DR KATRIN PHILIPPAR

Striking with HOT IRON

Dr Katrin Philippar is the coordinator of a multidisciplinary team seeking to combat iron deficiency in southern European soils

Could you outline the agronomical and economical impact of iron deficiency in crops?

Iron deficiency is a severe agronomic and economic problem, especially in the Mediterranean regions of Southern Europe, where intensive cultivation of fruit trees takes place on calcareous soils. Since iron is not readily available for plants in these high pH soils, farmers have to spend more than €50 million per year to provide iron fertilisers to fruit tree orchards. Worldwide, iron deficiency also affects other crop plants, such as soybeans (U.S.), rice (Asian countries) and peanuts (India).

What are the overall objectives of your research project?

The objective of 'HOT IRON - PLANT PROGROW' is to uncover regulatory processes linking iron homeostasis in the chloroplast (the most iron rich organelle in plant cells) and the cytosol with mechanisms of iron acquisition and transport. In a genome-, proteome-, and metabolome-wide approach, molecular targets in response to iron deficiency or toxicity are being identified in *Arabidopsis thaliana*. Results gained in this model plant are used to modify and improve growth conditions, as well as productivity of agronomical relevant crop species, eg. fruit trees. Furthermore, this should lead to the development of new fertilisers and crop management strategies to improve plant tolerance to iron deficiency.

To what extent is the work multidisciplinary and how does this help in achieving the project's goals?

The work is wholly multidisciplinary, involving molecular and cell biological tools, protein biochemistry and large scale analysis techniques (transcriptomics, proteomics,

metabolomics), as well as physiological and phenotypic screening procedures of mutant plant lines. In particular, the transfer to applied science (eg. testing of iron fertilisers on crop plants) and the embedding of field work is crucial for the achievement of the project's goals. Since our five partners have complementary methods and techniques, the discovery of new mechanisms and the transfer to field conditions are only achieved via a joint effort.

At what stage is the project?

The project is now in the last year of funding. To date, we have already produced significant findings across all three work packages, represented by eight original publications so far in internationally acknowledged, peer reviewed journals, and one contribution to a patent. The data collection of transcriptomics (LMU, INRA), proteomics (CSIC), metabolomics (CSIC, CIPAV), represented by our internal 'FeChlorosisDatabase' will be completed within the first half of 2012, and is planned to be published in a joint manuscript. Furthermore, our partner IPK has screened 4,000 mutant lines and identified two promising mutants severely affected in iron efficiency.

What is the market potential for this work?

Although the work is based on fundamental research, it is likely to have market potential. We are currently in the process of understanding how plants regulate their responses to iron deficiency and which genes, proteins or metabolites are involved. Until now, iron fertilisation treatments have not been knowledge based. They are quite crude, and just try to deliver a lot of iron in a form that is available for plants. The basic knowledge we aim to generate within 'HOT IRON' will likely allow the development of more targeted fertilisation approaches by exploiting and enhancing those mechanisms already used by plants.

Can you outline the impact you expect this work to have?

We are already impacting on the basic knowledge on plant iron deficiency physiology with our last publications. In the long term, this should lead to a better understanding of which iron efficiency mechanisms are relevant for what type of soil, and to the development of markers for breeding. In the near future, we expect to explore and develop new plant-friendly fertilisers.

Are there any further aspects of the project you wish to highlight?

One aspect we wish to emphasise is the establishment of tight collaboration and exchange of data and experience among the different 'HOT IRON' partners that turned out to work exceptionally well. In particular, the connection from basic research to applied science contributed to lift goals and perspectives to a new level.

Overcoming 'anaemia' in calcareous soils

Iron deficiency is a problem for plants that grow in alkaline soils, producing crops that are less nutritious. The European **HOT IRON** consortium is looking to uncover the mechanisms that control iron metabolism in such plants in an attempt to overcome this problem

DESPITE ITS STATUS as the fourth most abundant element in the Earth's crust, iron is often unavailable to plants that grow in calcareous (containing high levels of calcium carbonate) or alkaline soils, which compose around 30 per cent of the world's arable land.

This discrepancy is due to the reactive nature of iron, which combines with water or oxygen in alkaline soils under aerobic conditions to form hydroxides and oxides that fix the iron and make it unavailable for plants, thereby causing leaf chlorosis – characterised by a marked yellowing of the leaves, as well as reduced vegetative growth. This is a problem that is prevalent throughout the Mediterranean region in Europe, with citrus trees in Spain, Italy, and Portugal, and peach and pear trees in Spain, Italy, and Greece affected by iron deficiency that results in lower yields and poorer quality of fruit. These areas have high concentrations of such fruit trees in calcareous soils, and consequently farmers tend to supplement with iron-rich fertilisers.

FIGURE 1. Transmission electron micrograph (TEM) of *Arabidopsis thaliana* chloroplast (magnification 12,000x). © Professor Wanner, LMU

Iron fertilisers fall into several different categories of varying efficacy and price. Inorganic compounds are generally inefficient in alkaline soils, with most of the iron compounds being rapidly transformed into other insoluble chemicals that may also be harmful to the environment, especially those originating from industrial by-products. Synthetic fertilisers (iron chelates) on the other hand, are very expensive, and may also cause damage to the environment. Finally, natural iron complex fertilisers are unstable in soil and, hence, poorly suited as iron supplements. These problems have led researchers to attempt to elucidate the mechanisms regulating iron transport and uptake in the hope of both enhancing tolerance to iron deficiency in vulnerable plants, and to develop new fertilisers.

HOT IRON: RESTORING BALANCE IN IRON HOMEOSTASIS

HOT IRON – PLANT PROGROW is a consortium of European research institutions coordinated by Dr Katrin Philippar, from the Department of Biology I at the Ludwig-Maximilians-University of Munich (LMU, Germany), and includes partners from the Leibniz-Institut für Pflanzengenetik und Kulturpflanzenforschung (IPK, Germany), the Institut National de Recherche Agronomique (INRA, France), the Agencia Estatal Consejo Superior de Investigaciones Cientificas (CSIC, Spain), and the Centro de Investigación en Producción Animal y Vegetal (CIPAV, Spain).

The team is seeking to explain the regulatory processes that connect iron homeostasis in chloroplasts (the main iron store in plants, containing around 90 per cent of the iron in the leaves) and the cytosol of plant cells with the mechanisms that govern iron capture and transport. One of the puzzling phenomena associated with iron deficiency is characterised by the presence of high concentrations of iron in leaf veins of iron-deficient trees. This is known as the 'chlorosis paradox'. Philippar believes this may be due to bottlenecks in iron uptake and distribution in the plant, and this is a problem that can even be induced, as she relates: "Crops that were not affected by iron deficiency when grown in a traditional way, such as grapes and olives, can rapidly become susceptible when grown with novel technologies that force the growth and yield of the crop".

FIGURE 2. Screening of Arabidopsis mutant lines grown on iron-limiting, calcareous soil (IPK).

The search to understand iron homeostasis in plants, which is a process that involves a series of steps that include transport, utilisation, and storage mechanisms – each located in different plant organs, tissues, and sub-cellular components – has led to a focus on specific pathways within chloroplasts, which demand high levels of iron for photosynthesis and are thus prime candidates for such bottlenecks. However, the mechanisms for this at a molecular level are not fully understood, although an imbalance in iron cycling is believed to be a cause of limited plant growth or productivity.

HOT IRON – PLANT PROGROW

HOMEOSTASIS AND TRANSPORT OF IRON – TOWARD IMPROVING PLANT PRODUCTIVITY AND GROWTH

OBJECTIVES

To uncover regulatory processes linking iron homoeostasis in the chloroplast and the cytosol of plant cells with mechanisms of iron acquisition and transport.

PARTNERS

Dr Katrin Philippar, Ludwig-Maximilians-University Munich (LMU), Munich, Germany

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DR KATRIN PHILIPPAR studied Biochemistry (University of Hannover, Germany) and completed her doctoral thesis in Biology (research on Molecular Plant Physiology) 1999 at the University of Würzburg, Germany. Since 2004 she has held the role of group leader at from the Department of Biology I at the Ludwig-Maximilians-University of Munich (LMU, Germany) were she became a Private Lecturer for Botany and Cell Biology in 2010.

Crops that were not affected by iron deficiency when grown in a traditional way, such as grapes and olives, can rapidly become susceptible when grown with novel technologies that force the growth and yield of the crop

One of the main areas of focus for HOT IRON has been to elucidate the functional link between two chloroplast intrinsic proteins – ferritin, which stores iron, and PIC1, which transports it. These proteins are critical in controlling and regulating plant growth, biomass production, and seed yield. Furthermore, the role of citrate in long distance iron transport from the root to the shoots is under investigation. Previous research has shown that citrate acts as a chelating agent of iron (ie. complex the iron to allow transport), and *Arabidopsis* mutant species lacking the appropriate citrate transporters which load the iron into the xylem (the circulatory network in vascular plants) are consistently iron deficient. The HOT IRON team is now working on identifying new proteins that are involved in iron distribution and homeostasis within the plant, as well as on transcriptomic, metabolomic and proteomic deregulation upon iron deficiency and stress.

EUROCENTRIC BUT UNIVERSAL

The success of the project so far has been highly dependent on the collaborative efforts of the researchers from the various institutes involved. In addition to the competence of Philippar as the coordinator, other notable names to be mentioned include Professor Nicolaus von Wirén (IPK), who has been responsible for screening new genes that regulate iron transport and homeostasis by studying mutant plants growing in calcareous soil, and Dr Jean-Francois Briat (INRA), who has provided expertise on iron homeostasis in chloroplasts that is primarily mediated by ferritins, in addition to carrying out large-scale phenotype analyses of mutant plants that experience disturbed iron homeostasis. In Spain, Professor Javier Abadia (CSIC) has been invaluable for his knowledge regarding proteomics and metabolomics (especially the role of citrate in iron transport), and for his work in field trials on fruit trees. Last, but not least, is Dr Jose Maria Garcia-Mina (CIPAV), who has been the key partner from industry, and has been responsible for analysing plant hormones associated with iron homeostasis, and for developing new iron fertilisers that are being tested on cucumber plantlets. The various contributions of the partners have been coordinated through their annual meetings and day-to-day cooperation.

There has also been an exchange of their PhD students, as well as the establishment of a database – the FeChlorosisDatabase – which has been invaluable for the free exchange of knowledge between the groups. Funding is due to end later this year, and further results are eagerly awaited.

FIGURE 3. Extreme chlorosis and dwarfism in knockout mutants of the chloroplast iron-uptake protein PIC1 (3-week-old plantlet on agar medium, LMU).

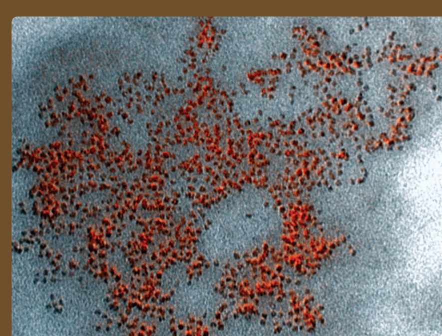

FIGURE 4. Electron spectroscopic imaging (TEM) picture of iron-ferritin clusters in leaf cells of pic1 knockout mutants (magnification 140,000x). © Professor Wanner, LMU