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Utilizing Innovative Materials Science Approaches to Enhance Bioremediation: Session III- Plants Fungal Based Bioremediation

A Novel strategy for Arsenic Phytoremediation

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EPA and NIEHS SRP Webinar
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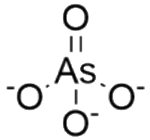
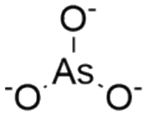
Purpose: Develop a genetics-based phytoremediation strategy for arsenic uptake, translocation, detoxification, and hyperaccumulation into the fast-growing, high biomass, non-food crop *Crambe abyssinica*.

❖ **The problem:** Arsenic (As) is highly toxic and As contamination is widely recognized as a global health problem. Phytoremediation is a cost-effective and ecologically friendly alternative to physical remediation methods. ***However, transport from soil to roots and subsequent translocation and accumulation into shoot biomass is limited.***

❖ **Our solution:** We will co-express several genes that control the transport, oxidation state, and binding of As for efficient extraction and hyperaccumulation of As into above-ground plant tissues. **Nanosulfur** will be utilized to modulate the bioavailability and phytoextraction of As from soil and to increase the storage capacity via enhanced sulfur assimilation.



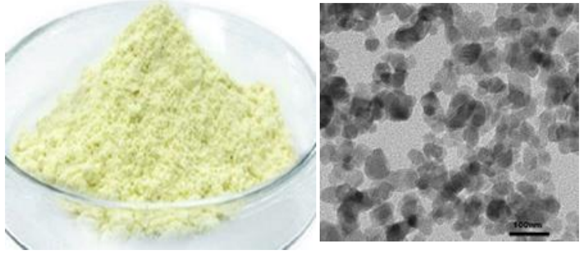
Arsenate



Arsenite

Inorganic As species are more toxic

Crambe abyssinica



Nanosulfur

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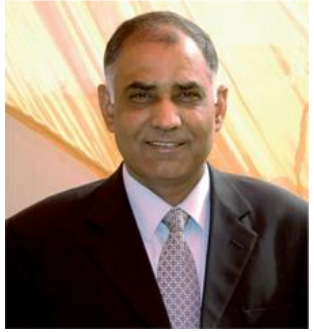
Our Team



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Arsenic ranks first on the Superfund List of Hazardous Substances

ATSDR

Department of Health and Human Services

Agency for Toxic Substances & Disease Registry

Toxic Substances & Health

- ATSDR Home
- ToxFAQs™
- ToxFAQs™ en Español
- Public Health Statements
- Toxicological Profiles
- ToxGuides
- Minimum Risk Levels
- Priority Data Needs
- MMGs
- MHMIs
- Interaction Profiles
- Priority List of Hazardous Substances
- Division of Toxicology

Home > CERCLA 2005 CERCLA Substance List

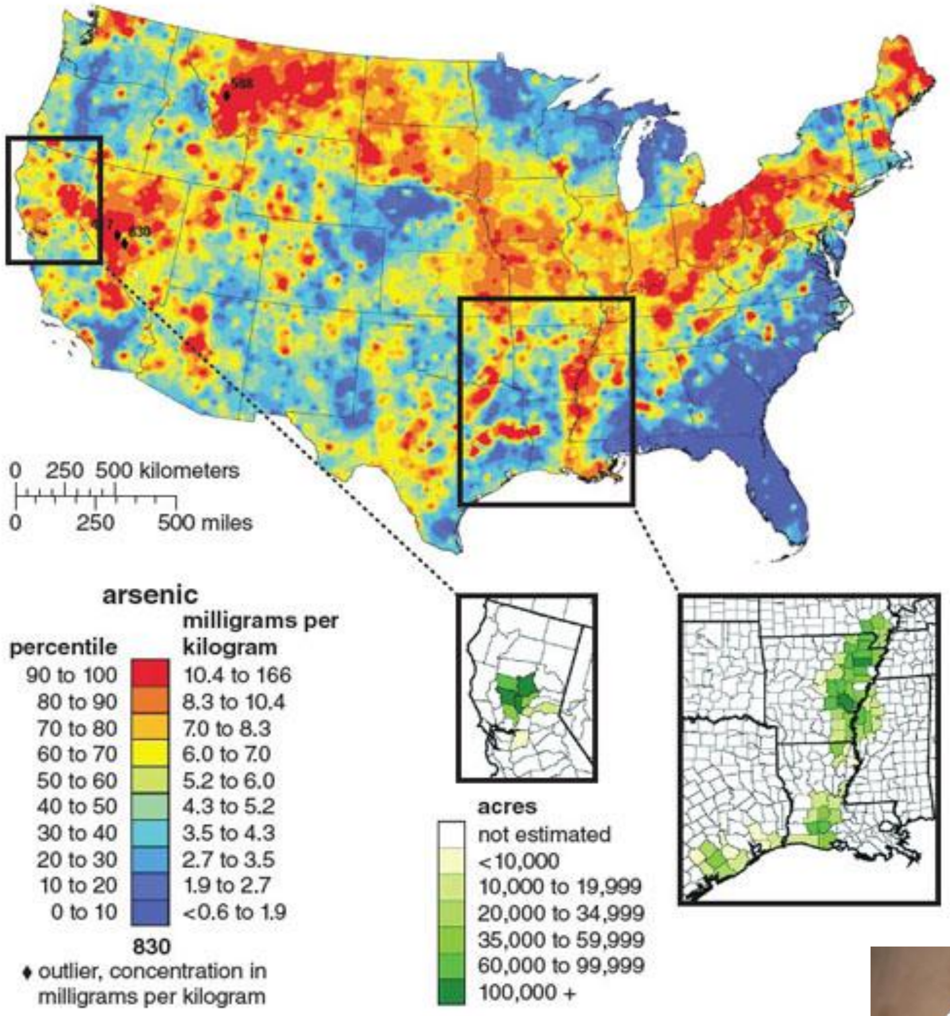
2005 CERCLA Priority List of Hazardous Substances

2005 RANK	SUBSTANCE NAME	TOTAL POINTS	2003 RANK	CAS #
1	ARSENIC	1668.56	1	007440-38-2
2	LEAD	1534.54	2	007439-92-1
3	MERCURY	1507.31	3	007439-97-6
4	VINYL CHLORIDE	1389.02	4	000075-01-4
5	POLYCHLORINATED BIPHENYLS	1371.60	5	001336-36-3
6	BENZENE	1353.53	6	000071-43-2
7	POLYCYCLIC AROMATIC HYDROCARBONS	1321.72	8	130498-29-2
8	CADMIUM	1321.47	7	007440-43-9
9	BENZO(A)PYRENE	1307.76	9	000050-32-8
10	BENZO(B)FLUORANTHENE	1263.06	10	000205-99-2

Lead arsenate being applied by hand to control boll weevils in the early 1900's

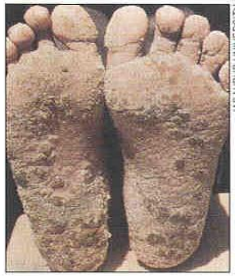
Arsenic Contamination in water and soil is widespread

Elevated level of As in top agriculture soil in USA

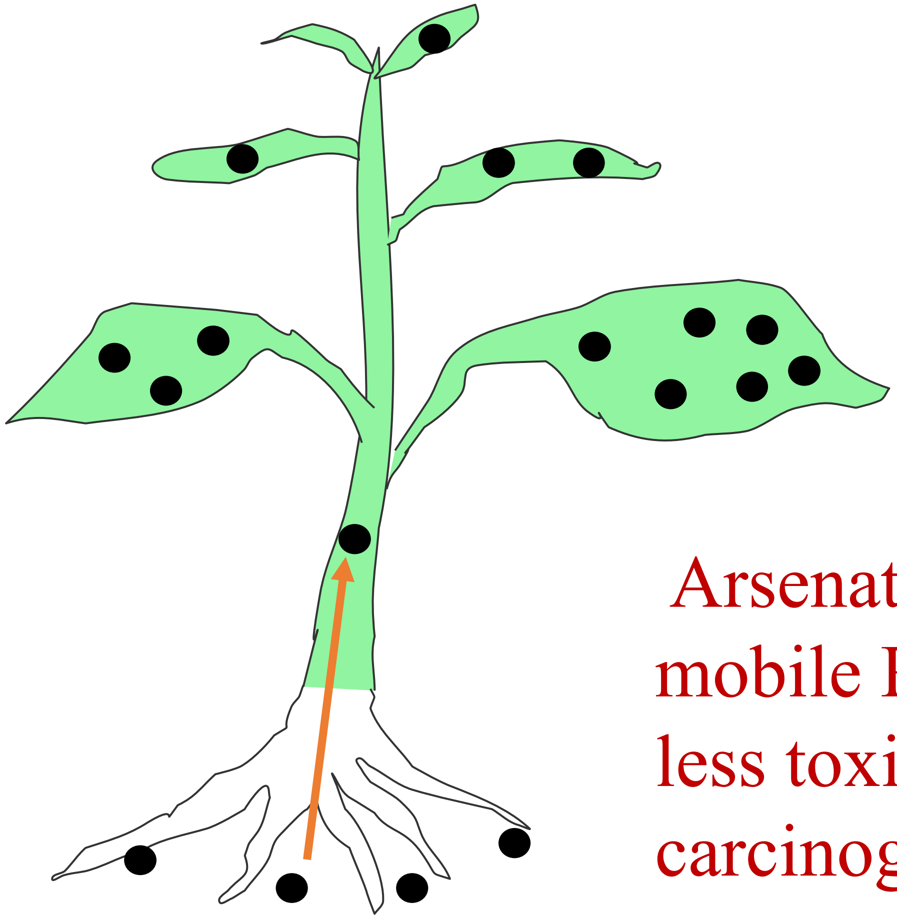


Organ system	Health effects
Skin	Hyperkeratosis of palms and soles Melanosis or depigmentation Bowen's disease Basal cell carcinoma Squamous cell carcinoma
Liver	Enlargement, Jaundice, Cirrhosis Non-cirrhotic Portal Hypertension
Nervous system	Peripheral neuropathy, Hearing loss
Cardiovascular system	Acrocyanosis Raynaud's Phenomenon
Respiratory system	Lung Cancer
Endocrine system	Diabetes mellitus and Goiter

- 56 million people in 25 states in USA are affected by arsenic contamination in drinking water.



Strategy for the phytoremediation of arsenic



Two major oxyanion forms of inorganic As in soil

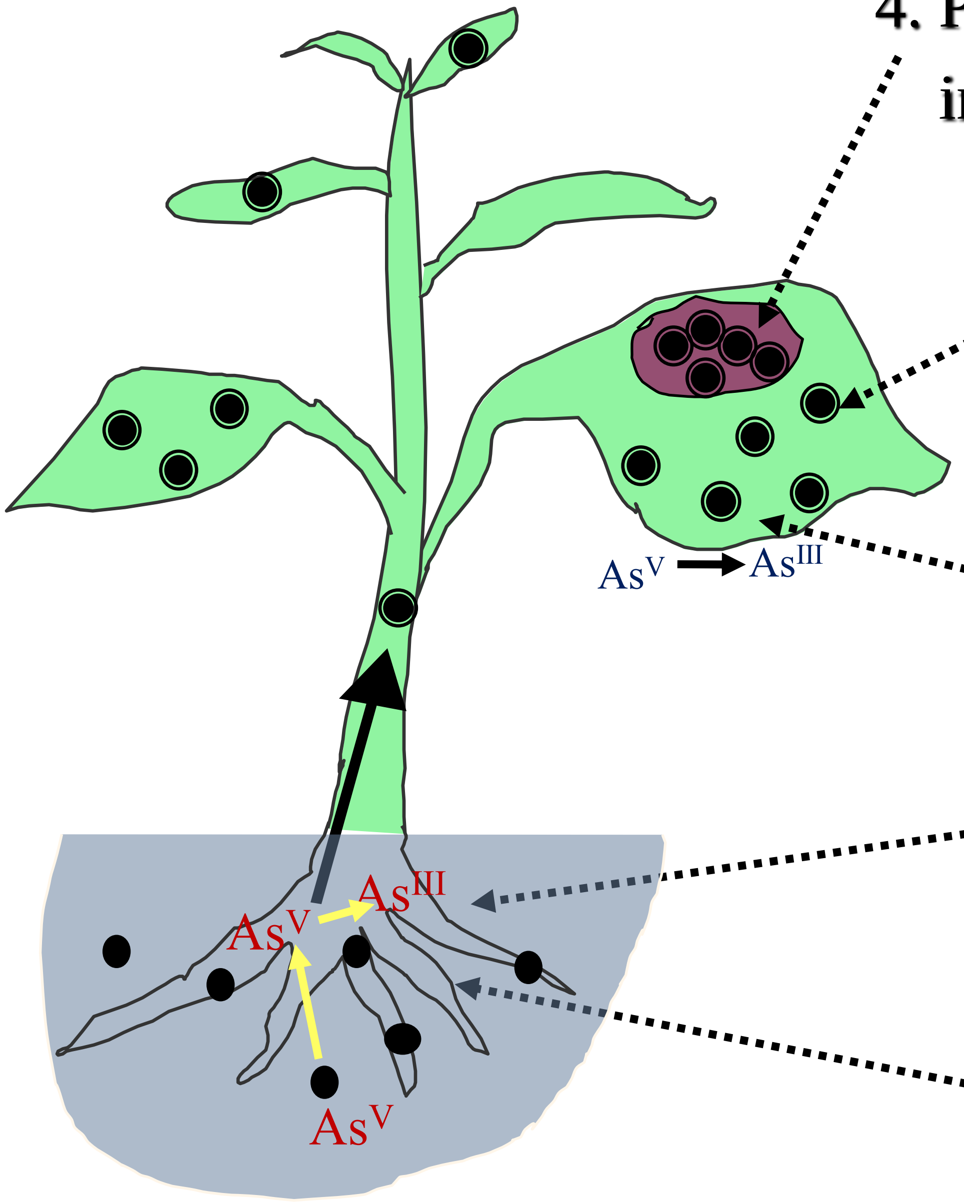
Arsenate(AsO_4^{-3}) mobile PO_4^{-3} analog & less toxic, but still carcinogenic

Arsenite (AsO_3^{-3}) thiol-reactive, immobile & more toxic

Arsenate (AsO_4^{-3})uptake via phosphate transporters

Arsenite(AsO_3^{-3}) uptake via silica transporters, Lsi1 and Lsi2

Genetics-based Strategy for the Phytoremediation of Arsenic



4. Pump (AsIII)-thiol complexes into vacuoles (GCP-YCF1, MRPs)

3. Detoxify & trap AsIII in thiol complexes (γ-EC, GSH, PCs)

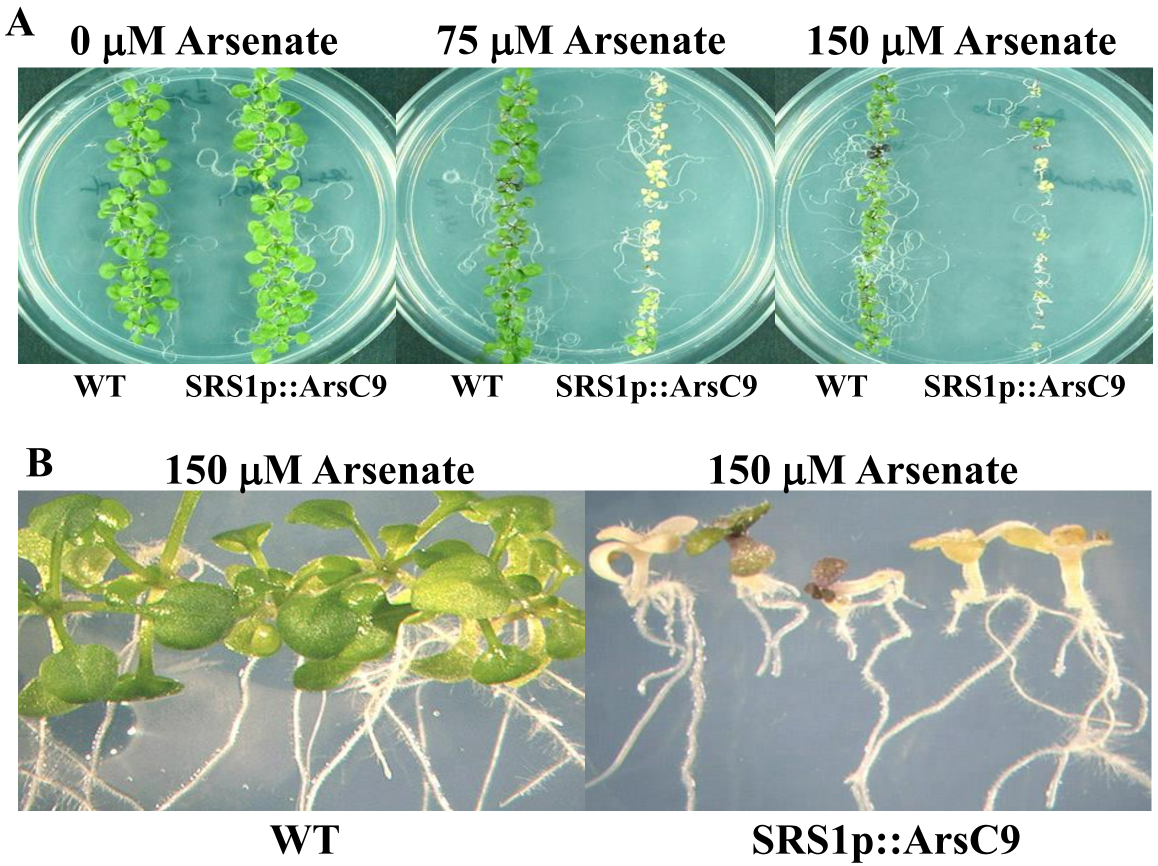
2. Reduction of AsV to AsIII in leaves- ArsC

5. Block endogenous AsV to AsIII reduction in roots (AtACR2)

1. Enhance AsV uptake (AtPTs)

Developing Proof of Concept for Arsenic Phytoremediation in Model Plant Arabidopsis

1. Light-induced *SRS1p::ArsC* expression in shoots confers arsenate sensitivity due to toxicity of AsIII in Arabidopsis

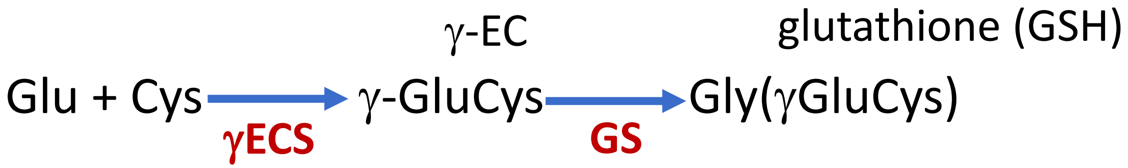


Arsenate (AsV)
(Less Toxic)

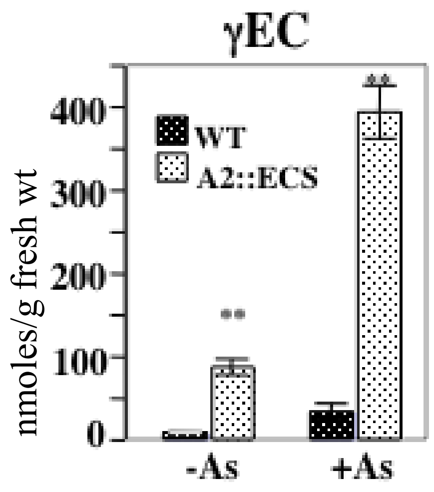
↓

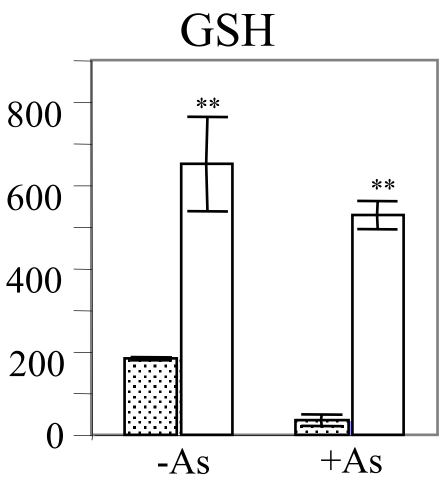
Arsenite (AsIII)
(More Toxic)

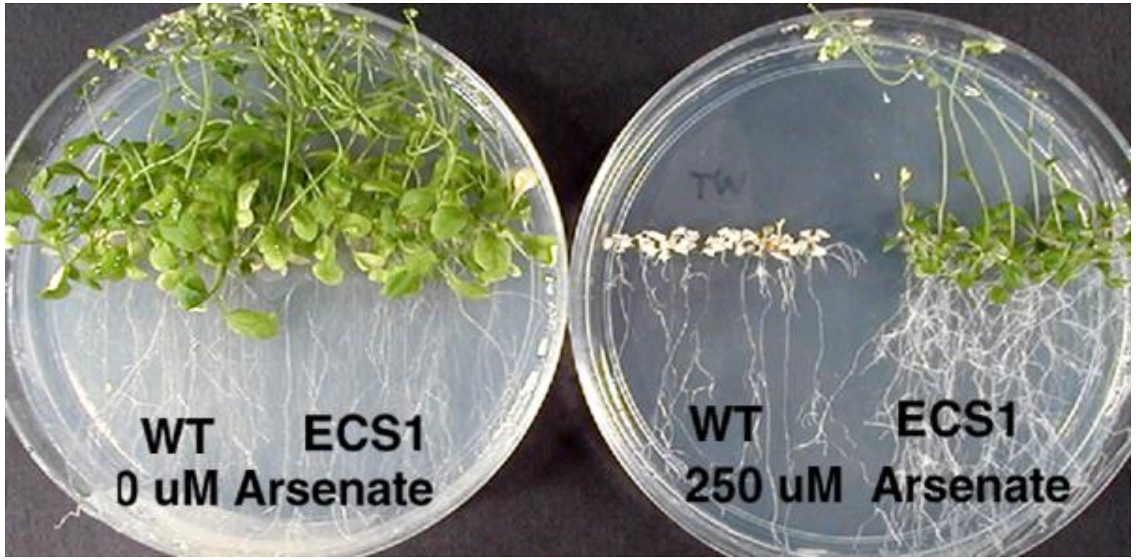
2. Overexpression of enzymes in the glutathione (GSH) biosynthetic pathway increased tolerance to arsenic and other toxic metals in plants



γECS = γ-glutamylcysteine synthetase
GS = GSH synthetase





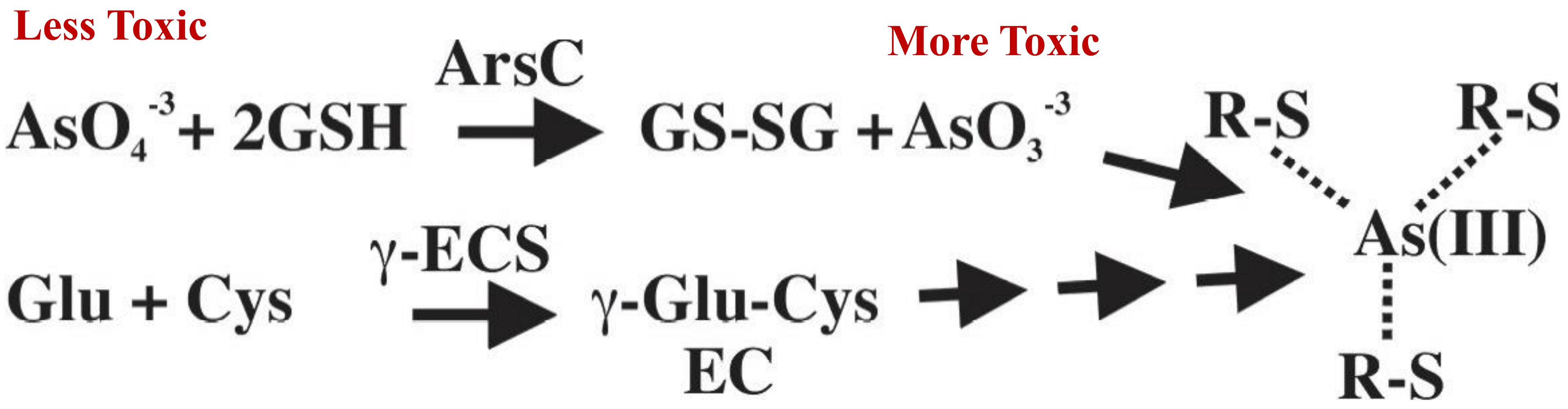


Constitutive expression of the bacterial *γ-ECS* gene from ACT2 promoter increased γ-EC and GSH levels and conferred strong tolerance to arsenate

Dhankher et al., 2002, Nature Biotech 20:1140-45
Li, Dhankher, Meagher et al., 2005, Environ. Toxicol. Chem. 24:1376-86
Paulose et al., 2013, Plant Cell, 25: 4580–4595

Combining the expression of SRS1p-ArsC and ACT2p-γECS significantly increased AsV tolerance and accumulation in aboveground biomass

For As detoxification and hyperaccumulation, we co-expressed bacterial arsenate reductase gene (ArsC) and γECS in leaves in tissue-specific manner (*ACTp::ECS* and *SRS1p::ArsC)*



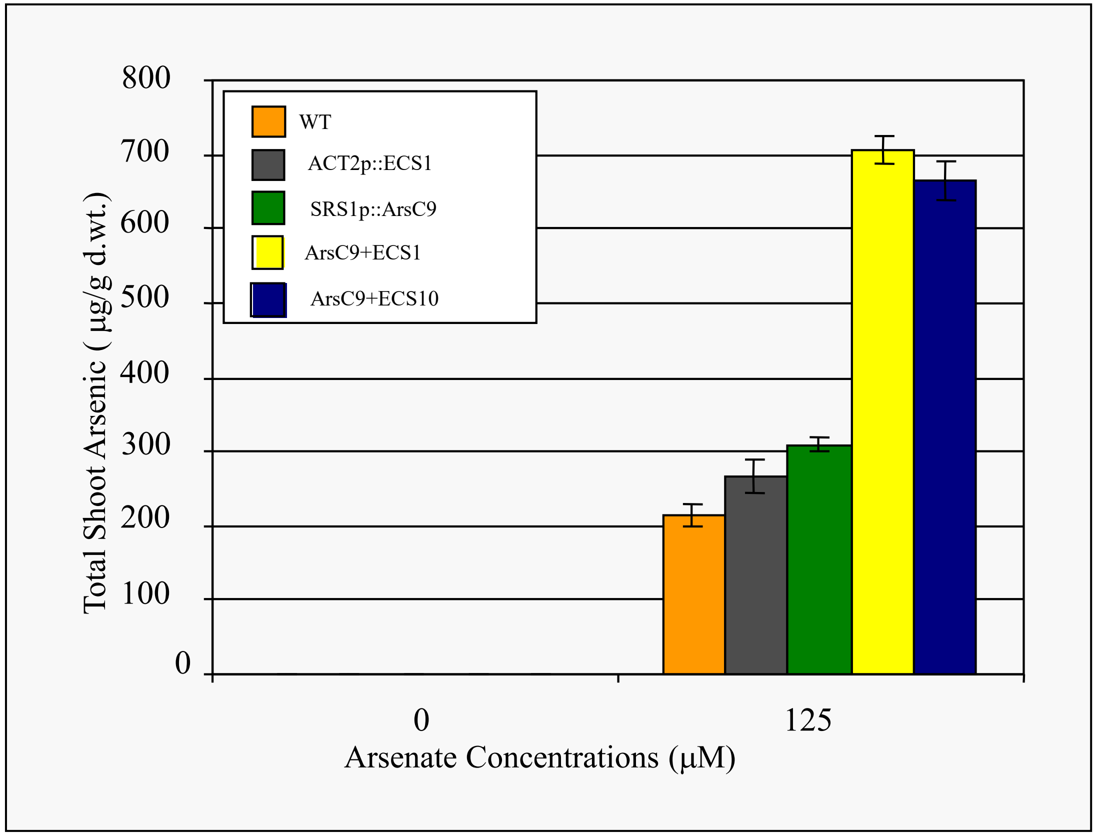
Combining *ACTp::ECS* and *SRS1p::ArsC* increased arsenate resistance and As hyperaccumulation

250 µM Arsenic

γECS

γECS+ ArsC

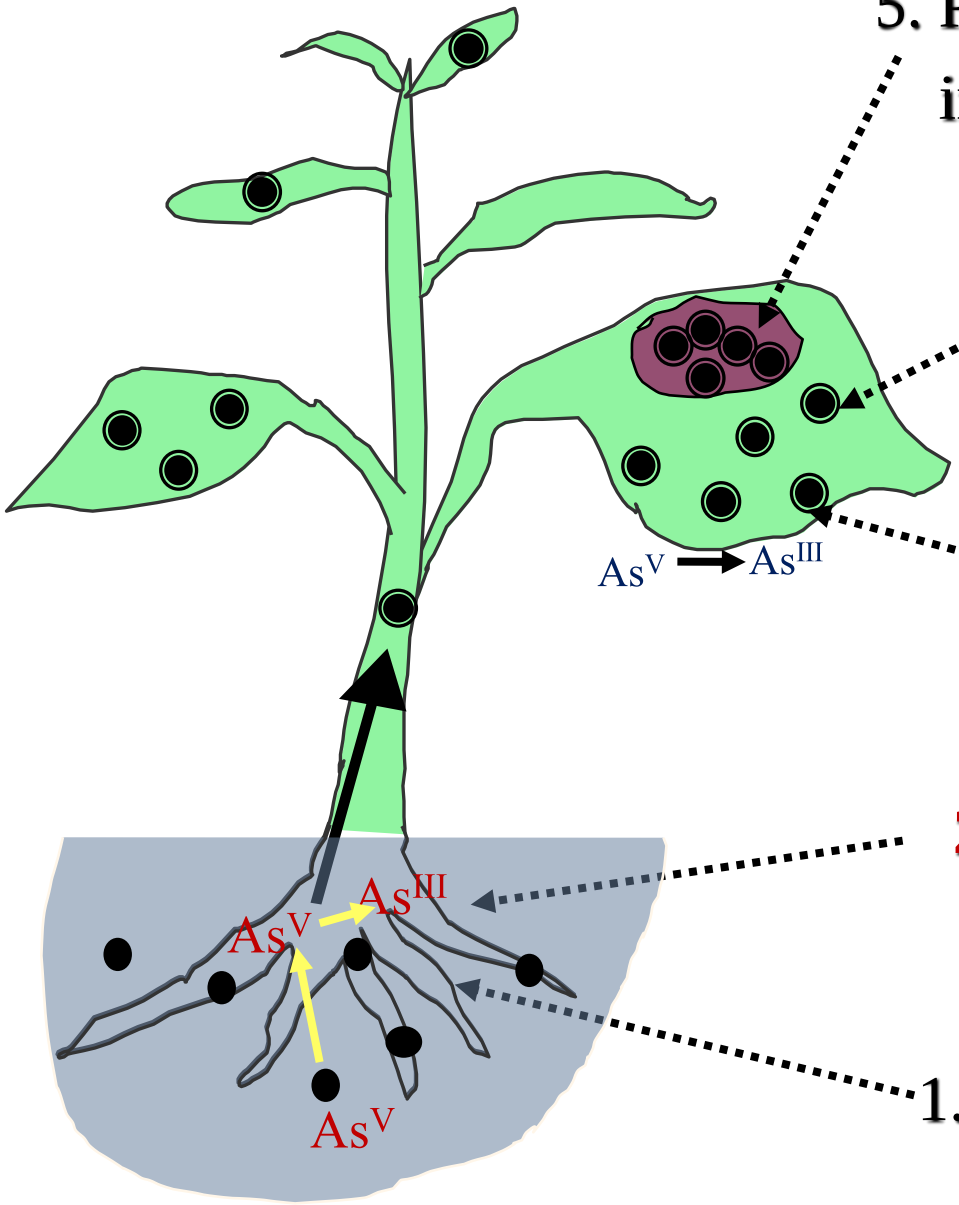
ArsC



ArsC+ECS double transformants accumulated **3-fold more As in shoots compared to controls**

Dhankher et al., Nature Biotech. (2002), 20:1140-1145

Genetics-based Strategy for the Phytoremediation of Arsenic



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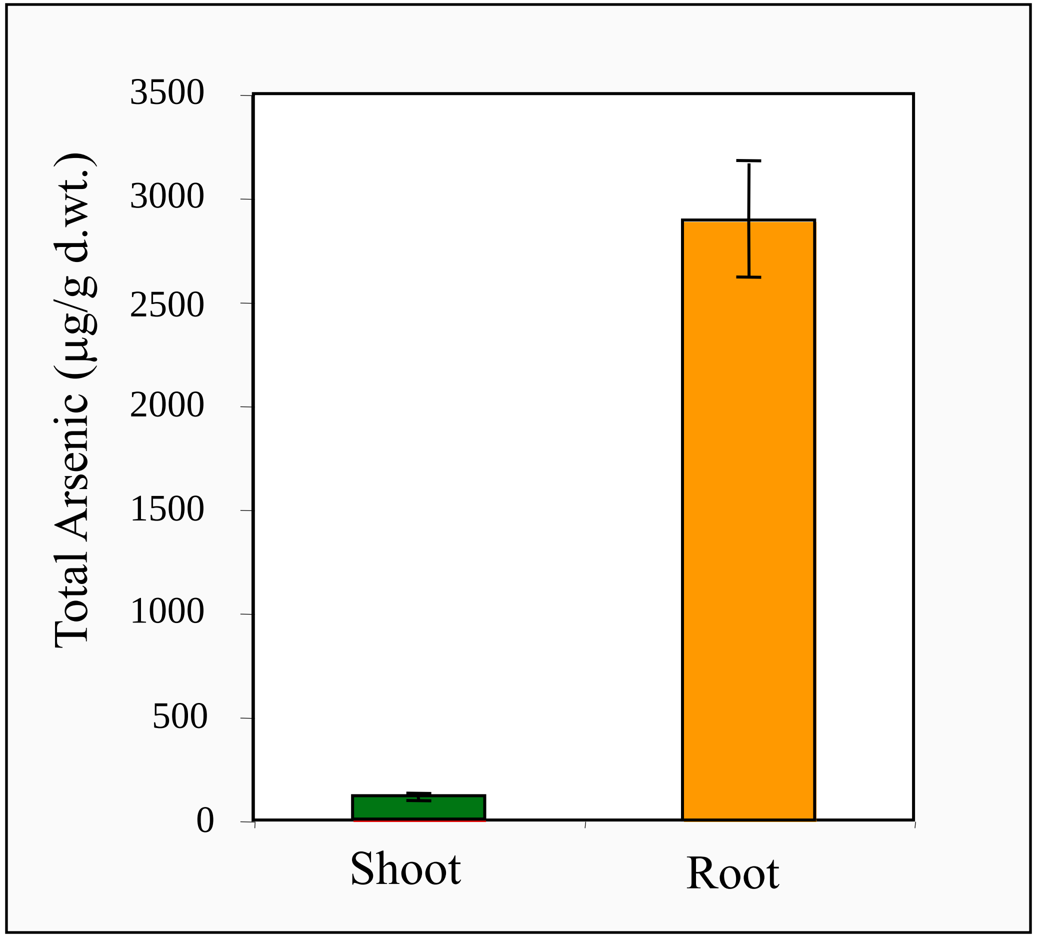
2. Block endogenous AsV reduction in roots (AtACR2)

1. Enhance AsV uptake (AtPTs)

A limitation to arsenic phytoremediation strategy

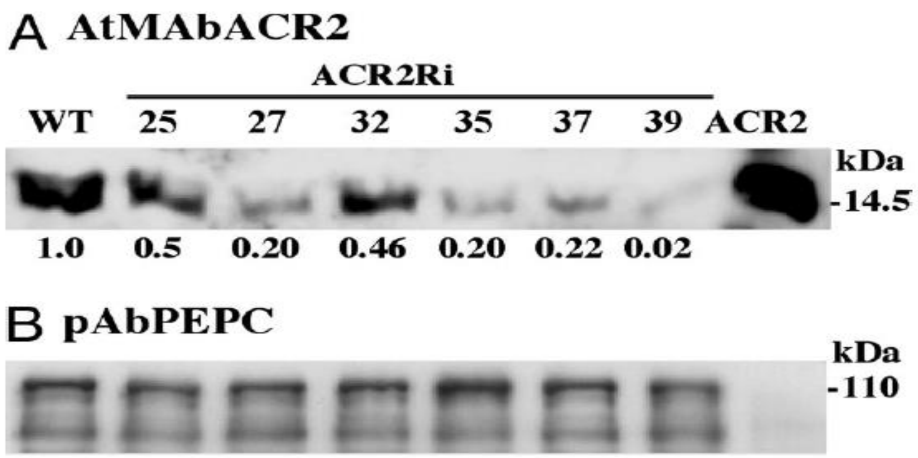


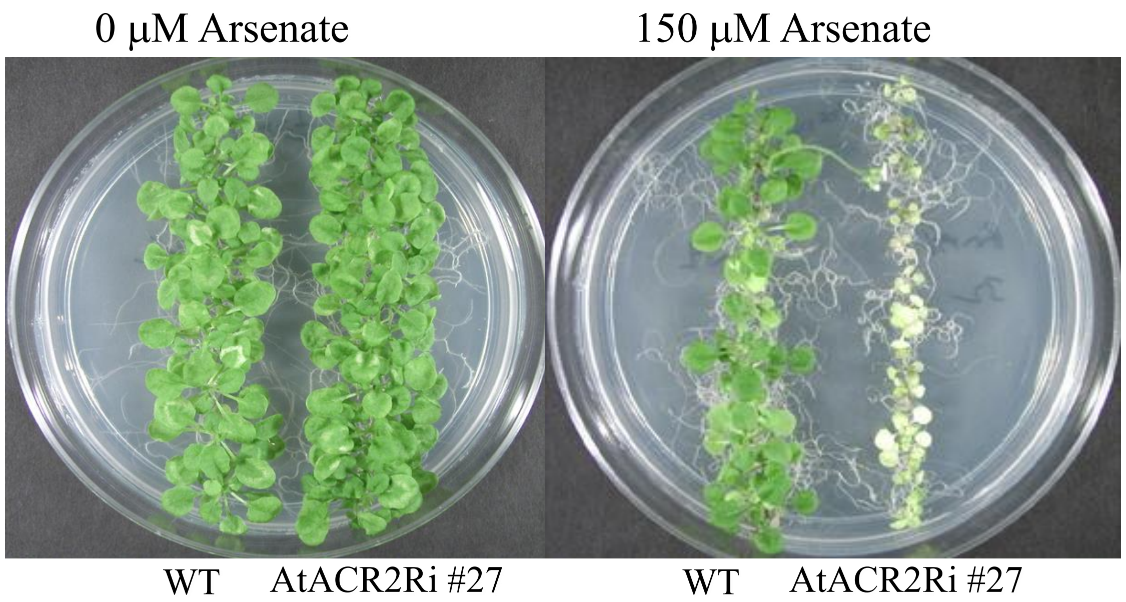
- Most of the arsenic taken up by plants is retained in roots. Only a fraction is translocated to shoots

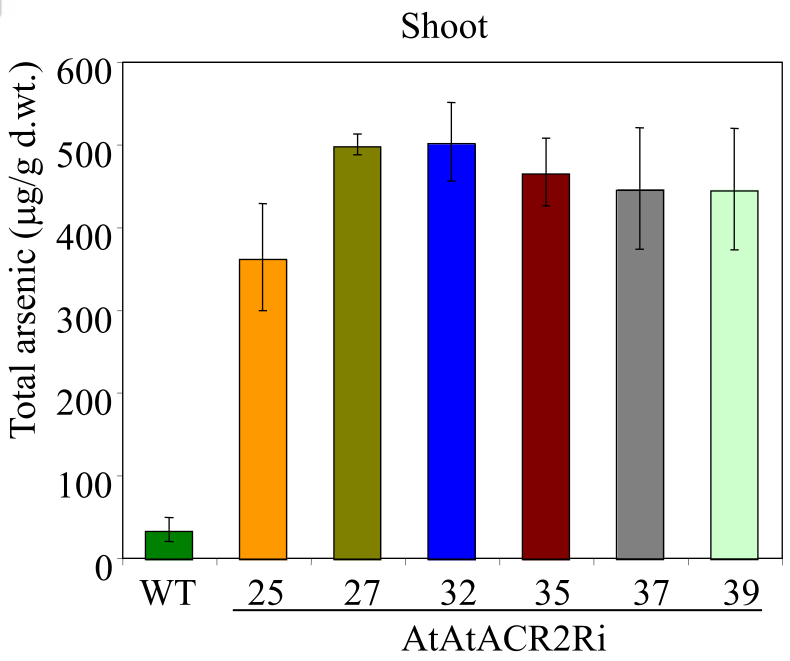
- Endogenous plant arsenate reductase enzyme reduce arsenate to arsenite and facilitate to trap arsenite belowground.

- Translocation of arsenate from roots to shoots can be enhanced by blocking the function of endogenous arsenate reductase (ACR2) enzyme in plants using RNA interference (RNAi) approach.

AtACR2 knockdown RNAi lines transported 10- to 15-times more arsenic to shoots and showed AsV sensitivity







Dhankher et al., (2006) PNAS 103: 5413-18

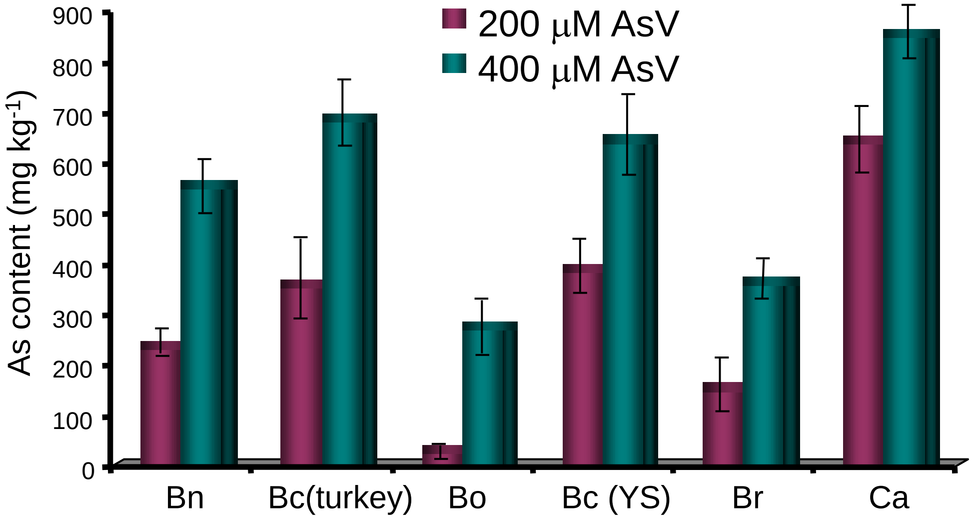
Taking Phytoremediation Strategy to Field: Engineering Non-Food, High Biomass, and Fast Growing Plants for Field Use

Crambe abyssinica:

An Ideal Crop for Phytoremediation and Biodiesel

- High biomass
- Inedible and odiferous industrial crop
- Short life cycle, only 40-45 days for max. biomass and 75-85 days for seed maturity
- Low agronomic practice requirements
- Can be grown as intercrop between rice and other crops
- **Does not out-cross with canola or other brassica sp. - no gene flow concern**

C. abyssinica *B. juncea*



Chhikara and Dhankher, Industrial Crops and Products 37: 457– 465 (2012)
Ma et al., 2015, Environ. Sci. Tech. 49: 10117-10126 (2015)

Technical Approach

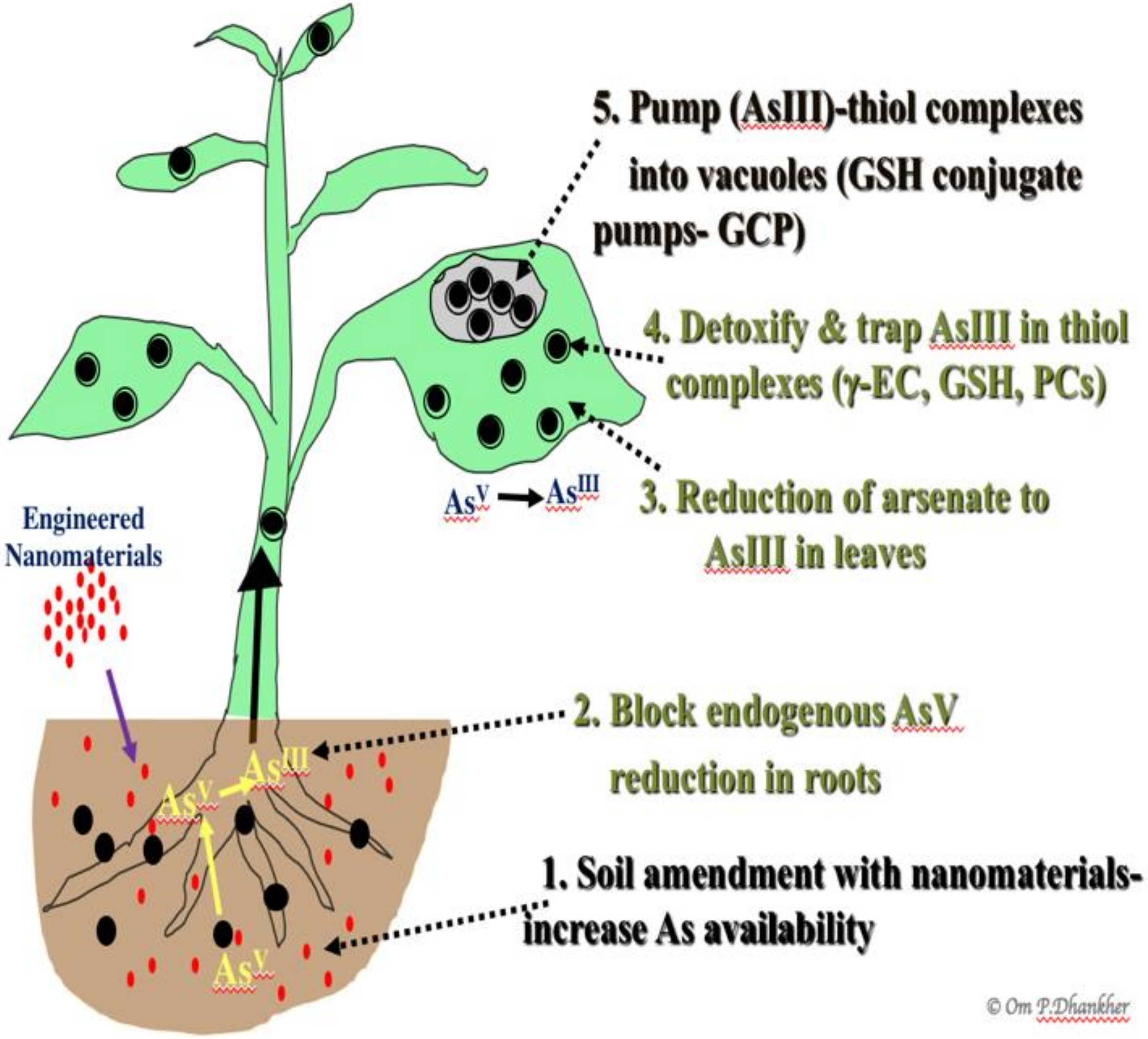
Specific Aims

Aim 1: Generate genetically engineered *Crambe abyssinica* lines co-expressing genes encoding arsenate reductases, GSH synthesis, glutathione-conjugate vacuolar transporter and RNAi suppression of *Crambe* endogenous arsenate reductase.

Aim 2: Evaluate the genetically enhanced *Crambe* lines for metal(loids) detoxification and hyperaccumulation in the above-ground tissues in hydroponic and tissue culture media in the laboratory conditions.

Aim 3: Application of nanosulfur for modulating the bioavailability, phytoextraction and accumulation of toxic metal(loids) from soil.

Aim 4: Conduct greenhouse study of selected genetically enhanced *Crambe* lines for arsenic phytoextraction on contaminated soils using nanosulfur.



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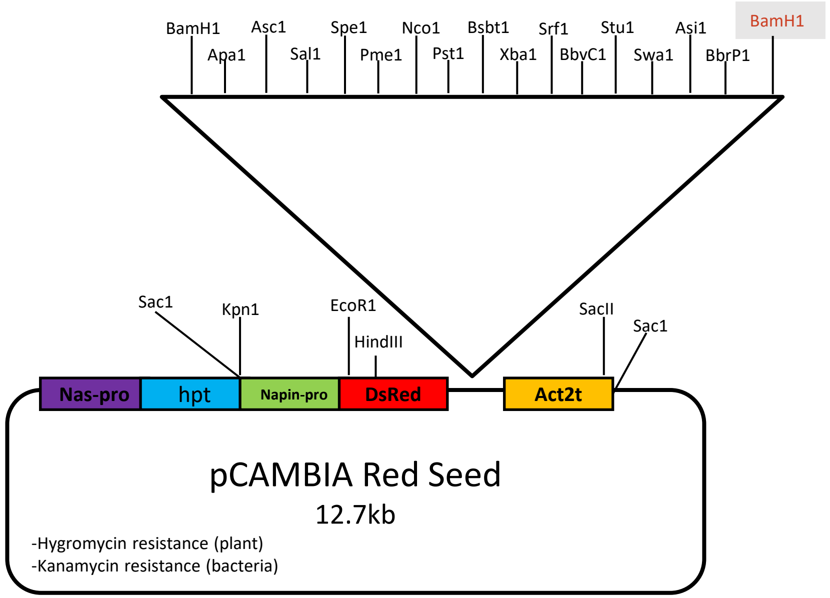
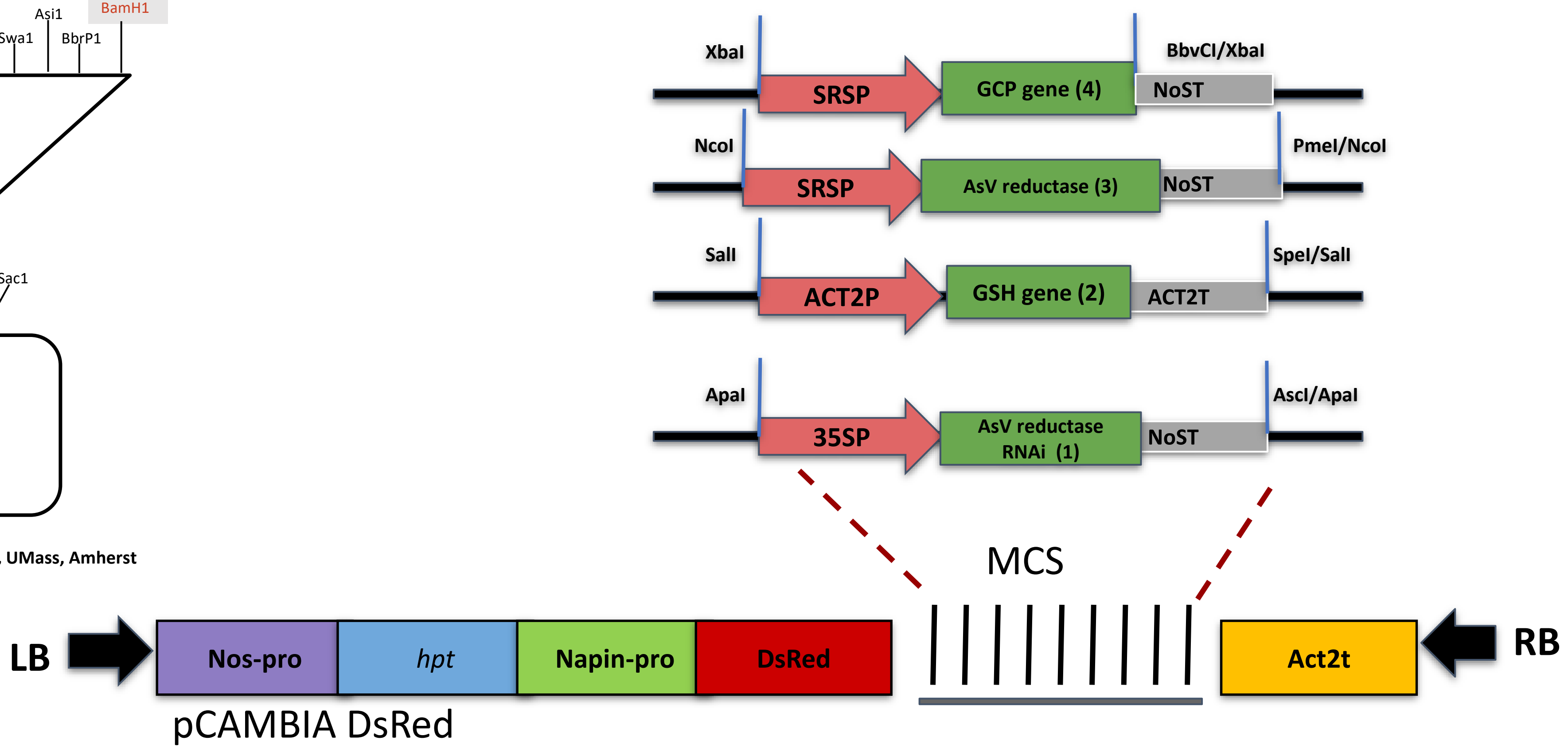
Objective 1: Generate genetically engineered *Crambe abyssinica* lines co-expressing genes encoding arsenate reductases, GSH synthesis, glutathione-conjugate vacuolar transporter and RNAi suppression of *Crambe* endogenous arsenate reductase.

Cloning and Stacking of all genes for tissue-specific expression into a single construct for developing transgenic Crambe for As tolerance and hyperaccumulation





Modified pCambia gene stacking vector

Figure. Maps of Synthetic Gene constructs in pCambia plant transformation vector.

Objective 2: Optimizing Crambe tissue culture protocol and generation of Crambe transgenics

Generation of Crambe transgenics

Obtaining and raising of Crambe transgenic workflow

Callus induction

↓

Agrobacterium infection

↓

Shoot regeneration and root formation

↓

Obtaining transgenic plantlets, transferring to soil, obtaining seeds

↓

Selection and raising for next generation to get homozygous transgenic lines

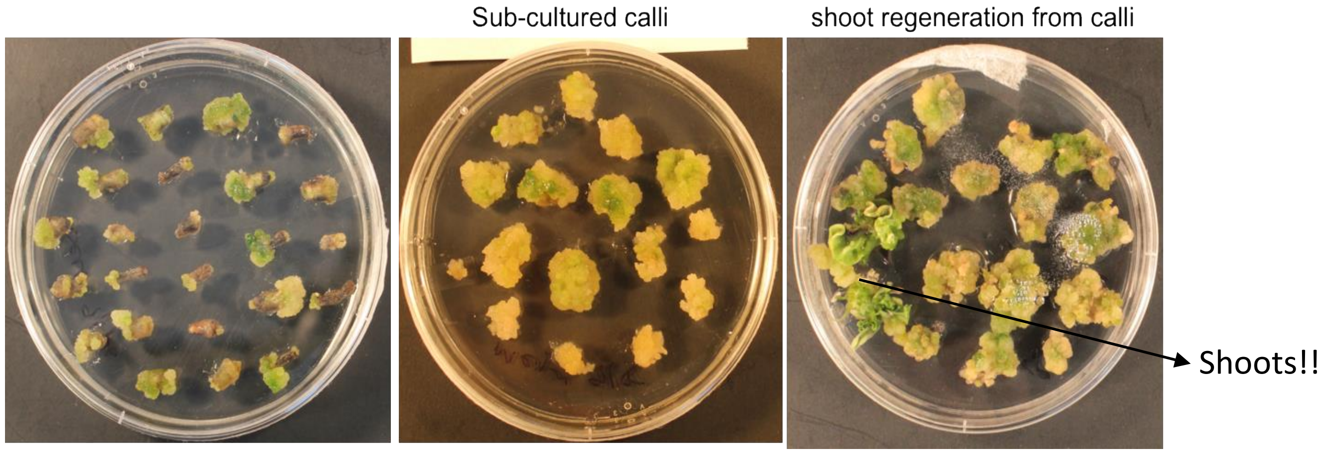
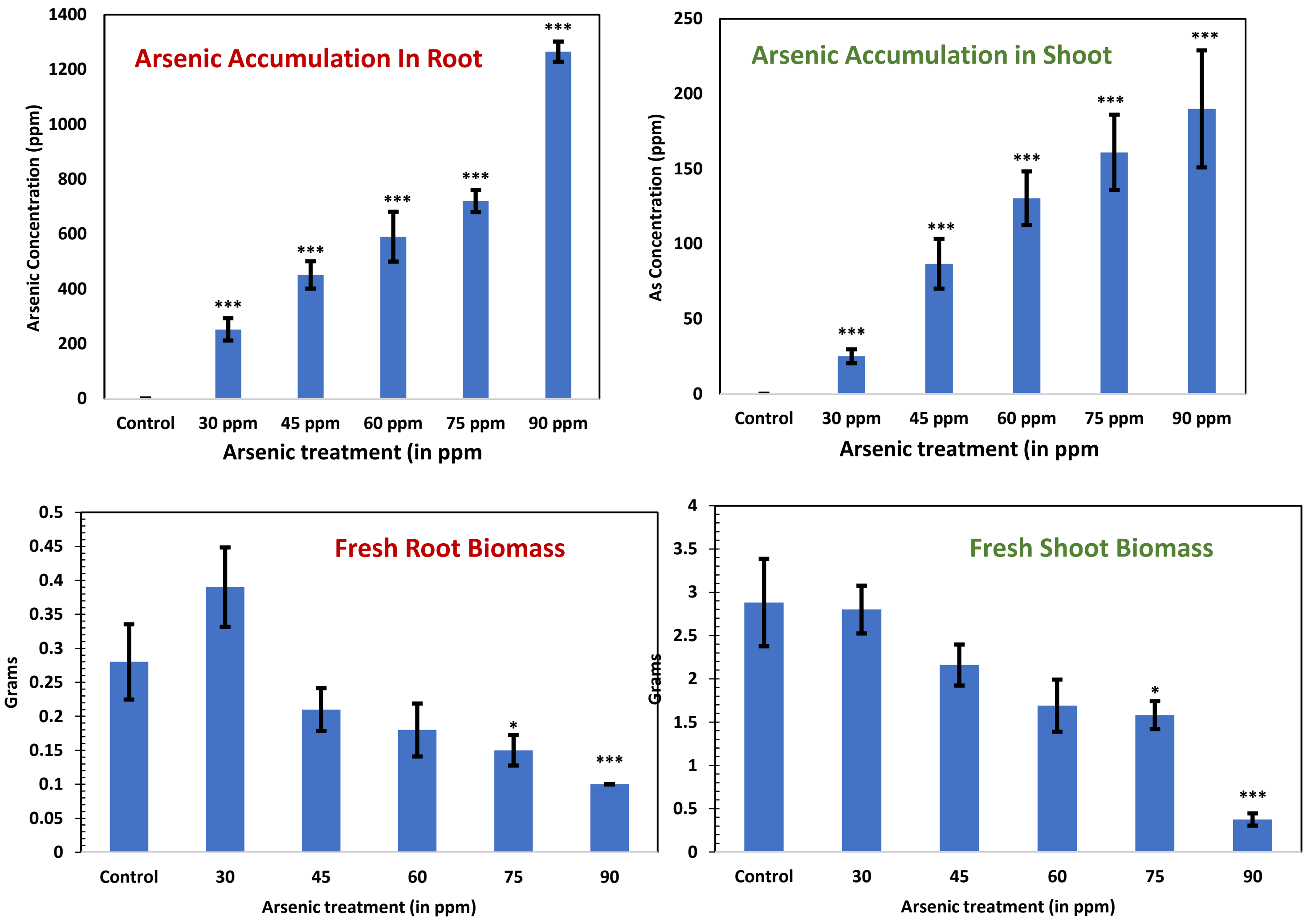


Figure. Optimization of callus inductions and shoot regeneration protocol for *Crambe abbyssinica.*

Objective 3: Optimization of nanomaterials application for modulating the bioavailability, phytoextraction and accumulation of arsenic from the soil

Analysis and optimization of AsV concentrations for tolerance and As accumulation in Crambe

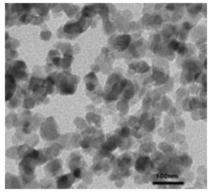


Arsenic accumulation in Crambe increases proportionally to arsenic concentration added to the soil.

Crambe biomass decreases proportionally to arsenic concentration added to the soil.

Figure. Dose-response curve analysis for As accumulation in Crambe

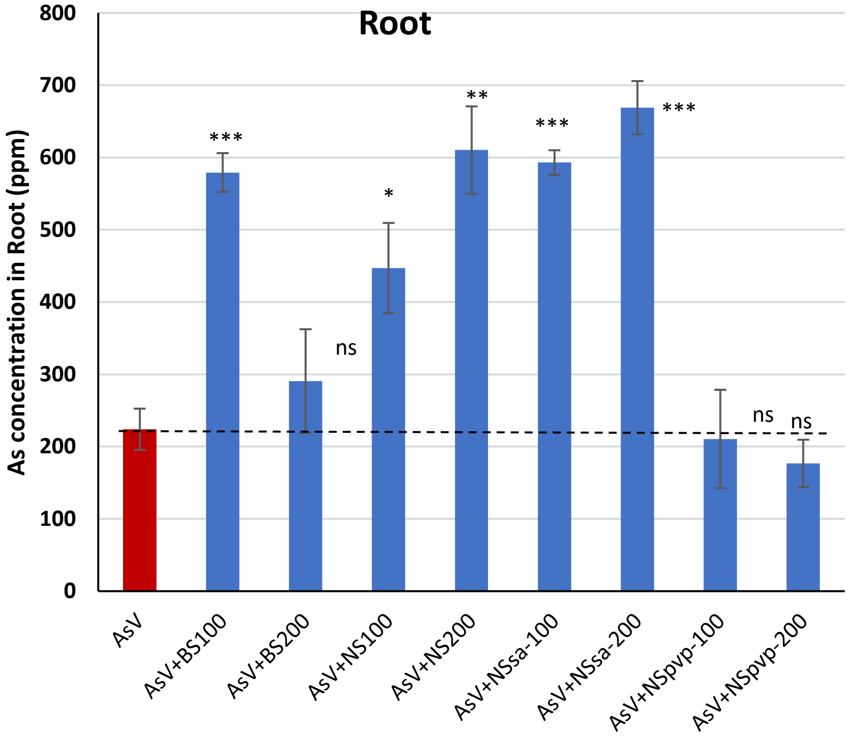
Objective 3: Optimization of nanomaterials application for modulating the bioavailability, phytoextraction and accumulation of arsenic from soil



Nanosulfur

Sulfur treatments mobilized more As in soil and increased the uptake in roots of wild type Crambe plants.

No.	Treatments
1	Control, 0 As and 0 S
2	AsV 60 ppm
3	BS100 + AsV60
4	BS200 + AsV60
5	NS30-100+AsV60
6	NS30-200+AsV60
7	NS30SA-100+AsV60
8	NS30SA-200+AsV60
9	NS30PVP-100+AsV60
10	NS30PVP-200+AsV60



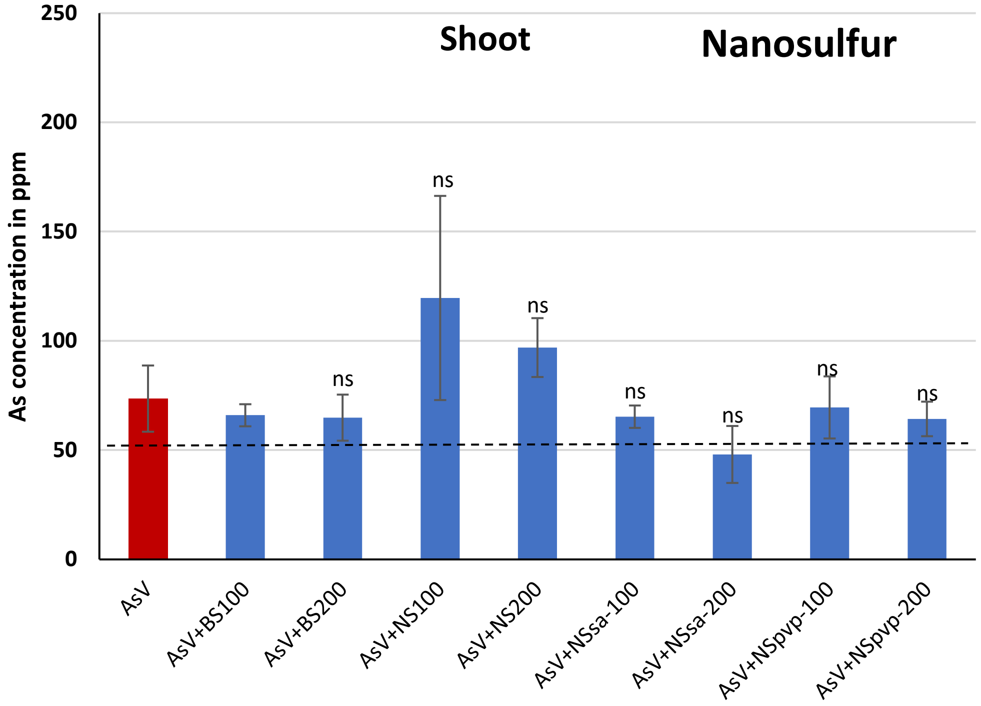
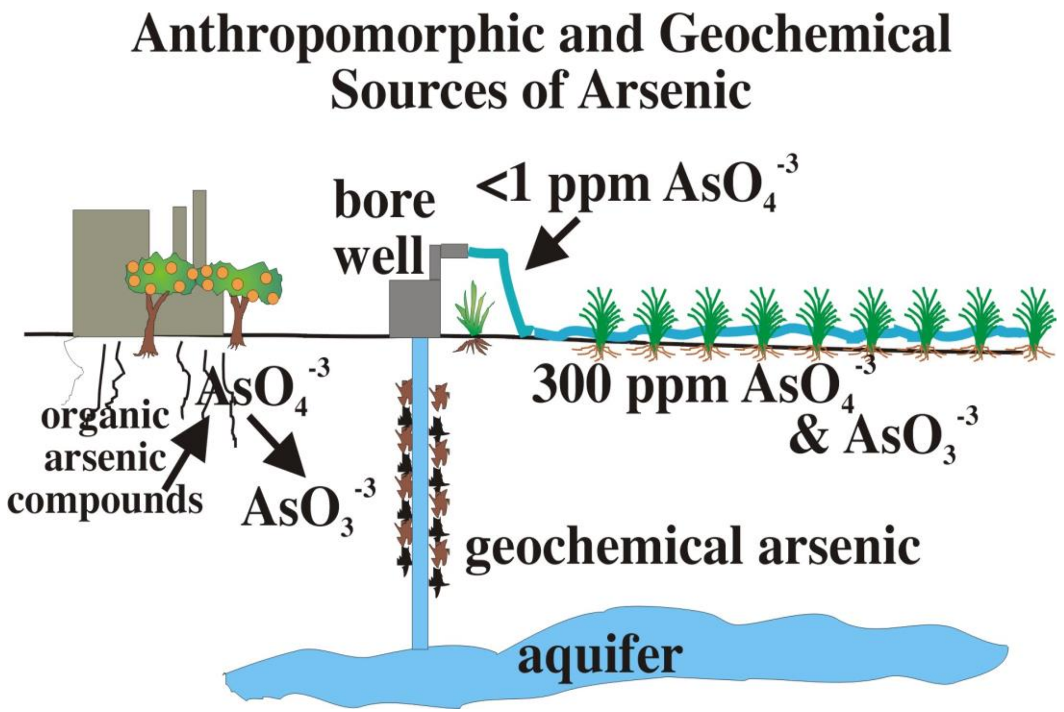
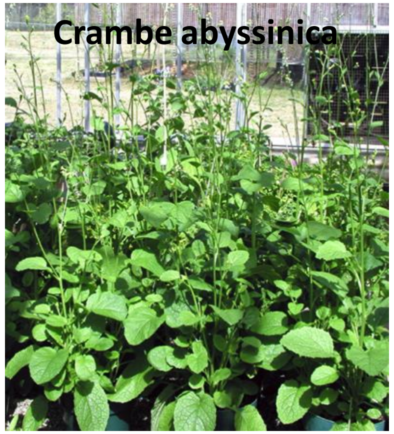


Figure. Effect of various Sulfur treatments for mobilization of As in soil and uptake in roots and shoots of wild type Crambe plants.

Crambe could be used as an ideal intercrop between rice and wheat cultivation for removing arsenic in the top layers of agricultural soil





Phytoremediation crops

Conclusions

- **As contamination in water and food crops is a serious human health concern.**
- **A proof of concept for efficient arsenic phytoremediation strategy was developed in model system Arabidopsis.**
- ***Crambe abyssinica* (a mustard family member) is an ideal fast growing, high biomass crop for phytoremediation of arsenic and other toxic metals.**
- **Transferring arsenic phytoremediation strategy to Crambe for field remediation.**
- **Soil amendments with engineered nanomaterials can modulate the bioavailability of As and increased uptake in plant tissues.**

Acknowledgements

Funding Support

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