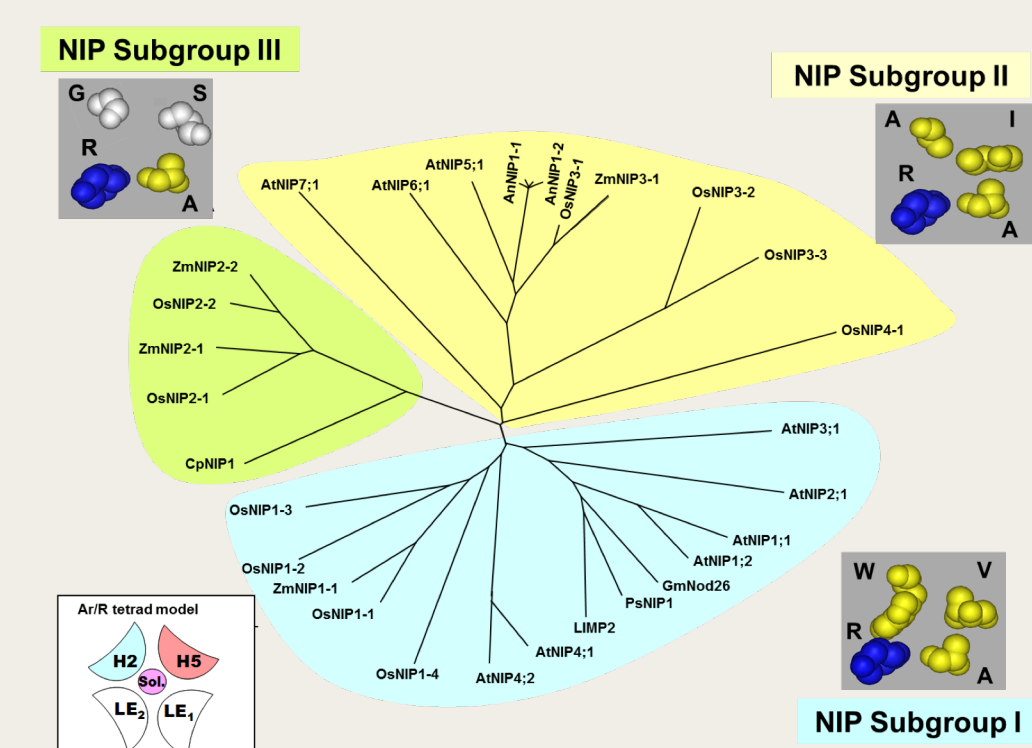
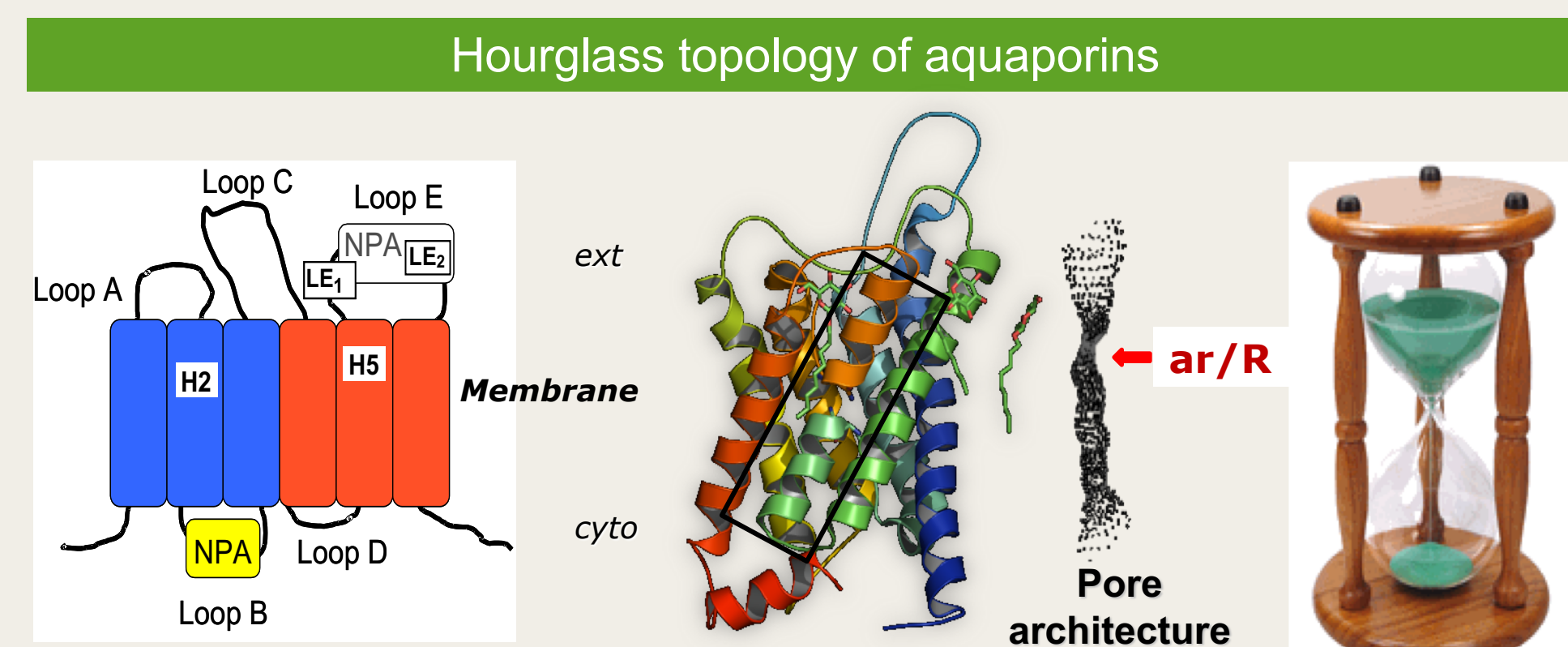
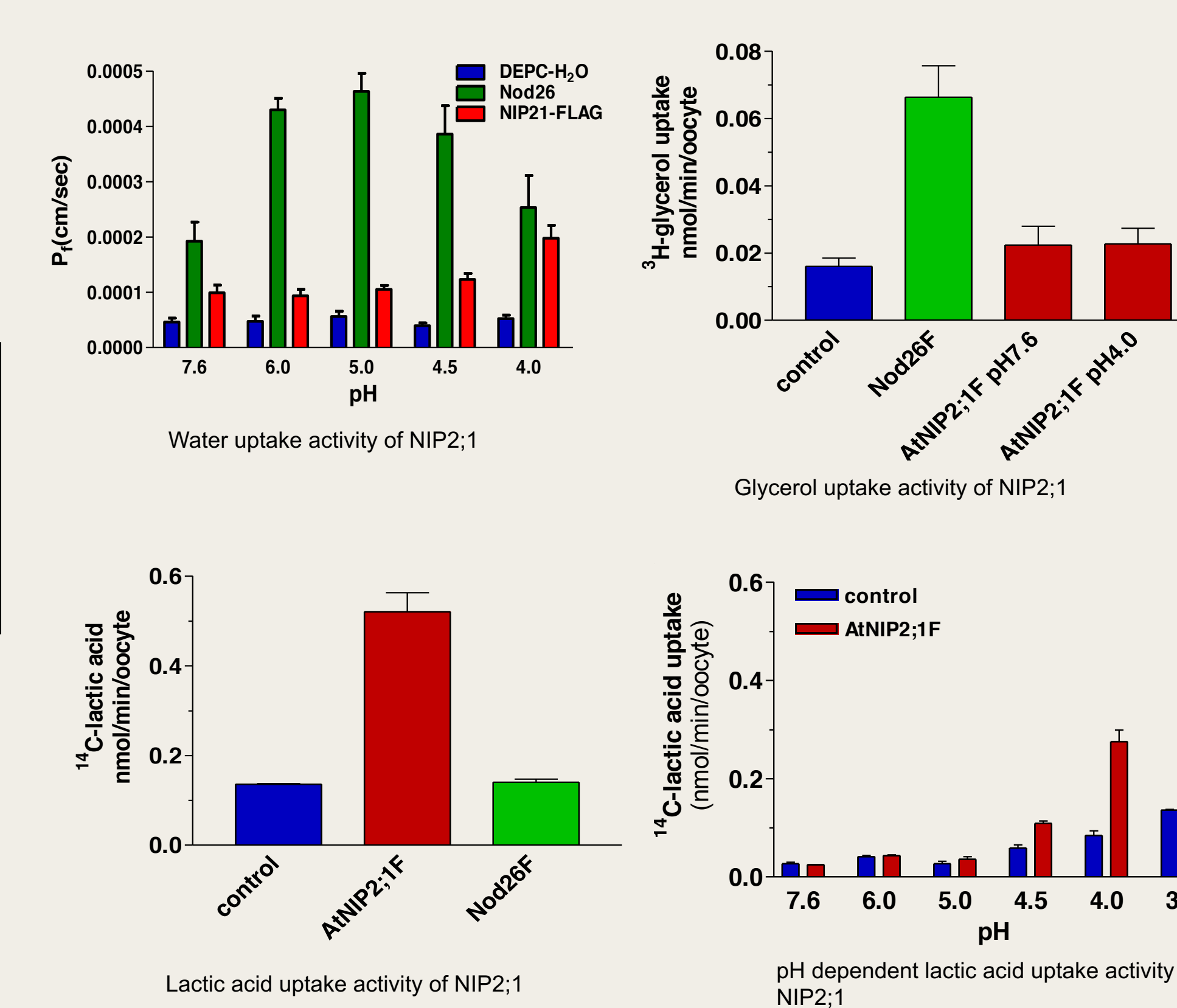


Figure. Different subgroups of plant specific NIP family. The plant specific NIP family is divided into three subfamilies based on composition of their Ar/R region. NIP I subfamily with a bulky Tryptophan transport water, ammonia and glycerol transport. NIP II family with Alanine transport boron and arsenite. NIP III family which is absent in *Arabidopsis* transport Silicon and Arsenite.

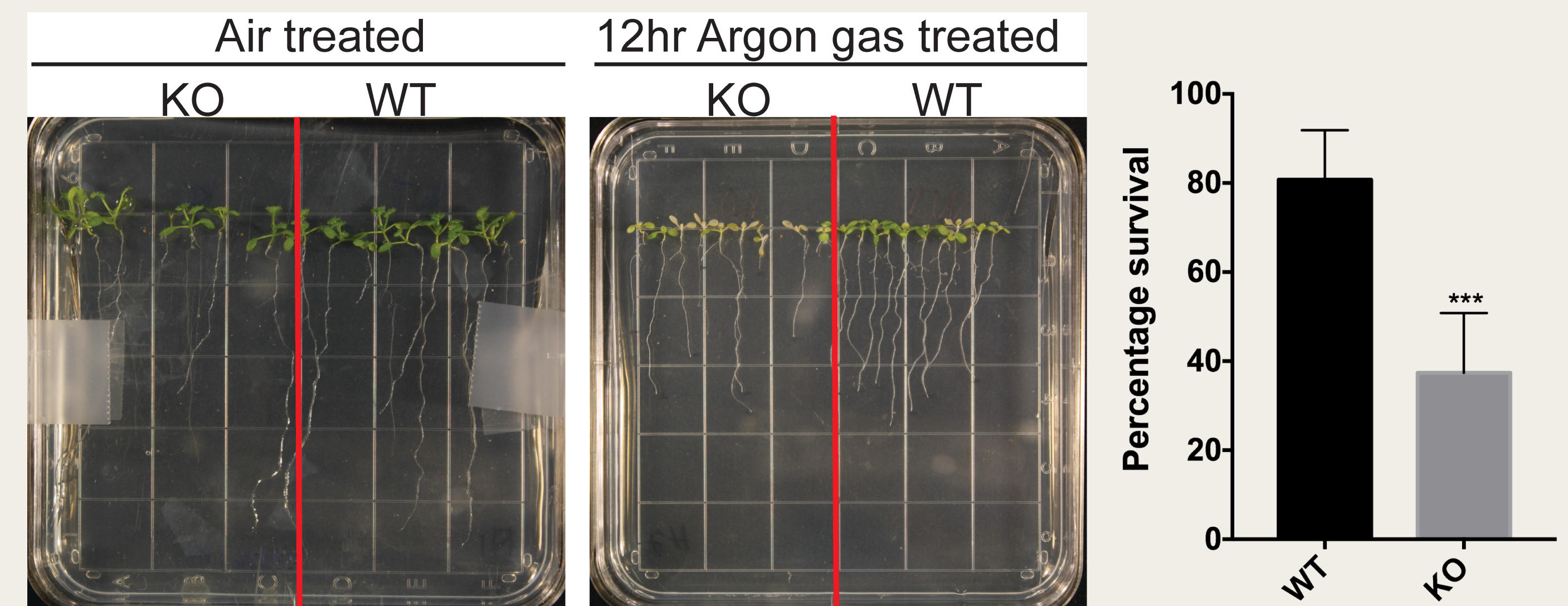
E) AtNIP2;1 transports lactic acid



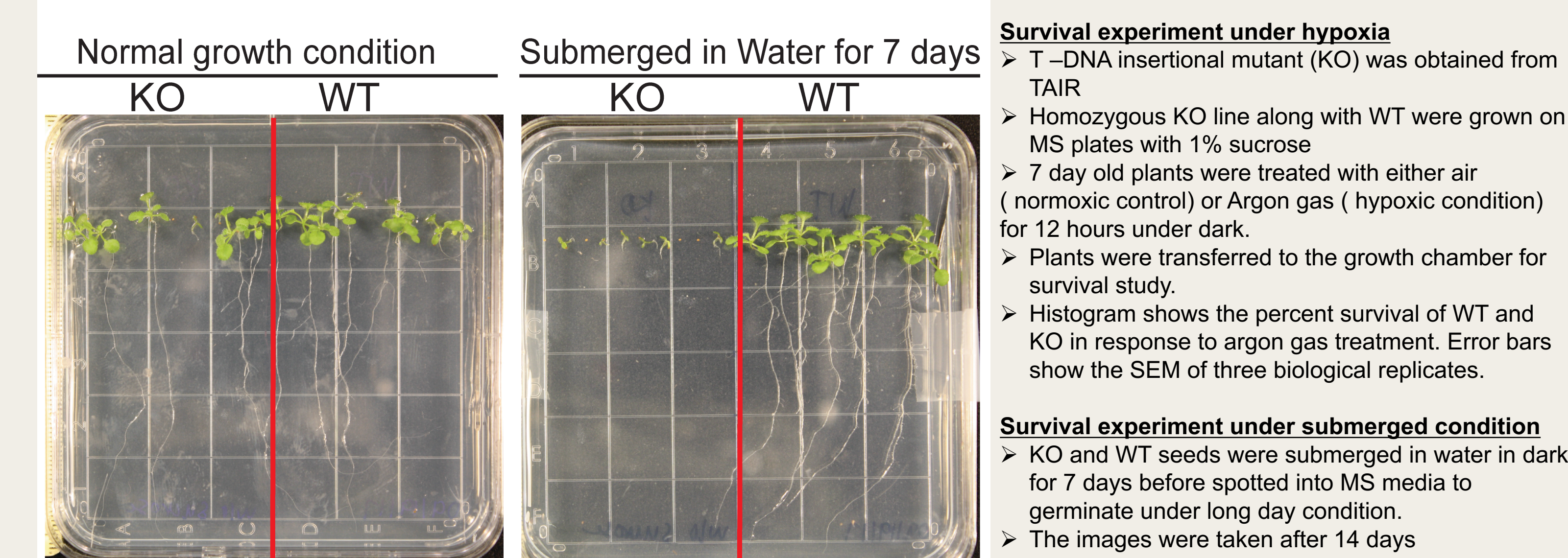
Lactic acid uptake activity of NIP2;1

Results

A) *AtNIP2;1* is indispensable for plant survival under hypoxia



B) *AtNIP2;1* is essential for seeds germination after flooding stress



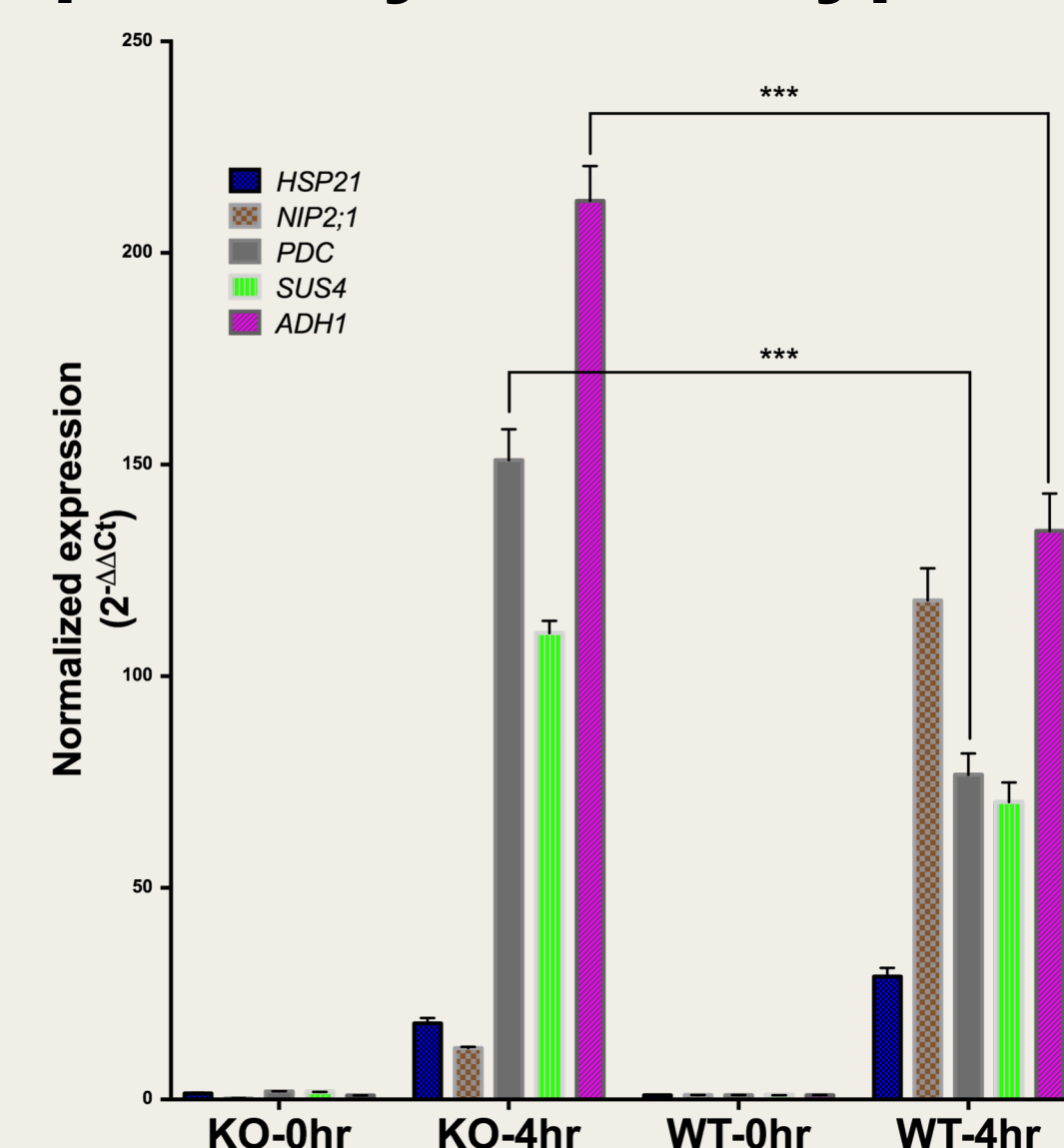
Survival experiment under hypoxia

- T-DNA insertional mutant (KO) was obtained from TAIR
- Homozygous KO line along with WT were grown on MS plates with 1% sucrose
- 7 day old plants were treated with either air (normoxic control) or Argon gas (hypoxic condition) for 12 hours under dark.
- Plants were transferred to the growth chamber for survival study.
- Histogram shows the percent survival of WT and KO in response to argon gas treatment. Error bars show the SEM of three biological replicates.

Survival experiment under submerged condition

- KO and WT seeds were submerged in water in dark for 7 days before spotted into MS media to germinate under long day condition.
- The images were taken after 14 days

C) Loss of *AtNIP2;1* induce alcohol fermentation pathway under hypoxia

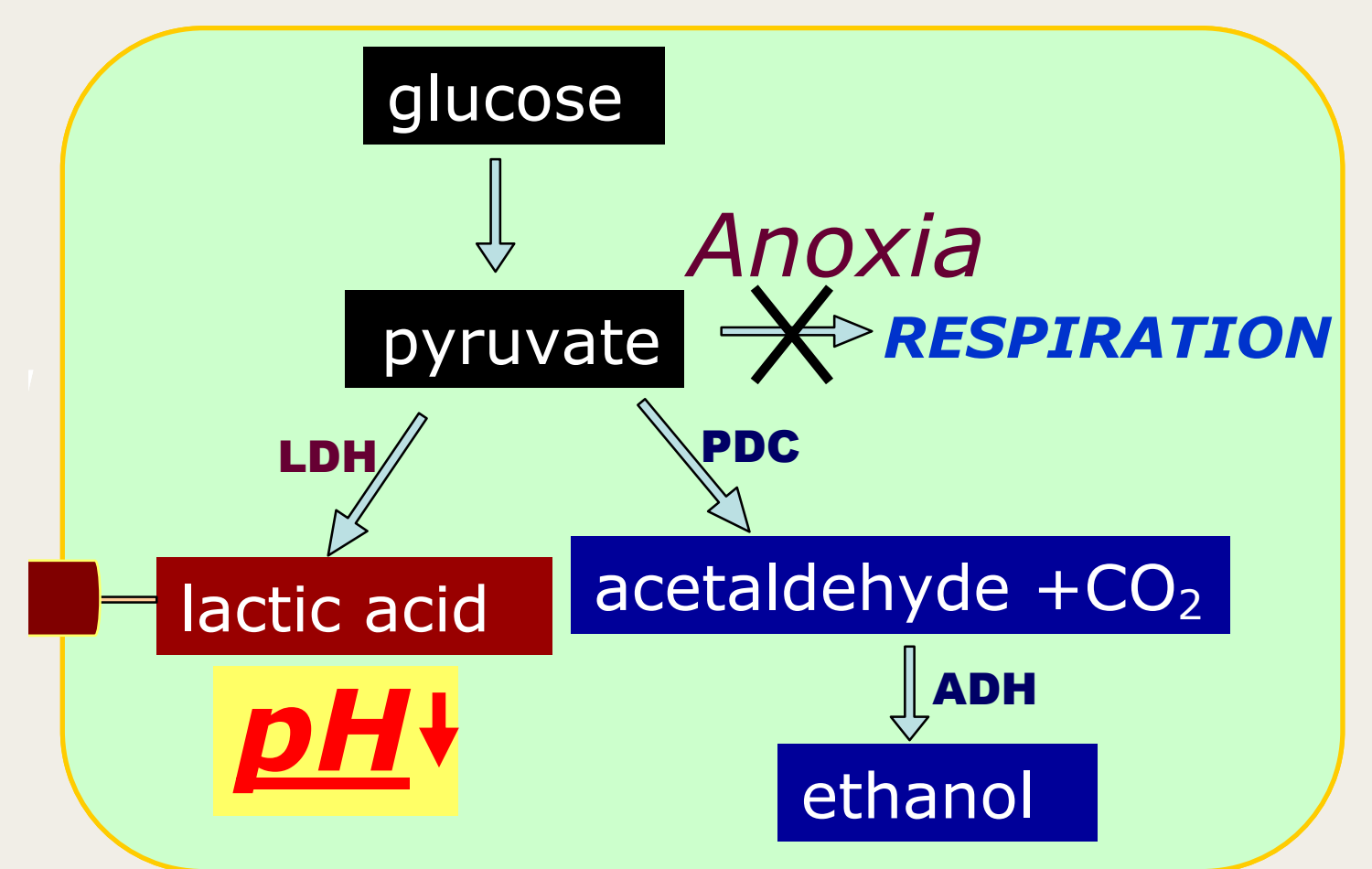


The qRT-PCR analyses was performed on cDNA generated from root tissues of WT and KO seedlings before (0hrs) and after 4 hours of hypoxia (4hrs). The expression of each gene was normalized to WT-0hrs, and *AtUBQ10* gene was used as internal standard for the experiment. Error bar represents the SEM of three biological replicates. The hypoxia treatment was performed by treating the seedling with argon gas. PDC: pyruvate decarboxylase; ADH1: Alcohol dehydrogenase; SUS4: Sucrose synthase; HSP21: Heat shock protein21.

Summary

- Flooding causes severe damage to plant development
- Flooding creates hypoxic/ anoxic environment that is detrimental for energy production
- Plants mostly rely on glycolysis for their energy production
- Anaerobic respiration produces lactic acid that lower the cytosol pH
- AtNIP2;1* is a core hypoxia gene upregulated by lack of oxygen.
- AtNIP2;1* transports lactic acid
- Loss of *AtNIP2;1* increases the plant susceptibility to hypoxia and flooding
- In absence of *AtNIP2;1* plants switch to alcoholic fermentation

D) Davies-Roberts model for plant adaptation to oxygen deprivation



Davies-Roberts Hypothesis suggests that:

- In hypoxia or anoxia, the energy catabolism is disrupted due to lack of oxygen
- Glycolysis is the principal source of ATP production
- To sustain the glycolysis, NAD⁺ is required
- Lactic acid fermentation provides the NAD⁺ for glycolysis
- Lactic acid leads to acidosis of the cytosol
- To prevent cytosol acidification, plants switch to alcoholic fermentation and/or lactic acid efflux is stimulated

Future studies

- Sub cellular localization of the NIP2;1 protein
- Post transcriptional and post translational regulation of the protein expression and function
- Differential expression of core hypoxia genes on loss of *AtNIP2;1*
- Metabolic profiling of loss of *AtNIP2;1* plants under normal and hypoxic condition