Characterization of Rice OsNIP1;1 for its Role in Arsenic Transport in Indian Mustard (*Brassica juncea*)

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INTRODUCTION

Arsenic is a naturally occurring toxic element that is present throughout the Earth's crust. There are two general categories of arsenic: organic and inorganic. Inorganic arsenic is the most detrimental and severe environmental contaminant ranking first on the Superfund List of Hazardous Substances due to its toxicity, frequency, and risk of human exposure. Long term exposure to inorganic arsenic often results in various deleterious health effects including hyperkeratosis, diabetes, cardiovascular diseases, and cancers. The most prevalent forms of arsenic in the environment are inorganic arsenite (As III) and arsenate (As V). The purpose of this research is to determine the role of rice aquaporin protein OsNIP1;1 in As tolerance/sensitivity as well as the transport of arsenic in *Brassica juncea* plants. Aquaporins are a family of small, integral membrane proteins whose primary function is to transport water and solutes across cell membranes. In addition to water, aquaporins also selectively transport dissolved particles such as CO2 and O2. The rice aquaporin protein OsNIP1;1 is categorized in the Nodulin 26-like protein subfamily has been observed to transport water as well as boron, silicon, and arsenic. *Brassica juncea* was chosen for the overexpression of OsNIP1;1 as *B. juncea* is widely implemented in the phytoremediation of arsenic due to fast growth, adequate mature biomass, accumulation of and tolerance to metalloids, and wide growth conditions. Furthermore, *B. juncea*, an edible mustard crop, is widely cultivated across Asia which is significantly affected by arsenic contamination. Increasing the crops tolerance to arsenic and decreasing the uptake of arsenic would greatly aid in the improvement of food safety.

METHODS

***Brassica juncea* Transformation**

- 855 bp amplicon encoding OsNIP1;1 was cloned in *pcambia1300* vector for plant pression.
- Hypocotyl explants were excised from 5 day old seedlings of Indian mustard (*Brassica juncea).*
- Explants were infected with agrobacterium tumefaciens GV3101 and co-cultivated for 2 days.
- The explants were screened on callus induction media for 3-4 weeks.
- The green calli were transferred to shoot induction / regeneration media containing 20 mg/L hygromycin and subsequent root induction / regeneration media.
- The fully regenerated plants were hardened off in soil and analyzed further.

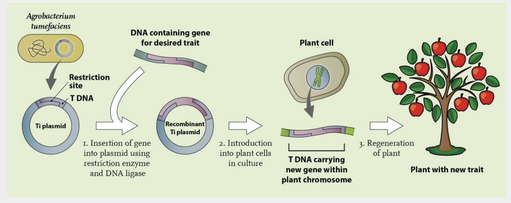


Figure 1: schematic depiction of genetic modification of plants with a recombinant plasmid DNA

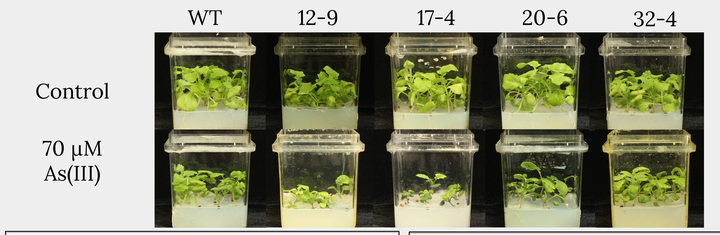
Phenotype Study

- All transgenic lines were germinated in both [½] MS media and 70 µM As(III) for 21 days.
  - Three Control magenta boxes & As(III) treated magenta boxes per line were used.
- All lines were then compared to their respective control as well as Wild Type plants.

Hydroponic Accumulation Study

- Wild type *Brassica juncea* and 4 homozygous transgenic lines were germinated in magenta boxes on ½ x MS media for 10 days.
  - Homozygosity of transgenic pllines was confirmed through germination with hygromycin.
- All lines were transferred to hydroponic system and treated with 70 µM As(III) for 7 days.
  - Hydroponic system was used to maintain clean root and shoot biomass for ICP-MS analysis.
  - ICP-MS analysis was performed on both shoot and root sample from all line to obtain arsenic concentration data.

RESULTS



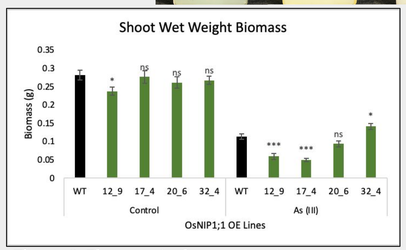


Figure 2. Phenotypic data comparing transgenic lines to the WT line show the transgenic lines are sensitive to As(III) compared to the WT line.

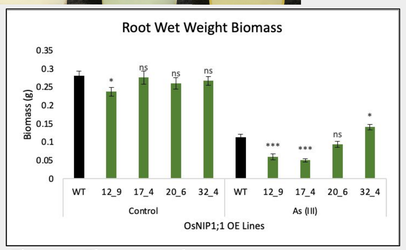
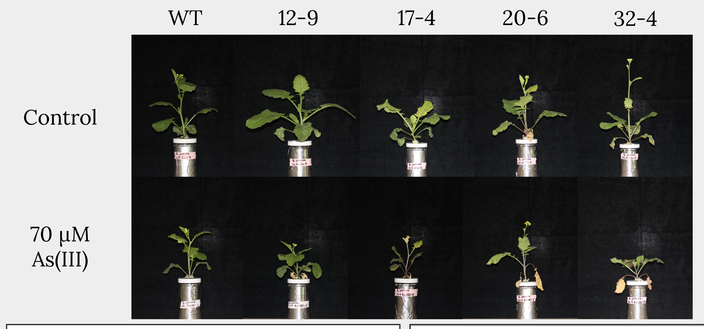


Figure 3: Phenotypic data comparing transgenic lines to the WT line show the transgenic lines are sensitive to As(III) compared to the WT line.



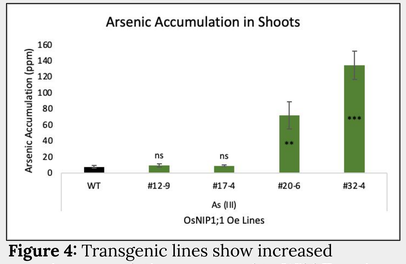


Figure 4: Transgenic lines show increased arsenic accumulation compared to Wild Type in shoots.

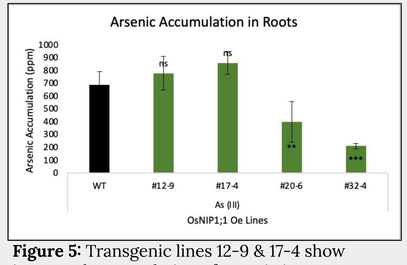


Figure 5: Transgenic lines 12-9 & 17-4 show increased accumulation of arsenic in roots compared to WT. Lines 20-6 & 32-4 show decreased accumulation of arsenic in roots compared to WT.

DISCUSSION

Phenotypic Study

- Transgenic lines 12-9 and 17-4 showed a significant decrease in biomass compared to the WT line in both shoots and roots when grown on AsIII.
- Transgenic lines 20-6 and 32-4 did not show a significant decrease in biomass compared to the WT line.
  - Transgenic line 32-4 showed a significant increase in shoot and root biomass when compared to the WT line.

Hydroponic Study

- Transgenic lines 12-9 and 17-4 showed no significant increase in arsenic accumulation or translocation from root to shoot when compared with the WT line.
- Transgenic lines 20-6 and 32-4 showed a significant increase in arsenic accumulation when compared to the WT line.
  - Additionally, transgenic lines 20-6 and 32-4 show a significant increase in translocation of arsenic from root to shoot when compared to the WT line.

Overall Discussion

- Initial data suggests that the transgenic lines are more sensitive to arsenic exposure compared to the WT line.
- The OsNIP1;1 protein contributes to the uptake and transport of arsenic in addition to water.
- Based on the results, down-regulation of the rice aquaporin protein OsNIP1;1 can be used to decrease the uptake and transport of arsenic plants in food crops. Such could be implemented in food crop to increase food safety by decreasing arsenic accumulation.
- Based on the results, *Brassica juncea* transgenic lines 20-6 and 32-4 could be utilized in the phytoremediation of arsenic as *B. juncea* is widely implemented for the phytoremediation of arsenic and transgenic lines 20-6 and 32-4 show both a significant increase in arsenic accumulation and translocation of arsenic from root to shoot.

FUTURE WORK

Study the effect of the combined overexpression of rice aquaporin protein OsNIP1;1 and plant arsenate reductases (ACR2 and HAC1) in *Brassica juncea* for their role in arsenic translocation and accumulation in plants.

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