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Selenium biofortification in rice

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Trace elements in food: applications with ICP-MS and ICP-MS/MS

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- ✧ Selenium Introduction
- ✧ Selenium biofortification in rice (article)
- ✧ Antimony introduction
- ✧ Effects of Selenium on Antimony uptake in rice
- ✧ Conclusions
- ✧ Future perspectives



Selenium (Se)

- an ambivalent element: essential for human nutrition but toxic at high concentration
- Se content of most of foods is very low, the Se requirement of the body can be satisfied with dietary supplements or with Se-enriched foods
- Se agronomic biofortification crop by selenate (Se(VI)) or selenite (Se(IV))
- Se(VI) → washed out by irrigation/rainfall
- Se(IV) → adsorbed by soil minerals
- Different Se incorporation in agriculture field (aqueous solution in soils or spraying on leaves)



Selenium in rice



- ❑ High Se concentration in roots than in shoots
- ❑ C-Se-C compounds being the dominant species of Se in all roots (Wang et al. 2015)

SELENITE (Se(IV))

- ❑ Se(IV) is transformed into organic Se in roots, limit translocation in shoots
- ❑ Selenite uptake into the plant may occur via phosphate transporters (Li et al. 2008) or passive diffusion (Shrift and Ulrich, 1969; Arvy, 1993) and/or a silicon (Si) influx transporter Lsi1 (OsNIP2;1) (Zhao et al., 2010).



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1st PART: Se biofortification in rice

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Article

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Selenium Biofortification in Rice (*Oryza sativa* L.) Sprouting: Effects on Se Yield and Nutritional Traits with Focus on Phenolic Acid Profile

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OBJECTIVE:

Evaluate Se accumulation in sprouts as organic and inorganic forms, and Se optimal concentrations and biofortification efficiency



Se experimental design

Rice (*Oryza sativa* L., cv. Selenio)



water

Se(IV) mg L⁻¹

Se(IV)_15

Se(IV)_45

Se(IV)_135

Se(IV)_405

Se(VI) mg L⁻¹

Se(VI)_15

Se(VI)_45

Se(VI)_135

Se(VI)_405

Roots

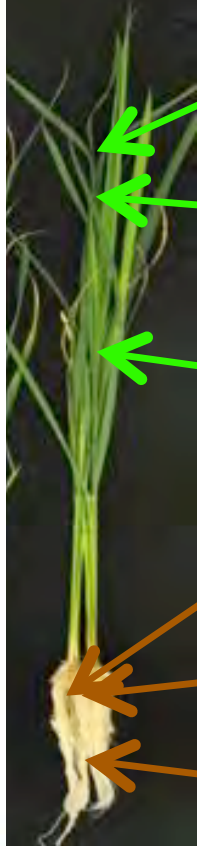
Shoots

Total Se
content

Se
speciation



Total selenium (tSe) content in rice roots and shoots



Shoot length was not affected by Se(IV), while it was always decreased by Se(VI).

tSe content increased in all Se treatments (except for Se(VI)₁₃₅ and Se(VI)₄₀₅)

Se(VI) application causes Se accumulation mainly in shoots

Se(IV) application causes Se accumulation mainly in roots

tSe content increased in all Se treatments.

Root length increased at low level of Se(IV) and Se(VI), while it decreased at higher Se concentrations especially in Se(VI).

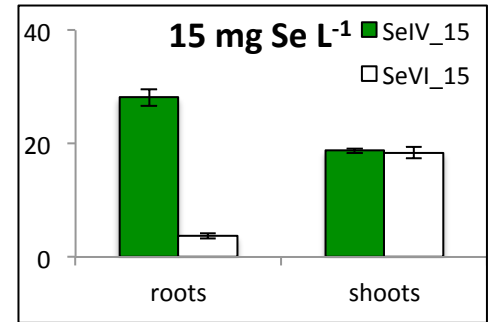
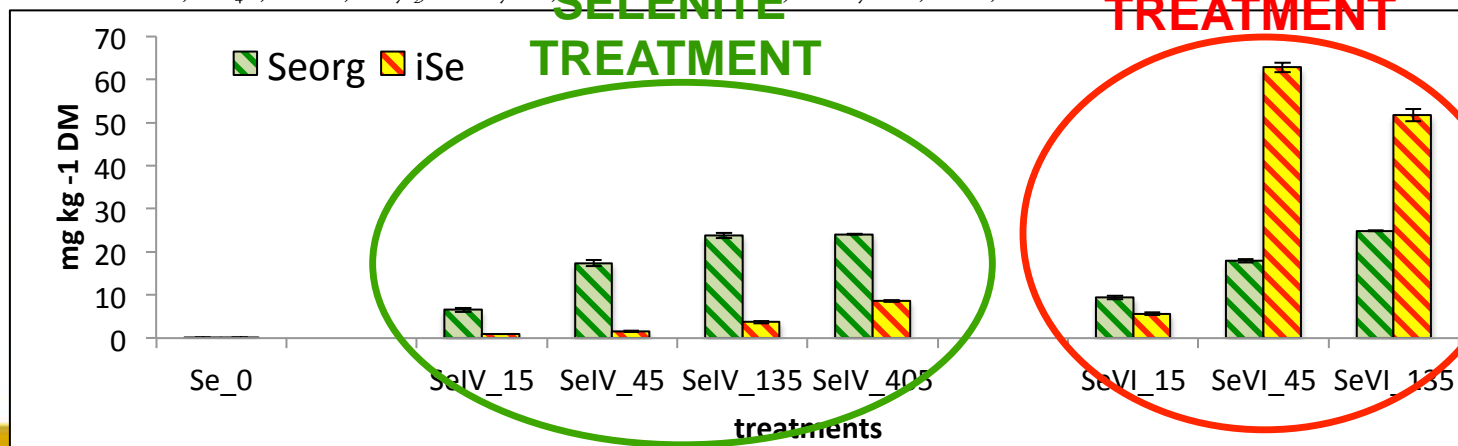




Table 3. Inorganic and Organic Se Species and Protein Content in Shoots of 10-Day Old Rice Sprouts^a

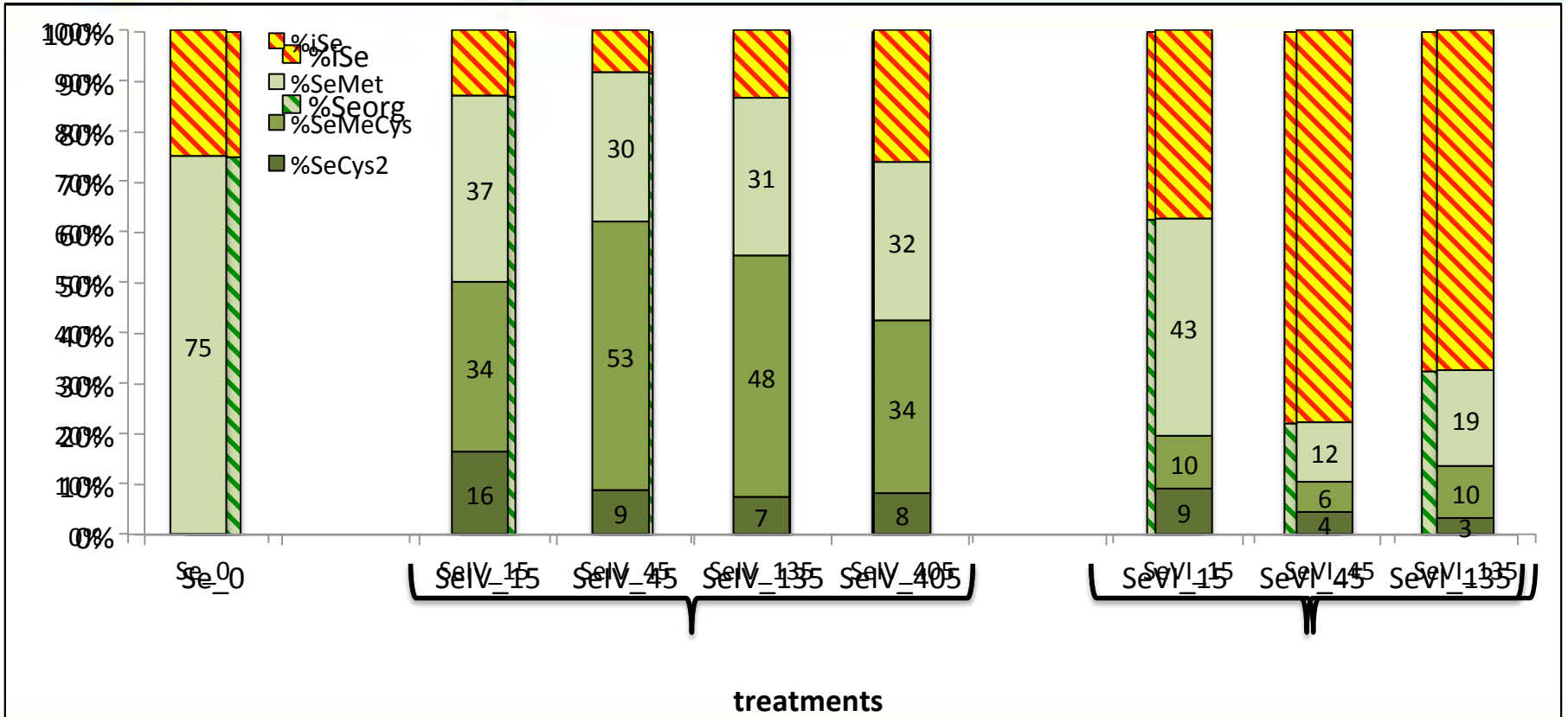
treatments	Se species ($\mu\text{g g}^{-1}$ DM)							proteins (mg BSA g^{-1} DM)
	inorganic species			organic species				
	SeO_3^{2-}	SeO_4^{2-}	total	SeCys ₂	SeMeSeCys	SeMet	total	
Se_0	0.02 (0.01) g	<LOD	0.02 (0.01) h	<LOD	<LOD	0.06 (0.01) h	0.06 (0.01) f	110 (1.75) b
SeIV_15	0.98 (0.03) f	<LOD	0.98 (0.03) g	1.24 (0.20) e	2.55 (0.13) f	2.80 (0.23) g	6.58 (0.50) e	107 (1.65) bc
SeIV_45	1.58 (0.16) e	<LOD	1.58 (0.16) f	1.65 (0.08) d	10.1 (0.28) c	5.62 (0.42) f	17.4 (0.75) c	130 (2.82) a
SeIV_135	3.62 (0.28) d	0.08 (0.01) d	3.70 (0.27) e	2.03 (0.08) c	13.2 (0.9) a	8.62 (0.44) d	23.8 (0.59) b	108 (0.49) bc
SeIV_405	8.49 (0.17) a	0.06 (0.02) de	8.55 (0.18) c	2.63 (0.19) b	11.2 (0.59) b	10.3 (0.45) b	24.1 (0.05) ab	103 (2.16) c
SeVI_15	1.66 (0.20) e	3.95 (0.11) c	5.61 (0.31) d	1.34 (0.09) e	1.57 (0.07) g	6.46 (0.37) e	9.37 (0.45) d	92.3 (1.37) d
SeVI_45	5.64 (0.24) c	57.2 (1.34) a	62.8 (1.10) a	3.55 (0.10) a	4.86 (0.09) e	9.51 (0.24) c	17.9 (0.35) c	78.5 (1.85) e
SeVI_135	7.59 (0.29) b	44.1 (1.54) b	51.7 (1.39) b	2.48 (0.12) b	7.81 (0.13) d	14.6 (0.12) a	24.9 (0.18) a	51.9 (3.32) f

^aObtained with distilled water (Se_0) or at 15, 45, 135, and 405 mg L^{-1} of sodium selenite (SeIV_15, SeIV_45, SeIV_135, and SeIV_405) and 15, 45, 135 mg L^{-1} of sodium selenate (SeVI_15, SeVI_45, SeVI_135). Average values for $n = 3$ independent replicates, each with 3 additional replicates, are reported. Standard deviations in brackets. Different letters within each column indicate statistically significant differences at $p < 0.05$. SeO₃²⁻, selenite; SeO₄²⁻, selenate; SeCys₂, selenocystine; SeMeSeCys, selenomethionine; SeMet, selenomethionine.



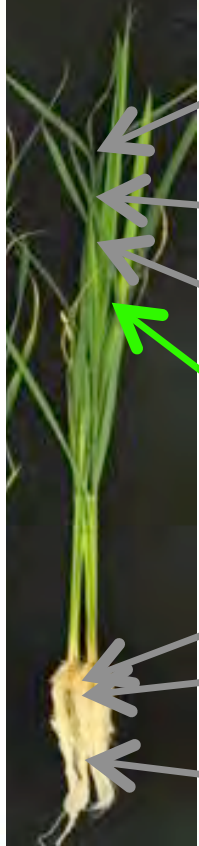


Organic (orgSe) and inorganic (iSe) selenium distribution in shoots (2/2)





Total Se content and speciation in rice roots and shoots



Shoot length was not affected by Se(IV), while it was always decreased by Se(VI).

tSe content increased in all Se treatments (except for Se(VI)₁₃₅ and Se(VI)₄₀₅)

Se(VI) application causes Se accumulation mainly in shoots

Higher values of OrgSe in Se(IV) treatment (SeMet and SeMeCys).
Higher values of iSe in Se (VI) treatment.

Se(IV) application causes Se accumulation mainly in roots

tSe content increased in all Se treatments.

Root length increased at low level of SeIV and SeVI, while it decreased at higher Se concentrations especially in SeVI.



Effects of Selenium on Antimony uptake in rice (*Oryza sativa* L.): speciation analysis

OBJECTIVES:

Investigate uptake and translocation of Sb in rice plants Se-biofortified or not, exposed to Sb(III) and Sb(V) at increased concentrations under hydroponic condition.

Study how Se influences/is influenced by the different Sb concentrations in the distribution of organic (SeCys₂, SeMeSeCys and SeMet) and inorganic forms [Se(IV), Se(VI)] of selenium from roots to shoots.



Sb

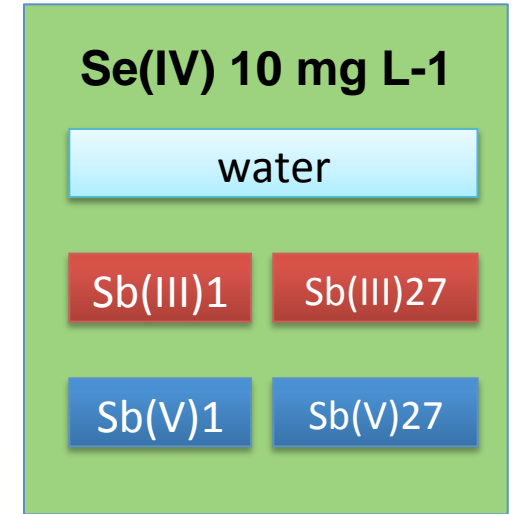
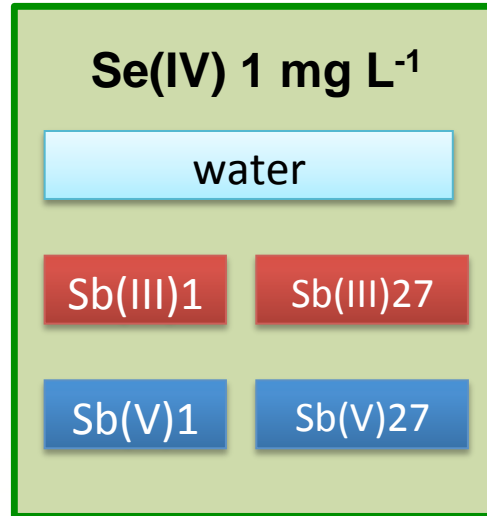
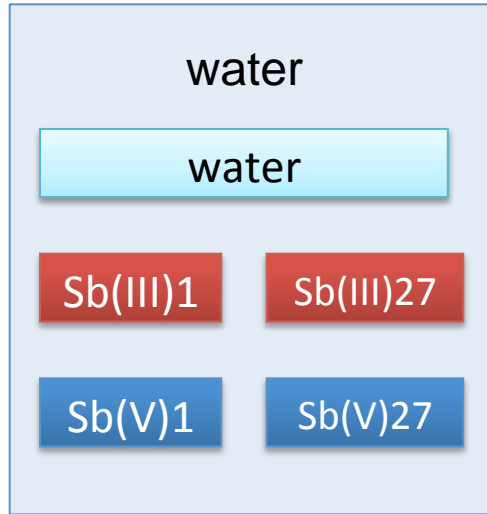


- priority pollutants
- Sb(III) and Sb(V)
- Sb(III) more toxic than Sb(V) species
- Sb(V) has octahedral structure, larger radius ($\text{\AA}=3.68$)
- Sb(V) is the predominant species in soil solution like $\text{Sb}(\text{OH})_6^-$.
- Sb(V) transport requires mediation by transporters
- Sb(III) cross cell membranes passively with water (aquaporins)



Se and Sb experimental design

Rice (*Oryza sativa* L., cv. Selenio)



7 days of Se biofortification

7 days of hydroponic contact of Sb species



Materials and method

Roots Shoots

Total Se, Sb
content

Se and Sb
speciation

0.25 g DM
+8 mL of ultrapure HNO_3 (65%w/w)
+2 mL H_2O_2 (30%w/w)

Microwave
digestion

Dilution with water
up to 20 mL

Filtration

ICP-MS
Octapole Reaction System



0.1 g FM
+4 mL of water
+1,5 mL protease

stirred in water bath
at 37 °C for 4 h

Centrifugation

Supernatant filtration

Ammonium
acetate
(gradient
analysis)

Na_2EDTA
+ KHP
(isocratic
analysis)

HPLC - ICP-MS



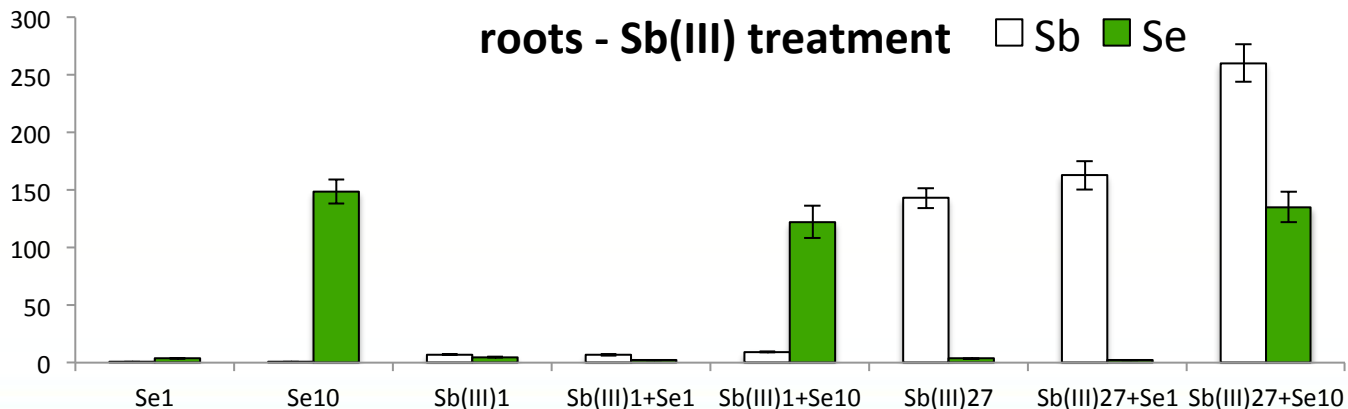
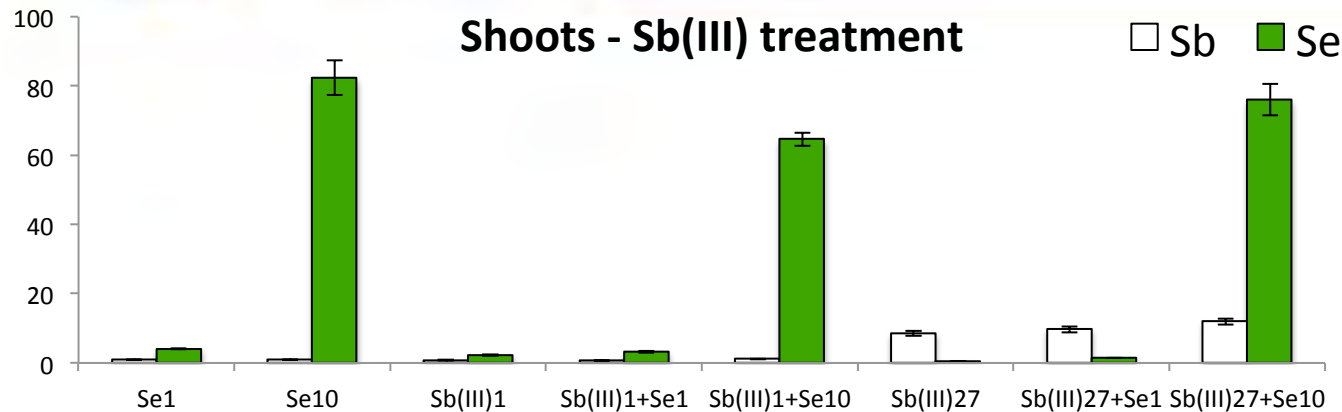


RESULTS

Sb and Se distribution in roots and shoots

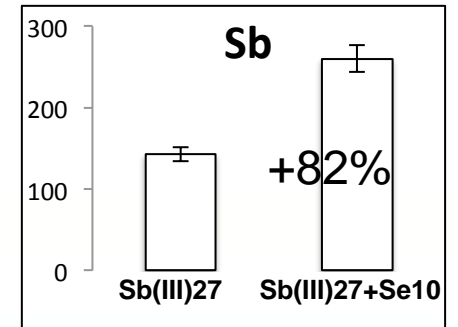
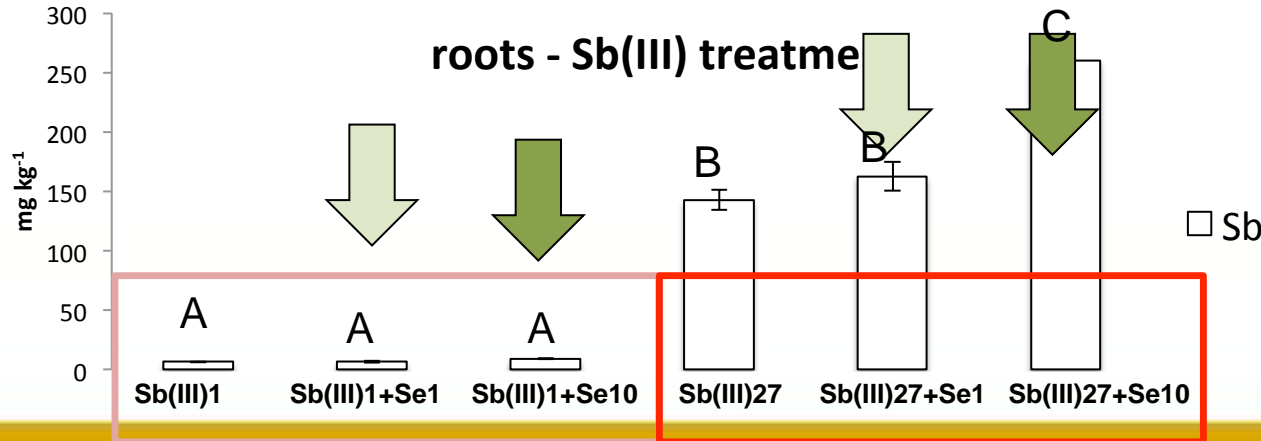
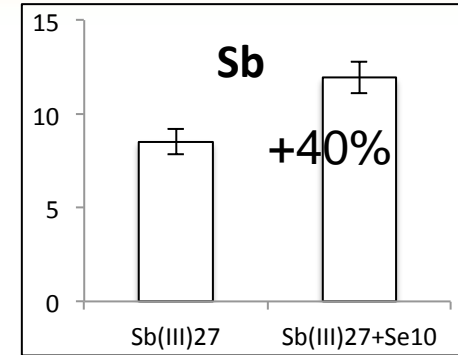
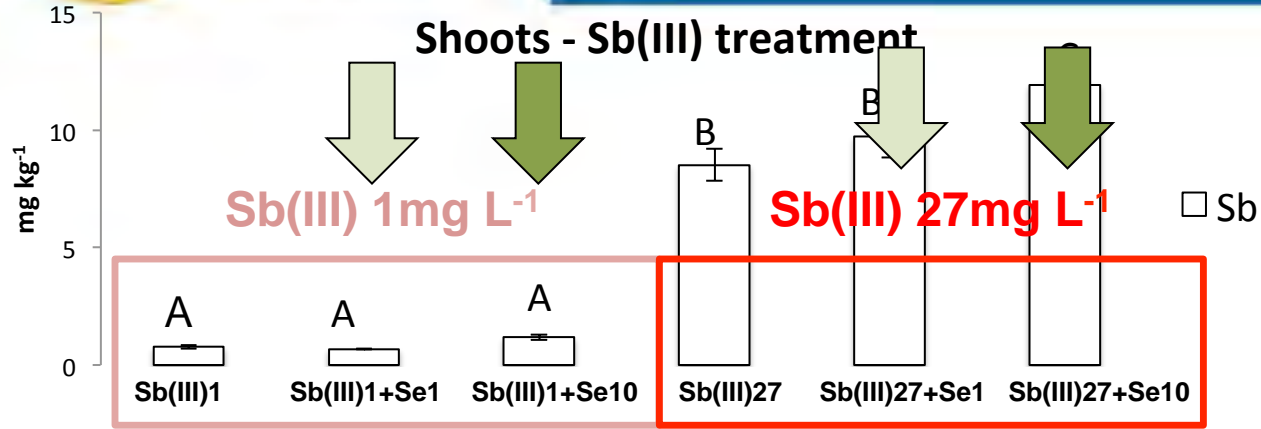


tSb and tSe distribution in roots and shoots (Sb(III)+Se in hydroponic solution)





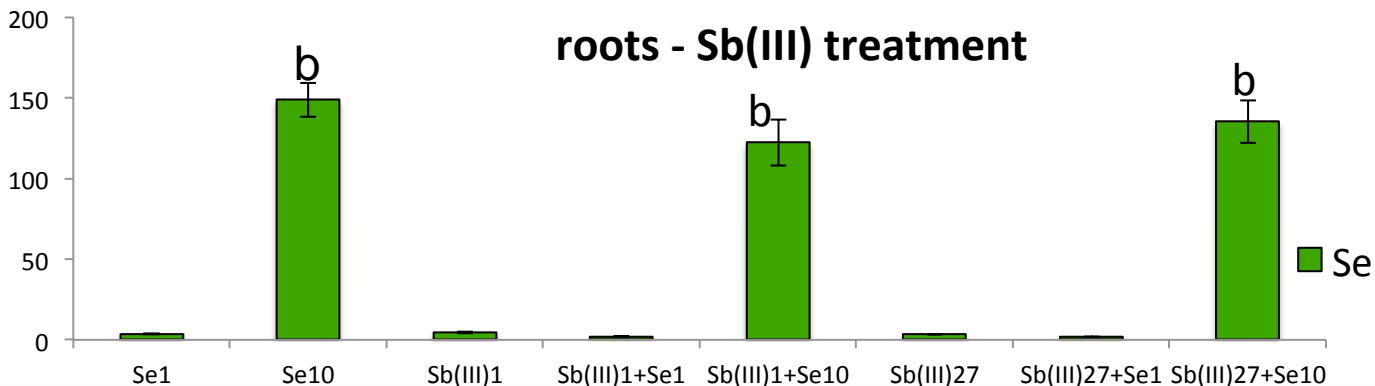
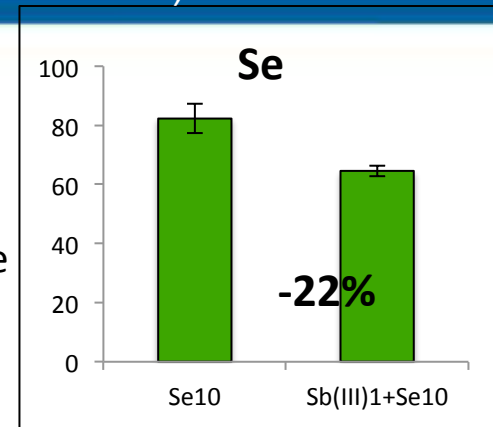
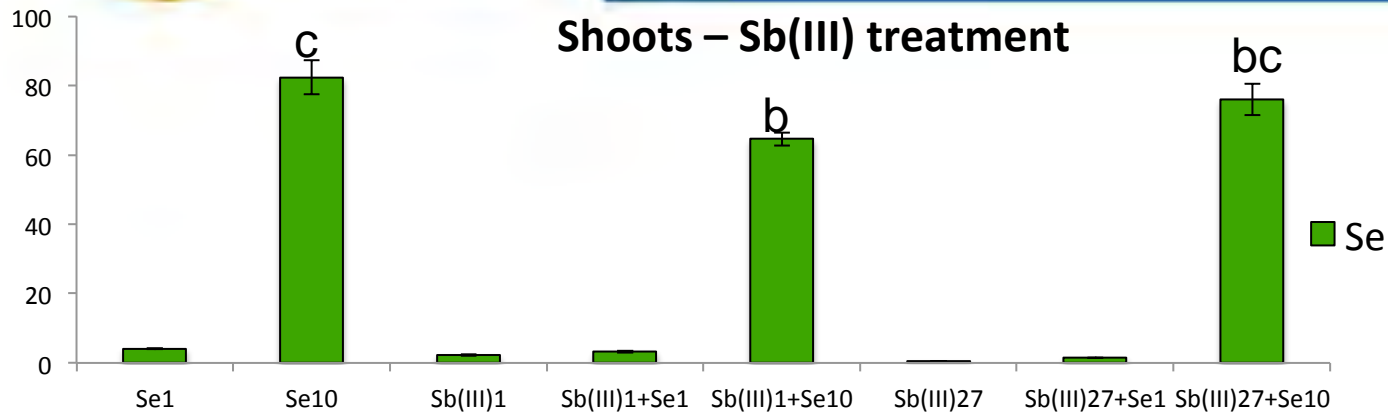
tSb distribution in roots and shoots (Sb(III)+Se in hydroponic solution)



SYNERGIC EFFECT

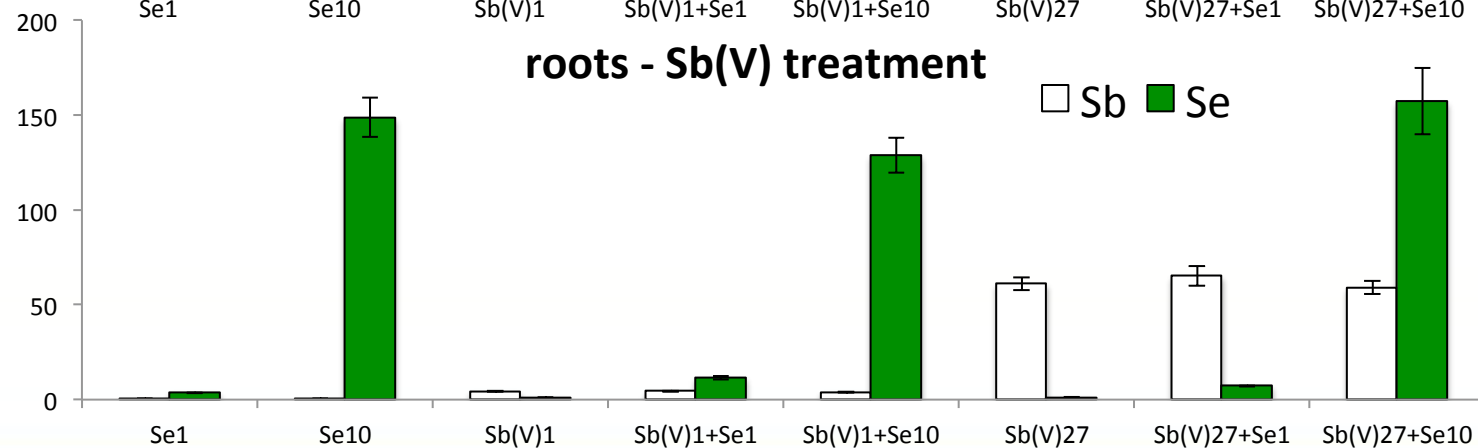
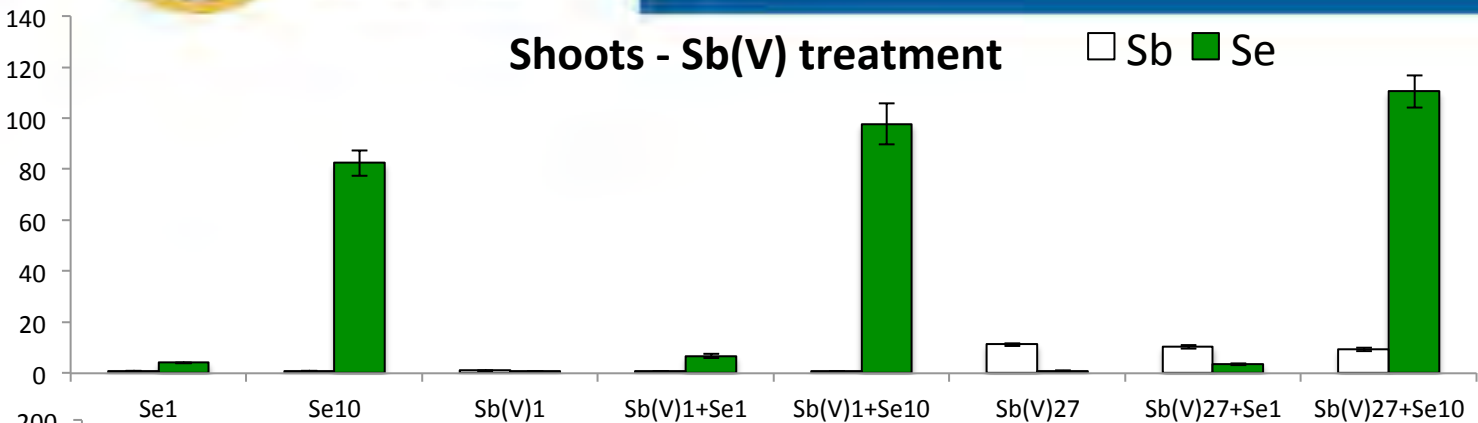


tSe distribution in roots and shoots (Sb(III)+Se in hydroponic solution)



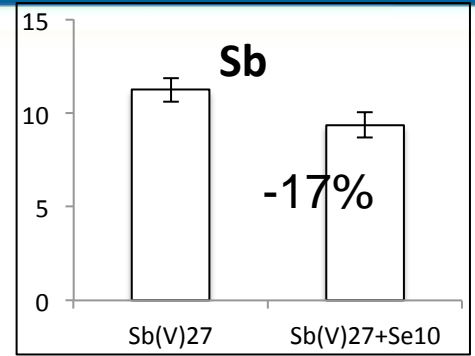
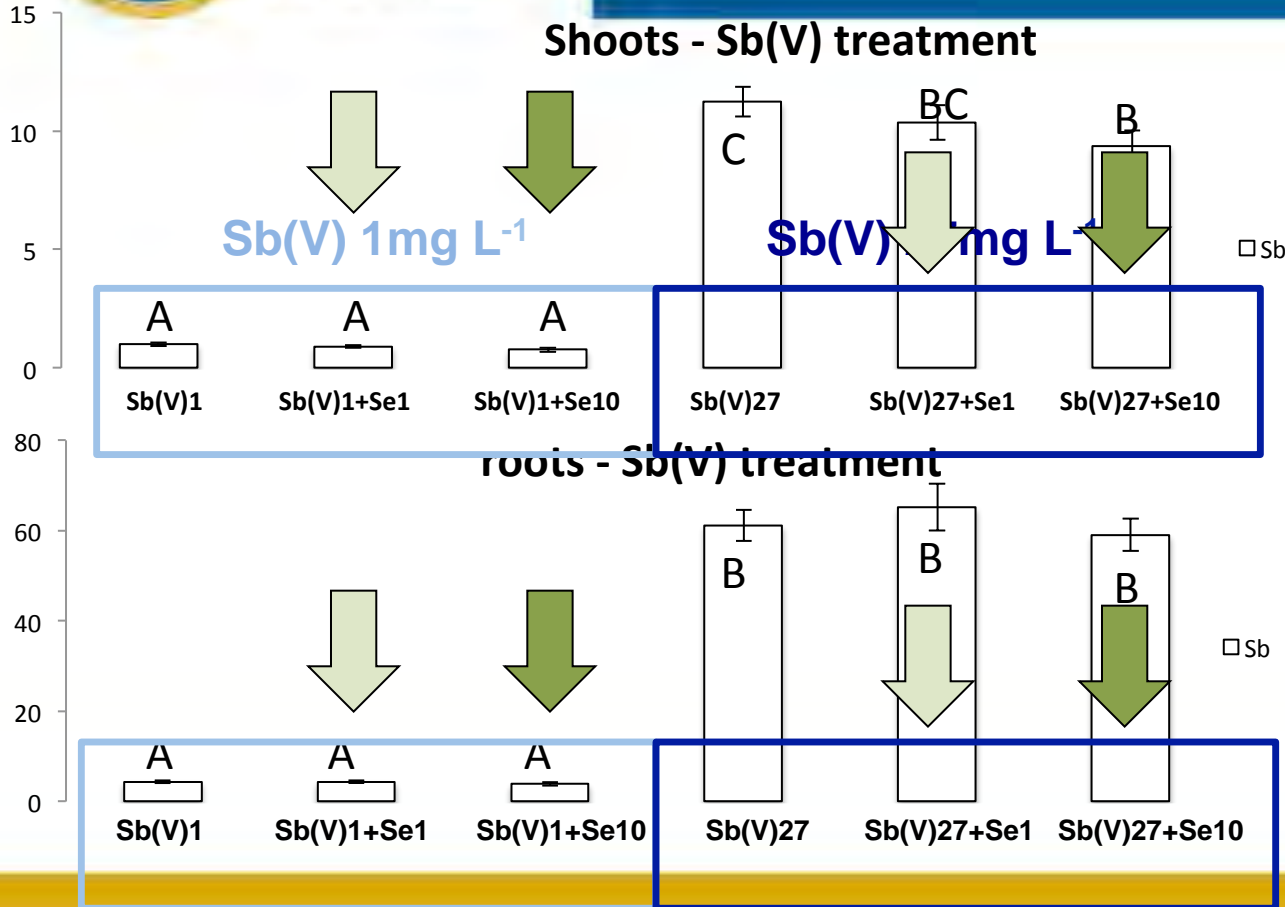


tSb and tSe distribution in roots and shoots (Sb(V)+Se in hydroponic solution)





tSb distribution in roots and shoots (Sb(V)+Se in hydroponic solution)

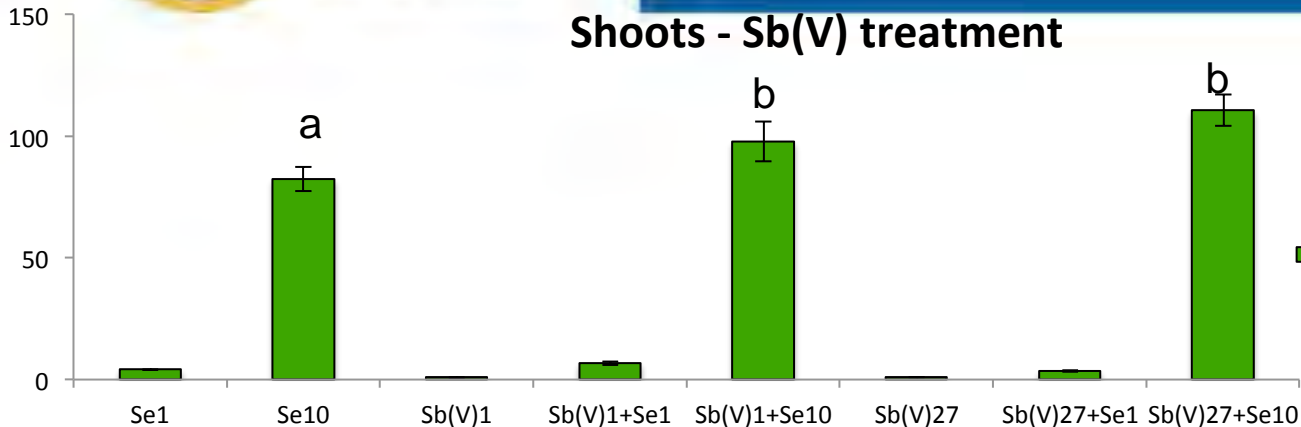


Antagonistic effect

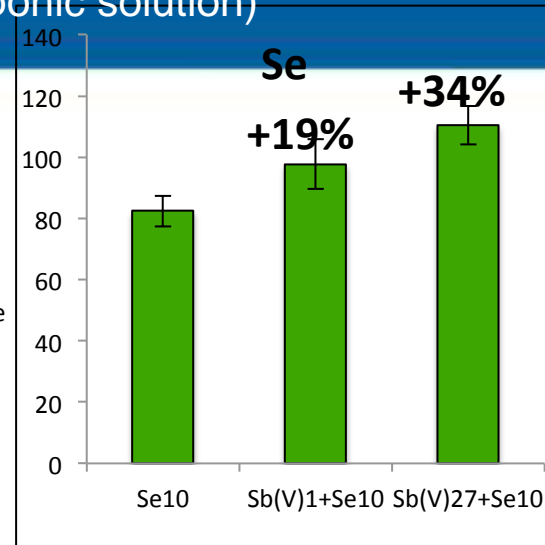
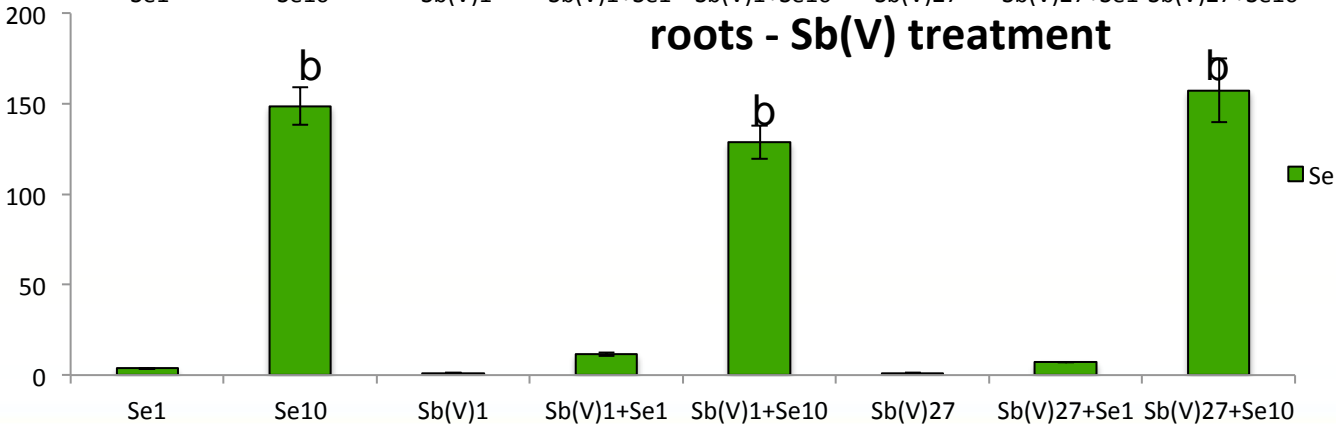


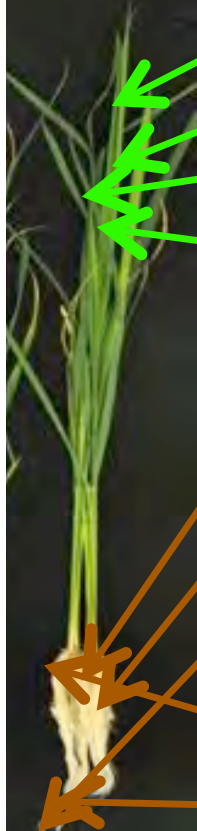
tSe distribution in roots and shoots (Sb(V)+Se in hydroponic solution)

Shoots - Sb(V) treatment



roots - Sb(V) treatment





tSb decreased with high level of Se (Sb(V)27+Se10) (antagonistic effect)

tSb increased with high level of Se (Sb(III)27+Se10) (synergic effect).

tSe increased with low and high Sb(V) levels

tSe decreased at low level of Sb(III)

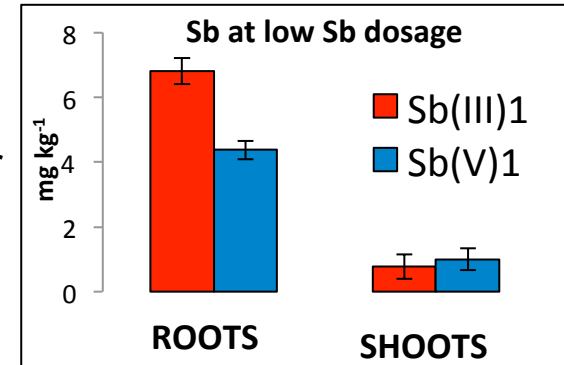
tSb content is higher in roots than shoots under Sb(III) and Sb(V) treatments.

Sb(III) is accumulated more easily than Sb(V)

tSe is higher in roots than shoots under Sb(III) and Sb(V) treatments.

tSb increased with high level of Se (Sb(III)27+Se10) (synergic effect).

Sb(III) and Sb(V) treatments don't influence tSe content





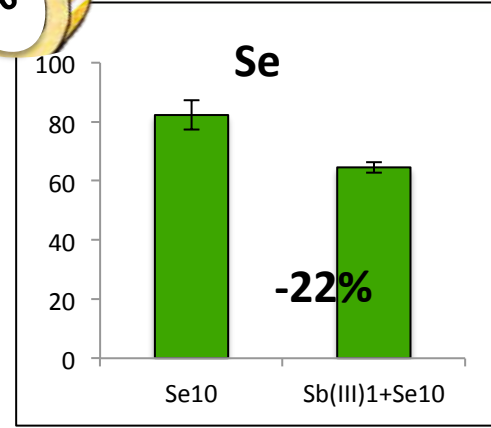
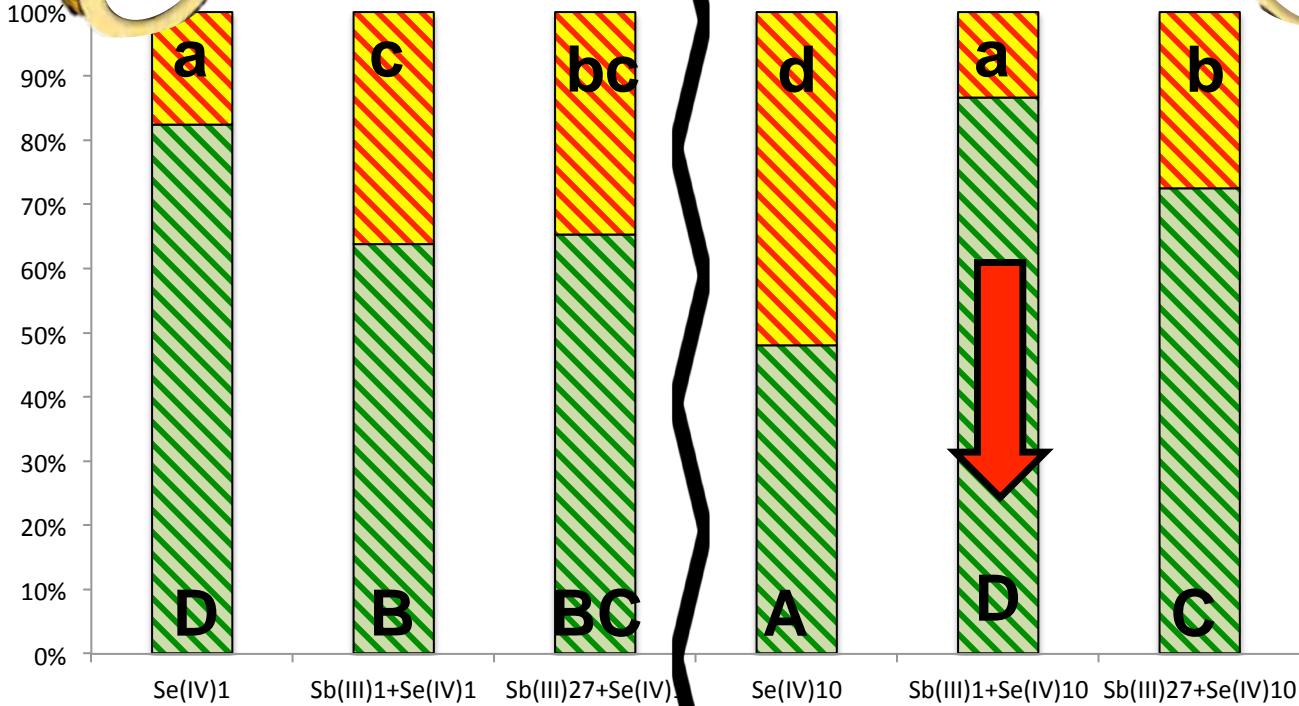
RESULTS

**Organic (orgSe) and inorganic (iSe) selenium
distribution**

Organic (orgSe) and inorganic (iSe) selenium distribution



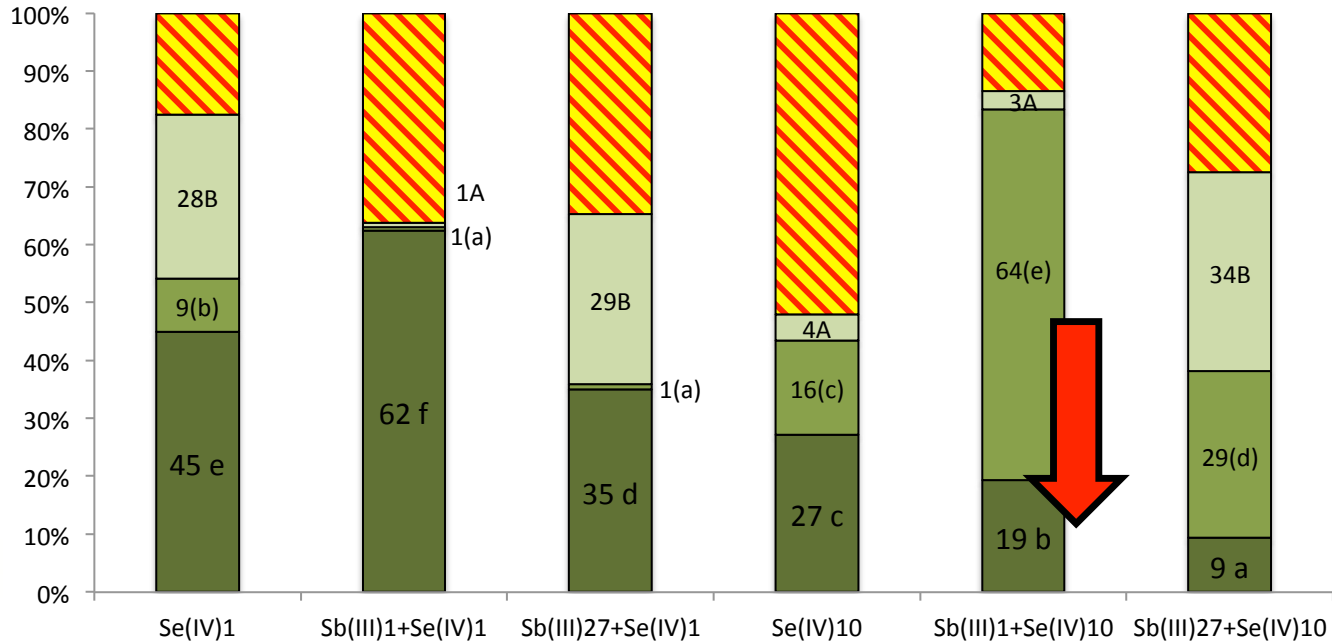
SHOOTS - Sb(III) treatment





SHOOTS – Sb(III) treatment

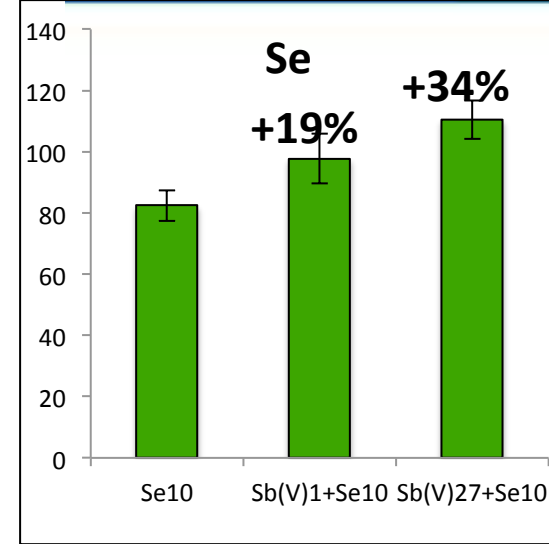
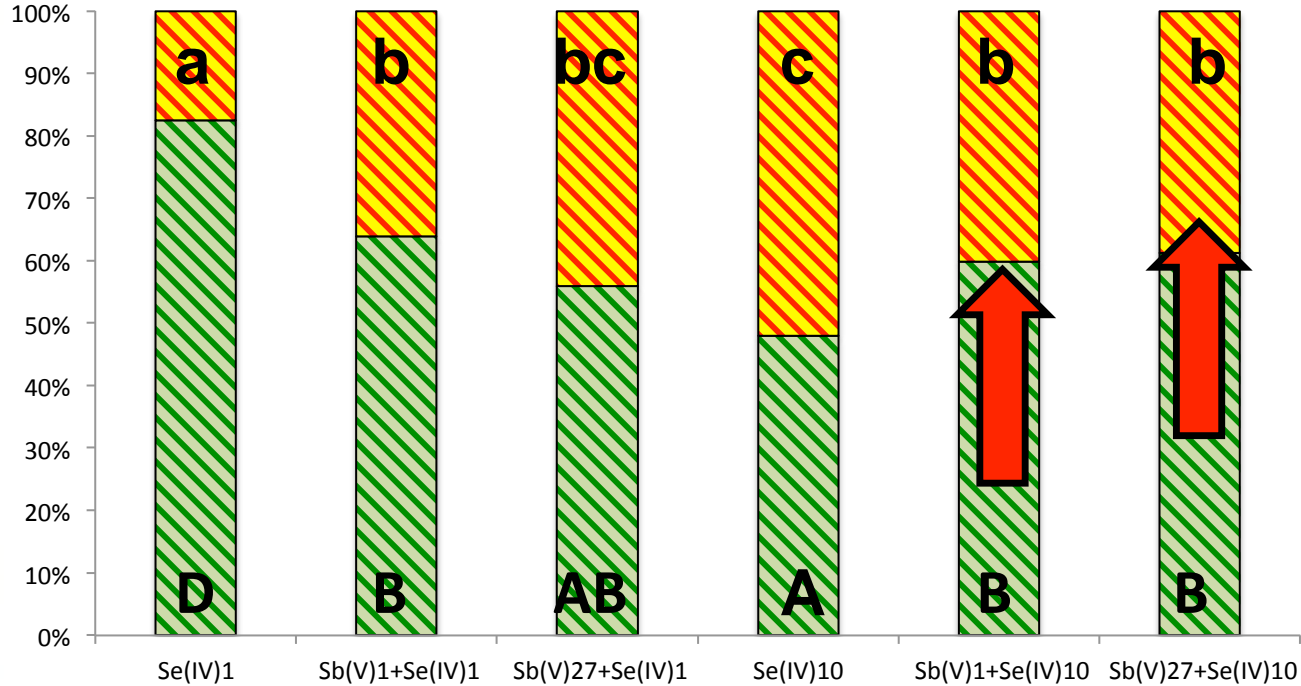
■ %iSe ■ %Met ■ %MeSeCis ■ %Cys





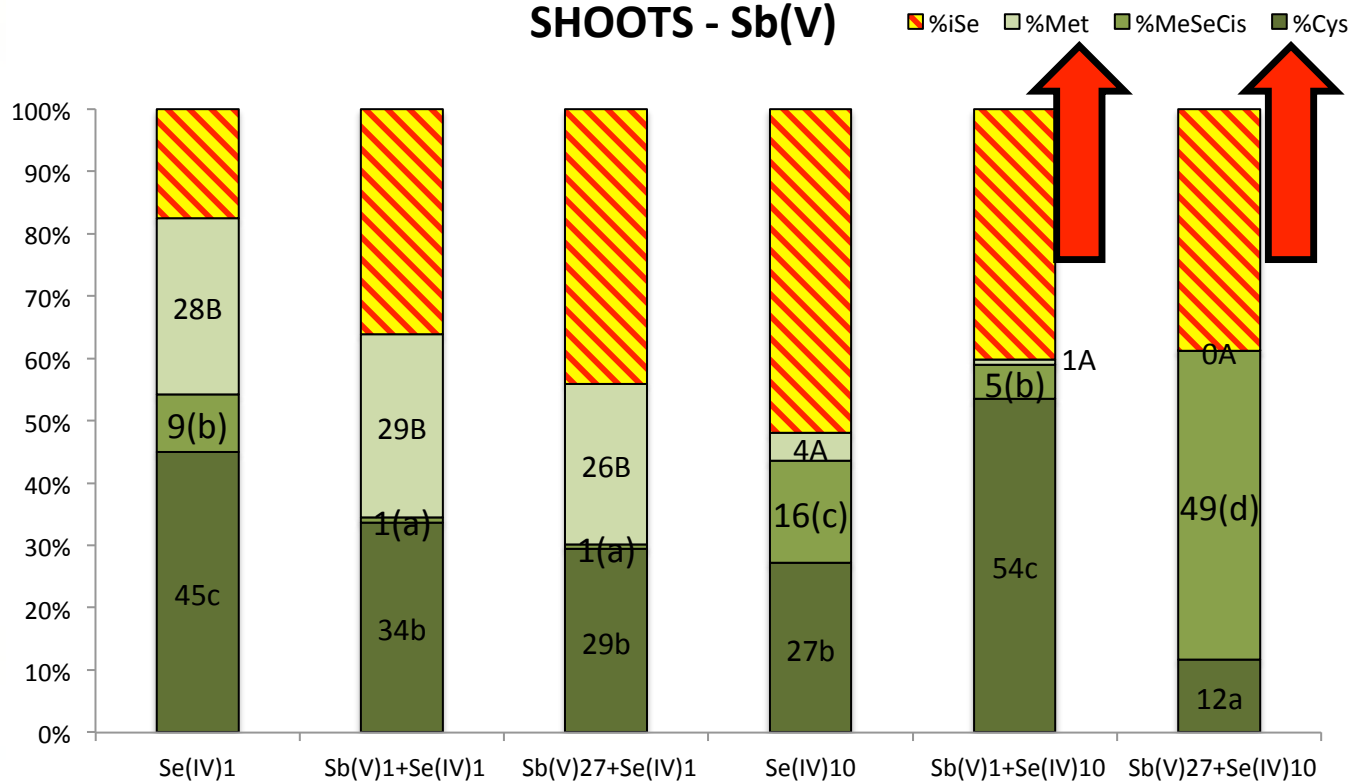
SHOOTS - Sb(V) treatment

■ % iSe ■ % orgSe





SHOOTS - Sb(V)





CONCLUSIONS

- Sb and Se are more stored in roots than in shoots
- Synergic effect of Se on Sb(III) in roots and shoots
- Antagonistic effect of Se on Sb(V) in shoots
- Sb treatments exhibit consequences on Se contents in shoots (negative with Sb(III) and positive with Sb(V))



Future perspectives

Improving knowledge about plant behaviour of other rice varieties

Deepening Sb influences on rice seedling in soil plots, until grains production.

Instrumental upgrade (different nebulizer for sensitivity improves, UHPLC-triple quad-ICP MS)

Thank You!

