

Table 1: Summary of the developed network around spectroscopic techniques during this Marie Curie project ( $\mu$ XRF: micro X-ray fluorescence,  $\mu$ XANES: micro X-ray absorption near edge structure spectroscopy,  $\mu$ PIXE: micro particle induced X-ray Emission,  $\mu$ FTIR: micro Fourier transformed infra-red spectroscopy)

| Subject                                | Place                                        | Country                    | Names                                 | Comments                                                                                                                                                                                 |
|----------------------------------------|----------------------------------------------|----------------------------|---------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| synchrotron                            | ESRF (European Synchrotron Radiation Source) | Grenoble, France           | Hiram Castillo-Michel                 | Pre-established collaboration                                                                                                                                                            |
| synchrotron                            | SLS (Swiss Light Source)                     | Villigen, Switzerland      | Daniel Grolimund, Vallerie-Ann Samson | Development of a cryo set-up for biological sample analysis                                                                                                                              |
| synchrotron                            | APS (Advanced Photon Source)                 | Chicago, United States     | Sophie Gleber                         | Experiment scheduled for the second part of 2015                                                                                                                                         |
| nuclear microprobe                     | CEA Saclay (Atomic Energy Commission)        | Saclay, France             | Suzy Surblé, Hicham Khodja            | Pre-established collaboration                                                                                                                                                            |
| nuclear microprobe                     | AIFIRA                                       | Bordeaux, France           | Stéphanie Sorieul                     | Pre-established collaboration                                                                                                                                                            |
| nuclear microprobe                     | RUBION                                       | Bochum, Germany            | Hans-Werner Becker, Detlef Rogalla    | Development of a micro-focused beam                                                                                                                                                      |
| nuclear microprobe                     | LIPSION                                      | Leipzig, Germany           | Juergen Vogt, Jan Meijer              | Development of a new collaboration                                                                                                                                                       |
| nuclear microprobe                     | Jožef Stefan Institute                       | Ljubljana, Slovenia        | Katarina Vogel-Mikus, Primož Pelicon  | Extensive knowledge on sample preparation                                                                                                                                                |
| LA-ICP-MS                              | Hannover University                          | Hannover, Germany          | Carla Vogt, Jan Thieleke              | Optimisation of Pb standard for accurate quantification                                                                                                                                  |
| nanoSIMS                               | Helmholtz Centre for Environmental Research  | Leipzig, Germany           | Hans Richnow, Hryhoriy Stryhanyuk     | Technical platform with cutting edge instruments                                                                                                                                         |
| nanoSIMS                               | UCLA (University of California Los Angeles)  | Los Angeles, United States | Markus Miethke                        | Development of a new collaboration                                                                                                                                                       |
| <i>irt1</i> project                    | RUB, Ruhr Universität Bochum                 | Bochum, Germany            | Maria Bernal                          | Distribution ( $\mu$ XRF) and speciation ( $\mu$ XANES) of Fe in <i>A. thaliana</i> mutant. In progress: imaging at the sub-cellular level (nanoSIMS, X-ray tomography)                  |
| <i>dez</i> project                     | RUB, Ruhr Universität Bochum                 | Bochum, Germany            | Scott Sinclair                        | Imaging ( $\mu$ XRF) and speciation ( $\mu$ XANES) determination of Zn in an <i>A. thaliana</i> mutant. Analysis of the mutation impact on bio-macromolecules distribution ( $\mu$ FTIR) |
| Role of HMA2 and HMA4 in seeds project | Copenhagen University                        | Copenhagen, Denmark        | Michael G. Palmgren, Lene Olsen       | Mapping of Zn in mutant seeds of <i>A. thaliana</i>                                                                                                                                      |
| FRD3 RNAi project                      | RUB, Ruhr Universität Bochum                 | Bochum, Germany            | Romario Melo                          | Distribution and quantification of elements ( $\mu$ PIXE) in an <i>A. halleri</i> mutant                                                                                                 |
| <i>A. thaliana</i> vs.                 | RUB, Ruhr                                    | Bochum,                    | Ricardo Stein                         | Elemental mapping ( $\mu$ XRF, $\mu$ PIXE),                                                                                                                                              |

|                                                                             |                        |                      |               |                                                                                                                                                                  |
|-----------------------------------------------------------------------------|------------------------|----------------------|---------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <i>A. halleri</i> & <i>A. halleri</i><br>diversity in the<br>field projects | Universtät<br>Bochum   | Germany              |               | quantification ( $\mu$ PIXE) of metals in <i>A. thaliana</i><br>and <i>A. halleri</i> plants. Determination of bio-<br>macromolecules distribution ( $\mu$ FTIR) |
| Pb imaging                                                                  | Bayreuth<br>University | Bayreuth,<br>Germany | Paula Pongrac | Joint projects to image Pb in different <i>A. halleri</i> plants                                                                                                 |

Table 2. Fe speciation in roots and shoots of WT and mutant seedlings grown on 50  $\mu$ M Fe as determined by linear combination fitting of experimental  $\mu$ XANES spectra (in %).

|                     | WT          |             |             | roz1        |             |             |
|---------------------|-------------|-------------|-------------|-------------|-------------|-------------|
|                     | Fe(0)       | Fe(II)      | Fe(III)     | Fe(0)       | Fe(II)      | Fe(III)     |
| LEAF                |             |             |             |             |             |             |
| trichome            | 29 $\pm$ 12 | 77 $\pm$ 16 |             | 21 $\pm$ 16 | 81 $\pm$ 18 |             |
| surface             | 20 $\pm$ 3  | 37 $\pm$ 1  | 44 $\pm$ 4  | 12 $\pm$ 1  | 23 $\pm$ 6  | 65 $\pm$ 6  |
| cell wall           | 31 $\pm$ 12 | 13 $\pm$ 3  | 56 $\pm$ 9  | 10 $\pm$ 1  | 15 $\pm$ 1  | 73 $\pm$ 0  |
| vein                | 10 $\pm$ 4  | 18 $\pm$ 6  | 74 $\pm$ 12 | 11 $\pm$ 4  | 20 $\pm$ 2  | 67 $\pm$ 6  |
| ROOT                |             |             |             |             |             |             |
| quiescent<br>center | 30 $\pm$ 16 | 70 $\pm$ 16 |             | 13 $\pm$ 13 | 88 $\pm$ 14 |             |
| epidermis           | 22 $\pm$ 3  | 51 $\pm$ 1  | 27 $\pm$ 4  | 21 $\pm$ 16 | 18 $\pm$ 6  | 62 $\pm$ 13 |
| cell wall           | 13 $\pm$ 18 | 86 $\pm$ 19 |             | 15 $\pm$ 6  | 86 $\pm$ 5  |             |

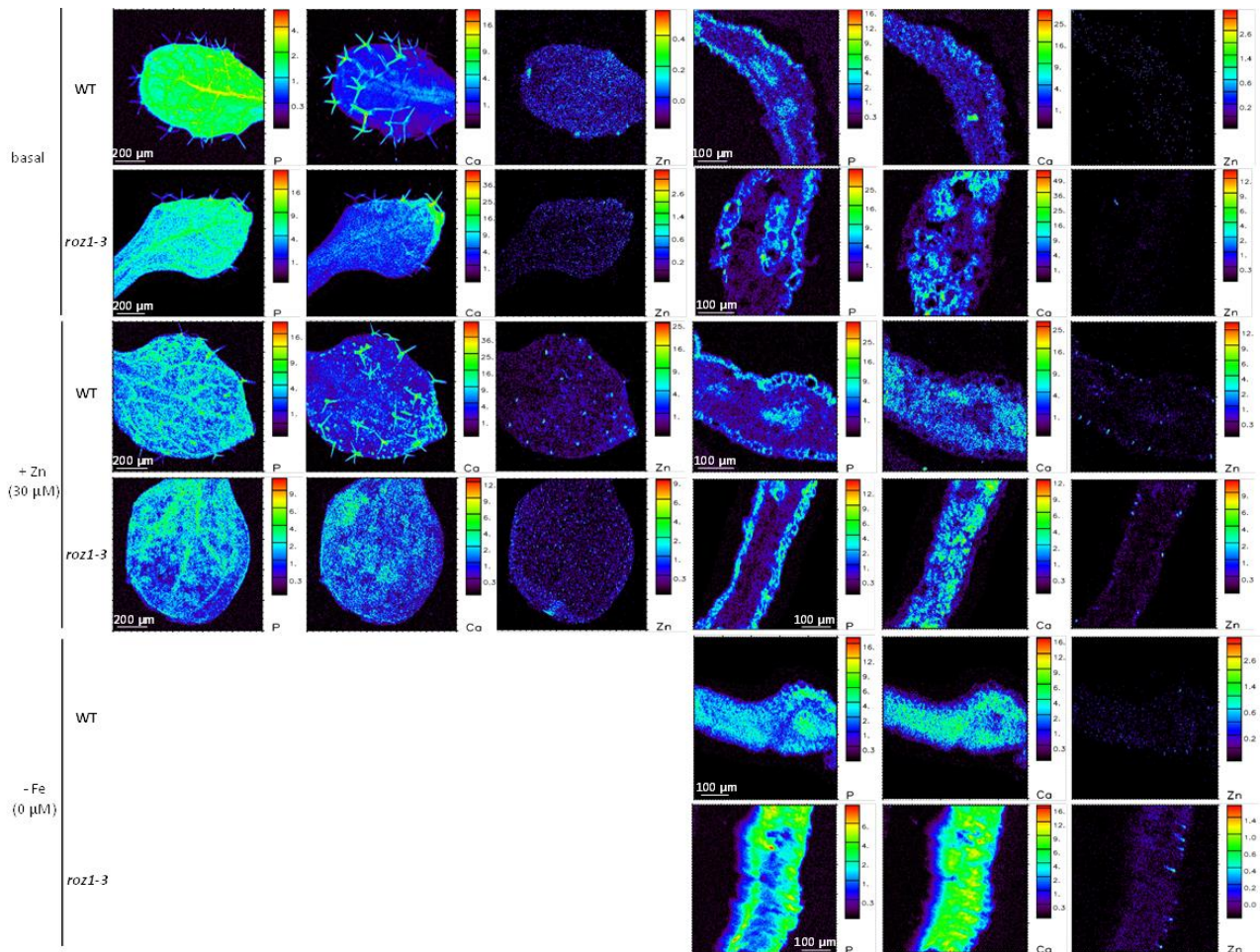


Figure 1.  $\mu$ PIXE analysis of *roz1-3* and WT leaves grown in three different media displaying P, Ca and Zn distributions. The color scale is in wt%.



Figure 2: WT and *roz1* phenotype on soil (sown on the same day).

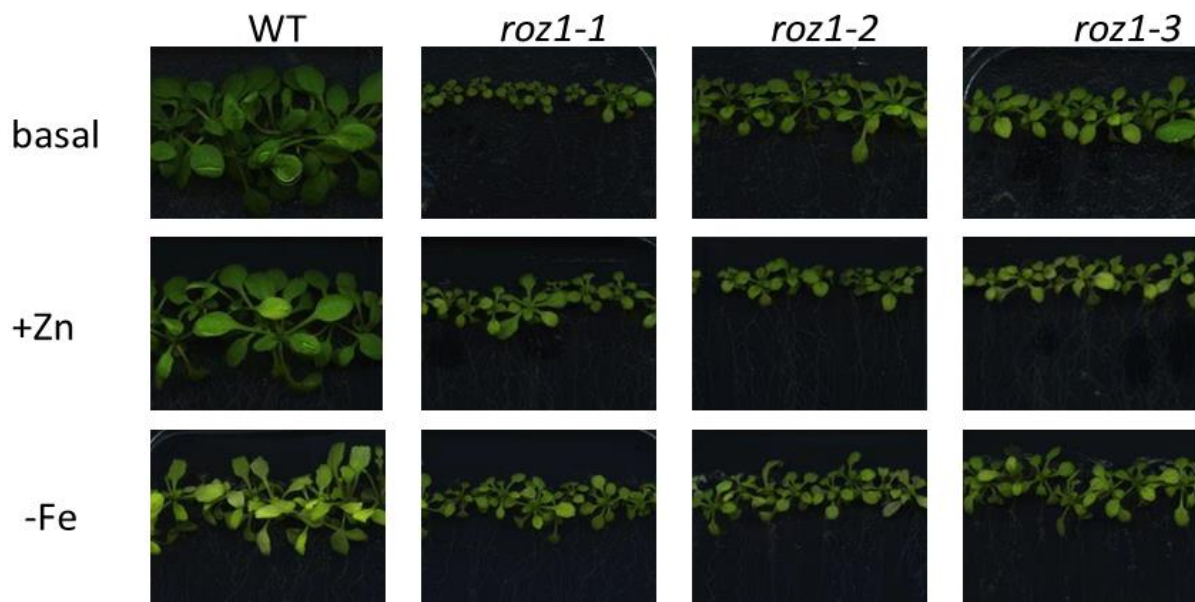


Figure 3: 4 day old mutant phenotypes in basal, high Zn (30  $\mu$ M) and no Fe (washed agar) conditions after 7 day exposure.

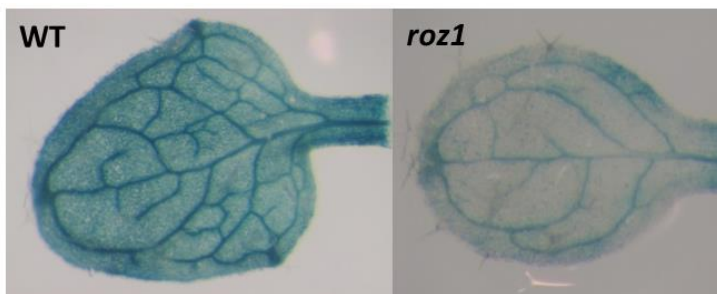


Figure 4: GUS staining of the first true leaf of WT and *roz1* 15 d old seedlings exposed for 7 days in high Zn condition

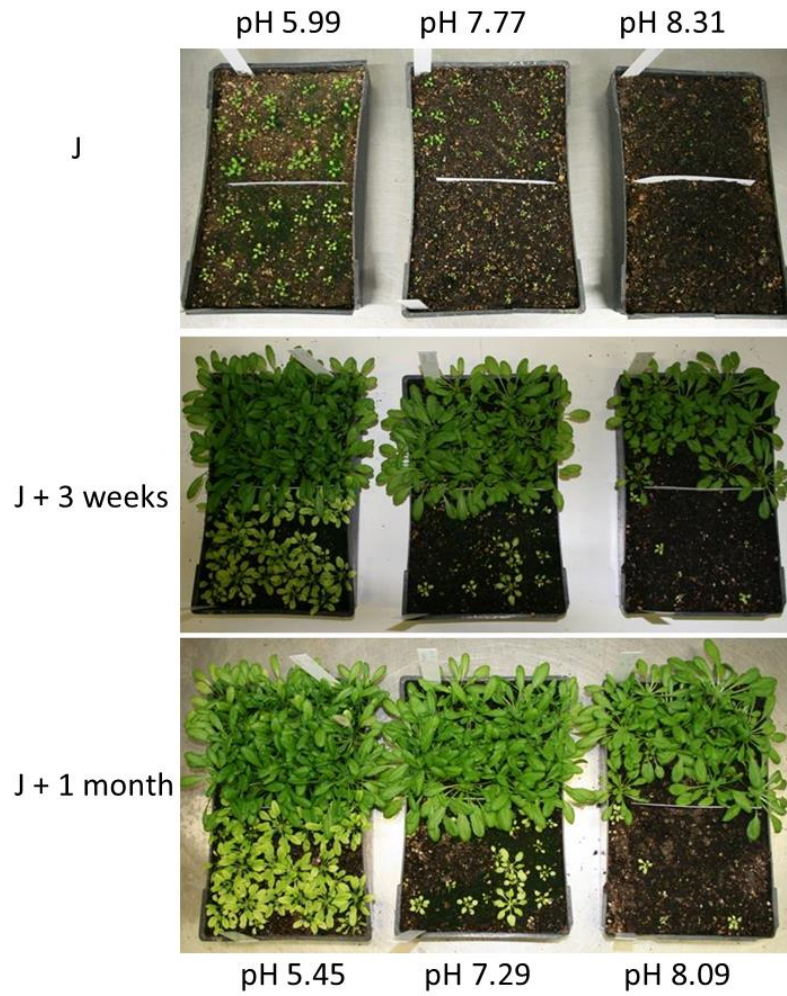


Figure 5: upper side of the tray *Columbia* and lower side *irt1*. Plants were sown on minitray soil (first column) or minitray with modified pH soil (second and third columns).