Research progress of iron isotopes in plants

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Abstract: *Iron is an essential element for plant life activities, and is deeply involved in the material cycle of plant-water-soil. Therefore, it is necessary to understand the iron isotope behavior and fractionation law of iron in the cycle of plant-water-soil, to trace the migration and circulation of iron in the surface biosphere, and to trace the relationship between the biosphere where plants are located and the various plastids in nature. Issues such as interaction play an important role. This paper summarizes the representation methods and basic storage information of iron isotopes, and summarizes two different strategies for absorbing iron from soils and their characteristics of iron isotope fractionation. The results showed that when strategy I plants absorb iron, they will reduce ferric iron first, and absorb light iron isotopes preferentially; while strategy II plants absorb iron after chelating iron without obvious fractionation; some plants can have two iron isotopes at the same time. an absorption mechanism. Iron isotopes have great application potential in the research on the absorption mechanism of iron by plants, and may also have great prospects in future research scenarios such as iron transport in plants and iron distribution in tissues and organs.*

Keywords: *Iron isotopes, Plants, Strategy I, Strategy II*

1. Introduction

Iron is a group VIII element of the fourth period of the periodic table and is a typical transition metal element with four stable isotopes ^{54}Fe(5.84%), ^{56}Fe(91.76%), ^{57}Fe(2.12%), ^{58}Fe(0.28%) [1]. Iron usually exists in nature in the form of elemental substance, +2 valence and +3 valence. Iron is the end point of fusion of stellar elements. It exists widely in the universe and is also one of the main constituent elements of various celestial bodies in the solar system [2]. In terrestrial planets such as the earth, iron will accumulate to form the core of the planet, and at the same time, hundreds of minerals formed by iron and other metals and non-metals are also widely distributed in the mantle, crust and other spheres. In addition, iron is also commonly used in various biochemical processes in microorganisms, animals and plants. For example, red blood in animals uses iron-containing hemoglobin for oxygen transport [3]. Iron has obvious isotopic differences before various reservoirs in nature, and can produce significant isotopic differences in celestial evolution, geochemistry, epigenetic environment, biochemistry and other processes. Therefore, iron isotopes have shown great potential in the research of these disciplines, attracting a large number of scholars to conduct research.

At present, the research work of iron isotope mainly focuses on the following aspects: 1. The iron isotope composition of the earth's reservoirs; Iron isotopic composition of the Earth and other celestial bodies in the solar system; 2. Mechanism of iron isotope fractionation in high-temperature geological processes such as mantle magmatic differentiation; 3. Fractionation mechanism of iron isotopes in surface low-temperature processes (such as weathering); 4. Trace paleontological activities and paleoenvironmental changes; 5. Mechanism of iron isotope fractionation in biological activities. At the beginning, the research work of iron isotope focused on the traditional geology and geochemistry. However, with the further development of iron isotope research, many scholars began to pay attention to the mechanism of iron isotope fractionation in biological activities, especially in plants. Combining with the mechanisms of iron absorption and utilization in animals and plants, researchers began to study these mechanisms and the change of iron isotopes in the process, and combined with the mechanism of iron isotope fractionation, to study the iron material cycle in water-soil-biosphere.

2. Representation of iron isotopes and reservoir information

In all isotopes of iron, ^{56}Fe has the highest content, ^{57}Fe is not interfered by the isotopes in the mass spectrometer, and ^{54}Fe has the lowest relative atomic mass. Therefore, the classical δ ^{56}Fe and δ ^{57}Fe represents iron isotope ratio element, as shown in Formula 1 and Formula 2 below:

$$\delta^{56}Fe = \left(\frac{\left({}^{56}Fe/{}^{54}Fe\right)_{Sam}}{\left({}^{56}Fe/{}^{54}Fe\right)_{Std}} - 1\right) \times 1000‰ \tag{1}$$

$$\delta^{57}Fe = \left(\frac{\left({}^{57}Fe/{}^{54}Fe\right)_{Sam}}{\left({}^{57}Fe/{}^{54}Fe\right)_{Std}} - 1\right) \times 1000‰ \tag{2}$$

The representation of the iron isotope value is the deviation of the relative standard material, so the selection of the iron isotope standard material is very important. In the early stage, some scholars represented by Beard et al. used the reported standard sample as mean igneous rocks (MIR) [4]. Subsequent studies have shown that there is significant fractionation of iron isotopes in igneous rocks. Thus, the most commonly used reference material for iron isotopes is IRMM-014(European Standards Agency). The two reference materials can be converted into each other, and the conversion relation is as follows: Equation 3.

$$\delta^{56}Fe_{IRMM-014} = \delta^{56}Fe_{MIR} + 0.09‰ \tag{3}$$

According to the analysis and calculation of different types of chondrites, the iron isotopic composition of the whole earth calculated by Dauphas et al. is (-0.016 ± 0.045) ‰[5]; The average iron isotope composition of the upper mantle is about 0.02 ‰ - 0.03 ‰[6]; According to the iron isotopic compositions of the average basic rock earth and high silica granite and their proportions in the continental crust, Poitrasson estimated that the iron isotopic compositions of the average continental crust and the average continental upper crust are about 0.073 ‰ and 0.082 ‰ respectively [7]. In seawater, iron mostly exists in dissolved state. Due to the oxygen-rich environment of the ocean, dissolved oxygen will soon precipitate or transfer out of the water area. The isotopic composition of iron in the open ocean is mainly controlled by the iron source area, and it is difficult to achieve global homogenization. Therefore, the δ56Fe of modern seawater varies greatly, ranging from -1.82 ‰ to 0.7 ‰[8]. Strategy I Plant δ ^{56}Fe ranges from -1.64 ‰ to -0.08 ‰, with an average of -0.71%; Strategy II Plant δ ^{56}Fe varies from -1.19 ‰ to 0.39 ‰, with an average value of -0.07 ‰[9].

3. Research progress of iron isotopes in plants

Iron is an essential nutrient for plant growth and development, and plays a key role in plant biochemical activities. Iron promotes the synthesis of chlorophyll and is an important constituent of a variety of important biological enzymes in plant cells. It participates in the respiration process of plant cells, the process of intracellular electron transfer, and the process of biological nitrogen fixation [10].Soil is the main carrier of plant growth and the main source of iron in plants. Exchangeable iron is dissolved in the soil and absorbed by the roots of plants. It is collected by the root cells of plants, and finally transported to the stems, leaves, fruits and other parts of plants through the xylem and other transport organs of plants. It is used by the cells in these places. The iron element in the soil is usually the main element with a high content. However, because the iron element mainly exists in the soil in a high valence state that is difficult to dissolve (iron hydroxide, iron oxide and other+3 valence compounds [11]), the content of easily soluble ferrous ions is very small, which is difficult to meet the needs of plants. Therefore, there are two mechanisms of utilizing+3 valent iron in plant groups. According to these two mechanisms, plants can be roughly divided into strategy I plants and strategy II plants [12, 13].

3.1. Strategy I Iron absorption mechanism in plants

Common strategy I plants are: dicotyledonous plants such as tomato, soybean, Peas, cucumbers and monocots except graminae [14]. The absorption system of strategy I consisted of several parts. The H^+ - ATpase pump system decreased soil pH and increased iron solubility in rhizosphere soil particles by secreting H^+ [15]. Even in more acidic soils, this system can be informed by iron deficiency signals and acidify the soil below Ph6. Ferric-chelate reductase oxidase (FRO), which reduces Fe^{3+} to Fe^{2+}, and NADPH dehydrogenase coupled with it. FRO is a class of transmembrane proteins that transfer intracellular NADPH electrons through heme functional groups to reduce chelated high-valence iron. And the ferrous iron transport system, which contains various iron transporters [16]. The absorption process of strategy I is that plants excrete H+ -ATpase and iron chelate reductase FRO2 on the surface of roots to acidify soil and reduce + 3-valent iron to + 2-valent iron, and then transport the dissolved + 2-valent iron to root cells through iron regulatory transport protein IRT1/IRT2, and finally to plant organs.

3.2. Strategy II Iron absorption mechanism in plants

Common strategy II plants include corn millet and other grasses [14]. The transfer substances dependent on strategy ii are relatively simple. Grasses have biological enzymes such as nicotinamide synthetase (NAS), nicotinamide aminotransferase (NAAT) and dideoxyglycolate synthetase (DMAS), which can synthesize S-adenosine methionate into lyseric acids through enzymatic reactions and secrete them in the root system [17].The absorption process of strategy II is as follows: the root cells of the plant secrete the glycolic acids to the extracellular via the glycolic acid family transporter 1 and chelate them with + 3-valent iron [18], and then the complex is transported to the cell by YS1, YSL and other transporters, and finally to the plant [19].

Charlson et al. [20] analyzed and found that strategy I and strategy II plants both contained strategy I iron absorption gene, but strategy I plants did not contain strategy II iron absorption gene, and the ancestor species of gymnosperms and monocots, Arabidopsis Thaliana, also did not contain strategy II gene. At the same time, studies also showed that strategy II pathway had higher Fe uptake efficiency than strategy I pathway, and was less affected by pH and soil water content changes. Therefore, it was speculated that strategy II was the Fe uptake pathway evolved from strategy I. Some plants can have both strategy I and strategy II iron absorption pathways, such as rice. It has been suggested that rice may adapt to changes in growing environment by adjusting iron uptake strategies, especially when iron supply changes significantly. When rice is in flooded environment, iron in the rhizosphere is abundant and may cause toxic effects due to excessive iron. Ferrous iron in solution enters root epidermal cells directly through divalent metal transporters. Under drought conditions, when iron mainly exists in the form of ferric hydroxide, the expression level of rice's ferric chelate reductase gene (OsFRO) is very low in the root [21], and the efficiency of strategy I pathway is too low. Therefore, rice root cells obtain iron through strategy II chelate pathway.

3.3. Iron isotope fractionation in two strategy plants

Guelke et al. [22]first discovered the fractionation of iron isotopes in plants. They found that the composition of iron isotopes was very different between strategy I and strategy II plants. Compared with soil, strategy I plants were significantly enriched in light iron isotopes, with fractionation values up to −1.6‰, and δ^{56}Fe values in plants were lighter in the upper part and heavier in the lower part. The isotopic values of Strategy II plants as a whole were close to those of soil, and the fractionation value of iron isotope was 0.2‰, and there was almost no difference in the isotopic composition of the whole plant.

Guelke-stelling et al. [23] studied other crops by artificial cultivation in the following research. The source of iron in plants is all EDTA-Fe (+3 valence) formulated manually. Typical strategy I plants and strategy II plants to choose between are soybeans and oats. Samples were collected at each growth stage of the plant. The results of the iron isotopes in both plants were similar to the previous ones. Both strategy I and Strategy II plants were enriched in light iron. The δ56Fe values in soybean plants became lighter according to the nutrient transport path from root to seed, reaching the minimum in seed (−2.5‰). However, the δ56Fe of oat was −0.5‰, which was slightly lighter, and there was little difference in iron isotopic composition within the plants. Guelke speculated that there was a reduction of ferric iron in the process of iron absorption and transport in strategy I, which resulted in a larger fractionation value. In the process of iron absorption by strategy II plants, chelation only had a slight effect on iron isotopes, and there was no iron REDOX process in the process of iron transport, so there was no difference in isotopic composition[24].

Kiczka et al. [25] determined the iron isotopic composition of three Alpine species (Oxyria digyna, Rumex scutatus, Agrostis gigantea). The results were as follows: Both strategy I and strategy II plants were enriched in light iron isotopes, and the enrichment degree of iron isotopes in strategy II was much smaller than that in strategy I plants, which was close to the ratio of soil. The fractionation value of total iron isotopes was -1.3‰ in strategy I plants and -0.2‰ in strategy II plants, which was similar to the result of Guelke [22]. Kiczka calculated the fractionation value of −1.7‰ caused by the selective absorption process in strategy I based on the measured values, which was close to the fractionation value of −1.5‰ caused by the dissimilatory reduction process of iron [26]. It was speculated that the selective absorption of root was mainly involved in the reduction and absorption process of iron.

Some plants, such as rice, exhibit unique differences in the isotopic composition of iron, which can combine both iron absorption strategies. Rice can transport Fe(III)-MA mixture through OsYSL15 transporter and absorb Fe2+ from soil through its own divalent iron transporter OsIRT1[27].Garnier et al. [28] measured the iron isotopic composition of soil-water-rice system in a detailed way. The fractionation of iron in rice plants reached −0.821‰, which was significantly different from the previous results that Strategy II plants were enriched with little or no light iron, but did not reach the fractionation value of typical Strategy I plant. Garnier et al. Garnier speculated that Strategy II might be the main mechanism of iron absorption in rice, and that rice roots would produce iron films. When rice roots absorb iron elements in the iron films, they absorb more light iron isotopes, while heavy iron isotopes tend to remain in the iron films.

For higher plants, there are two different ways to absorb iron: strategy I and II. However, in general, higher plants are enriched in light elements relative to soil, which is similar to the characteristics of other organisms in iron utilization. However, the isotopic fractionation of strategy I is greater than that of strategy II, and the isotopic composition of all parts in mechanism I is larger, while the isotopic values in strategy II are relatively uniform. This may be due to REDOX reactions in strategy I. The REDOX reaction of strategy I consumes more energy and has more complex steps than the chelation reaction of strategy ii, which may be one of the reasons for the higher absorption efficiency of strategy ii.

3.4. Iron isotope fractionation in other plants

It is not only higher plants that can produce significant fractionation of iron isotopes. Relatively simple algae can also produce isotopic fractionation, and since algae are abundant in water, the study of the isotopic composition and fractionation process of iron in algae also plays an important role in revealing the water-plant iron cycle. Michael et al. studied spring phytoplankton blooms in temperate zones [29]. At the beginning of the bloom, the isotopic ratios of dissolved iron in the mixed layer of water and algae at the top of the water body were lighter than those of solid particulate iron. The result of lighter isotopes may be due to the reduction of particulate iron by a combination of photochemical (dominant) and algal reduction processes. With the development of the bloom, the dissolved iron in the mixed layer of surface water and algae became heavier, which may indicate that the process of dissolved iron transfer was dominated by biological action.

Sun et al. studied Fe isotope fractionation of two algae species Chlorella Pyrenoidosa and Chlamydomonas Reinhardtii [30]. The two kinds of algae were cultured in the medium mixed with $FeSO_4$, and the culture experiments of different iron concentration gradients and sampling with the growth time of algae were carried out respectively. The experimental results show that in the use of algae and the process of iron metabolism, has obvious iron isotope fractionation phenomenon, but the cultivation of the concentration gradient experiment and the growth of two kinds of algae on the fractionation of iron element, sun and others speculate that this may be two different kinds of algae in intracellular iron concentration will take different strategies of iron absorption, At lower intracellular concentrations, the heavier Fe isotopes were preferentially absorbed, while at higher concentrations, the absorption strategy was switched to the lighter or equally heavy Fe isotopes.

Apparently, phytoplankton and algae produce significant fractionation of iron isotopes. Moreover, the iron isotope fractionation of the two groups showed great differences. Even the same group showed different isotope fractionation characteristics in different environments and growth processes. Further studies on the mechanism of iron fractionation in phytoplankton and algae may help us to shed more light on the utilization and recycling mechanism of iron in plants.

4. Conclusions

The fractionation of iron isotopes in plants is similar to that in nature, and the REDOX reaction may be an important factor leading to the fractionation of iron isotopes. Higher plants basically follow the two mechanisms of iron absorption, strategy I and strategy II. The overall iron isotope of higher plants is relatively light, but some higher plants follow different strategies to absorb and use iron with the change of environment and their own growth process, so the iron isotope values of different plants may vary greatly. Low plants can also produce obvious fractionation of iron isotopes during growth and development, and the mechanism of fractionation needs to be further studied. At present, the plant of iron isotope research is still relatively weak, but the iron isotope method for the research of plant iron biogeochemical behavior provides a new method, China railway isotope fractionation behavior can inversion water - plant - soil biogeochemical process of iron, for the biosphere, lithosphere iron cycle provides a new theoretical basis. Iron isotopes will have great application prospects in plant research.

Acknowledgements

This work was supported by the Internal Scientific Research Project of Shaanxi Provincial Land Engineering Construction Group Co., Ltd. (No. DJNY2021-31) and Innovation Capability Support Program of Shaanxi Province (No. 2021PT-053).

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