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Variation of Iron Content and Ferritin Distribution during Development Stage under Conditions of Iron Nutritional Status from Hydroponic Culture in Red Pepper(*Capsicum annuum* L.)

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ABSTRACT

Total iron content and ferritin distribution have been determined in red pepper(*Capsicum annuum* L.) during development stage under conditions of iron nutritional status from hydroponic culture. Color of the leaves become chlorotic on iron deficient and high concentration. The plant height on each iron concentration had retarding effect at concentration lower than 25μM and greater than 125μM. In normal green leaves, total iron content was almost constant with a mean value of 2.5μmole of iron/mg of dry matter, except at 63day, for which it increases slightly to 4μmole. However, iron content of chlorotic plants grew on iron free medium was not almost detectable. Also, In post chlorotic leaves(++Fe), iron content was evidently increased until 7days after transfer on liquid medium, but decreased from after 14days. Also, ferritin protein analysed total protein extracts prepared from leaves of different ages using antibodies raised against ferritin protein. Ferritin protein decreased progressively during the first week of germination and was not detectable in vegetative tissues. Ferritin protein in post chlorotic leaves was evidently strongly enhanced until 11days after transfer on liquid medium but decreased until the leves became chlorotic.

Key words: chlorotic, ferritin protein, iron content, red pepper, Western blot.

INTRODUCTION

The mechanism of iron transport in higher plants is not fully understood. Tiffin(1966) convincingly showed that iron is carried by citrate in the xylem but how it is transported in the phloem is not known. Among the essential nutriments, iron is great interest because of its role in important metabolic processes such as oxygen transfer, nitrogen fixation, electron transfer and DNA synthesis. Iron storage in living organisms is achieved by a class of multimeric (24-mer) proteins called ferritins (Theil, 1987). They are organized in hollow spheres able to accommodate a few thousand iron atoms inside their central cavity. Ferritins are known to sequester and thus detoxify iron taken up by cells which is not utilized for metabolic requirements. Under conditions of iron need, ferritin-Fe(Ⅲ) can be released by reduction for cellular use (Laulhere et al., 1990). Therefore ferritin are key proteins acting as a buffer for iron, protecting cell from a harmful concentration of free iron and regulating their immediate need. In the mammalian system, ferritin is known as the major iron storage protein. It is synthesized in response to iron and provides the cell with a mobilizable reserve(Munro and Linder, 1978; Aisen and Listowsky, 1980). The results of earlier work indicate that in plants ferritin acts not only as a storage for iron in seeds (Hyde et al., 1963), but also as a buffer molecular for iron in developing primary bean leaves (van der Mark

et al. 1981). Knowledge about the ferritin content and iron concentration in red pepper during development stage under conditions of iron nutritional status from hydroponic culture throughout its life cycle is clearly needed in order to start the study of the developmental regulation of ferritin synthesis in plants. In this paper, we investigated how ferritins are accumulation and degradation in red pepper during development stage under conditions of iron nutritional status from hydroponic culture.

MATERIALS AND METHODS

Seedlings of *Capsium annuum* L. cv. Chungyang were grown for 7days in darkness after germination, as described by Van der Mark et al.(1981). Subsequently, seedlings with a hypocotyl of about 5cm were transferred to 1 liter flasks containing 200ml nutrient solution with variable iron concentrations. The iron was supplied as ferric-sodium-ethylene-diaminetetraacetic acide (FeNaEDTA). Iron deficient plants were cultured by the iron-free medium(-Fe). The iron treatment plants was performed by replacing the iron-free medium with medium containing 125 M(+Fe) and 750 M(++Fe) FeNaEDTA respectively. A light regime of 16h light and 8h dark was used: the light intensity was 8,000lux and temperature 28℃. Different organs and tissues were harvested at different time(Fig. 1), frozen in liquid N2 and stored at -70℃. Total iron content was measured by recording absorbance of Fe^{2+}o-phenanthroline(0.02%) at 510nm, pH6.0(50mM acetic acid/NaOH buffer), using thioglycollic acid(Sigma) as a reducing agent. Total iron of the each tissues was determined after dry ashing according to Scott(1944). In order to determine firritin distribution from each tissues, total protein extraction procedure was done as described by Hurkman and Tanaka(1986). Soluble proteins were extracted from 500mg tissue, homogenized on ice with a polytron homogenizer in three volumes of 10mM sodium phosphate buffer (pH6.0) and 1% sodium bisulfate as a reducing agent, except that 0.1% o-phenanthroline and 20mM EDTA were added to the extraction buffer in order to prevent ferritin reactions leading to possible ferritin degradation during sample preparation (Laulhere et al., 1988). The homogenate was centrifuged at 14000 × g for 10 min. The protein content was measured as described by the method of Bradford(1976). Prior to fractionation on SDS-PAGE, protein was denatured at 100℃ for 3min in a solution containing 100mM dithiothreitol(DTT), 2% SDS, 50mM Tris-HCl(pH6.8), 10%(v/v) glycerol, 0.1% bromphenol blue. Electrophoresis was performed at 25mA in 12% polyacrylamide gel containing 0.1% SDS (Laemmli, 1970) and electroblotted to Immobile-P membrane (Milipore, Bedford, MA). Blots were blocked for 1hr in phosphate buffered Saline(PBS) containing 3% skim milk and incubated with polyclonal anti-Ferritin antibody (1:1000 dilution) for 1hr. After washing buffer (PBS+ 0.5% Triton X-100) three times for 10min. each, the blot was treated with alkaline phosphatase-conjugated goat anti rabbit antiserum and developed with BCIP/NBT substrate solution.

RESULTS

Red pepper plants were grown under conditions of iron nutritional status from hydroponic culture, as shown schematically in Fig. 1. The growth was effected on FeNaEDTA concentration. Color of the leaves became chlorotic on iron deficient and high concentration(Fig 2 upper). The plant height on each iron concentration was presented in Fig. 3. Iron had retarding effect at concentration lower than 25 M and greater than 125 M. Total iron content and ferritin protein of normal green, chlorotic and post

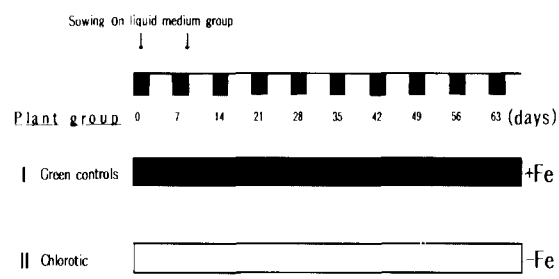

Fig. 1. Schematic representation of hydroponic culture of red peppers. Sowing after germination, on perliter with iron free-medium. Transfer to liquid medium with 125 M FeNaEDTA(Green control), without FeNaEDTA(Chlorotic) and 750 M FeNaEDTA(Post chlorotic).

Fig. 2. Leaf color grown on liquid medium without FeNaEDTA (Chlorotic)(A) and with 125 M FeNaEDTA(Green control)(B).

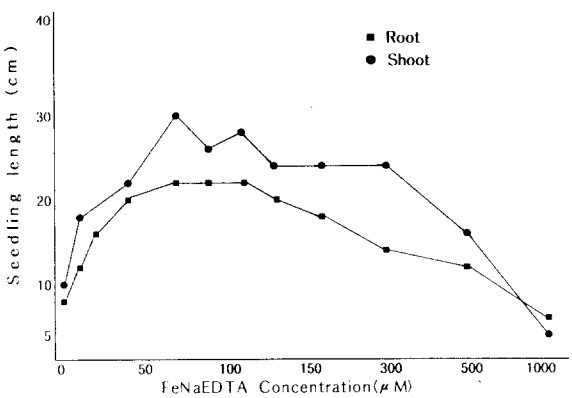

Fig. 3. Shoot and root growth inhibition of red pepper seedling grown for 21days after germination in the hydroponic culture containing various FeNaEDTA concentration

chlorotic plants were presented to Table 1. In 7days normal green leaves after transfer on liquid medium(+Fe), amount of the total iron was showed 1.8mol/mg FW.

Table1. Iron content and ferritin protein levels in the leaves of green(+Fe), chlorotic(-Fe) and post chlorotic(++Fe) red pepper plants. The plants were grown as shown in Fig. 1, Total Iron content and ferritin were determined as described in the Methods section. (FW = Fresh weight)

	Day. of Harvest	Total Iron (nmol . mg FW)	Ferritin (% Total protein)
Normal Green	14	1.8	0.26
Chlorotic	14	0.3	0.01
Post chlorotic	14	5.1	0.61
	14	5.9	0.64
	18	7.4	0.96
	20	5.6	0.80
	22	3.0	0.81

However, chlorotic leaves cultured by the iron-free medium(-Fe) was showed a low amount with 0.03mol/mg FW. In post chlorotic leaves(++Fe), iron content was evidently strongly enhanced, accompanied by the synthesis of ferritin until 11days after transfer on liquid medium, but decreased from after 11days. This course of the total iron content in the first secondary leaves after transfer on liquid medium was determined. In order to study the storage of iron in more detail, iron content on the basis of dry weight was determined among green control plants, chlorotic, and post chlorotic plants during development stage(Fig. 4).

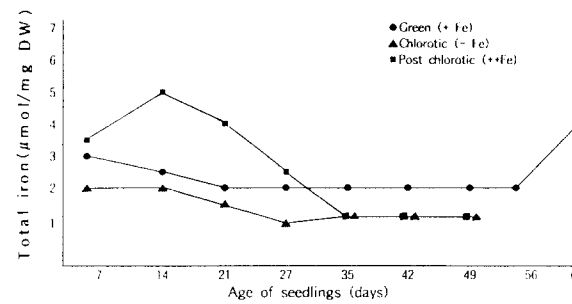

Fig. 4. Total iron contents on the basis of dry weight in growing leaves of normal green, chlorotic and post-chlorotic plant

In normal green leaves, total iron content was almost constant with a mean value of 2.5 mole of iron/mg of dry matter, except at 63 day, for which it increases slightly to 4 mole. However, iron content of chlorotic plants grew on iron free medium was not almost detectable. Also, In post chlorotic leaves(++Fe), iron content was evidently

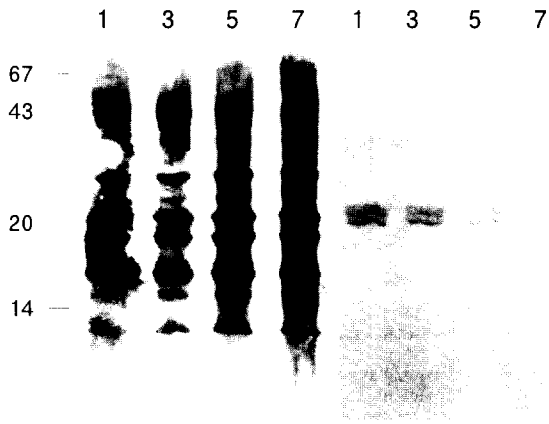

Fig. 5. Disappearance of ferritin from the tissues during the early stages of germination. M: molecular markers, Line: days after germination. (A): Total protein, (B):Immunoblot of a duplicate of (A).

increased until 7days after transfer on liquid medium, but decreased from after 14days. So it can be concluded that the iron in leaf was accumulated at early stage after iron plus, transported on green tissues. In order to determine whether these variations of iron content influence the accumulation of ferritin during development in red pepper leaves, ferritin was analysed with total protein extracts prepared from leaves of different ages using antibodies raised against ferritin protein. Fig. 5 shows that ferritin was present with same amount until 3days after germination. However, ferritin concentration decreased clearly in leaves during the first 7days after germination. Also ferritin protein was detectable in leaves until 3days after transfer on liquid medium containing iron, but all development stage investigated in this experiment, no ferritin protein was detectable(not shown data). However, in post chlorotic leaves, ferritin protein in 11days after transfer on liquid medium was evidently strongly enhanced but decreased until the leaves became chlorotic leaves. From the these results, ferritin protein shows processes and disappears during germination and is not detectable in vegetative tissues.

DISCUSSION

Generally, the presence of ferritin in plant tissues is considered to be associated with high iron levels in the xylem(Seckbach, 1969) or with the absence of the active photosynthetic apparatus, like in seeds, flowers, roots and virus infected cells(Whatley, 1977; Seckbach, 1972). In this paper, we examined the iron content and the ferittin distribution in red pepper leaves during development when grown in hydroponic cultures under conditions of continuous iron stress and iron supply. Under conditions of the iron stress ferritin was virtually absent. When chlorotic plants are supplied with an excessive iron pulse in the roots, early stage was a strong accumulation of iron in the leaves which was accompanied by ferritin synthesis. However, only part of the iron becames ferritin bound. The larger part remains easily accessible for transport from the leaves to other parts of the plants. Presumably, this part of accumulated iron never entered the mesophyll cells but was stored in or around the vessels. This picture of soluble iron stored in or around the vessels shortly after iron donation corresponds to data from Branton and Jacobson(1962). With microradioautographic procedures they localized iron in leaf tissue and found that incoming iron accumulated rapidly in the vascular boundless and moved slowly to the surrounding mesophyll. Critrate could play a role in this storage, since it has been reported to be the main iron transporter in plants(Cataldo et al., 1988). Also, in yeast, a large amount of iron can be stored in the chelated form in small unidentified molecules located in the vacuoles (Theil, 1987). Supplying surplus iron to iron deficient plants leads to the induction of ferritin synthesis; the environmental induction could be controlled at the transcriptional level(Proudhon et al., 1989).

In this paper, we suggest that ferritin may act as a buffer for iron in a situation where the iron in flux is not properly turned to the immediate need of the tissue and may serve to protect the cell from the harmful overdose of free iron. However, the low but significant level of ferritin iron in normal leaves indicates that its buffering function may be of a general nature. The relationship between iron content of the culture medium and the level of ferritin iron in leaves could mean that individual leaves may overcome a temporary deficiency of iron during growth without harmful effects on the synthesis of the chloroplast

system.

Also, In post chlorotic leaves(++Fe), iron content was evidently increased until 7days after transfer on liquid medium, but decreased from after 14days.

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