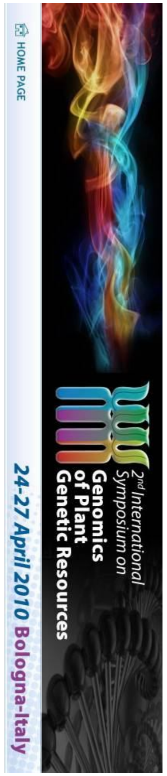
Effects of *At*FRO2 expression in the **nutritional enhancement of soybean (*Glycine max.* L)**



Marta W. Vasconcelos

April 27th 2010

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Nutrition

“the science of food and it´s relation with health”

Macronutrients

carbohydrates, proteins, fats, macro minerals and water

Micronutrients

vitamins and trace elements

Micronutrient Malnutrition

- Iron
- Zinc
- Iodine
- Vitamin A

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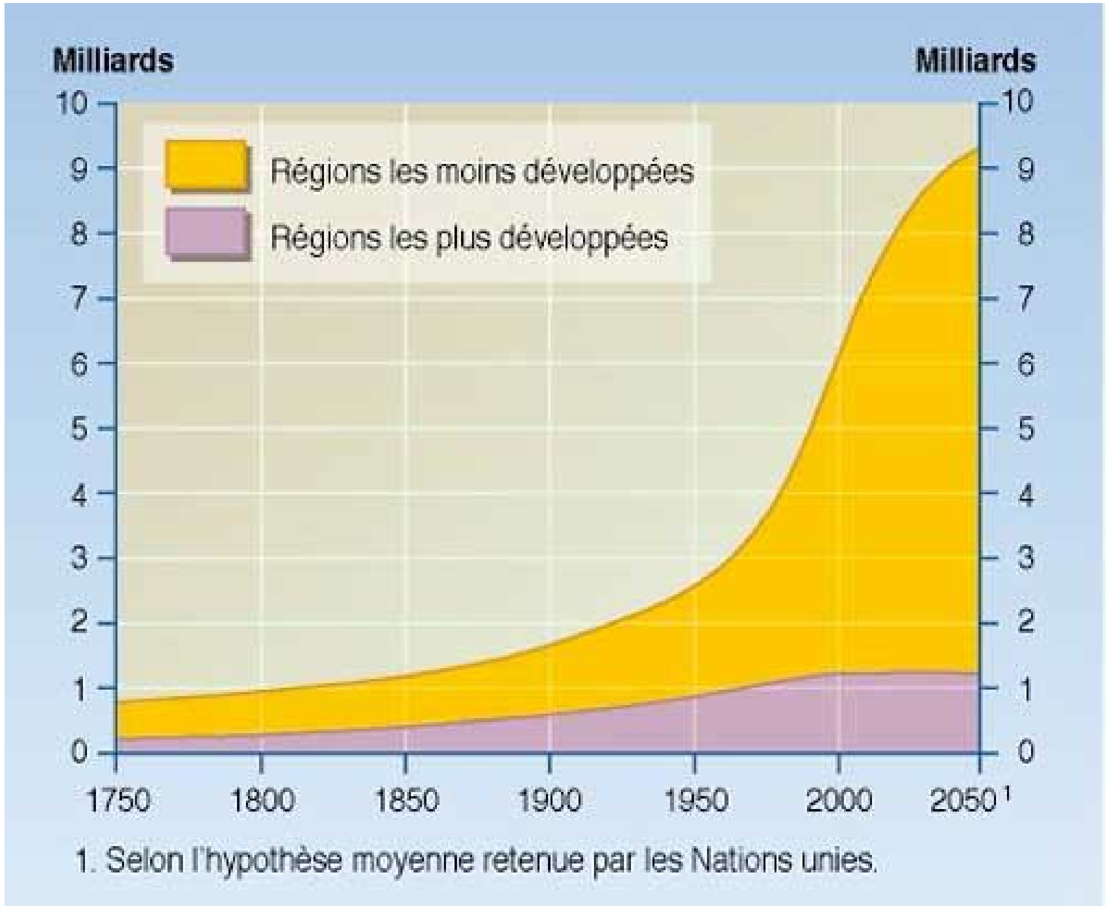


Source : *World population 1998, The World at Six Billion* (octobre 1999) et *World Population Prospects: The 2000 Revision*, (février 2001), Nations unies, Département des affaires écono-miques et sociales, Division de la population, New York.

The best way to avoid micronutrient deficiencies is by way of a varied diet, rich in vegetables, fruits and animal products

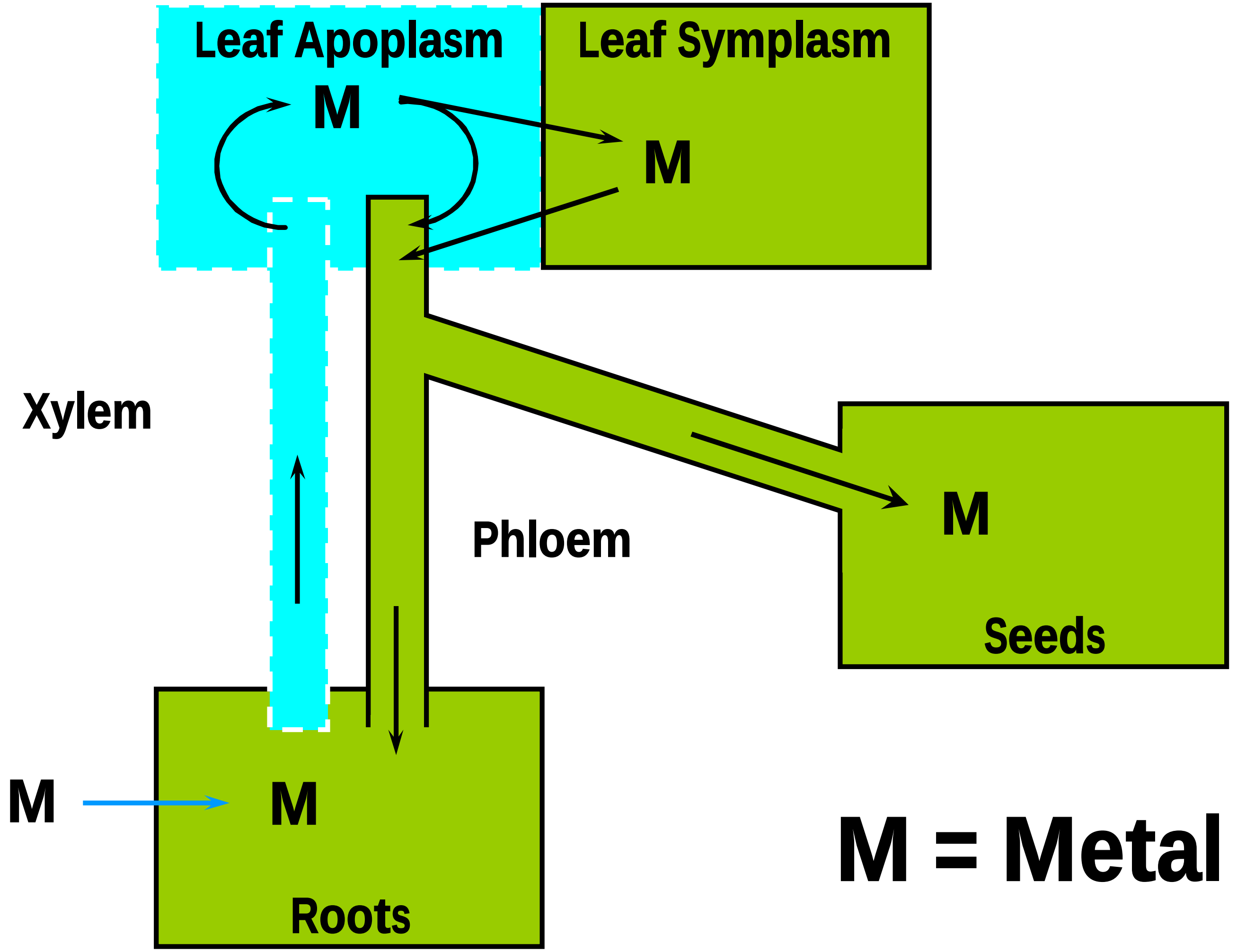
The second best approach, especially for those who cannot afford a varied diet, is by way of nutrient-dense staple crops

"Biofortification"

A sustainable strategy to engineer and/or breed for staple crops with elevated Fe, Zn and pro-vitamin A concentrations.

Minerals Must be Acquired from the Soil Environment and then Transported to and Packaged Within the Edible Tissues.

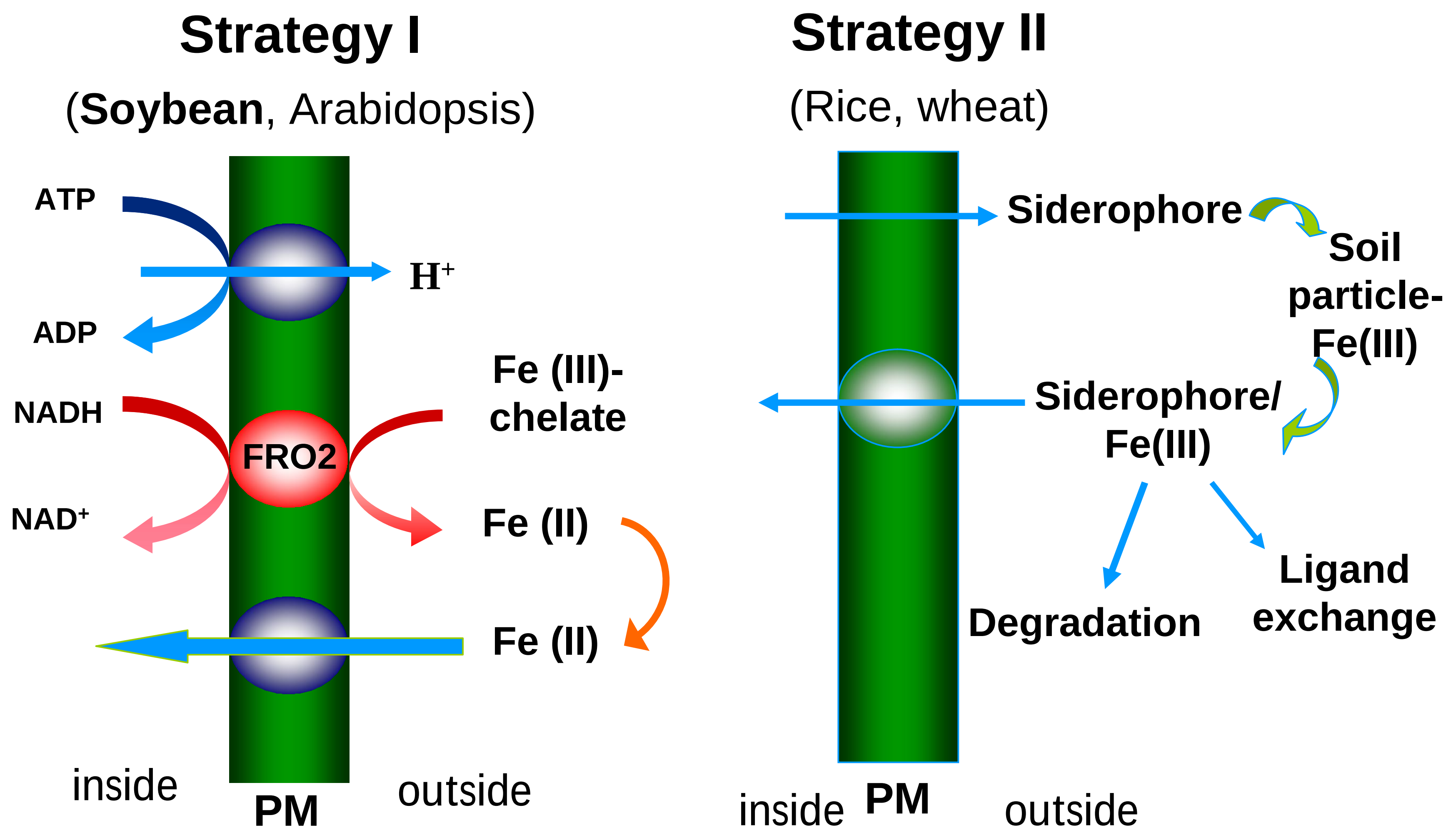
Pathways for Whole-Plant Metal Transport



Important Gene Groups for Metals in Plants

- Biosynthetic genes
  - Phytosiderophores, Nicotianamine
- Divalent metal transporters
  - ZIPs, NRAMPs, CE (cation efflux) family, etc.
- Metal-chelate transporters
  - YSL (Yellow-Stripe Like) family members
- Metal storage
  - Ferritin

Iron absorption mechanisms in plants



Soybean (*Glycine max.*)

First grown as a crop in China 5000 years ago (one of the 5 sacred plants-rice, wheat, barley and millet)

First shipment to the US: 1804

First commercial crop: planted in 1929

Now: essential source of protein and oil with a multitude of uses in both human food and animal feeds, with numerous industrial applications

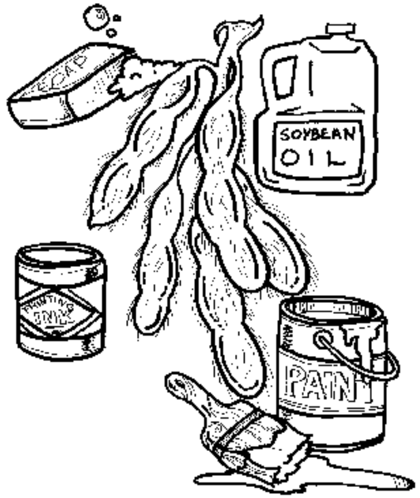
The many uses of soybean

Edible uses

- Seed
- Tofu
- Miso
- Flour
- Bread
- Candy
- Crackers
- Coffee
- Butter
- Milk
- Sprouts
- Cooking oil

Not-so edible uses

- Biodiesel
- Textiles
- Crayons
- Plastics
- Paint
- Insecticide
- Paper
- Rubber
- Cosmetics
- Electrical insulation
- Soap/Shampoos



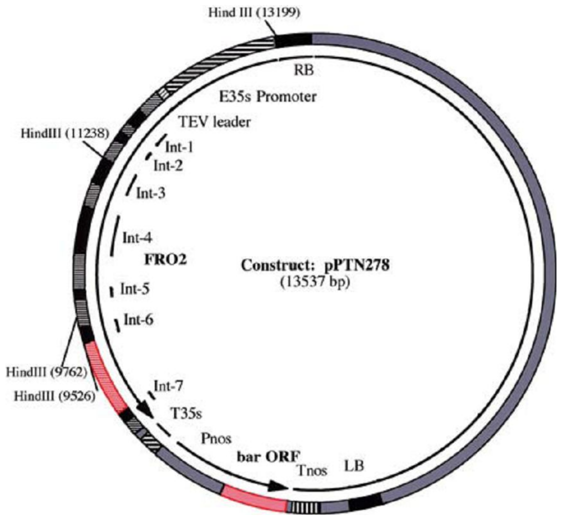
The problems

1. Limited growth in iron deficient soils
2. Low iron absorption limits amount that can be transported to the seeds (edible portion)

Materials

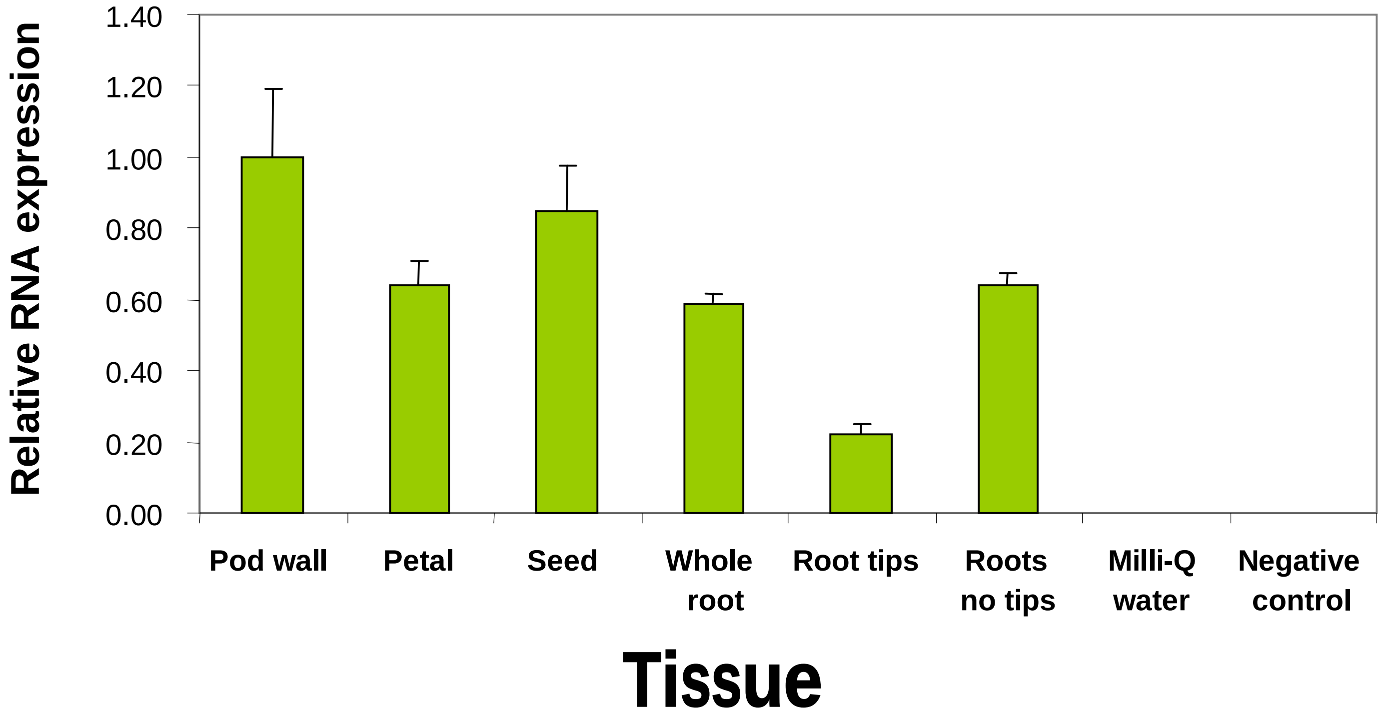
Agrobacterium transformation of soybean with *AtFRO2* gene

38 transgenic lines with ***AtFRO2 (2 parental lines)***



Vasconcelos *et al* Planta (2006) 224:1116–1128

AtFRO2 expression in different tissues



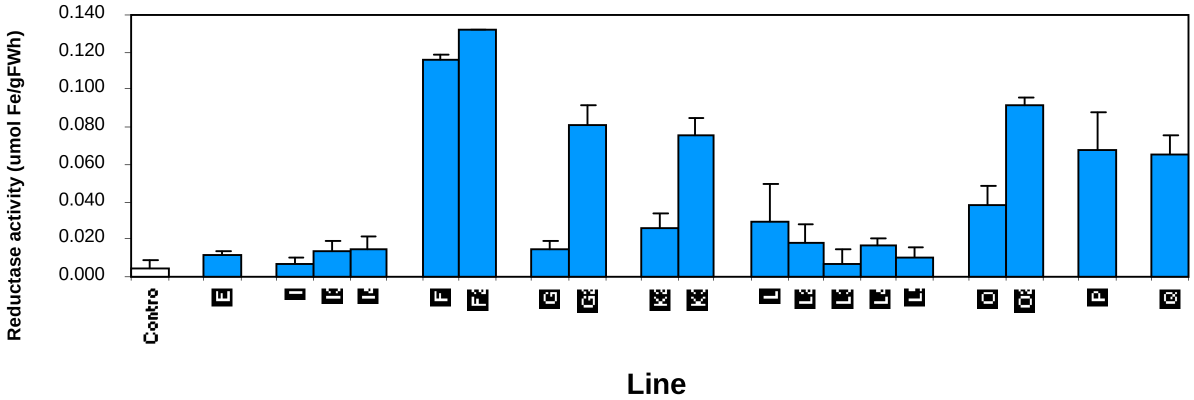
Soluble assay for quantification of reductase activity

Reductase:

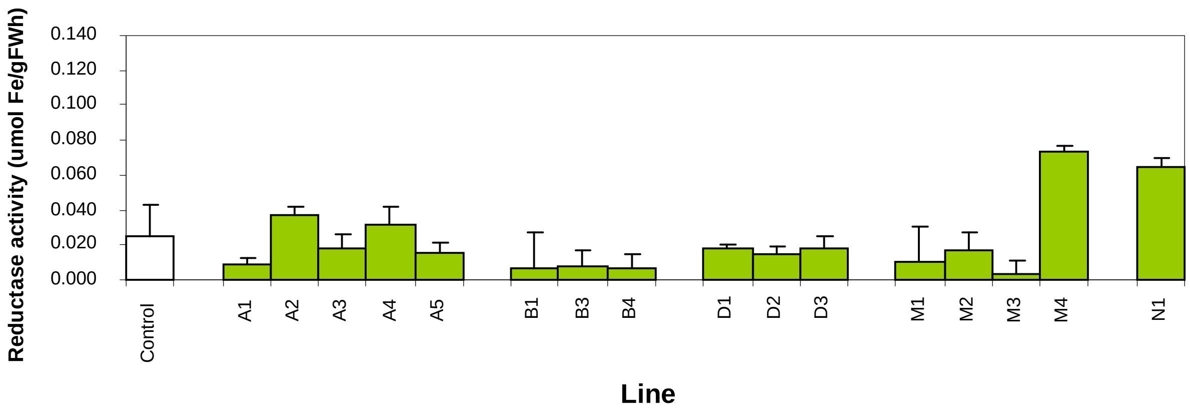
$$\frac{\text{Av. Abs}_{535} \text{ x Vol. Sol/1000}}{\text{0.02214 x Root FW x Assay min/60}} = \mu\text{mol Fe/ g FW h}$$

Iron reductase activity

392-3 line: 23 X higher activity vs. control



Parental line Thorne



Parental line A32

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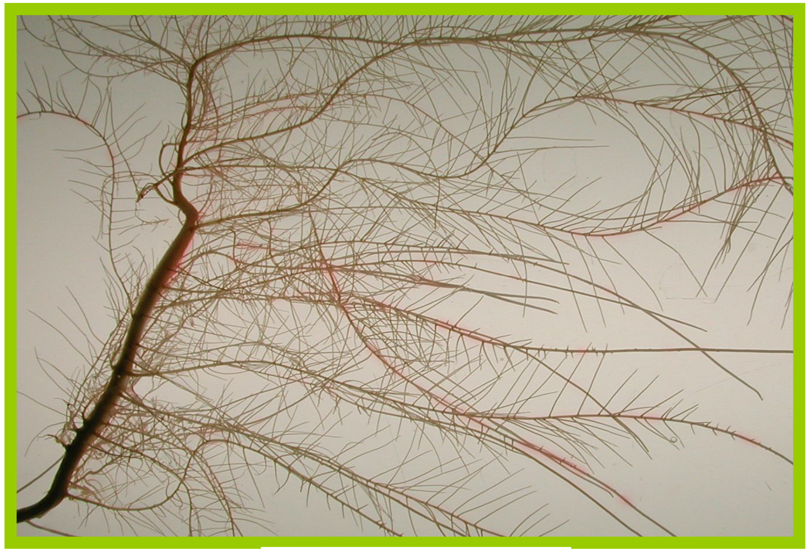
Localization of reductase activity

392-3

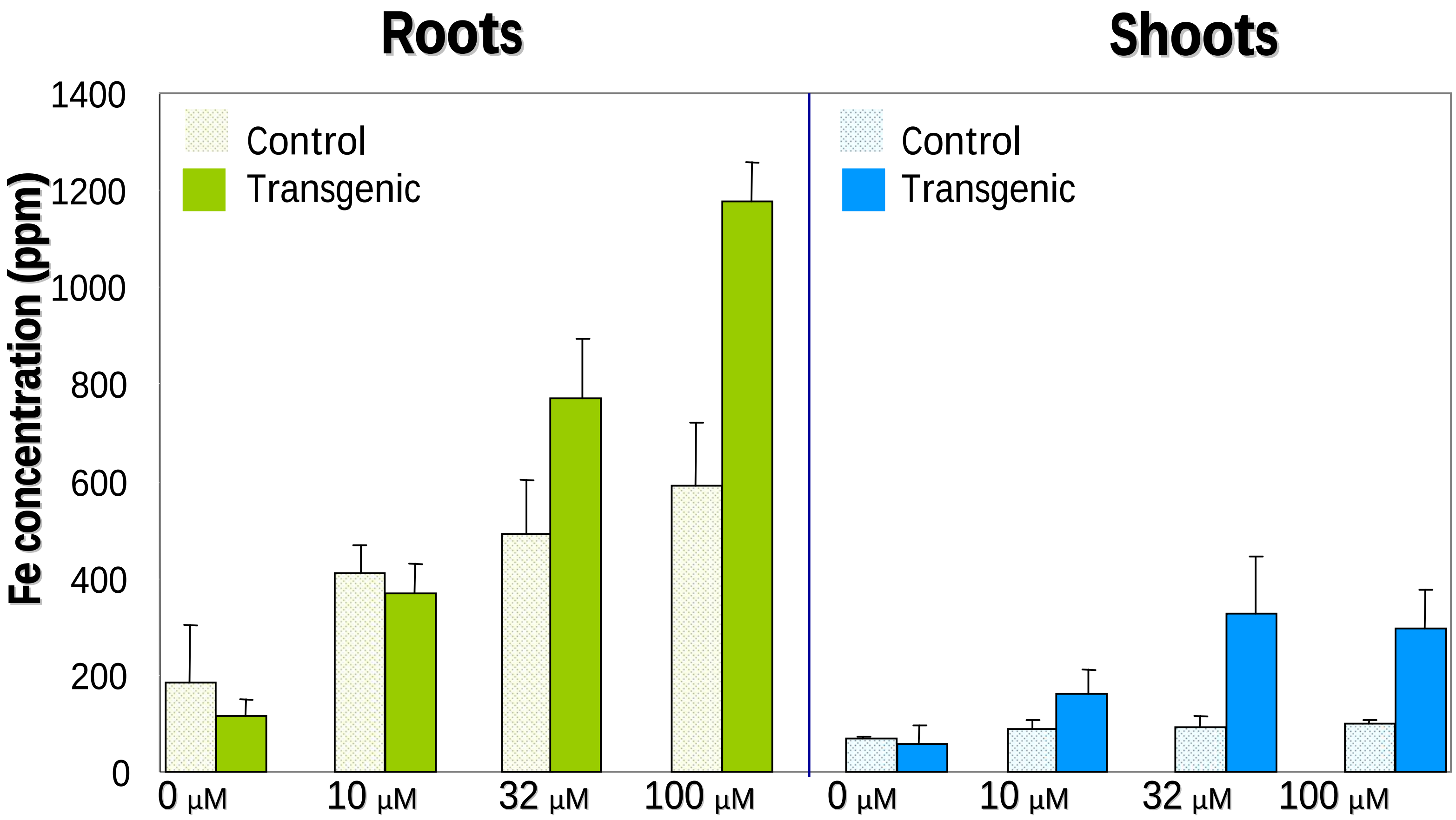


Control

Xylem nutrients

Mineral (µM)	*392-3*	*Wild-type*
Fe	98±5	86 ± 20
Mn	2.9 ± 0.5**	0.9 ± 0.3
K	9606 ± 560*	7395 ± 463
P	1303 ± 165*	596 ± 75
Zn	178.05 ± 34*	88.54 ± 8

Fe concentration at 14d



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Effects of *At*FRO2 expression in the **nutritional enhancement of soybean (*Glycine max.* L)**

Micronutrients

Mineral (µg.g-1)	Shoots		Roots		Stems	
	392-3	*Wt*	*392-3*	*Wt*	*392-3*	*Wt*
Fe	**766 ± 31****	196 ± 8	**582 ± 25****	201 ± 11	**94± 6****	41 ±3
Mn	**188 ± 8****	65 ± 2	**258 ± 13****	122 ± 10	**258 ± 13****	122 ± 10
K	**30575±1306***	27244 ± 389	**51266± 2875***	46430± 7979	**46004 ±2467***	33692± 2556
P	**6912 ± 105***	4658 ± 34	**17490 ± 880***	12284 ± 738	**6106 ± 393***	4558 ± 195
Zn	**283 ± 6****	136 ± 3	**346 ± 11****	100 ± 4	**149 ± 9****	56 ± 3

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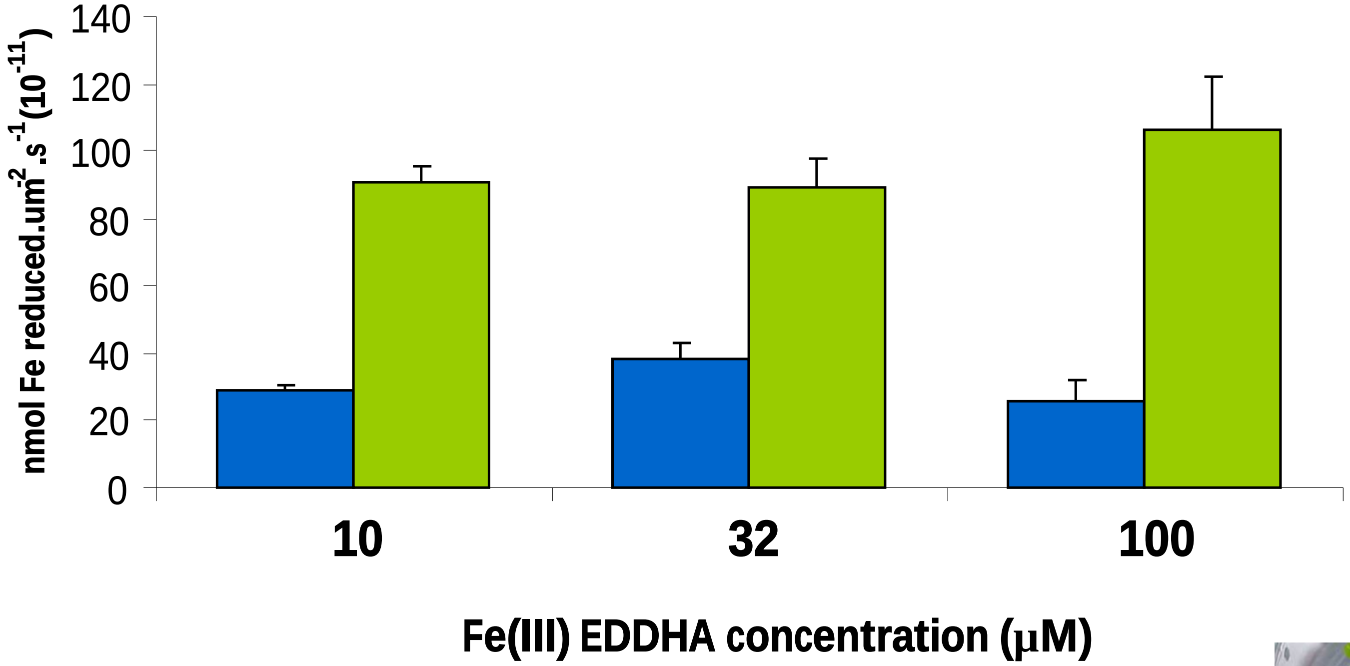
Collection stage	Fe (µM)	Shoot Wt	Shoot 392-3	Pericarp Wt	Pericarp 392-3	Seeds Wt	Seeds 392-3
I	10	**241** ± **13**	379 ± 32**	59 ± 7	69 ± 2	90 ± 2	94 ± 2
	32	**260** ± **14**	287 ± 35	74 ± 6	61 ± 2	93 ± 1	104 ± 2**
	100	**430** ± **34**	734 ± 106*	58 ± 4	125 ± 3***	105 ± 7	116 ± 1**
II	10	**291** ± **8**	308 ± 16	58 ± 2	58 ± 4	93 ± 2	118 ± 4**
	32	**234** ± **15**	308 ± 39*	65 ± 6	53 ± 2	94 ± 3	104 ± 3**
	100	**193** ± **6**	1073 ± 24***	54 ± 4	135 ± 5***	89 ± 1	111 ± 5**
III	10	**126** ± **2**	147 ± 19	38 ± 1	52 ± 2***	92 ± 1	112 ± 4**
	32	**153** ± **3**	294 ± 14***	39 ± 1	63 ± 1***	103 ± 1	110 ± 3**
	100	**435** ± **18**	1142 ± 69***	49 ± 1	120 ± 4***	96 ± 2	112 ± 3**

FC-R activity measured *in vivo* in protoplasts



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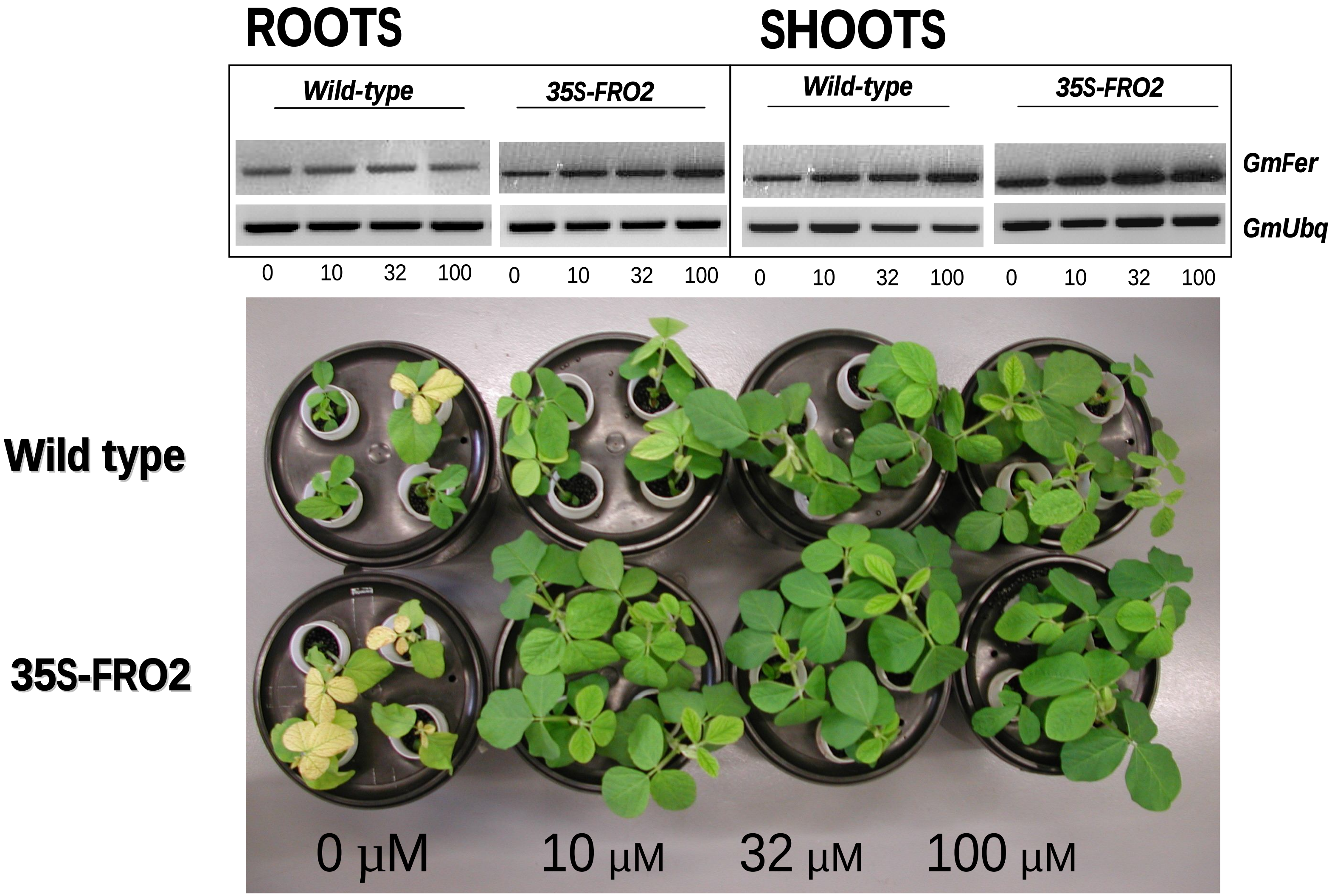
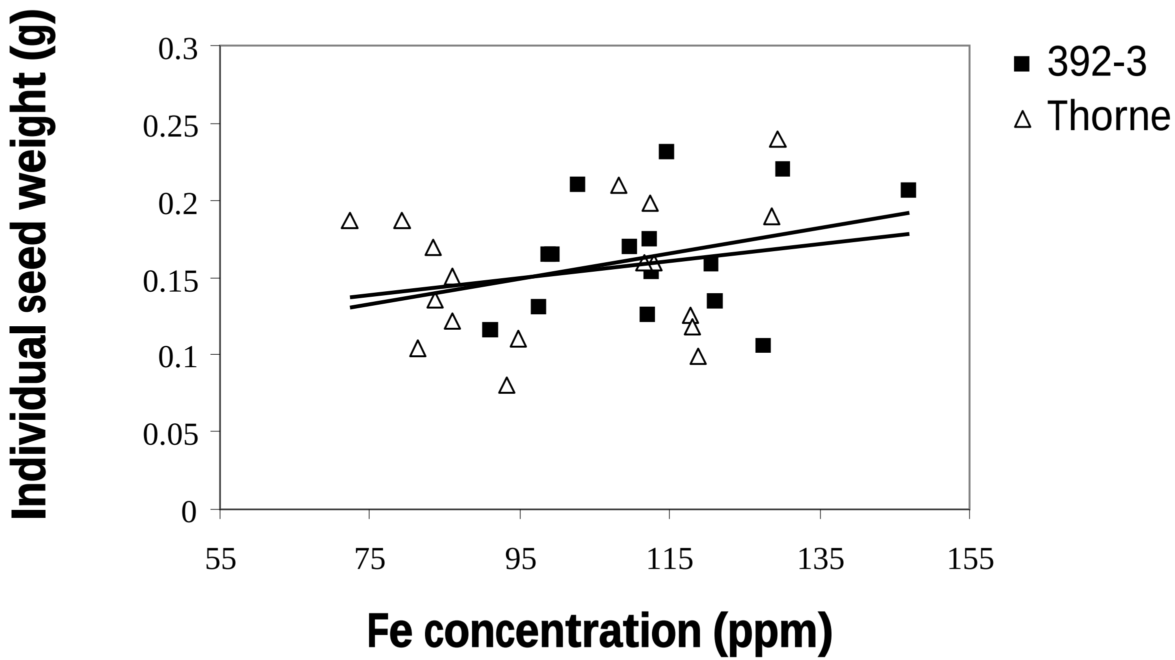


Fe concentration vs. transported dry matter



CONCLUSIONS

- Seed iron levels increased by 10%
- Leaf, pericarp and pod wall iron concentrations increased
- Protoplasts from transgenic leaves had three (3)-fold higher reductase activity
- *Ferritin* expression levels were higher in transgenic leaves
- Mn, K, P, Zn: significantly higher concentrations in leaves, pod walls, roots and xylem sap
- Zn concentrations were also higher in the transgenic seeds
- Ubiquitous role of the iron reductase in plant mineral dynamics?
- Biofortification tool for green leafy vegetables?

Acknowledgments

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THANK YOU FOR YOUR ATTENTION