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


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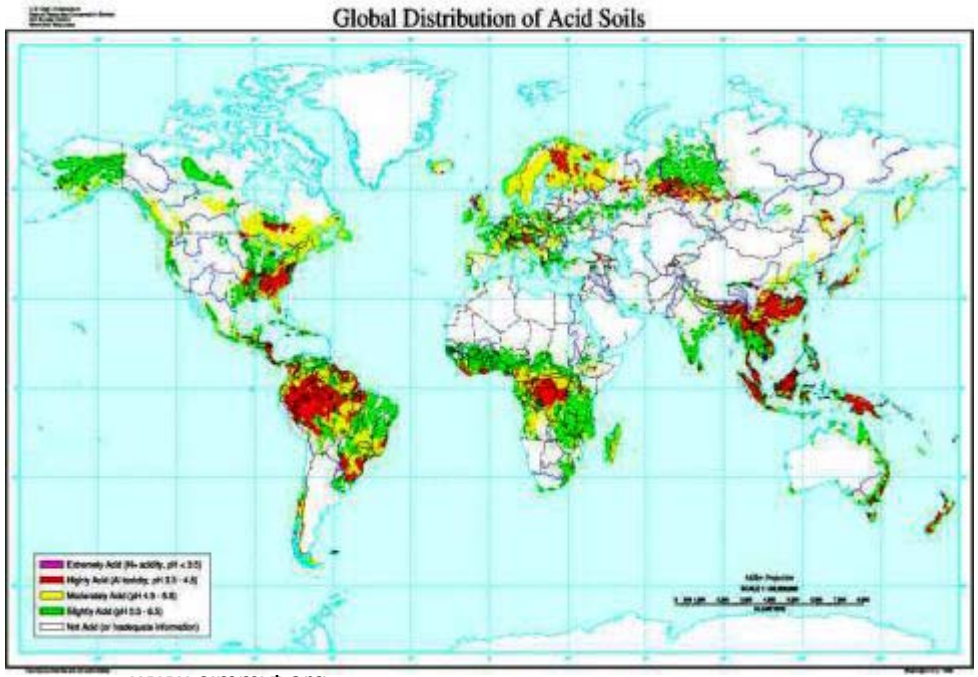
# Manganese (Mn) availability

- 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

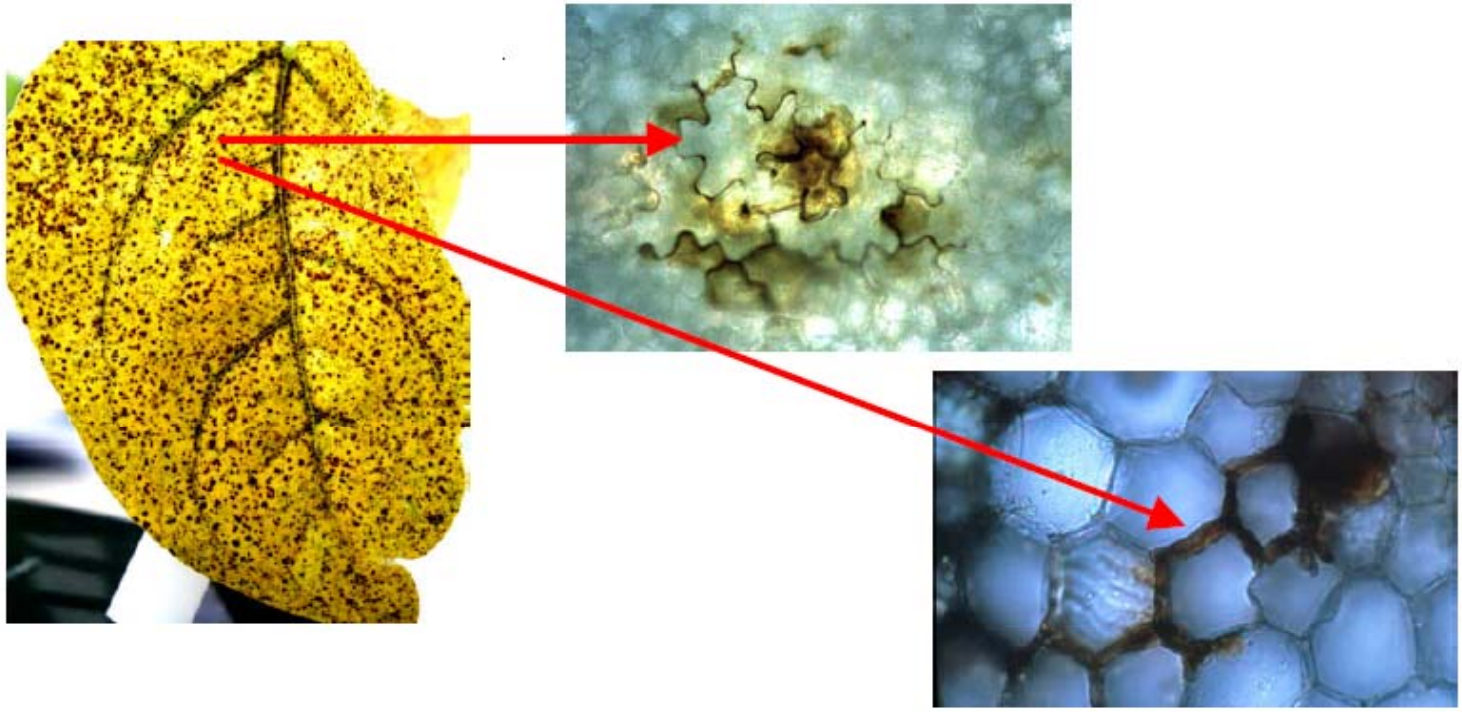
→ Yield decline

# 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



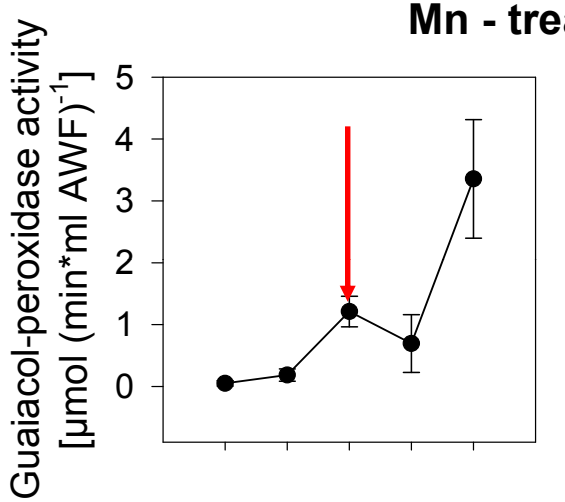
Wissemeier und Horst, 1992, Plant and Soil, 143: 299-309

Polyphenols und Mn - oxides

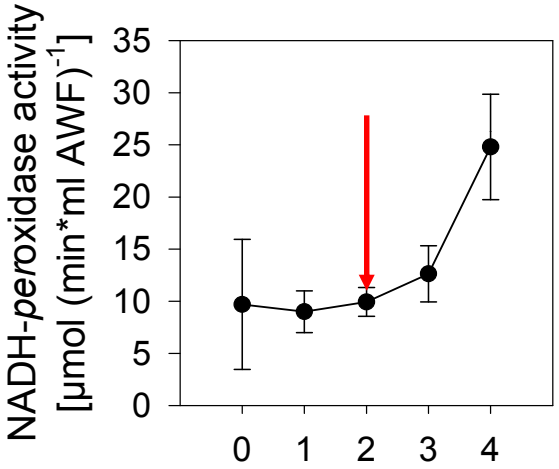
# Toxicity symptom - development coincides with increase in apoplastic peroxidase activities

**Mn - treatment: 50 µM**

**$H_2O_2$ – consuming guaiacol – peroxidase activity**

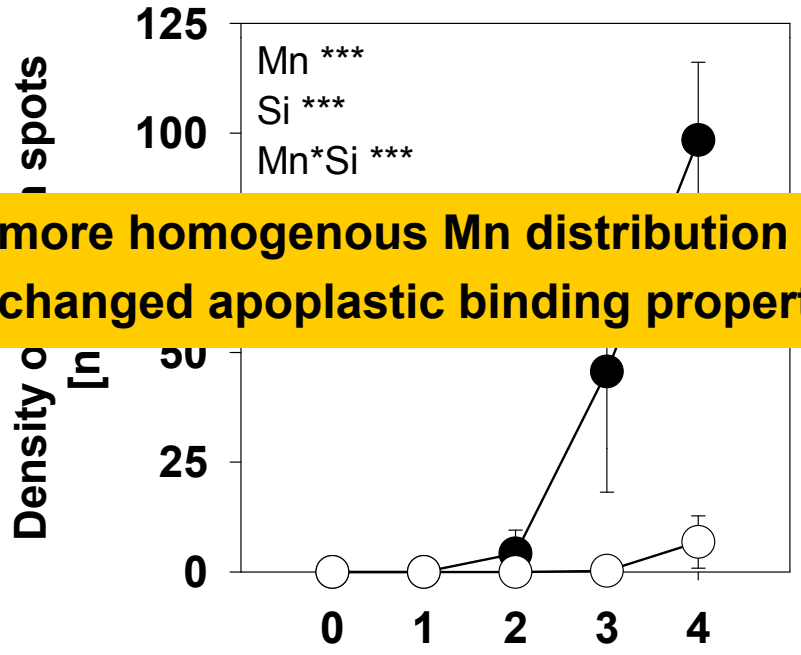



**$H_2O_2$ - producing NADH - *per*oxidase activity**



# 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:

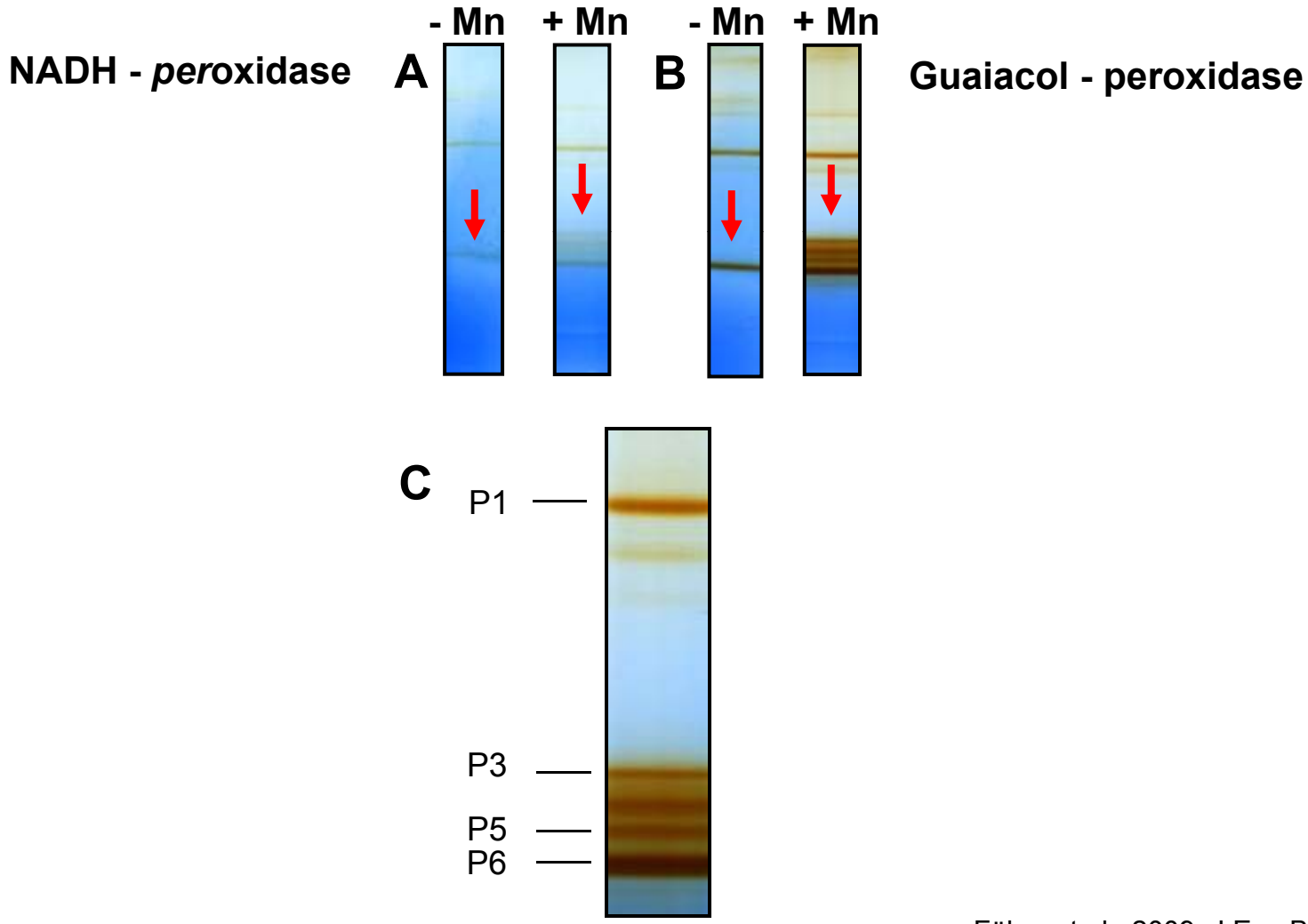
- Mn - sensitivity?
- Mn - tolerance?
  - Si - mediated Mn - tolerance?
  - genotypic Mn - tolerance?

# 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**

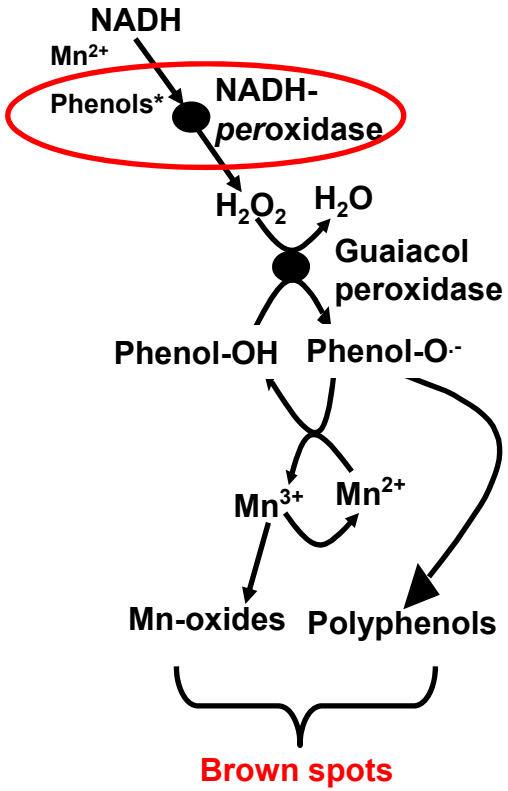
# Excess Mn changes the apoplastic peroxidase - isoenzyme profile of TVu 91 (s)






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

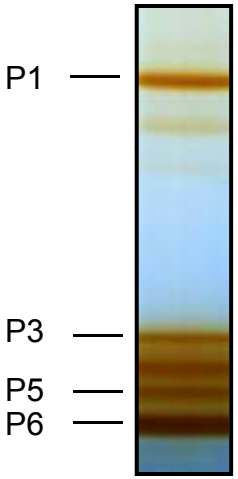
# The NADH-*per*oxidase activity requires phenols as co-factors

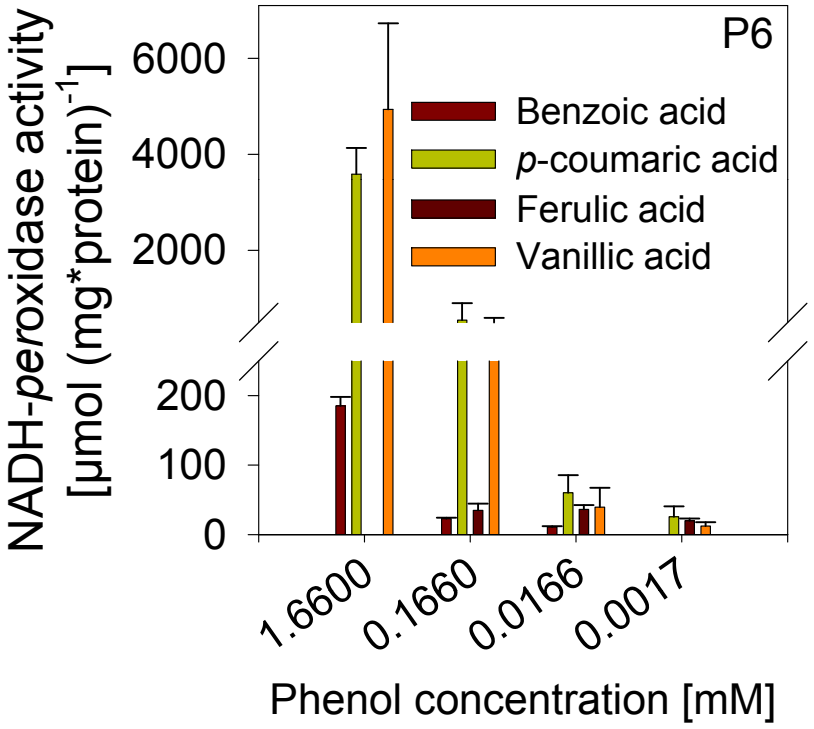





Modified after Fecht-Christoffers et al., 2007, In: *The apoplast of higher plants: compartment of storage, transport, and reactions.* 307-322

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



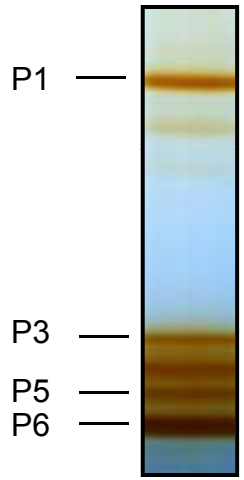



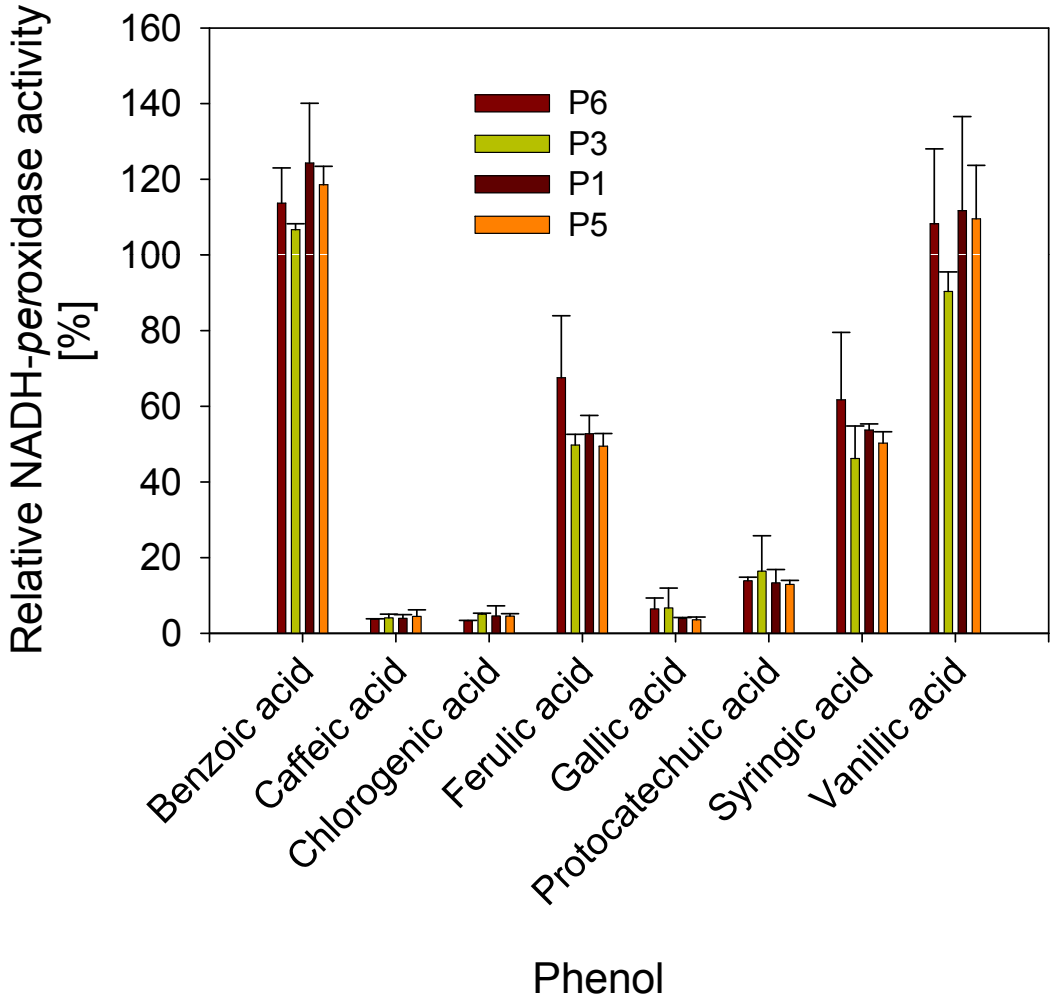

Reaction schemes similar for all isoenzymes



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

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

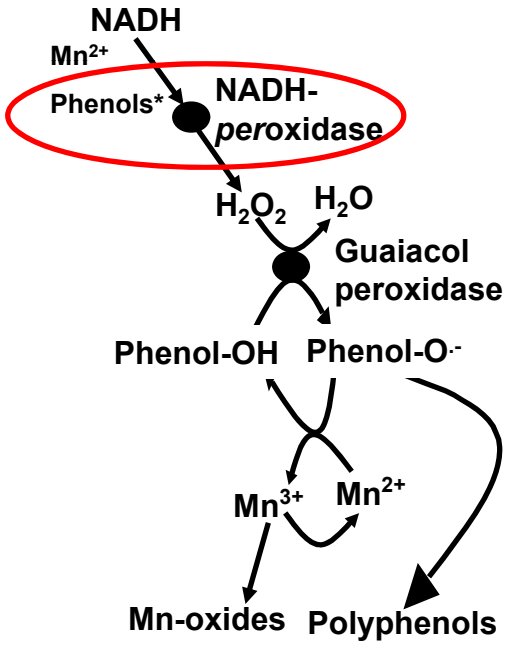





Führs et al., 2009, J Exp Bot, 60:1663-78

# Phenols affect the NADH - *per*oxidase activity

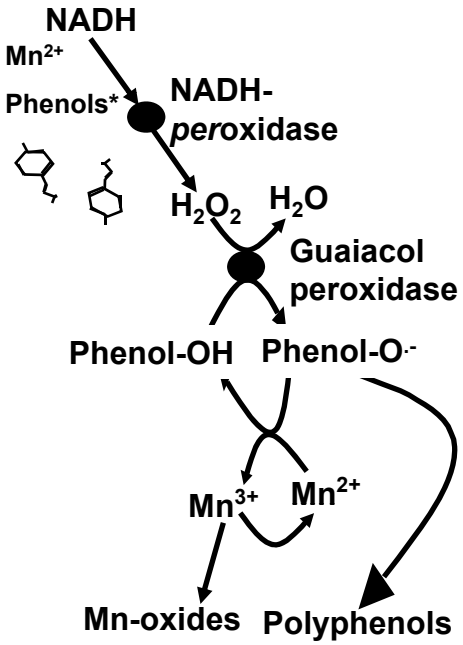


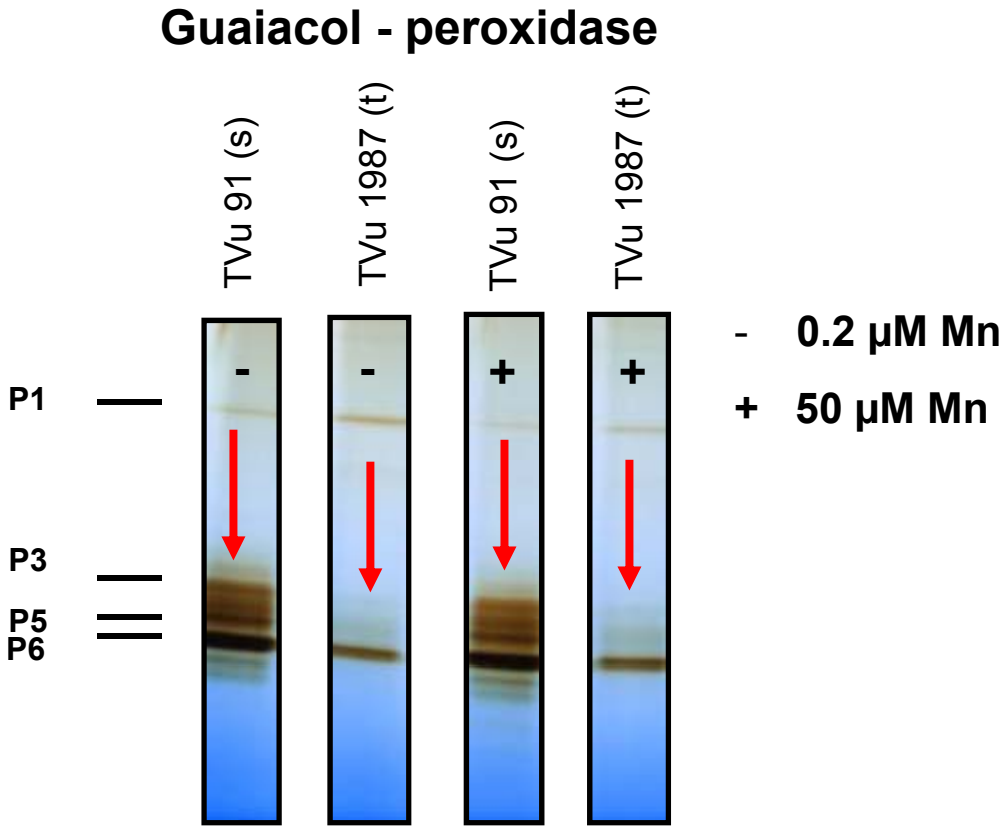

- phenol identity

- phenol concentration

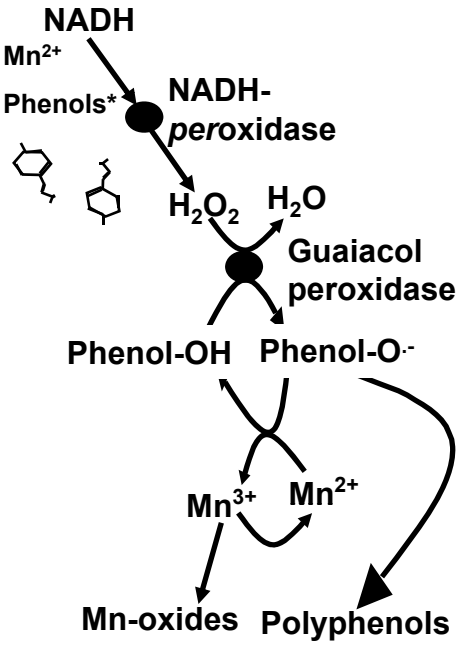
- phenol composition

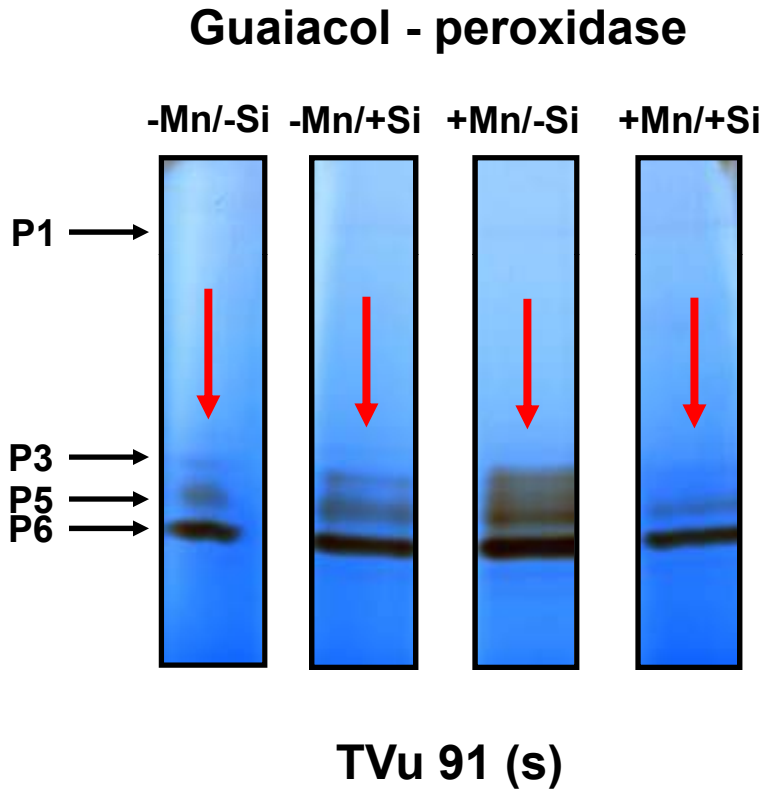
# There are genotypic differences in the apoplastic peroxidase – isoenzyme profile



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

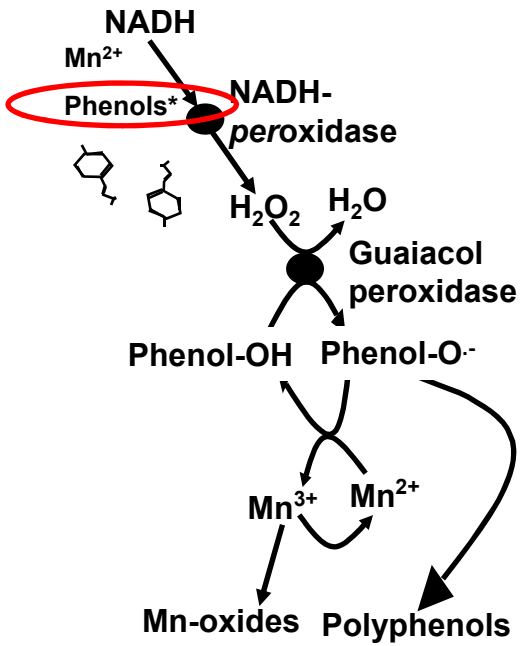




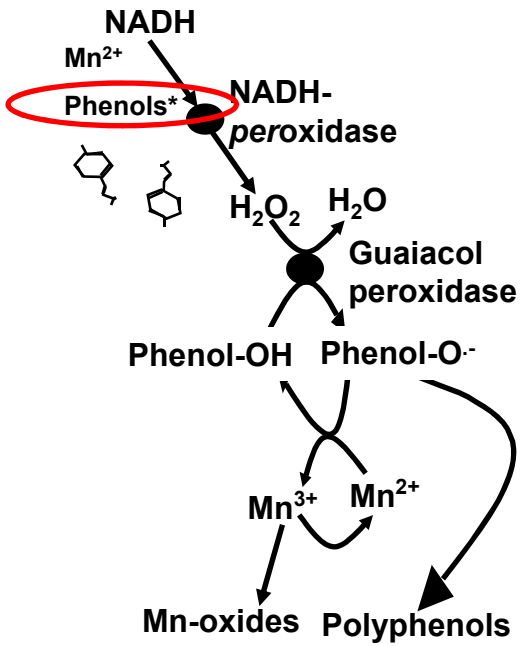

Führs et al., 2009, J Exp Bot, 60:1663-78

# 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**

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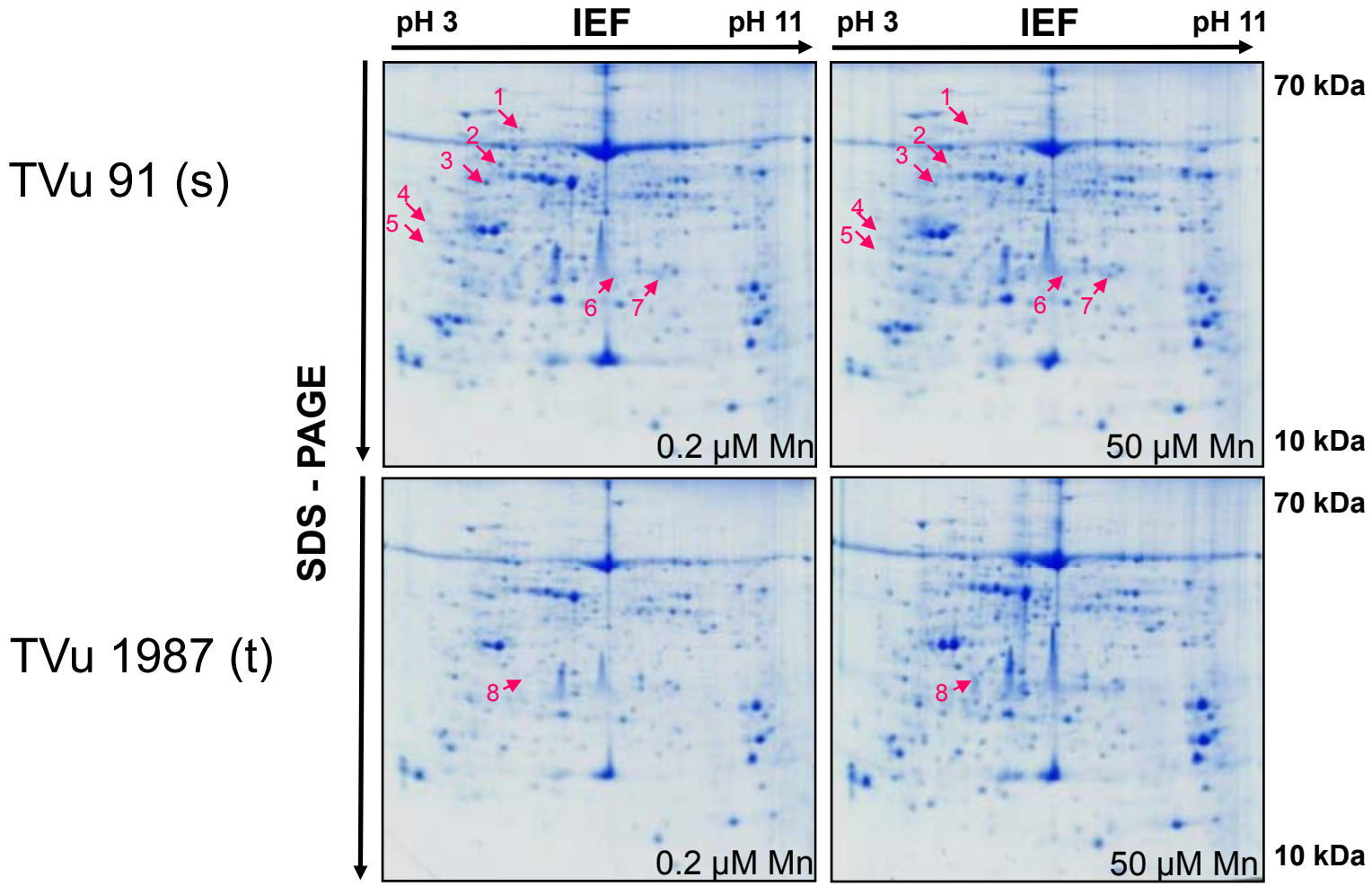
- ferulic acid
- **in TVu 91 (s) reduced by excess Mn**
  - **sensitivity effect**
- **in both genotypes increased by excess Si**
  - **tolerance effect**



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)






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

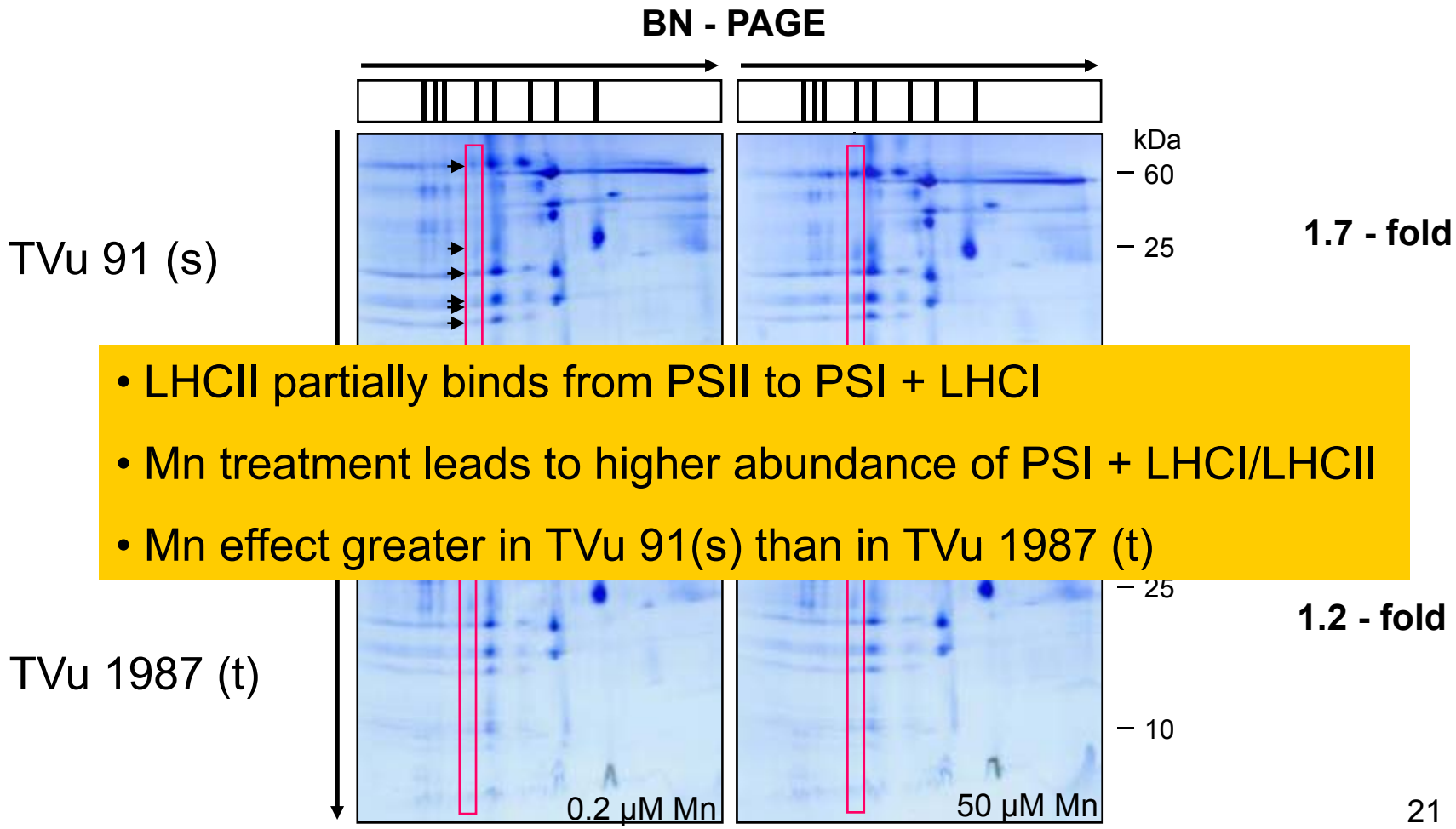
# Proteins affected by long - term elevated Mn supply in TVu 91 (s) und TVu 1987 (t)

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



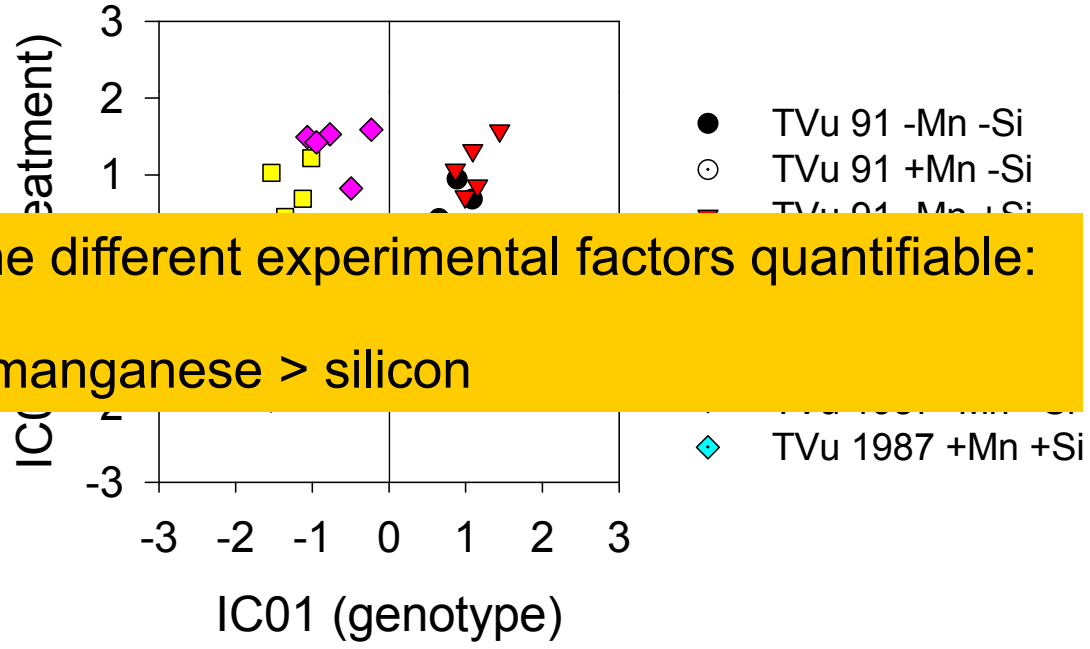



Führs et al., 2008, Proteomics 8: 149–159

# 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)

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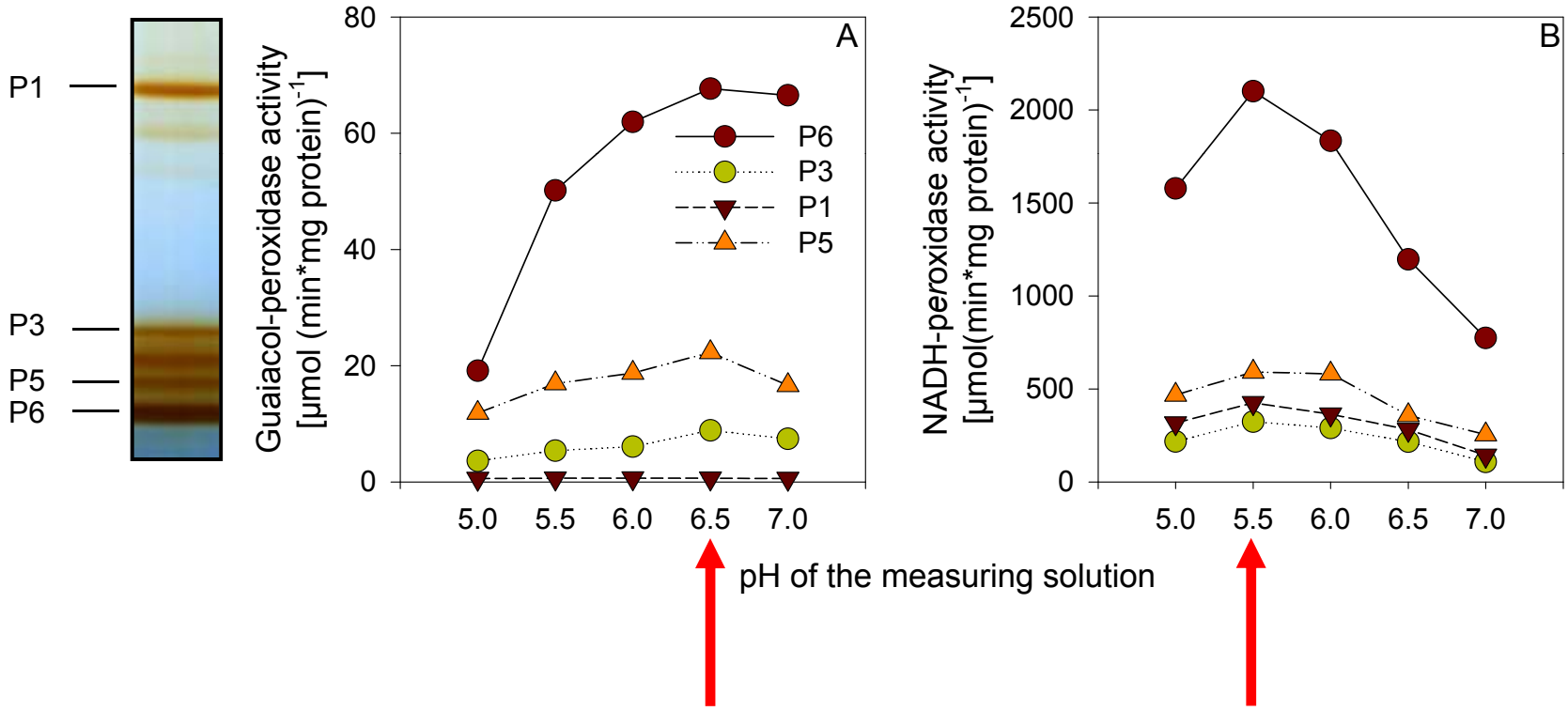
# The guaiacol-peroxidase and the NADH-*per*oxidase activity have different pH optima



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