NOVEL APPROACHES FOR THE CONTROL OF IRON CHLOROSIS IN FRUIT TREE CROPS

Exploitation Report

Version 2, January 2000

Contract AIR3 CT94-1973

FINAL REPORTING

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FINAL REPORTING-EXPLOITATION REPORT

Project Title

NOVEL APPROACHES FOR THE CONTROL OF IRON CHLOROSIS IN FRUIT TREE CROPS

Organisations involved

P1: Co-ordinator, Consejo Superior de Investigaciones Científicas, Estación Experimental de Aula Dei, Zaragoza, Spain.
P2: Unidade de Ciencias e Tecnologías Agrarias, Universidade do Algarve, Faro, Portugal ("UCTA-U.AL").
P3: National Agricultural Research Foundation, Pomology Institute. Naoussis, Greece ("NARF").
P4: Heinrich-Heine-Universität Düsseldorf, Institut für Oekologische Pflanzenphysiologie, Düsseldorf, Germany ("UNI-DU").
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P6: Universitá degli Studi di Bologna, Dipartimento di Colture Arboree, Bologna, Italia ("UB-DCA").

1 Description of the results and comparison of the innovative aspects to the state of the art

The results obtained during the project have shown that **chlorosis has major negative impacts in fruit yield and quality** in different fruit species grown in the Mediterranean area. An increase in chlorosis symptoms led not only to severe reductions of total fruit yield per tree, but also affected adversely fruit size and quality. These new data emphasise the importance of controlling chlorosis in fruit tree crops in Southern Europe. No such clear data on the effect of Fe chlorosis on fruit yield and quality were available before the project.

The results found in the project have established the principles to perform the **early diagnosis (prognosis) of Fe chlorosis in trees** from the mineral analysis of tree flowers. Two mineral analysis parameters in flowers, the concentration of Fe and the K/Zn ratio, have been shown in the project to be well correlated with tree chlorosis later in the year. This suggests

that the analysis of flowers of any previously non-analysed orchard or tree could be useful for estimating the future chlorosis status, as well as an index for assessing the Fe level of trees in the previous year. The **Fe flower concentration** was proposed before the project as a tool for the prognosis of chlorosis in fruit trees. Our results have demonstrated that the correlation between Fe in fruit tree flowers and the leaf chlorophyll concentration later in the season is often significant. We have found that a new parameter, the **K/Zn ratio in flowers** may offer advantages over the Fe flower concentration for the prognosis of tree chlorosis later in the year. The K/Zn ratio in flowers is well correlated to chlorosis every year. However, and conversely to what happens with Fe, the average concentration of K and Zn in flowers as well as the K/Zn ratio have quite consistent values from year to year.

Agronomic means for controlling Fe chlorosis are still viewed with great interest by fruit growers. Since Fe chelates were introduced, there has been little effort in the research of alternative means for controlling the chlorosis. Our work has demonstrated that alternatives do exist and in the future may be included among the routine practices of managing fruit trees. The results found in the project have established that some agronomic practices, such as applying foliar sprays with inorganic Fe sources, growing graminaceous plants fertilised with Fe in the orchard and adding new Fe-containing products to the soil, could be as effective as synthetic Fe-chelates in controlling chlorosis in fruit trees. Alternatives to Fe chelates are of great importance in orchards that follow the guidelines of "Biological Production" or "Integrated Production".

Experiments carried out in the project have shown that **foliar sprays of cheap Fe salts** such as Fe sulphate could be effective in controlling chlorosis. Of course several applications need to be made during the growing season, and more work has to be done to optimise the effects of foliar sprays in the regreening of leaves and minimising the possible

deleterious effects in fruit quality in each crop. Also, some **agronomic practices**, such as growing graminaceous plants near the trees concomitantly to the addition of inorganic Fe salts to the soil or using new commercial compounds such as blood meal, are promising for controlling chlorosis. The proposed techniques, however, would need also validation under the integrated production management schemes used by commercial growers.

Research done in the project has also shown the importance of the Fe pools inactive in the chlorotic leaves of fruit trees, since when chlorosis was not too severe re-greening may occur by adopting strategies that remobilise Fe. The well-accepted phenomenon that chlorotic and green leaves have similar total Fe concentration, which has been termed "Fe paradox", indicates the existence of Fe pools in the chlorotic leaf which are somehow inactive. Data from our experiments demonstrate that sprays aiming to activate the Fe pools in the chlorotic leaf are effective, although rarely caused a full recovery from Fe chlorosis. This indicates that in a chlorotic leaf part of the Fe is inactivated outside the mesophyll cells.

Data obtained in this project have permitted to establish a **new protocol for screening tests** based on physiological characteristics associated to tolerance to Fe chlorosis in fruit trees. We have produced consistently increases (20-fold) in the root ferric chelate reductase activity in tolerant fruit tree rootstocks, by re-supplying plants grown without Fe for several days with a small amount of Fe(III)-EDTA. Furthermore, we have found that this protocol may be used in screening assays to select rootstock genotypes tolerant to Fe chlorosis, as shown from preliminary comparisons of several genotypes differing in chlorosis tolerance. The proposed new protocol could be used to assess in the future the germplasm available for chlorosis tolerance. The works made with fruit tree rootstocks before the project showed that the root ferric chelate reductase activity was not always induced by Fe deficiency, and the reason for that was not known.

The possible use of **somaclonal variation methods** has given promising results with pear genotypes. These results are still preliminary but open a new way of finding tolerance to chlorosis in fruit trees.

The works developed within the project have provided light on several physiological changes induced by Fe deficiency in plants. The biochemical characteristics of the **root ferric chelate reductase enzyme** have been extensively studied on the level of intact roots *in vivo*, and on the level of purified plasma membranes *in vitro*. Our finding that the enzymatic characteristics depend on the measuring pH has changed the view on the root ferric chelate reductase enzyme.

A standardised **root tip test** has been developed to estimate root ferric chelate reductase activity *in vivo*. This test could be also useful in screening programs.

The biochemical characteristics of the **leaf mesophyll ferric chelate reductase enzyme** have been studied at the level of mesophyll tissue *in vivo*, intact protoplasts and purified plasma membranes *in vitro*. The *in vivo* leaf ferric chelate reductase activity has been confirmed to be light dependent. The pH dependence of the ferric chelate reductase enzyme in isolated protoplasts is different from that of isolated plasma membranes, with its optimal pH being closer to that of the apoplastic pH. Both the intrinsic decrease in ferric chelate reductase activity per protoplast surface and a possible shift in the pH of the apoplastic space could be responsible for the immobilisation of physiologically inactive Fe pools in chlorotic leaves. The knowledge on this enzyme before the project was very limited, with only one paper published on that issue.

A very important finding is that the natural complexes of citrate and malate with ferric Fe are good substrates for the ferric chelate reductase enzyme, both in mesophyll tissue and in

isolated plasma membranes. This opens new possibilities for applying these compounds to control Fe chlorosis in field conditions, as already shown in kiwifruit.

Knowledge has been gained during the project on the molecular changes in **proteins induced by Fe deficiency** in leaf and root tissue. The isolation and partial characterisation of the FC-R from spinach leaves has been carried out during the project. Other approaches such as 2-D electrophoresis of root tip proteins have led to the identification of a series of polypeptides which are candidates to play a role in the Fe-efficiency responses of plants to Fe deficiency. It is expected that these polypeptides will be characterised in the near future.

The new knowledge on the photosynthetic changes induced by Fe deficiency has provided the background data to understand one of the characteristics less studied of Fe chlorosis, the light dependence of the deficiency. The changes in PS II efficiency in Fe-deficient leaves have been characterised in studies carried out in the project.

Finally, we have demonstrated in the project that a photosynthetic parameter, the chlorophyll content estimated from **SPAD readings**, is an excellent tool to be used as an **indicator of Fe chlorosis**. This parameter, once properly calibrated for a given species, is far better than other indicators such as chlorophyll fluorescence, mineral content and visual ratings. We have used the SPAD apparatus in most experiments developed in the project, including chlorosis control experiments.

2 Description of the practical applications

1. - Chlorosis has major negative impacts in fruit yield and quality in different fruit species grown in the Mediterranean area. This emphasises the importance of controlling chlorosis in fruit tree crops in Southern Europe.

2. - Early diagnosis (prognosis) of Fe chlorosis in trees could be made at least from two mineral analysis parameters in flowers, the concentration of Fe and the K/Zn ratio.

3. - Alternatives to Fe-chelates do exist and in the future may be included among the routine practices of managing fruit trees. Agronomic practices such as applying foliar sprays with inorganic Fe sources, growing graminaceous plants fertilised with Fe in the orchard and adding new Fe-containing products to the soil could be as effective as synthetic Fe-chelates in controlling chlorosis in fruit trees.

4. - A new protocol for screening tests based on physiological characteristics associated to tolerance to Fe chlorosis in fruit trees has been developed. The proposed new protocol, which induces root ferric chelate reductase activity in Fe deficiency-tolerant fruit tree rootstocks, could be used to assess germplasm for chlorosis tolerance.

5. - The possible use of somaclonal variation methods has given promising results with pear genotypes.

6. - A standardised root tip test has been developed to estimate root ferric chelate reductase activity *in vivo*.

7. - The natural complexes of citrate and malate with ferric Fe are good substrates for the leaf ferric chelate reductase enzyme. This opens new possibilities for applying these compounds to control Fe chlorosis in field conditions.

8. - The new knowledge on the photosynthetic changes induced by Fe deficiency has provided the background data to understand one of the characteristics less studied of Fe chlorosis, the light dependence of the deficiency.

9. - A photosynthetic parameter, the chlorophyll content estimated from SPAD readings, is an excellent tool to be used as an indicator of Fe chlorosis.

3 Exploitation plan

3.1 Patents or registered designs

None.

3.2 Means of exploitation

Several activities designed to disseminate the results of the project have been or are going to be developed in the near future. This includes the following:

3.2.1. - **Project brochure**. A diptych-type brochure has been produced by the Coordinator, indicating some of the practical results found in the project (see draft enclosed to the cover letter). Once reviewed by the Commission Services this diptych would be sent to the extension services of the different countries for dissemination.

3.2.2. - **Web Project page**. A new web page has been created (www.spicom.es/chloropage). This page is expected to contain the Project information summarised in the brochure, papers already published by Project participants and the text of the final Project Report.

3.2.3. - **Dissemination papers**. An effort will be made to publish some summary papers in farmer-oriented Journals, such as the two recently published in the "Rivista di Frutticoltura e di Ortofloricoltura" and in "Phytoma" (see numbers 24 and 29 in list **4.1** below).

3.2.4. - **Joint Posters in Symposia**. Presentation of joint posters summarising the results of the project will be made in the next Plant Nutrition Symposia of Cairo (2000) and Hanover (2001).

4 Information of publications

From the works developed in the project we have published research and technological papers (see list **a** below and attached Project Publications Collection), and several Theses (see list **c** below). Recently we have submitted for publication a number of new papers (see list **b** below).

Since a large part of the work is still unpublished, it is expected that at least during two years there will be a continuous flow of papers being sent for publication. In this context, we shall do an effort to publish results in publications oriented to growers.

4.1 Published Research and Technical Papers

1 **Tagliavini M, Rombolà AD 1995** Nuove prospettivi puor superare la clorosi ferrica negli alberi da frutto. **Rivista di Frutticoltura** 9, 11-21.

2 **Susín S, Abadía A, González-Reyes JA, Lucena JJ, Abadía J 1996** The pH requirement for *in vivo* expression of the Fe-deficiency-induced "turbo" ferric chelate reductase. A comparison of the Fe deficiency-induced Fe reductase activities of intact plants and isolated plasma membrane fractions in sugar beet (*Beta vulgaris* L.). **Plant Physiology** 110, 111-123.

3 **Tagliavini M, Scudellari S, Marangoni B, Pelliconi F, Valli S 1997** Valutazione di metodi alternativi ai chelati per il controllo della clorosi ferrica nell'actinidia. In: **Proceedings Convegno Nazionale sulla Coltura dell'Actinidia,** Faenza, Italia, 197-202.

4 **Marangoni B, Tagliavini M, Toselli M 1997** La chlorosi ferrica del pesco: conoscenza, prevenzione e terapia. In: **Proceedings of the XXII Convegno Peschicolo,** Cesena, Italia, 108-113.

5 **Pestana M, Ferreira P, Correia PJ, David M, de Varennes A, Faria** EA. 1997 Efeito da clorose férrica em porta-enxertos de citrinos: estudo de alguns parâmetros fisiológicos. **Actas Horticultura** 18, 44-51.

6 **Nedunchezhian N, Morales F, Abadía A, Abadía J 1997** Decline in photosynthetic electron transport activity and changes in thylakoid protein pattern in field grown iron deficient peach (*Prunus persica* L.). **Plant Science** 129, 29-38.

7 **Sanz M, Belkhodja R, Toselli M, Montañés L, Abadía A, Tagliavini M, Marangoni B, Abadía J 1997** Floral analysis as a possible tool for the prognosis of iron deficiency in peach. In: **Mineral nutrition and fertilizer use for deciduous fruit crops**. **Acta Horticulturae** 448, 241-245. Val J, Montañés L, Monge E eds. ISBN 90-6605-759-9.

8 **Morales F, Grasa R, Abadía A, Abadía J 1998** The iron "chlorosis paradox" in fruit trees. **Journal of Plant Nutrition** 21, 815-825.

9 **Morales F, Abadía A, Abadía J 1998** Photosynthesis, quenching of chlorophyll fluorescence and thermal energy dissipation in iron-deficient sugar beet leaves. **Australian Journal of Plant Physiology** 25, 403-412.

10 **Rombolà AD, Tagliavini M, Scudellari D, Quartieri M, Malaguti D, Marangoni B 1998** La clorosi ferrica delle piante arboree da frutto: aspetti generali e strategie di cura. **Proceedings Technical Meeting "Novel approaches to Mineral Nutrition of Fruit Trees", Notiziario Tecnico CRPV** 54, 35-50.

11 **Bassi D, Tagliavini M, Rombolà AD, Marangoni B 1998** Il progetto di selezione di portinnesti clonali per il pero, serie Fox. **Rivista di Frutticoltura e di Ortofloricoltura** 4, 17-19.

12 **Rombolà AD, Brüggemann W, Tagliavini M, Moog PR 1998** Meccanismi biochimici di tolleranza alla clorosi ferrica in Actinidia (*A. deliciosa*). In: **Proceedings of 4th "Giornate Scientifiche della Società Orticola Italiana ".** pp. 395-396.

13 **Rombolà AD 1998** Report sul "9th International Symposium on Fe Nutrition and Interaction in Plants". **Notiziario SOI di Ortoflorofrutticoltura** 6, 201-203.

14 **Belkhodja R, Morales F, Quílez R, Abadía A, Abadía J 1998** Iron deficiency causes changes in chlorophyll fluorescence due to the reduction in the dark of the photosystem II acceptor side. **Photosynthesis Research** 56, 265-276.

15 **Belkhodja R, Morales F, Sanz M, Abadía A, Abadía J 1998** Iron deficiency in peach trees: effects on leaf chlorophyll and nutrient concentrations in flowers and leaves. **Plant and Soil** 203, 257-268.

16 **González-Vallejo EB, Susín S, Abadía A, Abadía J 1998** Changes in sugar beet leaf plasma membrane Fe(III)-chelate reductase activities mediated by Fe-deficiency, assay buffer composition, anaerobiosis and the presence of flavins. **Protoplasma** 205, 163-168.

17 **Abadía J 1998** Absorción y transporte de hierro en plantas. In: Actas del VII Simposio Nacional-III Ibérico sobre Nutrición Mineral de las Plantas. pp. XIII-XXIV. Ediciones de la Universidad Autónoma de Madrid, ISBN 84-8497-927-X.

18 **Gogorcena Y, Abadía J, Abadía A 1998** Inducción in vivo de la reductasa de patrones frutales de *Prunus persica* L. In: Actas del VII Simposio Nacional-III Ibérico sobre Nutrición Mineral de las Plantas. pp. 27-32. Ediciones de la Universidad Autónoma de Madrid, ISBN 84-8497-927-X.

19 **Morales F, Abadía A, Abadía J 1998** Mecanismos de protección frente al exceso de luz en hojas deficientes en hierro. In: Actas del VII Simposio Nacional-III Ibérico sobre Nutrición Mineral de las Plantas. pp. 101-106. Ediciones de la Universidad Autónoma de Madrid, ISBN 84-8497-927-X.

20 **López-Millán AF, Morales F, Abadía A, Abadía J 1998** Implicaciones metabólicas en la respuesta bioquímica a la deficiencia de hierro en remolacha (*Beta vulgaris* L.). In: Actas del VII Simposio Nacional-III Ibérico sobre Nutrición Mineral de las Plantas. pp. 143-148. Ediciones de la Universidad Autónoma de Madrid, ISBN 84-8497-927-X.

21 **González-Vallejo EB, Abadía A, Herbik A, Stephan U, Rémy R, Abadía J 1998** Determinación de patrones polipeptídicos de raíz de remolacha (*Beta vulgaris* L.) en condiciones de deficiencia de Fe. In: Actas del VII Simposio Nacional-III Ibérico sobre Nutrición Mineral de las Plantas. pp. 119-124. Ediciones de la Universidad Autónoma de Madrid, ISBN 84-8497-927-X.

22 **Grasa R, Morales F, Abadía A, Abadía J 1998** Contenido foliar de nutrientes en árboles de melocotonero y pérdida de los mismos por abcisión y poda. In: Actas del VII Simposio Nacional-III Ibérico sobre Nutrición Mineral de las Plantas. pp. 131-136. Ediciones de la Universidad Autónoma de Madrid, ISBN 84-8497-927-X.

23 **Pestana M, Gonçalves DA, de Varennes A, Faria, EA 1999** The recovery of citrus from iron chlorosis using different foliar applications. Effects on fruit quality. In: **Improved Crop Quality by nutrient management.** D. Anaç, D. Martin-Prével eds. pp 201-204. Kluwer Academic Publishers, Dordrecht, The Netherlands.

24 **Rombolà AD, Quartieri M, Scudellari D, Marangoni B, Abadía J, Tagliavini M 1999** Strategie di cura della clorosi ferrica in frutticoltura integrata. **Rivista di Frutticoltura e di Ortofloricoltura** 5, 59-64.

25 **González-Vallejo EB, González-Reyes JA, Abadía A, López-Millán AF, Yunta F, Lucena JJ, Abadía J 1999** Reduction of ferric chelates by leaf plasma membrane preparations from Fe-deficient and Fe-sufficient sugar beet. **Australian Journal of Plant Physiology** 26, 601-611.

26 **Abadía J, Morales F, Abadía A 1999** Photosystem II efficiency in low chlorophyll, iron-deficient leaves. **Plant and Soil** 215, 183-192.

27 **Igartua E, Grasa R, Sanz M, Abadía A, Abadía J 2000** Prognosis of iron chlorosis from the mineral composition of flowers in peach. **Journal of Horticultural Science and Biotechnology** 75, 111-118.

28 **Gogorcena Y, Abadía J, Abadía A 2000** Induction of in vivo root ferric chelate-reductase activity in the fruit tree rootstock *Prunus amygdalo-persica*. **Journal of Plant Nutrition** 23, 9-21.

29 **González-Vallejo EB, Morales F, Abadía A, Abadía J 2000** Iron deficiency decreases the Fe(III)-chelate reducing activity of leaf protoplasts. **Plant Physiology** 122, 337-344.

30 **Sanz M 2000** El diagnóstico nutricional en agricultura y viabilidad de programas de fertilización. **Phytoma** 114, 44-50.

31 **Morales F, Belkhodja R, Abadía A, Abadía J 2000** Photosystem II efficiency and mechanisms of energy dissipation in iron-deficient, field-grown pear trees (*Pyrus communis* L.) **Photosynthesis Research** 63, 9-21.

32 **M Tagliavini, Abadía J, Rombolà AD, Abadía A, Tsipouridis C, Marangoni B 2000** Agronomic means for overcoming Fe chlorosis in deciduous fruit plants. **Journal of Plant Nutrition** 23, in press.

33 **Rombolà AD, Brüggemann W, Tagliavini M, Marangoni B, Moog PR 2000** Iron source affects Fe reduction and re-greening of kiwifruit (*Actinidia deliciosa*) leaves. **Journal of Plant Nutrition** 23, in press.

34 **Abadía J, Tagliavini M, Grasa R, Belkhodja R, Abadía A, Sanz M, Faria EA, Tsipouridis C, Marangoni B 2000** Using the flower Fe concentration for estimating crop chlorosis status in fruit tree orchards. A summary report. **Journal of Plant Nutrition** 23, in press.

35 **Morales F, Belkhodja R, Abadía A, Abadía J 2000** Energy dissipation in iron-chlorotic, field-grown pear. **Journal of Plant Nutrition** 23, in press.

4.2 Papers Submitted

36 **Pestana M, Correia PJ, de Varennes A, Abadía J, Faria EA 2000** Effectiveness of different foliar applications to correct for iron chlorosis in citrus grown on a calcareous soil. Submitted to **Journal of Plant Nutrition**.

37 **Pestana M, Correia PJ, de Varennes A, Faria EA 2000** Relações entre a composição mineral de flores de laranjeira e algumas características morfofisiológicas dos frutos. Submitted to **Revista das Ciências Agrárias**.

38 **Pestana M, Correia PJ, de Varennes A, Abadía J, Faria EA 2000** The use of floral analysis to diagnose the nutritional status of orange trees. Submitted to **Journal of Plant Nutrition**.

39 **Pestana M, David M, de Varennes A, Abadía J, Faria EA 2000** Responses of Newhall Orange Trees to Iron Deficiency in Hydroponics: Effects on Leaf Chlorophyll, Photosynthetic Efficiency and Root Ferric Chelate Reductase Activity. Submitted to **Journal of Plant Nutrition**.

40 **Larbi A, Morales F, López-Millán A.F., Gogorcena Y, Abadía A, Moog PR, Abadía J 2000** Reduction of Fe(III)-chelates by mesophyll leaf disks of sugar beet. Multi-component origin and effects of Fe deficiency. Submitted to **Planta**.

41 **Zouari M, Abadía A and Abadía J 2000** Iron is Required for the Induction of Root Ferric Chelate Reductase Activity in Fe-deficient Tomato. Submitted to **Journal of Plant Nutrition**.

42 **López-Millán A.F.,, Morales F, Abadía A and Abadía J 2000** Changes induced by iron deficiency in the composition of the leaf apoplastic fluid from field-grown pear (Pyrus communis L.) trees. Submitted to **Journal of Experimental Botany**.

43 **López-Millán AF, Morales F, Andaluz A, Gogorcena Y, Abadía A, de las Rivas J, Abadía J 2000** Protective mechanisms in roots of iron deficient sugar beet: changes in carbon assimilation and oxygen use. Submitted to **Plant Physiology**

44 **López-Millán AF, Morales F, Abadía A, Abadía J 2000** Effects of iron deficiency on the composition of the leaf apoplastic fluid and xylem sap in sugar beet. Implications for iron and carbon transport. Submitted to **Plant Physiology**

45 **López-Millán AF, Morales F, Abadía A, Abadía J 2000** Changes induced by Fe deficiency and Fe resupply in the organic acid metabolism of sugar beet (Beta vulgaris L.) leaves. Submitted to **Physiologia Plantarum**

4.3 Theses

1 **Grünewald, Sussanne** (1996) **Diploma Thesis** (Diplomarbeit). Eisenchelatreduktion durch Mesophyllzellen. Heinrich-Heine-Universität Düsseldorf, Germany.

2 **Zouari, Mohamed** (December 1996) **Master Thesis**. Reponses radiculaires face à la deficience en fer chez differents genotypes de tomate et de betterave. International Center for Advanced Mediterranean Studies-Instituto Agronómico Mediterráneo de Zaragoza (CIHEAM-IAMZ), Spain.

3 **Chatti, Jameleddine** (January 1997) **Master Thesis**. Quelques changements de la composition chimique de la sève du xylème sous deficience en Fe chez la tomate, le pêcher et l'amandier. International Center for Advanced Mediterranean Studies-Instituto Agronómico Mediterráneo de Zaragoza (CIHEAM-IAMZ), Spain.

4 **Valli, Stefano** (1998) **Tesi di Laurea in Scienze Agrarie (Master).** Sviluppo di metodi alternativi ai chelati per superare la clorosis ferrica nell'actinidia. Dipartimento di Coltture Arboree- Facoltà di Agraria , Università di Bologna, Italia.

5 **Fidalgo, Carolina** (February 1998) **Trabajo Fin de Carrera** (Diploma). Efecto de distintos factores en la corrección de clorosis férrica en peral. Escuela Universitaria Politécnica de Huesca. Universidad de Zaragoza, Spain.

6 **García-Laviña**, **Pilar** (February 1998) **Trabajo Fin de Carrera** (Diploma). Tratamientos foliares para la corrección de la clorosis férrica. Escuela Universitaria Politécnica de Huesca. Universidad de Zaragoza, Spain.

7 **Belkhodja, Ramzi** (July 1998) **PhD Thesis** (Tesis Doctoral). Evaluación de la tolerancia a estreses ambientales en plantas cultivadas mediante técnicas de fluorescencia de clorofila, análisis de pigmentos y contenido mineral. Universidad de Lleida, Spain.

8 **Burg, Sascha** (1998) **Diploma Thesis** (Diplomarbeit). Characterization of the reducing capacity of iron-deficient and non-deficient bean roots. Heinrich-Heine-University Düsseldorf, Germany.

9 **Folli, Federico** (1998) **Tesi di Laurea in Scienze Agrarie (Master) -** Diagnosi e prevenzione della clorosi ferrica nelle colture arboree da frutto. Dipartimento di Coltture Arboree- Facoltà di Agraria , Università di Bologna, Italia.

10 **Rombolà, Adamo Domenico** (1998) **PhD Thesis** (Tesi di Dottorato di Ricerca). Aspetti fisiologici e biochimici della clorosi ferrica in Actinidia (*A. deliciosa*). Università di Bologna, Italia.

11 **González-Vallejo, Elena B** (May 1999) **PhD Thesis** (Tesis Doctoral). Caracterización de mecanismos de adquisición de Fe en plantas superiores. Universidad de Zaragoza, Spain.

12 **Wagner, Volker** (1999) **PhD Thesis.** Isolation of plasma membrane-bound ferric chelate reductase from spinach leaves. Heinrich-Heine-University Düsseldorf, Germany (to be presented in July).

13 **Larbi, Ajmi** (June 1999) **Master Thesis**. Effet de la chlorose ferrique sur la réduction de fer par le mésophyle de feuilles de la betterave a sucre (*Beta vulgaris* L.) et du pêcher (*Prunus persica* L.). International Center for Advanced Mediterranean Studies-Instituto Agronómico Mediterráneo de Zaragoza (CIHEAM-IAMZ), Spain.

14 **López-Millán, Ana Flor** (2000) **PhD Thesis** (Tesis Doctoral). Adquisición y transporte a larga distancia de hierro en las plantas. Universidad de Zaragoza, Spain (to be presented in April 2000).

15 **Weyrauch, Katharina** (2000) **PhD Thesis**. Isolation of plasma membrane-bound ferric chelate reductase from iron deficient bean roots. Johann Wolfgang Goethe-University Frankfurt, Germany (to be presented in May 2000).

5 Supplementary investments

Some supplementary funds were obtained from the Spanish National Research Plan at the beginning of the project to acquire related durable equipment.

6 Dissemination activities other than publications

Results of the project have been disseminated so far by means of communications to technical and scientific meetings (see list **d** below), formal presentations to the agroindustrial sector (see list **e** below) and technological offers already sent to the IRC centres (see list **f** below).

Other way of disseminating the results has been through the continuous flow of visits of farmers, agricultural companies and agricultural schools to the partner Institutes in Faro (Portugal), Zaragoza (Spain), Bologna (Italy) and Naoussa (Greece). In these visits results have been disseminated by providing reprints and preprints of the work carried out, as well as by discussing details of the work.

6.1 Communications to Technical and Scientific Meetings

1995

1 ***International Conference on Bioiron.*** *Asheville, North Carolina, USA, April 1995.*

Belkhodja R, Morales F, Quílez R, Abadía A, Abadía J The redox state of the photosystem II acceptor side in iron-deficient sugar beet (*Beta vulgaris* L.) leaves: Evidence for an incomplete plastoquinone reoxidation in the dark (Communication /Poster).

2 ***8th Congress of Algarve.*** *Faro, Algarve, Portugal, April 1995.*

Varennes de A, Faria EA, Pestana M, Quelhas dos Santos J The project "Novel approaches for the control of iron chlorosis in fruit tree crops" (Communication/Poster).

3 ***17th Congress of the Greek Horticultural Society****.*

Tsipouridis K, Ferios I, Stilianidis D Evaluation of 59 peach varieties for iron chlorosis tolerance (Communication /Poster).

4 ***XXII Convegno Peschicolo.*** *Cesena, Italia, September 1995.*

Marangoni B, Tagliavini M, Toselli M Iron chlorosis of peach trees: causes, prevention and methods of control (Communication).

1996

5 ***Third International Symposium on Mineral Nutrition of Deciduous Fruit Trees.*** *Zaragoza, Spain, July 1996.*

Sanz M, Belkhodja R, Toselli M, Montañés L, Abadía A, Tagliavini M, Marangoni B, Abadía J Floral analysis as a possible tool for the prognosis of iron deficiency in peach (Communication /Poster).

6, 7 ***Symposium of the German Botanical Society.*** *Düsseldorf, Germany, August 1996.*

Grünewald S, Brüggemann W, Moog PR Vergleichende Charakterisierung der in-vivo Eisenchelat-Reduktion durch Mesophyll unterschiedlich eisenversorgter Dicotyledonen. Poster P-4.012. page 78 (book of abstracts of the symposium).

Wagner V, Brüggemann W, Moog PR

Charakterisierung der Plasmalemma-gebundenen Eisenchelat-Reduktaseaktivitaet von Spinat (Spinacea oleracea). Poster P-13.033. page 312 (book of abstracts of the symposium).

8 ***Convegno Nazionale sulla Coltura dell'Actinidia.*** *Faenza, Italia, September 1996.*

Tagliavini M, Scudellari S, Marangoni B, Pelliconi F, Valli S. Alternatives to iron chelates for the control of Fe chlorosis in kiwifruit (Communication).

1997

9-16 ***9th International Symposium on Iron Nutrition and Interactions in Plants.*** *Hohenheim, Germany, July 1997.*

Abadía J, Tagliavini M, Abadía A, Sanz M, Tsipouridis C, Araujo-Faria E, Marangoni B Using the flower Fe concentration for estimating crop chlorosis status in fruit tree orchards. A summary report (Communication/Keynote Lecture).

Tagliavini M, Abadía J, Abadía A, Tsipouridis C, Marangoni B. Alternatives to Fe-chelates for overcoming fruit tree iron chlorosis in Mediterranean countries (Communication/Keynote Lecture).

López-Millán AF, Abadía A, Abadía J Organic acid concentrations in the apoplast of iron-sufficient and iron-deficient sugar beet (*Beta vulgaris* L.) (Communication /Poster).

Morales F, Belkhodja R, Abadía A, Abadía J Photosystem II photochemical efficiency and mechanisms of energy dissipation in the leaf of iron-deficient, field-grown pear (*Pyrus communis* L.) (Communication /Poster).

González-Vallejo EB, Abadía A, González-Reyes JA, Abadía J Characterization of the Fe(III)-chelate reductase activities of plasma membrane preparations isolated from leaves of iron-sufficient and iron-deficient sugar beet (*Beta vulgaris* L.) (Communication /Poster).

Moog PR, Grünewald S Ferric reduction by intact mesophyll cells (Communication /Poster).

Wagner V, Moog PR Biochemical characterization of plasma membrane-bound Ferric chelate reductase activity isolated from spinach leaves (Communication /Poster).

Rombolà AD, Brüggemann W, Tagliavini M, Moog PR Iron deficiency in kiwifruit (*Actinidia deliciosa*): Responses of roots and leaves (Communication /Poster).

17 ***II Congresso Iberoamericano, III Congresso Ibérico de Ciências Hortícolas.*** *Vilamoura, Portugal, March 1997.*

Pestana M, Ferreira P, Correia PJ, David M, de Varennes A, Faria EA. Efeito da clorose férrica em porta-enxertos de citrinos: estudo de alguns parâmetros fisiológicos (Communication /Poster).

1998

18 ***International Symposium on Plant Nutrition.*** *Hohenheim, Germany, February 1998.*

Abadía J, Morales F, Abadía A Photochemical efficiency in low-chlorophyll, Fe-deficient leaves (Communication/Keynote Lecture).

19 ***4th Giornate Scientifiche della Società Orticola Italiana.*** *Sanremo, 1-3 April 1998.*

Rombolà AD Meccanismi biochimici di tolleranza alla clorosi ferrica in Actinidia (*A. deliciosa*) (Oral Communication).

20 ***International Conference "Plasma membrane redox systems and their role in biological stress and disease".*** *Antwerp, Belgium, April 1998.*

González Vallejo EB, Abadía A, González-Reyes JA, Lucena JJ, Abadía J Fe(III)-chelate reductase activities of plasma membrane preparations affected by iron deficiency in sugar beet (*Beta vulgaris* L.) leaves. (Communication/Poster).

21-22 ***XIth International Congress on Photosynthesis.*** *Budapest, Hungary, August 1998.*

Morales F, Abadía A, Abadía J Photosynthetic induction in iron-deficient sugar beet leaves: a time-resolved, laser-induced chlorophyll fluorescence study (Communication/Poster).

Dauborn BE, Moog PR Iron deficiency under low light causes changes in the capacity of the antioxidative system in *Phaseolus vulgaris* L (Communication/Poster).

23 ***International Workshop on Photosynthesis under Biotic and Abiotic Stress. Stress Sinergisms in Plants.*** *Tata, Hungary, August 1998.*

Morales F, Abadía A, Abadía J Photosynthetic induction in iron-deficient sugar beet leaves: a time-resolved, laser-induced chlorophyll fluorescence study (Communication/Poster).

24-29 ***VII Simposio Nacional-III Ibérico sobre Nutrición Mineral de las Plantas.*** *Madrid, España, September 1998.*

Abadía J Absorción y transporte de hierro en plantas (Keynote speech).

Gogorcena Y, Abadía J, Abadía A Inducción in vivo de la reductasa de patrones frutales de *Prunus persica* L. (Oral Communication).

Morales F, Abadía A, Abadía J Mecanismos de protección frente al exceso de luz en hojas deficientes en hierro (Oral Communication).

López-Millán AF, Morales F, Abadía A, Abadía J Implicaciones metabólicas en la respuesta bioquímica a la deficiencia de hierro en remolacha (*Beta vulgaris* L.) (Communication/Poster).

González-Vallejo EB, Abadía A, Herbik A, Stephan U, Rémy R, Abadía J Determinación de patrones polipeptídicos de raíz de remolacha (*Beta vulgaris* L.) en condiciones de deficiencia de Fe (Communication/Poster).

Grasa R, Morales F, Abadía A, Abadía J Contenido foliar de nutrientes en árboles de melocotonero y pérdida de los mismos por abcisión y poda (Communication/Poster).

1999

30 ***10º Symposium Internacional. La sanidad de los frutales en condiciones Mediterráneas.*** *Valencia, España, November-December 1999.*

Sanz M El diagnóstico nutricional en agricultura y viabilidad de programas de fertilización (Keynote speech).

2000

31-33 ***International Symposium on the Optimization of Plant Nutrition.*** *Cairo, Egypt, April 2000.*

Abadía J, Abadía A, Faria EA, Pestana M, Tsipouridis C, Moog PR, Brüggemann W, Negueroles J, marangoni B, Tagliavini M Results of the European Project "Novel Approaches for the Control of Iron Chlorosis in Fruit Trees (Poster).

Gogorcena Y, Abadía J, Abadía A A new protocol that can be used as a tool in screening fruit tree rootstocks for tolerance to iron chlorosis (Poster).

Pestana M, Correia PJ, de Varennes A, Abadía J, Faria EA Iron chlorosis in two *Citrus* rootstocks: effect on some physiological parameters (Poster).

34 ***10th International Symposium on Iron Nutrition and Interactions in Plants.*** *Houston, U.S.A., May 2000.*

Abadía J, López-Millán AF, Abadía A Apoplastic organic acids and their role in the availability of iron for leaf cells (Keynote speech).

6.2 Other formal presentations to the agro-industrial sector

1995

1 ***Meeting with the Public field advisors of Regione Emilia Romagna.*** *Ferrara, Sala Convegni del Centro Operativo Ortofrutticolo.*

Tagliavini M "Iron chlorosis in fruit tree crops".

2 ***Joint Project Meeting in Naoussa. Dissemination Report.*** *Naoussa, Makedonia, Greece.*

Tsipouridis C "Results with practical economical interest".

1997

3 ***Fruttiflor exhibition.*** *Faenza, Italia, October 10, 1997.*

B Marangoni, Tagliavini M "Strategies for overcoming the Fe chlorosis in deciduous fruit trees".

4 ***Consorzio Provinciale Agrario di Ravenna.*** *Ravenna, Italia, November 14, 1997.*

Tagliavini M "Summary results of the iron chlorosis project".

5 ***Consorzio Provinciale Agrario di Forli Cesena-Rimini.*** *Forli Cesena-Rimini, Italia, November 28, 1997.*

Tagliavini M "Summary results of the iron chlorosis project".

6 ***Center of Educational Training (K.E.K.).*** *Kopanos, Naoussa, Greece (2 days).*

Tsipouridis C Lecture including results of the iron chlorosis project.

7 ***Center of Educational Training (K.E.K.).*** *Lamia, Greece (2 days).*

Tsipouridis C Lecture including results of the iron chlorosis project.

8 ***Center of Agricultural Education (K.E.T.E.).*** *Veria, Greece (2 days).*

Tsipouridis C Lecture including results of the iron chlorosis project.

9 ***Center of Educational Training (K.E.T.E.).*** *Rhodes, Greece (2 days).*

Tsipouridis C Lecture including results of the iron chlorosis project.

1998

10 ***Demonstration activities of C.R.P.V, Cesena.*** *Ferrara, June 1998.*

Scudellari D "Visit by field technicians of the Emilia-Romagna Region to a pear trial managed with strategies alternative to Fe chelates ".

11 ***Cooperative P.A.F. of Faenza and UNICOOP.*** *Faenza, October 6 1998*

Rombolà AD, Tagliavini M "Results of trials carried out under the chlorosis project".

12 ***Center of Educational Training (K.E.K.).*** *Kopanos, Naoussa, Greece (2 days).*

Tsipouridis C Lecture including results of the iron chlorosis project.

13 ***Center of Educational Training (K.E.K.).*** *Lamia, Greece (2 days).*

Tsipouridis C Lecture including results of the iron chlorosis project.

14 ***Center of Agricultural Education (K.E.T.E.).*** *Veria, Greece (2 days).*

Tsipouridis C Lecture including results of the iron chlorosis project.

15 ***Center of Educational Training (K.E.T.E).*** *Rhodes, Greece (2 days).*

Tsipouridis C Lecture including results of the iron chlorosis project.

16 ***Escuela Universitaria Politécnica de Huesca,*** *Huesca, Spain.*

García-Laviña, P and Fidalgo, C Lecture including results of the iron chlorosis project.

17 ***Escuela Superior de Ingenieros Agrónomos,*** *Universidad de Lleida, Spain.*

Belkhodja, R Lecture including results of the iron chlorosis project.

1999

18 ***Center of Educational Training (K.E.K.).*** *Kopanos, Naoussa, Greece (2 days).*

Tsipouridis C Lecture including results of the iron chlorosis project.

19 ***Center of Educational Training (K.E.K.).*** *Lamia, Greece (2 days).*

Tsipouridis C Lecture including results of the iron chlorosis project.

20 ***Center of Agricultural Education (K.E.T.E.).*** *Veria, Greece (2 days).*

Tsipouridis C Lecture including results of the iron chlorosis project.

21 ***Center of Educational Training (K.E.T.E).*** *Rhodes, Greece (2 days).*

Tsipouridis C Lecture including results of the iron chlorosis project.

22 ***I Jornadas Hydro de Fertirrigación,*** *Zaragoza, Spain.*

Sanz M Fertirrigación (Lecture including results of the iron chlorosis project).

23 ***Day of Science,*** *Faro, Portugal (24 November).*

Faria, EA Poster including results of the iron chlorosis project.

24 ***Citriculture Meeting,*** *Feira de Silves, Portugal (1-5 December).*

Faria, EA Lecture including results of the iron chlorosis project.

2000

25 ***Jornadas Técnicas sobre Fruticultura. Actualización de Técnicas de Cultivo en Frutales. Escuela Universitaria Politécnica de la Almunia de Doña Godina****. 24 February. Zaragoza, Spain.*

Sanz M Diagnóstico nutricional (Lecture including results of the iron chlorosis project).

7 Further support through technology transfer organisations

Part of the most practically oriented results of the project have been sent to the corresponding IRC centres for dissemination among member states. The technological offers disseminated so far are as follows:

7.1 Technological offers sent to IRC

Flower analysis for early diagnosis of nutritional status of fruit trees.
From Innovation Relay Centre-Zaragoza, Spain.
(Reference 250199)
Posted January, 1999

Low input treatments to control iron chlorosis.
From Innovation Relay Center- ICR IRENE, c/o ENEA Bologna, Italy.
Posted January, 1999

Effectiveness of Different Foliar Applications to Correct for Iron Chlorosis in Citrus Grown on a Calcareous Soil.
From Innovation Relay Center Faro, Portugal.
Posted January, 1999

Screening for iron chlorosis tolerance.
From Innovation Relay Centre Hessen /Rheinland –Pfalz, Germany
Posted January, 1999

8 Dissemination by the Commission services

A short information on the project could be accessed through the Cordis web page.