

# Isolation of the cDNA Responsible for Iron-Reductase and Characterization of its Activity in Cucumber (*Cucumis sativus*)

A. N. WYNN<sup>1</sup>\* and B. M. WATERS<sup>2</sup>

<sup>1</sup>Natural Science Department and <sup>2</sup>Biology Department McMurry University, Abilene, TX

**Introduction:** In order to accumulate essential metals, plants have developed strategies for transport into cells. In dicots, Iron (Fe) is reduced from Fe (III) to Fe (II) before it is taken into the cell. In *Arabidopsis*, the protein needed for reduction is encoded by the gene *FRO1*.

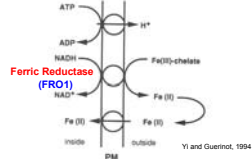


Fig. 1. Model of Fe acquisition processes in dicots and non-grain monocots. Under Fe deficiency, acidification, Fe(III) reduction, and Fe uptake activities are up-regulated.

Identifying the genes responsible for Fe uptake will allow further research into developing plants that could stockpile Fe. In addition, genetic markers could be developed to identify cultivars better suited to accumulating Fe.



Fig. 2. Localization of ferric reductase (FeR) activity of an Fe deficient pea root.

## Objectives:

1. To screen cucumbers (*Cucumis Sativus*) cultivars for Fe-reductase (FeR) activity
2. To identify the cDNA that corresponds to the FeR.
3. To examine the expression of *FRO1* in the roots and stems of Fe deficient and sufficient cucumber.

## Materials and Methods:

Germinated cucumber seedlings were grown for 4 days in Fe sufficient conditions, then switched to solutions of either -Fe or +Fe, for 1 to 4 days.



Fig. 3. Hydroponic apparatus for growing cucumber plants.

In most experiments, three roots from each treatment were excised, and placed in vials containing a Ferrozine assay solution (Fig. 4). After 20 minutes the absorbance at 562 nm was determined, and FeR activity was calculated (nmol/h/g).

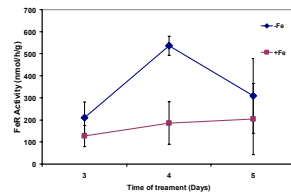
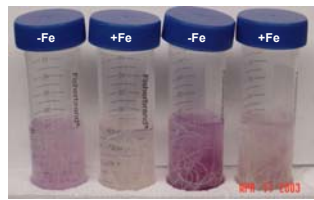


Fig. 5. Ferric reductase assay of +Fe and -Fe cucumber roots (± s.e.). The maximum level of FeR occurred on day 4, therefore all further measurements were taken on day 4.

## Results and Discussion:

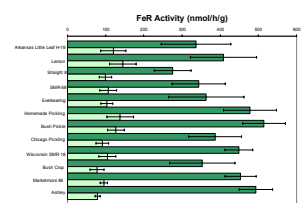


Fig. 6. FeR activity of cucumber cultivars (± s.e.) in +Fe and -Fe growth conditions. Trials were repeated between 4 and 6 times.

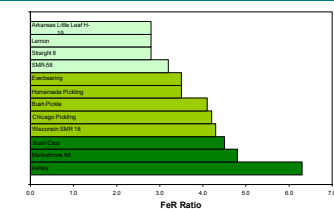
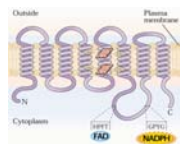


Fig. 7. Data from Fig. 6 converted to ratio of FeR (-Fe/+Fe). Examination of ratios allowed for categorization of cultivars as low capacity reducers (pale green), intermediate capacity reducers (chartreuse), or high capacity reducers (dark green).

## Cloning a new ferric reductase gene

Degenerate primers based on conserved sequences of known ferric reductase proteins were used for RT-PCR to amplify a partial cDNA of *CsFro1* from mRNA from Fe deficient cucumber roots.



Buchanan et al. (eds), 2000

Fig. 8. Model of known ferric reductase proteins.

Gene specific primers were designed and RACE-PCR was performed to amplify the ends of the cDNA. A full length cDNA was cloned, and the sequence obtained was submitted to a BLAST search to compare it with other FRO genes.

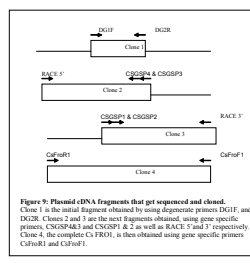


Figure 9. Plasmid cDNA fragments that got sequenced and cloned. Class 1 is the initial fragment obtained by using degenerate primers DGEF and DCEB. Classes 2 and 3 are the next fragments obtained, using gene specific primers, CS2SP4-3 and CS2SP1 & 2 as well as RACE 5 and 7 respectively. Class 4, the complete *CsFRO1*, is then obtained using gene specific primers CsFroR1 and CsFroF1.

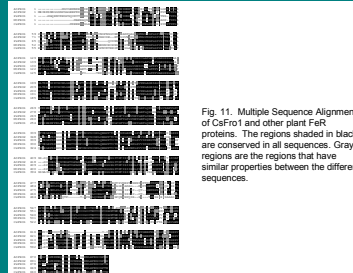


Fig. 11. Multiple Sequence Alignment of *CsFro1* and other plant FeR proteins. The regions shaded in black are conserved in all sequences. Grey regions are the regions that have similar properties between the different sequences.

Table 1. Conservation of deduced protein sequence between *CsFro1* and other ferric reductase proteins. Percentage identity and similarity were determined by the BLAST algorithm.

	% Identity	% Similarity
<b>PsFro1</b>	<b>56</b>	<b>74</b>
<b>MtFro1</b>	<b>57</b>	<b>74</b>
<b>AtFro1</b>	<b>51</b>	<b>71</b>
<b>AtFro2</b>	<b>48</b>	<b>69</b>

Fig. 12. Phylogram including *CsFro1* and similar proteins identified from GenBank by BLAST. *CsFro1* was most similar to *Fro1* from garden pea (*PsFro1*) and barrel medic (*MtFro1*).

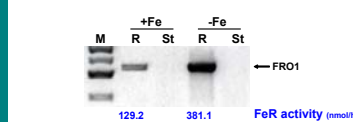
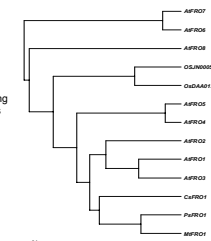


Fig. 13. *CsFRO1* expression in roots and stems. Roots were assayed to confirm expected FeR activity before RNA extraction on day 4. RT-PCR was performed using RNA from +Fe and -Fe roots and stems. *FRO1* shows higher expression in -Fe roots. There was no expression detected in the stems under either sufficient or deficient conditions.

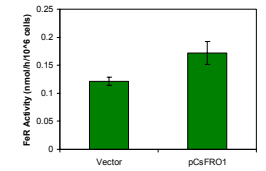


Fig. 10. Functional expression of *CsFro1* in yeast. *CsFRO1* was cloned into a yeast expression vector. Transformed cells were assayed for FeR (± s.d.).

## Conclusions:

- Significant genetic variability of FeR was observed among the cucumber cultivars.
- When FeR activity levels were elevated, *CsFro1* mRNA was more abundant.
- Comparing the *CsFro1* sequence to other FROs indicates that it is quite similar to other FeR proteins.
- Increased FeR activity in yeast that have the *CsFRO1* insert, reinforces that *CsFRO1* is the full-length clone of the gene responsible for FeR activity in Cucumber.
- *FRO1* was expressed in the roots at increased levels in the plants grown under -Fe conditions.

## Future Directions:

- Differences in FeR activity could be a function of polymorphisms in the *FRO1* gene of the different cultivars. Identifying polymorphisms could yield more information about FeR in the cultivars.
- Do some polymorphisms result in a change in protein sequence that alters function?
  - Do some polymorphisms alter *Fro1* protein levels?
  - Do some cultivars have higher *FRO1* expression?
  - Genetic markers could be based on polymorphisms.

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