# Can leaf apoplastic manganese and silicon concentrations explain Si-enhanced manganese tolerance of *Vigna unguiculata* (L.) ?

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## Introduction and Objektive

First visible Mn toxicity symptoms are dark-brown spots on older leaves (Fig. 1), which represent local accumulations of oxidised Mn and phenols. Silicon nutrition increases the Mn tolerance of Vigna unguiculata (Fig. 2). The objective of this study was to gain further information on the influence of Si-induced modification of Mn binding properties of the cell walls on the Mn concentration in the apoplast and on the expression of Mn toxicity symptoms.

*Fig. 1: Mn toxicity symptoms of cowpea*

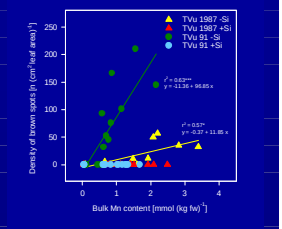
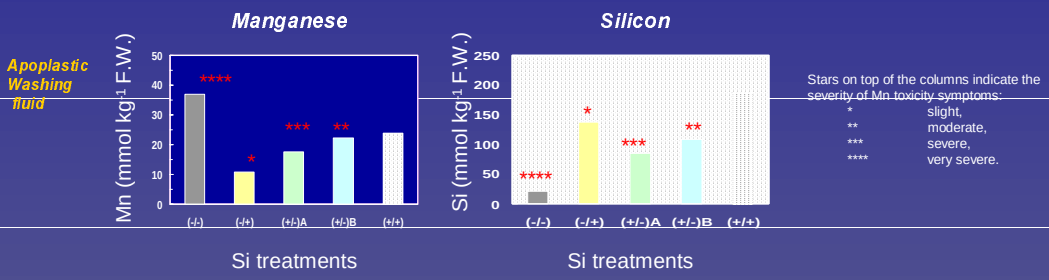



*Fig. 2 Relationship between density of brown spots and Mn content in the leaf tissue*

## Results

***Silicon nutrition reduces Mn concentration in the AWF by enhanced Mn binding to the cell wall***




Stars on top of the columns indicate the severity of Mn toxicity symptoms:

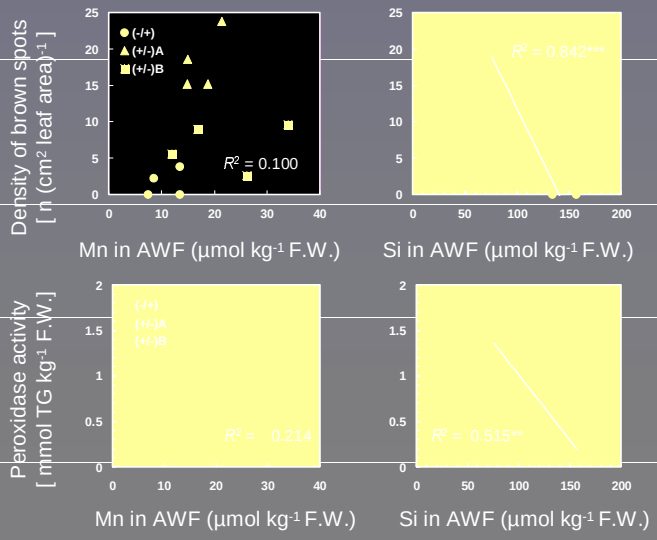
| | |
|---|---|
| * | slight, |
| ** | moderate, |
| *** | severe, |
| **** | very severe. |

**Manganese concentrations in fractions prepared from the 2nd oldest leaf**

| Mn levels (µM) | Si treatments | Fractions: AWF | | Buffer | | DTPA | | MnO | | HF/Res | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | (µmol Mn kg⁻¹ F.W.) | | | | | | | | | |
| 0.2 | - | 0.318 | (0.7) | 23.0 | (49.1) | 9.86 | (21.0) | 2.59 | (5.5) | 11.1 | (23.7) |
| 0.2 | + | 0.196 | (0.3) | 25.1 | (42.0) | 12.4 | (20.8) | 2.78 | (4.7) | 19.2 | (32.2) |
| 10 | - | 4.55 | (1.5) | 258 | (86.5) | 21.2 | (7.1) | 5.83 | (2.0) | 8.51 | (2.9) |
| 10# | + | 3.71 | (0.9) | 325 | (81.0) | 41.8 | (10.4) | 12.1 | (3.0) | 18.6 | (4.6) |
| 50 | - | 21.2 | (1.9) | 917 | (83.6) | 98.0 | (8.9) | 36.4 | (3.3) | 23.6 | (2.2) |
| 50# | + | 11.9 | (0.8) | 1190 | (81.8) | 166 | (11.4) | 51.5 | (3.5) | 34.9 | (2.4) |

# ⁼With Mn toxicity symptoms  * Values in parentheses indicate % distribution of Mn to each fraction.

***Mn toxicity symptoms and peroxidase activity are correlated with the Si concentrations but not with the Mn concentrations in the AWF***







## Materials and Methods

***Cultivation***

| Treatments Si (mM) | Days after transplanting: 0~17 | 18~20 | 21~27 |
|---|---|---|---|
| | Mn 0.2 µM | | Mn 50 µM |
| (-/-) | 0 | 0 | 0 |
| (-/+) | 0 | 0 | 1.43 |
| (+/-)A | 1.43 | 0 | 0 |
| (+/-)B | 1.43 | 1.43 | 0 |
| (+/+) | 1.43 | 1.43 | 1.43 |

***Fractionation of Mn and Si in the 2nd oldest leaf***

***Apoplastic washing fluid (AWF)***
vacuum infiltration with 50 mM MES-Tris buffer, pH 6.5

***Buffer soluble fraction (Buffer)***
homogenization with 50 mM MES-Tris buffer, pH 6.5

***DTPA extractable fraction (DTPA)***
extraction by the buffer solution containing 5 mM DTPA and 10 mM $CaCl_2$, pH 7.3, 2 hr.

***Mn oxides fraction (MnO)***
extraction by 0.1 M $NH_2OH$·HCl, pH 2, 0.5 hr.

***HF soluble / residual fraction (HF/Res)***
extraction by 1 M HCl and 2.3 M HF mixture (1:2), 24 hr., and dry ashing (500 °C)

## Conclusions

**Two factors contribute to Si-enhanced Mn tolerance in cowpea :**

- **decrease of the free Mn in the apoplast through binding to Si incorporated in the cell walls.**
- **detoxification of free Mn in the apoplast through soluble Si thus maintaining the reduced state of the apoplast.**