**Abstract**

Nodulin-26-like proteins (NIPs) are a plant specific aquaporin subfamily known for transporting water, glycerol, metabolites and other uncharged solutes at different parts of the plant. AtNIP2;1 is one of the NIP family 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 cope with, 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 hypoxic 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.

**B) Anaerobic respiration**

\[ \text{O}_2 \] 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 absence of \[ \text{O}_2 \] depleted, cells switched to the anaerobic method of ATP generation.
- And shuts down the high energy consuming 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 further accumulates lowering the cytosolic pH, hence toxic for the cell.
- To overcome, the lactic acid biotransformation, 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 induce certain set of genes in response to low oxygen conditions.
- 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 the core hypoxia gene.
- The expression of AtNIP2;1 is most predominant in roots. The expression induced further under hypoxic conditions.

**E) AtNIP2;1 transports lactic acid**

Water uptake activity of NIP2;1

Lactic acid uptake activity of NIP2;1

Glycerol uptake activity of NIP2;1

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

- In hypoxia or anoxia, the energy metabolism is disrupted due to lack of oxygen.
- Glycolysis is the principal source of ATP production.
- The production of cytosolic lactic acid is increased.
- Lactic acid fermentation provides the NAD+ for glycolysis.
- Lactic acid leads to acidosis of the cytosol.
- In the absence of AtNIP2;1 plants switch to alcoholic fermentation when oxygen deprivation is prolonged.

**Results**

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

- **Air treated:** KO WT
- **12hr Argon gas treated:** KO WT

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

- **Normal growth condition:** KO WT
- **Submerged in Water for 7 days:** KO WT

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

- **AtNIP2;1 KO WT**

**D) Davies-Roberts hypothesis suggests that:**

- In hypoxia or anoxia, the energy metabolism is disrupted due to lack of oxygen.
- Glycolysis is the principal source of ATP production.
- The production of cytosolic lactic acid is increased.
- Lactic acid fermentation provides the NAD+ for glycolysis.
- Lactic acid leads to acidosis of the cytosol.
- AtNIP2;1 loss of function results in alcoholic fermentation and lactic acid efflux is stimulated.