Proteomic and Metabolomic Analysis of Manganese Sensitivity and Tolerance in the Tropical Legume Cowpea (*Vigna unguiculata* L.)

Hendrik Führs
Institute for Plant Nutrition
Faculty of Natural Sciences
Leibniz University Hannover

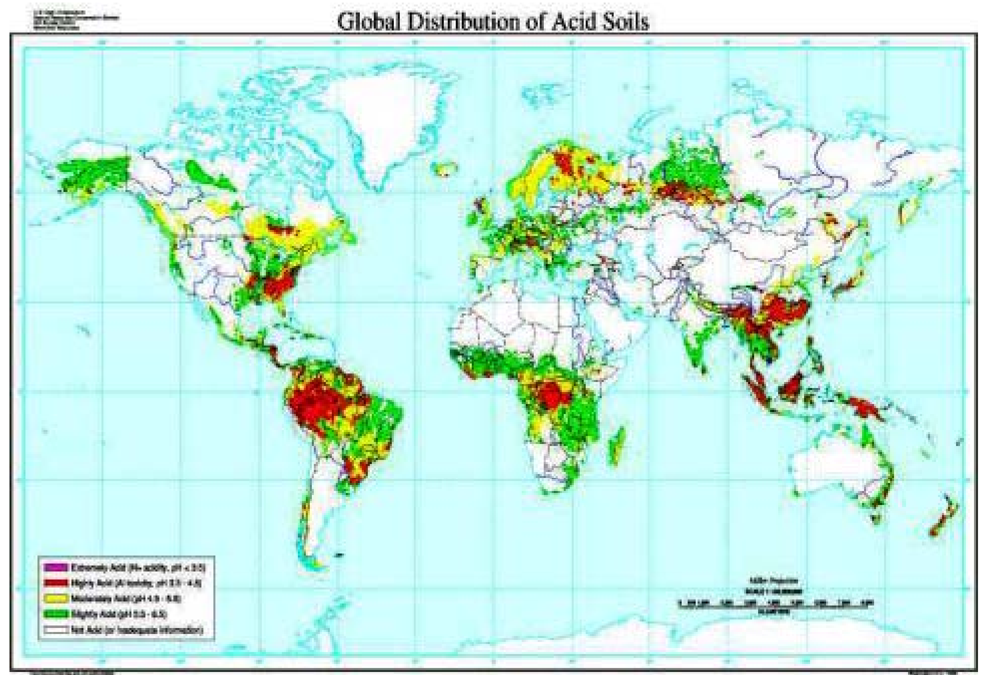
Manganese (Mn) availability

- **essential micronutrient**
- **plant availability dependent on pH und redox potential**

$$MnO_2 + 4H^+ + 2e^- \leftrightarrow Mn^{2+} + 2H_2O$$

→ **increased plant Mn availability**

  - **acid, insufficiently drained soils**
  - **low redox potential**
  - **typical for tropics and subtropics**



www.cimmyt.org

Mn toxicity symptoms

- starting on older leaves
- brown spots
- chlorosis
- necrosis
- leaf shedding

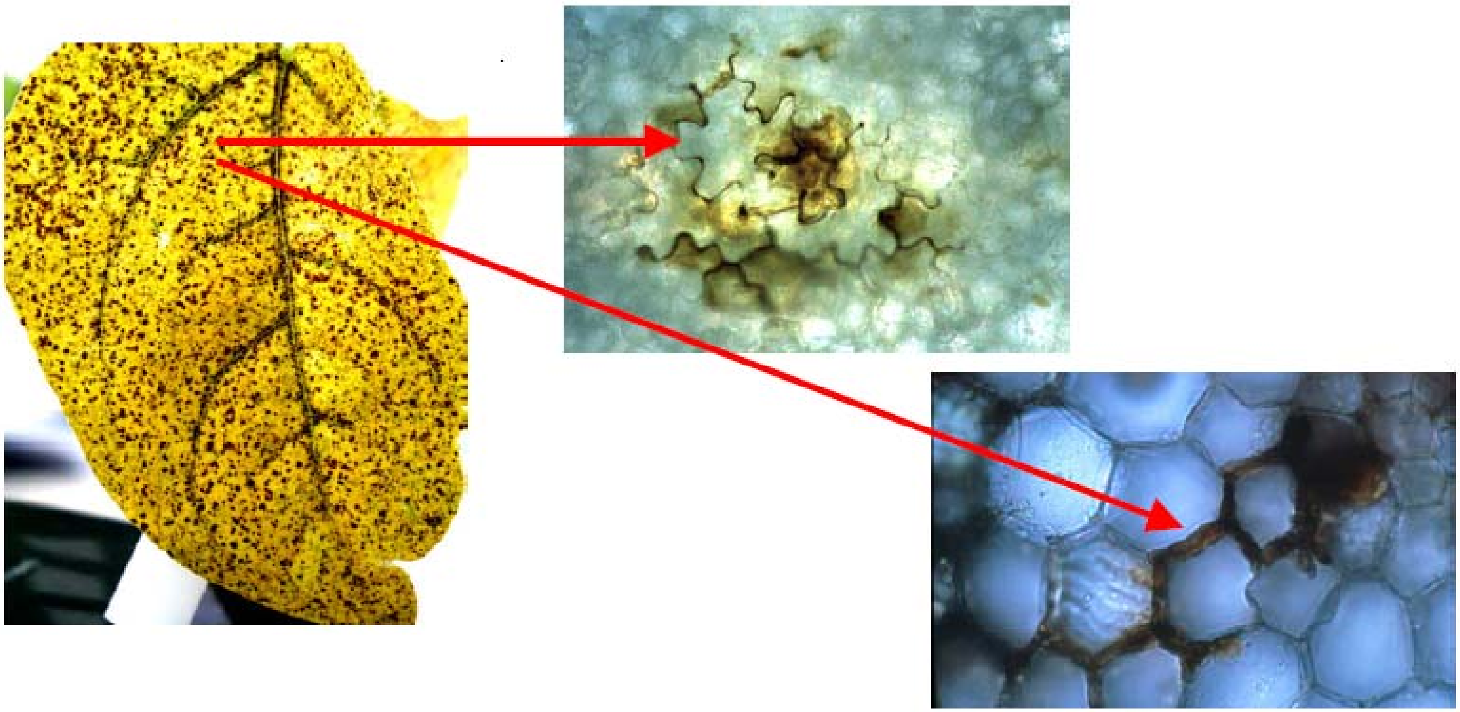
→ Yield decline

Mn sensitivity and tolerance

- **Great inter - and intraspecific variability**
- **Mn tissue tolerance**
- **Internal Mn compartmentation via transporters**
  - **Vacuole** (Hirschi et al., 2000, Delhaize et al., 2003, 2007)
  - **ER** (Delhaize et al., 2003, Wu et al., 2002)
  - **Golgi Apparatus** (Peiter et al., 2007)

No differences in Mn compartmentation between cowpea genotypes differing in Mn tolerance

Composition of typical Mn toxicity symptoms



Wissemeier und Horst, 1992, Plant and Soil, 143: 299-309

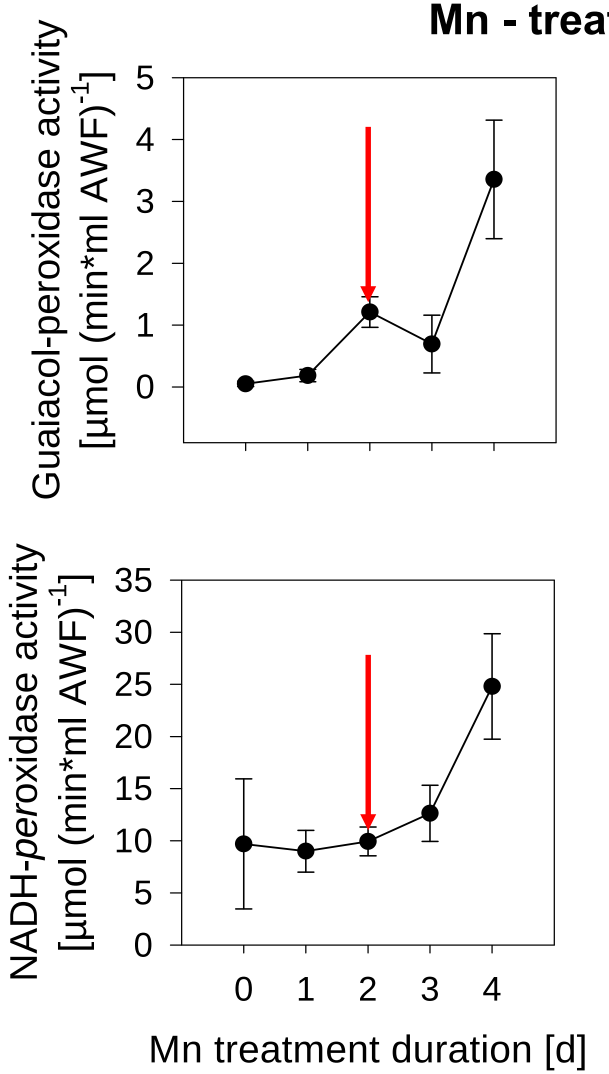
Polyphenols und Mn - oxides

Toxicity symptom - development coincides with increase in apoplastic peroxidase activities

Mn - treatment: 50 µM

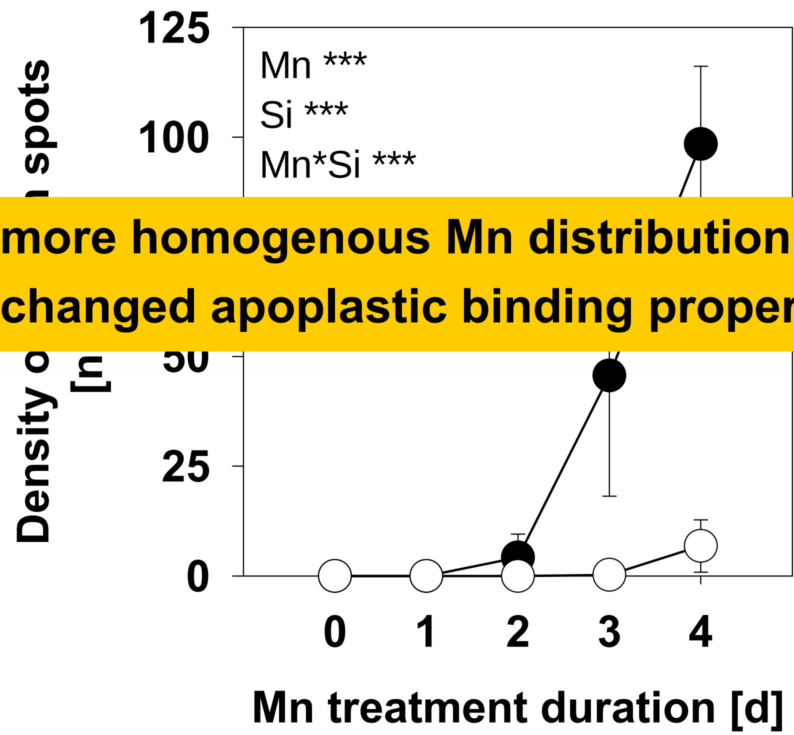
H_2O_2 – consuming guaiacol – peroxidase activity

H_2O_2 - producing NADH - *per*oxidase activity



Silicon (Si)

- **„plant beneficial“**
- **Silicon reduces and delays Mn toxicity development**



- **more homogenous Mn distribution in leaves**
- **changed apoplastic binding properties**

Mn treatment: 50 µM

Führs et al., 2009, J Exp Bot, 60:1663-78

Which factors lead to:

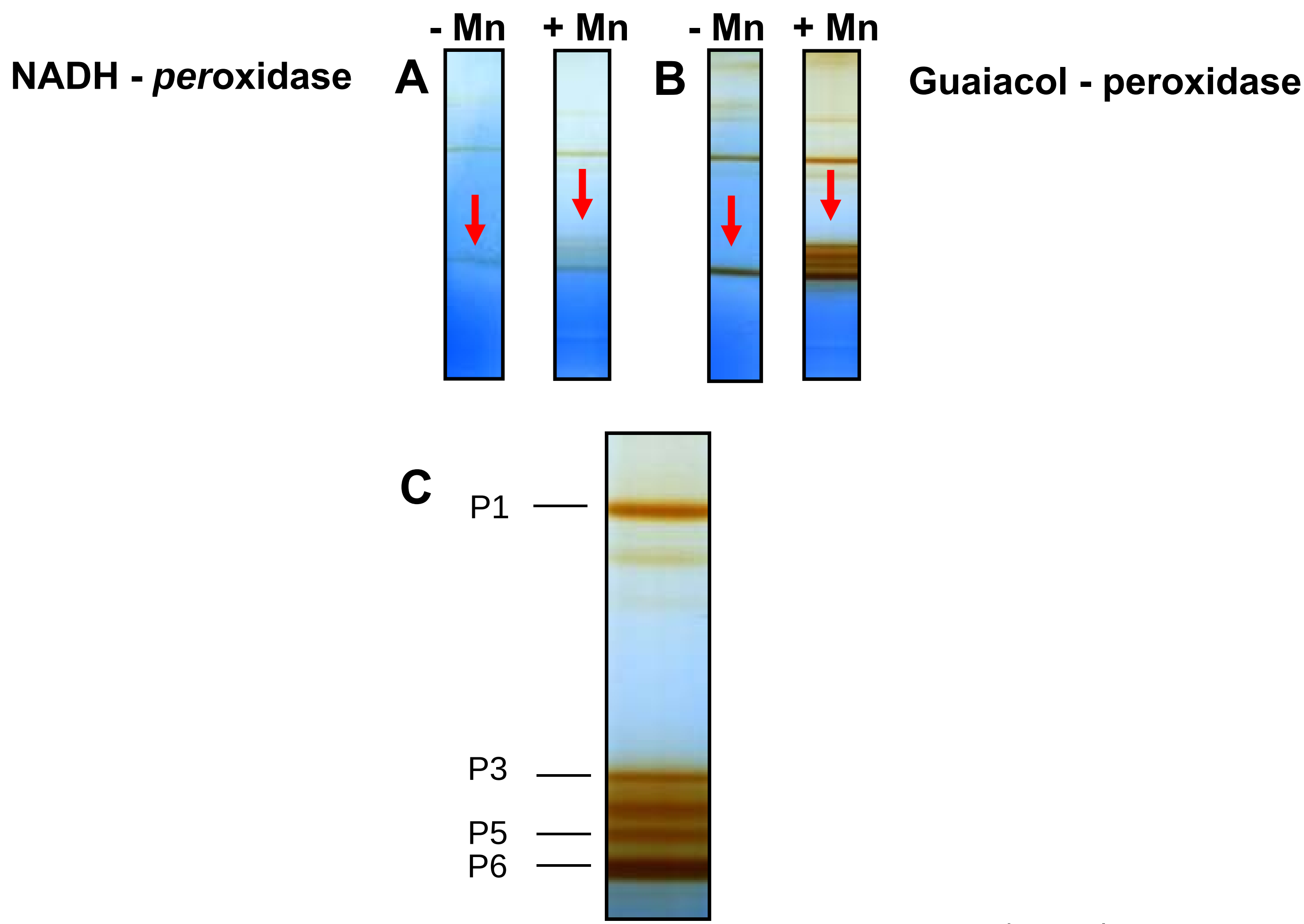
- Mn - sensitivity?
- Mn - tolerance?
  - Si - mediated Mn - tolerance?
  - genotypic Mn - tolerance?

Genotypes and treatments

- Mn - sensitive genotype TVu 91 (s)
- Mn - tolerant genotype TVu 1987 (t)
- Si - supply constitutive
  - (Aerosil© → fumed SiO_2)
- Mn treamtent
  - 50 µM Mn for 3 - 6 d

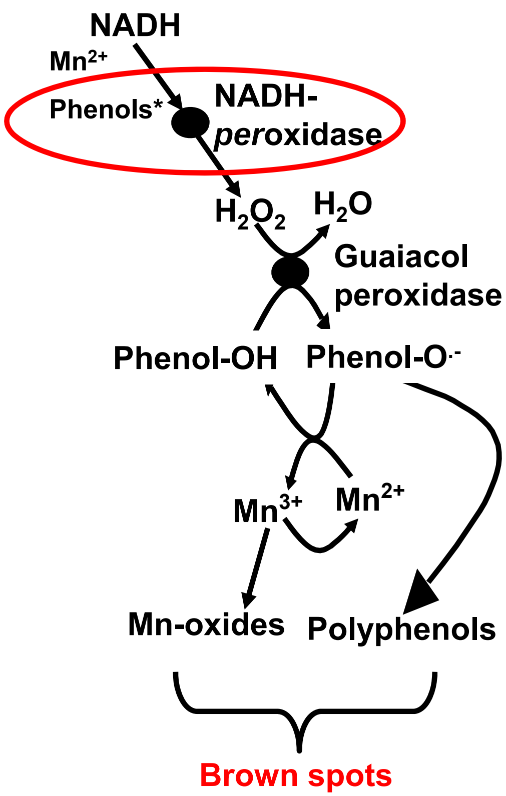
TVu 1987 **TVu 91**

Excess Mn changes the apoplastic peroxidase - isoenzyme profile of TVu 91 (s)



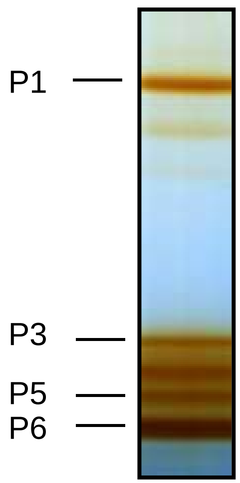
Führs et al., 2009, J Exp Bot, 60:1663-78

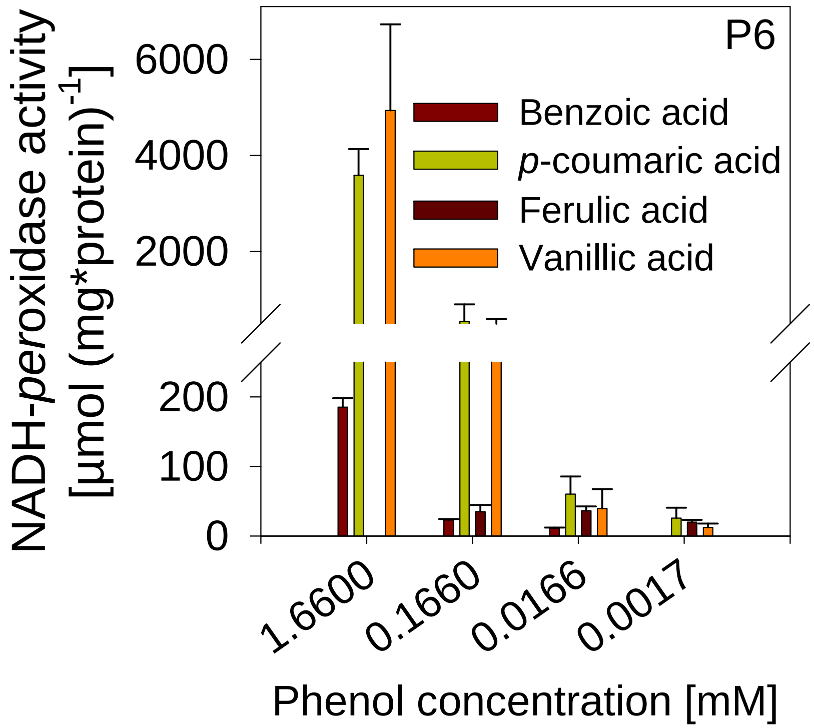
The NADH-*per*oxidase activity requires phenols as co-factors



Modified after Fecht-Christoffers et al., 2007, In: *The apoplast of higher plants: compartment of storage, transport, and reactions.* 307-322

The phenol identity and concentration specifically affect the NADH-*per*oxidase activity

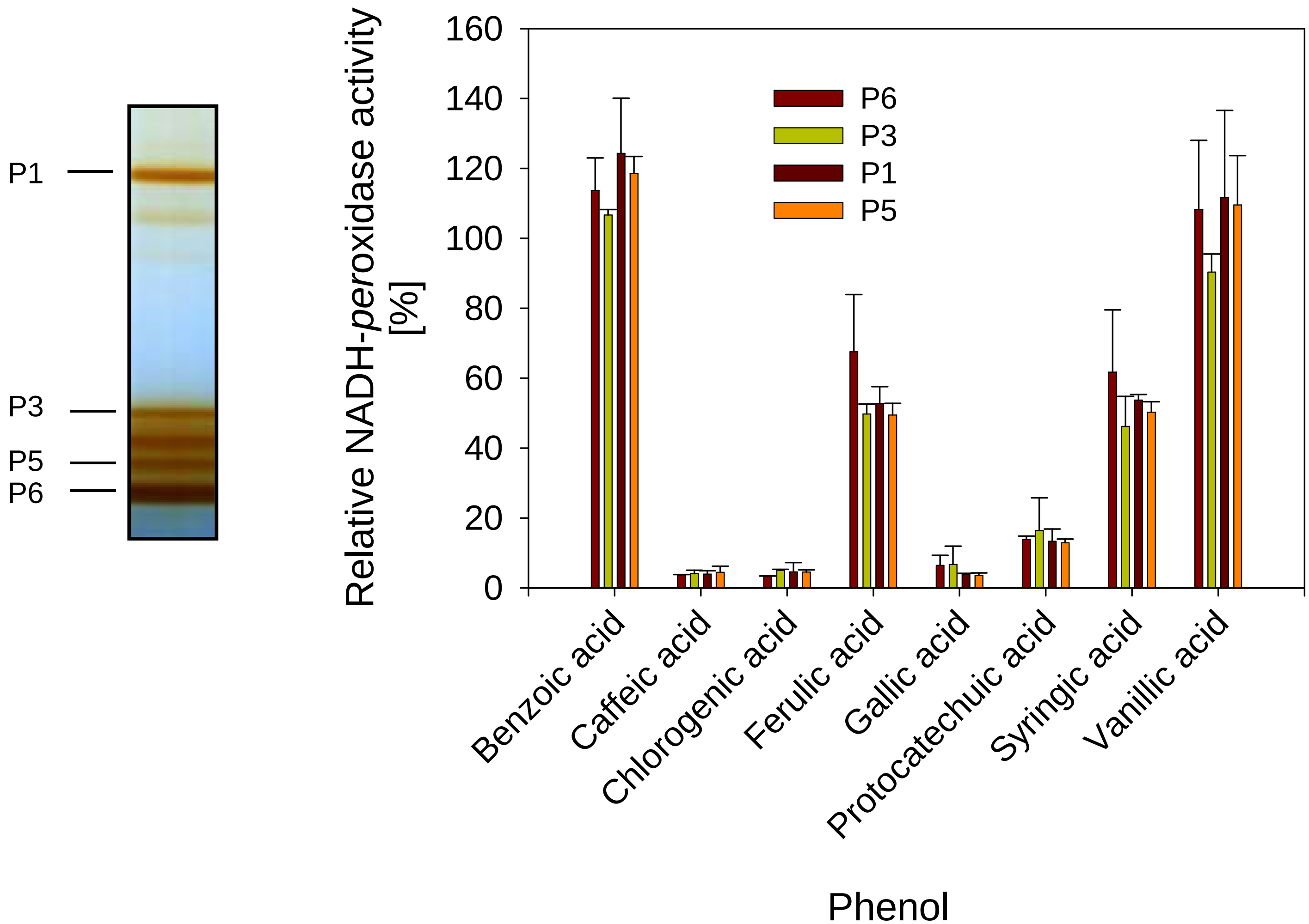




Reaction schemes similar for all isoenzymes

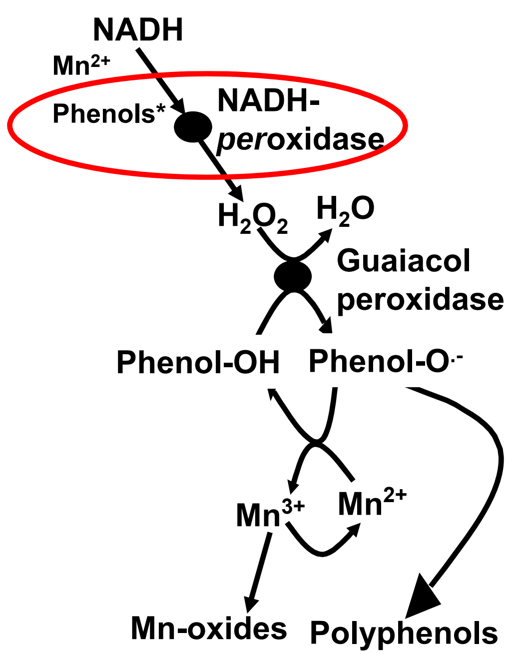
Führs et al., 2009, J Exp Bot, 60:1663-78

The phenol composition impacts on the induction of the NADH-*per*oxidase activity



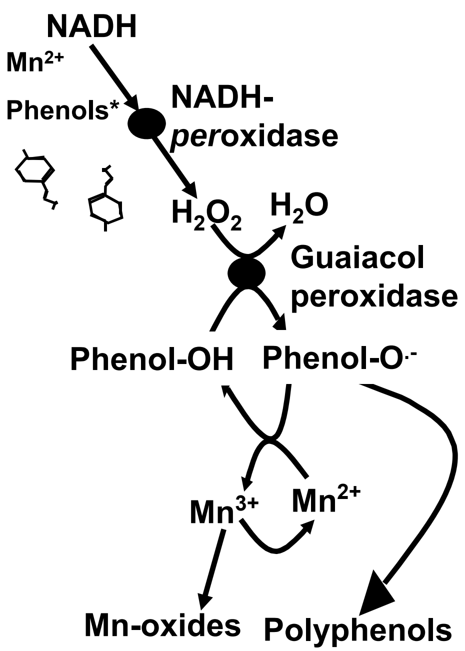
Führs et al., 2009, J Exp Bot, 60:1663-78

Phenols affect the NADH - *per*oxidase activity

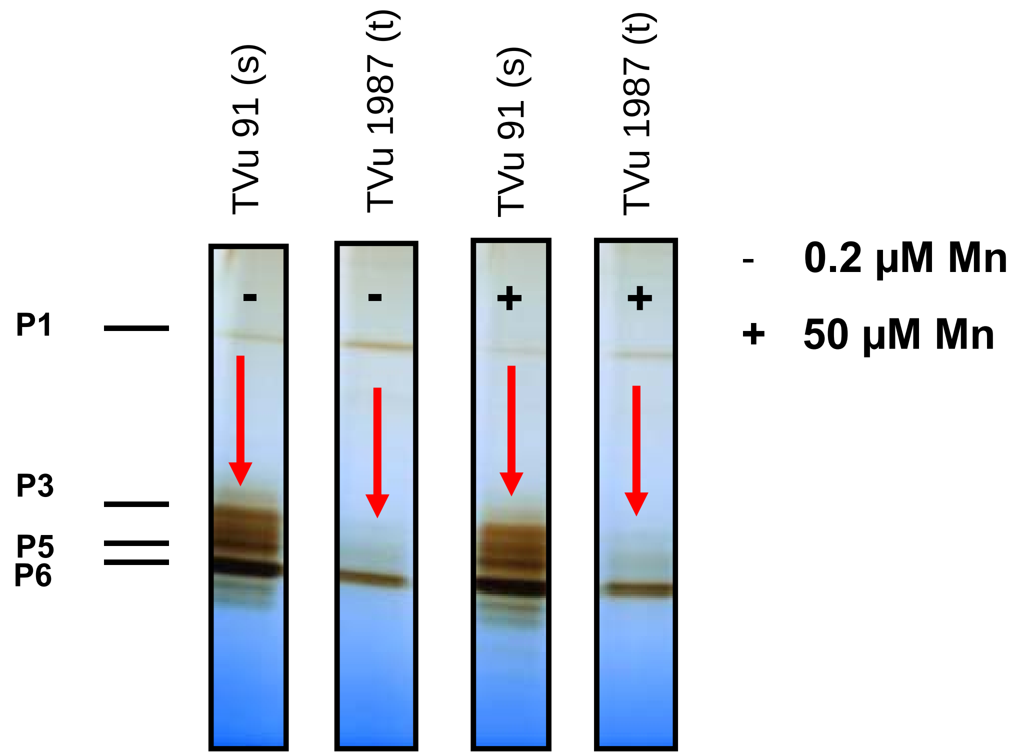


- phenol identity
- phenol concentration
- phenol composition

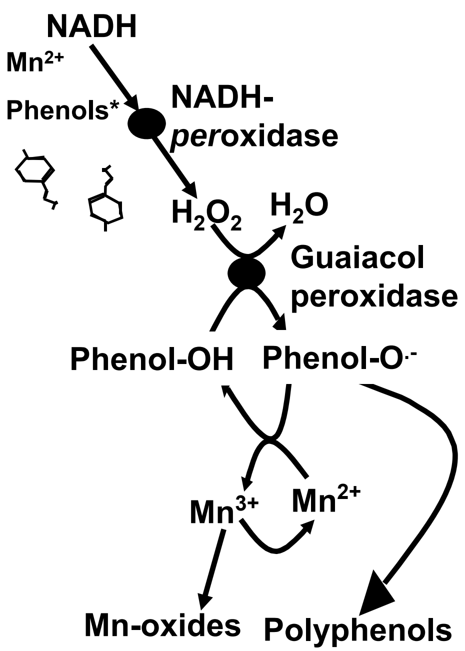
There are genotypic differences in the apoplastic peroxidase – isoenzyme profile



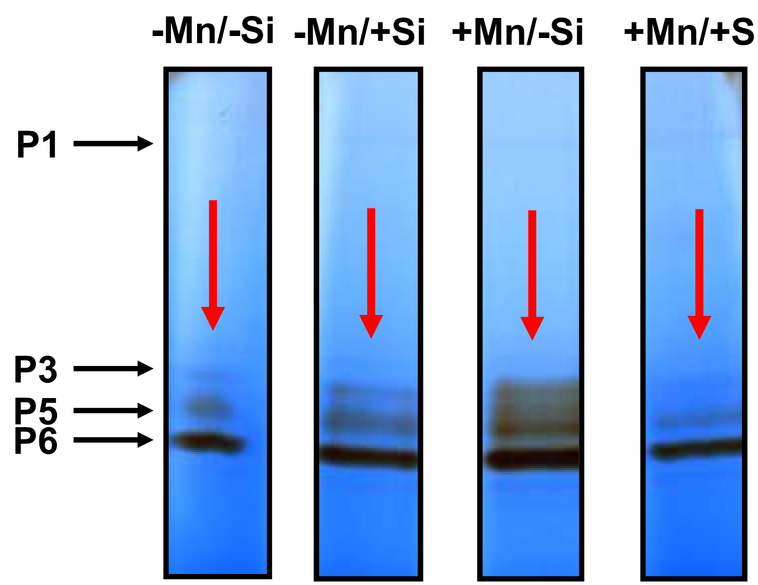
Guaiacol - peroxidase



Si reduces the Mn - induced accumulation of peroxidase - isoenzymes in the apoplast



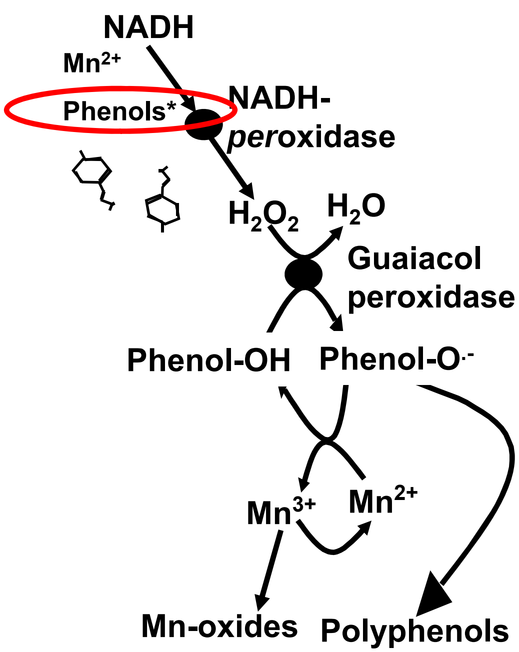
Guaiacol - peroxidase



TVu 91 (s)

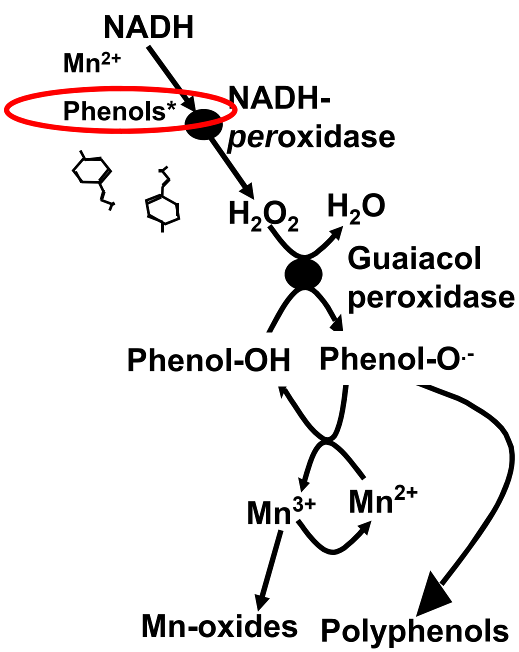
Führs et al., 2009, J Exp Bot, 60:1663-78

Through „metabolite profiling“ identified apoplastic phenols



- *p*-hydroxybenzoic acid
- sinapic acid
- benzoic acid
- *p*-coumaric acid
  - enhances NADH-*per*oxidase activity
- ferulic acid
  - inhibits NADH-*per*oxidase activity

Mn and Si supply induce changes in the apoplastic phenylpropanoid pool



- *p*-coumaric acid
- **in TVu 1987 (t) reduced by excess Mn**
  - **tolerance effect**

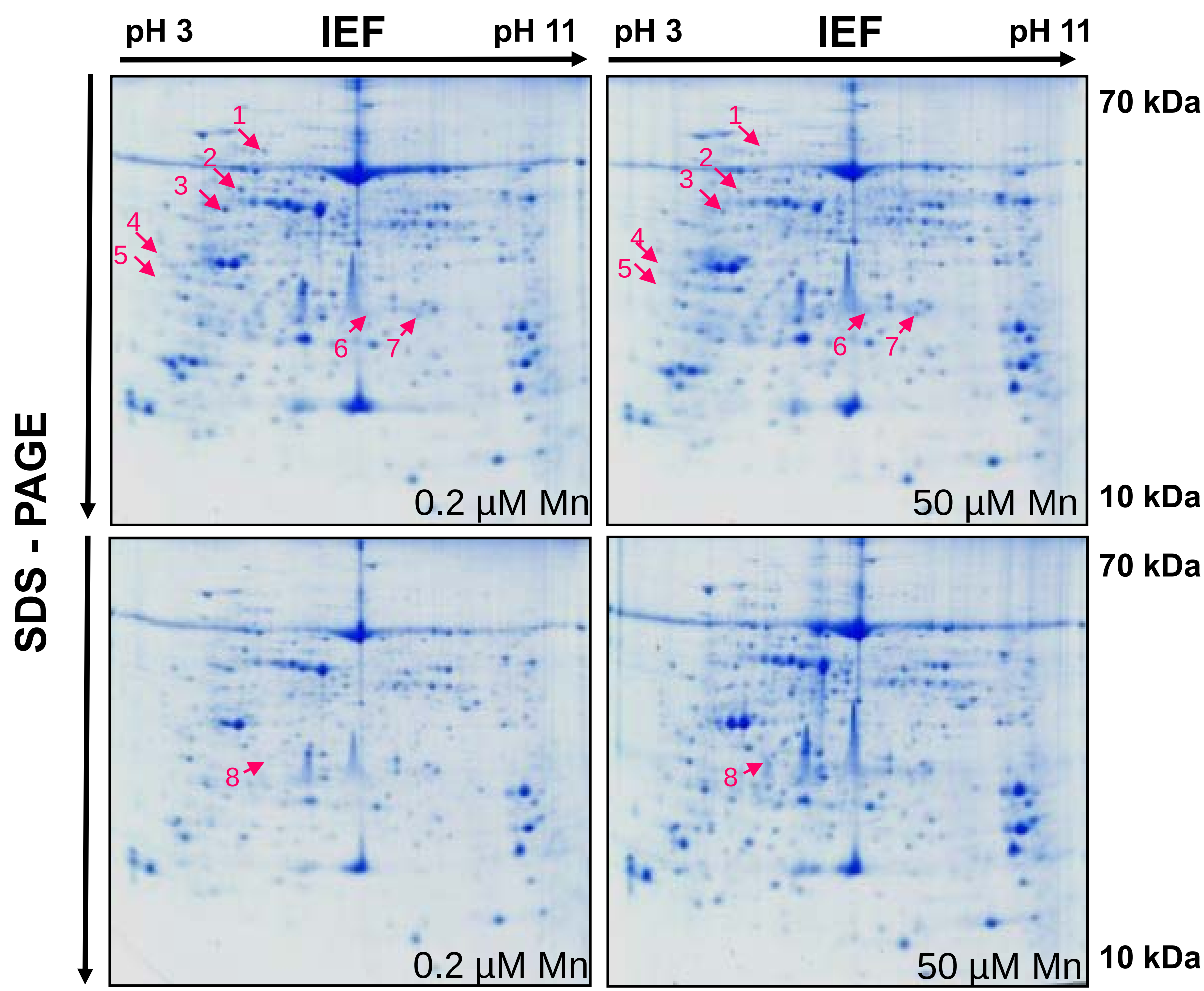
- ferulic acid
- **in TVu 91 (s) reduced by excess Mn**
  - **sensitivity effect**
- **in both genotypes increased by excess Si**
  - **tolerance effect**

Führs et al., 2009, J Exp Bot, 60:1663-78, modified

Symplastic changes in the total proteome after long - term elevated Mn supply to TVu 91 (s) und TVu 1987 (t)

TVu 91 (s)

TVu 1987 (t)



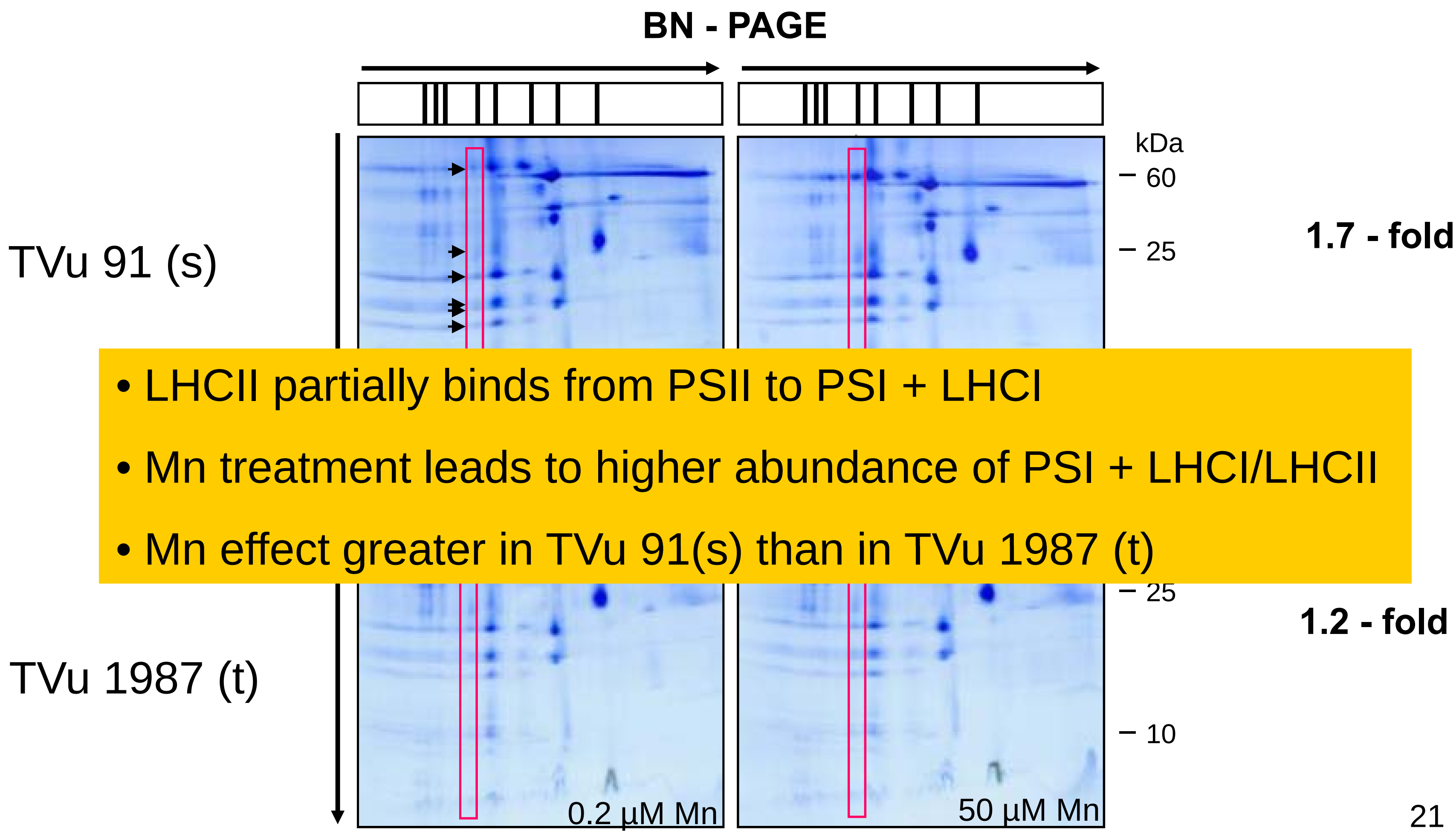
Führs et al., 2008, Proteomics 8: 149–159

Proteins affected by long - term elevated Mn supply in TVu 91 (s) und TVu 1987 (t)

N0.	Name	+Mn/-Mn ratio	
1	RubisCO-binding protein, beta subunit	0.38	TVu 91
2	RubisCO activase	0.48	TVu 91
3	Phosphoribulokinase	0.49	TVu 91
4	Oxygen-evolving enhancer protein 1	n.q.	TVu 91
5	Pathogenesis-related protein P4	n.q.	TVu 91
6	Putative beta6 proteasome subunit	2.03	TVu 91
7	Pathogenesis-related protein 5-1	2.46	TVu 91
8	Oxygen-evolving enhancer protein 2	3.80	→ TVu 1987

Führs et al., 2008, Proteomics 8: 149–159

Long – term Mn supply leads to changes in the photosynthetic protein complex composition: state transitions



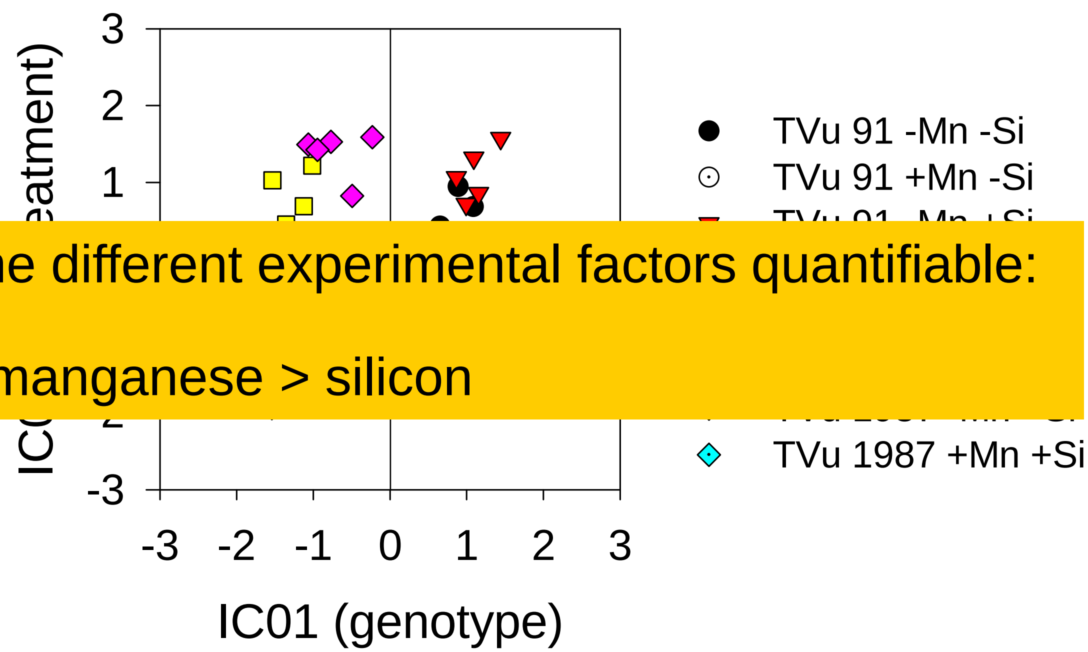
- LHCII partially binds from PSII to PSI + LHCI
- Mn treatment leads to higher abundance of PSI + LHCI/LHCII
- Mn effect greater in TVu 91(s) than in TVu 1987 (t)

Führs et al., 2008, Proteomics 8: 149–159

Photosynthetic changes induced by long - term elevated Mn supply

- State transitions
  - cyclic electron transport
  - increased ATP production at the cost of NAD(P)H provision
- Calvin cycle
  - less NADPH → RubisCO activity maintaining proteins are reduced

„Metabolite profiling“: Mn and Si modify the total leaf metabolome of TVu 91 (s) and TVu 1987 (t)



The impact of the different experimental factors quantifiable:

→ genotype > manganese > silicon

Specific metabolites affected by the experimental factors: indirect effects of photosynthesis

- Carbohydrate metabolism: **glucose / fructose** (primary photosynthesis products)
- Carbohydrate metabolism: **sucrose** (transport form)

- Amino acid metabolism: **asparagine** (N-storage and transport form)
- Amino acid metabolism: **aspartic acid** (N-storage and transport form)

Specific metabolites as affected by the experimental factors: antioxidative state

- Symplastic antioxidants: **ascorbate**
- Symplastic antioxidants: **dehydroascorbate**
- Symplastic and apoplastic antioxidants: **organic acids**

Summary and Conclusion

- **Mn - sensitivity**
  - **photosynthesis**
  - **peroxidases and their modulation by phenolics**
  - **protein degradation / general stress response**

- **Si - mediated Mn tolerance**
  - **not only by changed apoplastic binding capacity/homogenous distribution**
  - **Si constitutively changes the metabolome**
  - **peroxidase – isoenzyme profile**
  - **NADH - *per*oxidase activity-modulating phenolics**

- **Genotypic Mn tolerance**
  - **Great differences in the metabolome between the genotypes**
  - **Some genotypic differences of Mn - treated plants in specific metabolite pools probaly due to changed / affected photosynthesis**
  - **Preformed genotypic differences in the proteomic and metabolomic antioxidative state of the cells**

Thank you!

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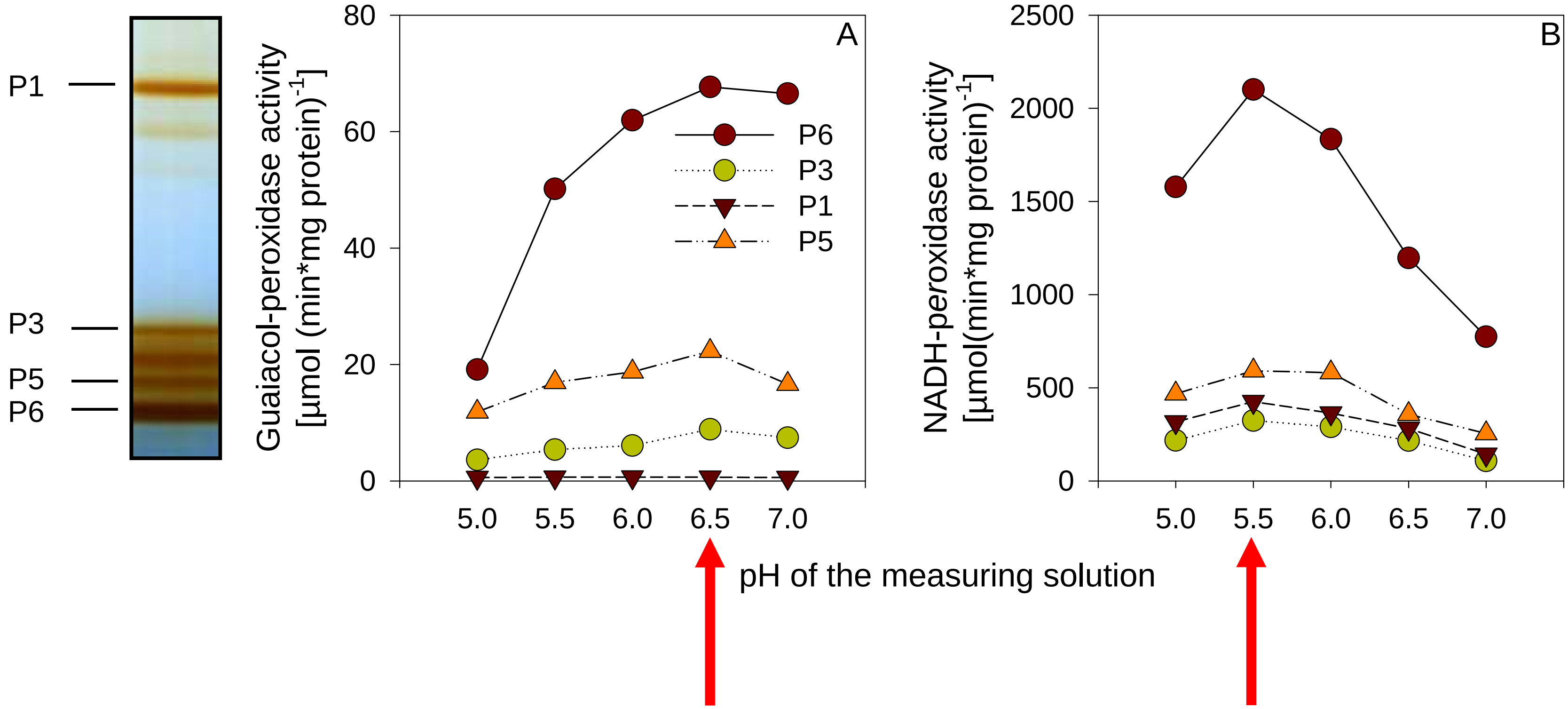
The guaiacol-peroxidase and the NADH-*per*oxidase activity have different pH optima



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