Roots – unnoted giants

Differential plasma membrane H^+ ATPase activity of closely related acidophil and basophil plants

Gymnasium Bäumlihof 3I

1.Introduction

Our planet is covered with a thin layer of soil and plants. As soon as there are no plants erosion and desertification begins. Agriculture was essential for the Neolithic revolution and the beginning of human culture and until today the nutrition of the world population is dependent on agriculture, breeding of appropriate food plants and fertilizer. Especially the accessibility of nitrogen as NO_3^- or NH_4^+ is essential to achieve an appropriate yield. Hence plants and their roots together with the soil form a web of life of incredible importance. Some of plant roots mysterious interactions with the soil are well known [3,4].

Uptake of inorganic components by roots

Plants need amongst others inorganic components to be able to grow. So that they can take in the minerals, they have to exchange cations with the soil solution, as most soil particles are negatively charged and therefore the positive charged cations like K^+, Ca^{2+}, Mg^{2+} stick to the particles. Therefore, the plant root releases H^+ through the activity of a membrane bound H^+ ATPase to displace the adsorbed minerals. This process leads to an acidification of the rhizosphere. It is possible to monitor this acidification with the help of a pH indicator [1, 4]. Negatively charged anions like NO_3^- do not bind to the negatively charged soil particles and are therefore lost by leaching [3].

Root activity even influences xylem transportation. Driving forces for this transport are transpiration and root pressure. In the root minerals are actively transported into the xylem leading to an accumulation of osmotic active substances and therefore, to a flow of water into the xylem. This increasing pressure causes water to be squeezed into the sprout sometimes leading to the formation of guttation drops at the tip of the leaves.

Acidic and alkaline soil

Soil can have very different pH and this specifically affects plant nutrient availability by controlling the chemical forms of the nutrient. Acidic earth is detrimental for some plants because it can contain toxic amounts of aluminium. Alkaline soils have a high saturation of base cations (K^+, Ca^{2+}, Mg^{2+} and Na^+). So each plant seem optimised to the pH of the soil it is growing. In this research project we were interested if closely related plants with different soil preferences (acidophil and basophil) exhibit a differential plasma membrane H^+ ATPase activity. In addition we wanted to proof that the pH change of the rhizosphere is really dependent on H^+ ATPase activity and not an effect of CO_2 release from respiration

2. Material and Methods

In these experiments five different plants were used: *Erica carnea*, *Calluna vulgaris*, *Lathyrus vernus*, *Lathyrus linifolius* and *Glechoma hederacea* . The first three were bought in a local market garden for wild flowers, *G. hederacea* were found around our school building and *L. linifolius* was searched in the Elsässer Hard.

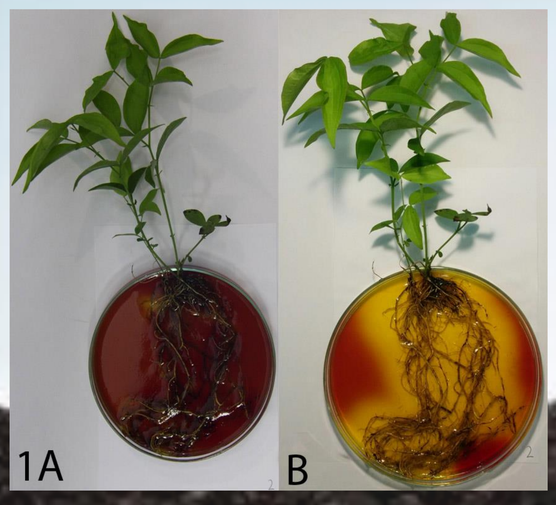
We documented the change of pH (dependent variable) around the roots of the closely related plants *E. carnea* (basophil) and *C. vulgaris* (acidophil) as well as of the closely related plants *L. vernus* (basophil) and *L. linifolius* (acidophil) (independent variable) in order to test if basophil and acidophil plants react differently. We observed the colour change of the pH indicator bromocresol purple during 24 hours.

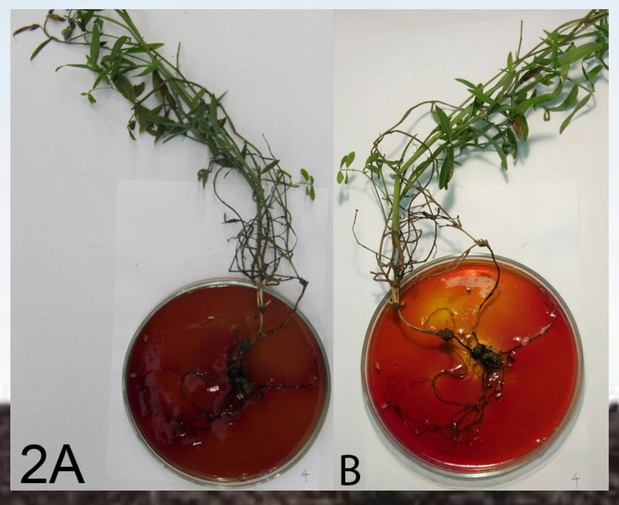
For our experiment we first washed the roots carefully and embedded them in 1% agarose media containing $(NH_4)_2SO_4$ (5 mmol/l) (controlled variable) and bromocresol purple (pH 5-6.6) (60 mg/l): agarose was melted by boiling, the pH adjusted with 0.1 NaOH to pH 6 so that the colour of the indicator was just purple and before pouring into a petri dish, the mixture was allowed to cool down to 45° C. Plants were kept under a cold light for 24 hours at room temperature and all shown pictures were taken after 24 hours (all controlled variables)

To proof that the H^+ export is dependent of a plasma membrane H^+ ATPase we used a special prepared petri dishes with a silicon wall that separated the dish in two halves. In one half we poured the same agarose solution as in the experiment above and in the other half we poured agarose solution including 1 mmol/l sodium orthovanadate (independent variable). The pH of the solution containing vanadate was adjusted with 0.1M HCl until it had the exact same colour as the pH of the control solution without Vanadate. Vanadate is a plasma membrane H^+ ATPase inhibitor[5]. The experiment was done with *G. hederacea* and the roots of one plant was partly in the media containing vanadate and partly in the media without vanadate. Hence we had the control in one and the same plant (controlled variable)! Plants were kept at room temperature and at constant light. Colour change of the pH indicator bromocresol purple was documented after 48 hours (dependent variable).

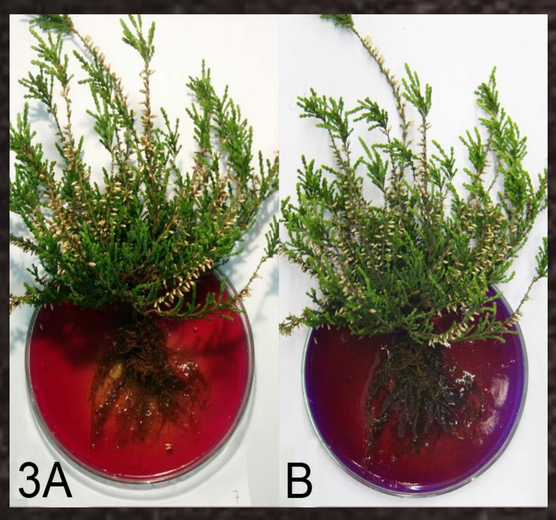
Each experiment was done in three replica hence we used 15 plants in total

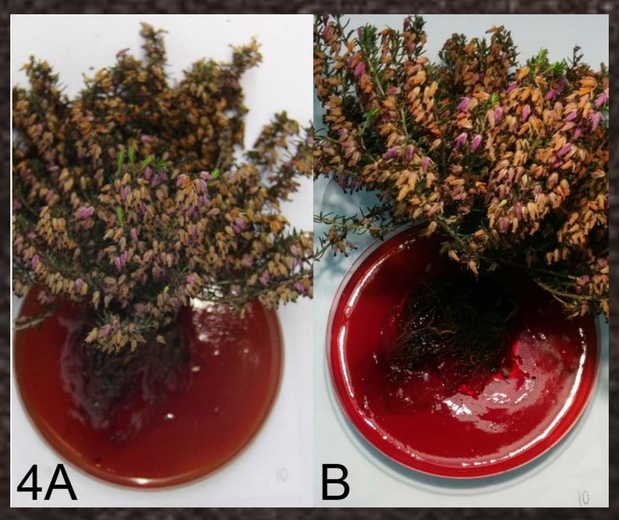
3. Results



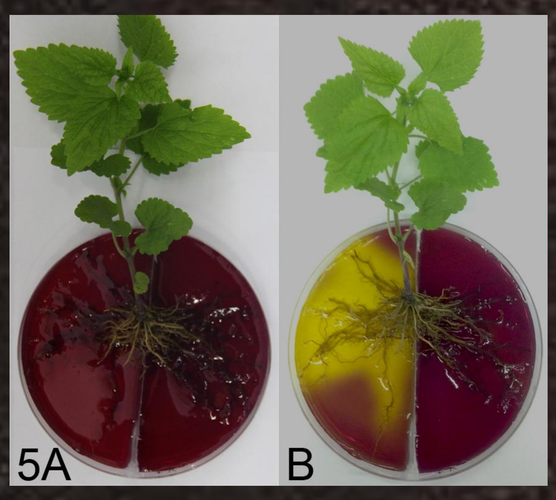


The basophil *Lathyrus vernus* (1A, B)[2] is closely related to the acidophil *Lathyrus linifolius*: (2A, B)[2]. Fig. 1A shows a *L. vernus* plant with the roots imbedded into the agarose at the beginning of the experiment. After 24 hours a clear acidification of the rhizosphere is recognisable: Bromocresol purple turned yellow and therefore, the pH is below 5. Fig. 2A shows a *L. linifolius* at the beginning of the experiment. After 24 hours (2B) bromocresol purple showed some regions turning yellow but the effect is marginal compared to the colour change in *L. vernus* (1B).





The basophil *Erica carnea* (3A, B)[2] is closely related to the acidophil *Calluna vulgaris* (Fig. 4A, B)[2]. Fig. 3A shows a *E. carnea* plant with the roots imbedded into the agarose at the beginning of the experiment. After 24 hours (3B) no change in the colour of bromocresol purple was detectable. Fig. 4A shows a *C. vulgaris* plant at the beginning of the experiment. After 24 hours (4B) a slight alkalisation of the rhizosphere is recognizable: Bromcresol purple turned to a deep purple. The colour change is not immediately around the roots but more at the edge of the petri dish visible.



Glechoma hederacea (5A, B)[2] was imbedded in a petri dish which to separate chambers in a way that the roots are partly in the two chambers. The right chamber containing in addition to $(NH_4)_2SO_4$ (5 mmol/l) and bromocresol purple (pH 5-6.6) (60 mg/l) 1 mmol/l sodium orthovanadate, a strong H^+ ATPase inhibitor. The left chamber was without inhibitor. Fig. 5A shows the plant at the beginning of the experiment, Fig 5B after 48 hours. A clear acidification of the rhizosphere is recognizable in the chamber without vanadate (colour change to yellow around roots), whereas there is now colour change in the chamber with vanadate. Hence, vanadate inhibits effectively.

4. Discussion

There are three different ways of N-Nutrition in plants: NH_4^+ or NO_3^- uptake and N_2 fixation by symbiotic *Rhizobium* bacteria. The energy requirements are very different depending on the starting molecule being least for NH_4^+ and most for N_2. NH_4^+ is taken up by acidification of the rhizosphere through the activity of the ATPase. Hence NH_4^+-nutrition is called physiological acid-nutrition [4]. In contrary to that NO_3^- -nutrition is caused by a cotransport of NO_3^- and H^+, leading to a alkalisation of the rhizosphere. Hence NO_3^- -nutrition is called physiological alkali-nutrition [4]. The preferences of the nutrition is dependent on plant species as well as on soil acidity. Usually a mixed nutrition is ideal. NO_3^- in an acidic soil is preferred as the H^+-stress gets reduced [4].

In the here presented experiments only NH_4^+ is available for plant nutrition. Hence the basophil *L. vernus* acidify the rhizosphere (1A, B) whereas the acidophil *L. linifolius* used to NO_3^--nutrition and alkalisation did not change its rhizosphere (2A ,B). Hence, it might take longer or is not possible to change the physiology of nutrition from NO_3^- to NH_4^+ for the acidophil *L. linifolius*.

It is surprising that the basophil *Erica* (3A, B) did not change the pH of the rhizosphere as it would be expected that *Erica* exhibits NH_4^+-nutrition. *Calluna* (4A, B) shows a alkalisation of the environment. Hence, it might be that *Calluna* tries to cotransport NO_3^- and H^+ despite the fact that there is no NO_3^- in the media leading to a slight alkalisation.

It would be interesting in a next experiment to test the same plant species (e.g. corn) in a NH_4^+ or a NO_3^- medium containing bromocresol purple. It would be expected that alkalisation happens under NO_3^--nutrition and acidification under NH_4^+-nutrition. In addition this experiment could then be repeated with acidophil and basophil *Lathyrus* plants.

Our second experiment (5A, B) clearly indicates that vanadate inhibits the ATPase and that the acidification in solely due to its activity and not based on cellular respiration and CO_2 accumulation.

In a next experiment it would be interesting if ATPase is involved in NH_4^+-nutrition and NO_3^--nutrition and hence can block acidification and alkalisation of the rhizosphere.

5. References

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