Accumulation and fractionation of rare earth elements (REEs) in wheat: controlled by phosphate precipitation, cell wall absorption and solution complexation

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Abstract

Previous studies on rare earth element (REE) bioaccumulation have focused on their accumulation rate and fractionation, but the processes involved remain unclear. In this study, the accumulation and fractionation of REEs in wheat (Triticum aestivum L.) were investigated using solution culture with exogenous mixed REEs. A decrease in REE contents was observed from the roots to the tops of wheat. Significant fractionations of REEs were found in wheat organs as compared to the exogenous mixed REEs. Middle REE (MREE, the elements from Sm to Gd) enrichment and an M-type tetrad effect (an effect that can cause a split of REE patterns into four consecutive convex segments) were observed in the roots, which were probably caused by phosphate precipitation of REEs in/on the roots and absorption of REEs to root cell walls. Light REE (LREE, the elements from La to Eu) and heavy REE (HREE, the elements from Gd to Lu) enrichments were observed in the stems and leaves, respectively, accompanied by conspicuous W-type tetrad effects (an opposite effect to the M-type tetrad effect) in the REE patterns. HREE enrichment decreased from the older to the younger leaves and increased upwards within a single leaf. It is suggested that the solution complexation that occurred in the xylem vessels plays an important role in REE fractionations in the above-ground parts of wheat.

Key words: Accumulation, fractionation, rare earth elements (REEs), the tetrad effect, Triticum aestivum L., wheat.

Introduction

The rare earth elements (REEs) comprise a group of 17 trivalent metallic elements with similar chemical properties. They vary in relative atomic mass from 139 (lanthanum) to 175 (lutetium). Also commonly included in the group are scandium (45) and yttrium (89). Lanthanum, the most well known, has the useful property of being electron opaque compared with other Group III metal cations (e.g. Al3+, Ga3+, Sc3+) (Robards and Robb, 1974). Laboratory experiments have dealt with the potential for REEs to replace and compete with Ca for binding sites on proteins, and with effects on membrane stability (Brown et al., 1990). Previous studies have also identified some notable similarities in the early reactions by primary roots to REE3+ and to Al3+ (Bennet and Breen, 1992; Ishikawa et al., 1996; Kataoka et al., 2002). This enables REEs potentially to be ultra-structural tracers for AI toxicity to plants.

There is an increasing interest in the bioaccumulation processes of REEs due to the wide application of REEs in a variety of non-nuclear industries and agriculture, resulting in possible environmental contamination (Choppin et al., 1986; Wang et al., 2001). Meanwhile, due to their unique chemical structures as a group of elements, they may be used to trace the sources of inorganic elements in plants (Fu et al., 1998, 2001; Fu and Tasuku, 2000) and the
behaviour of actinides for risk assessment of radioactive waste disposal (Brookins, 1989; Miekeley et al., 1994).
Investigations into the bioaccumulation characteristics of REEs have been carried out in recent years as sensitive techniques such as inductively coupled plasma-mass spectrometry (ICP-MS) and neutron activation analysis (NAA) have become available (Ichihashi et al., 1992; Wyttenbach et al., 1994, 1998; Fu et al., 1998, 2001; Fu and Tasuku, 2000; Liang et al., 2001; Wei et al., 2001). Concentrations of REEs in plants are extremely variable, with about 700 ng g⁻¹ La reported in one species of fern (Matteuccia) (Fu et al., 1998), but it can be less than 10 ng g⁻¹ La in the needles of Norway spruce (Wyttenbach et al., 1994).

Possible reasons for the differences in REE concentrations in plants include differences of REE concentrations in soils and differences of plant species (Miekeley et al., 1994; Fu et al., 2001). Like other trace elements, REE concentrations are elevated in roots and they decrease in the order of roots>leaves>seeds (Wyttenbach et al., 1998). However, the reasons for this kind of variation are not clear, and more investigation into the accumulation of REEs in plants is needed.

For decades, geologists have recognized that REEs are very useful tracers in studies of geochemistry because of their generally coherent and predictable behaviour (Henderson, 1984). The property of REEs as tracers can also be applied to explain the mechanisms of element intake by plants (Fu et al., 1998). For example, based on REE patterns, Fu et al. (2001) suggested that silicate particles in soils might be a significant source of inorganic elements for plants. However, this may be complicated by the fractionation among individual REEs.

Despite extensive studies on REE uptake and transport in plants, little attention has been paid to the REE fractionation in bioaccumulation processes. The results of no or no obvious fractionation were often claimed, but they were most probably due to analytical difficulties or uncritical use of the term ‘no fractionation’ (Wyttenbach et al., 1998). Fractionations of REEs in biogeochemical processes have been observed, for example, fractionation between light REEs (LREEs, La–Eu) and heavy REEs (HREEs, Gd–Lu) (Wyttenbach et al., 1998; Wei et al., 2001), non-redox-sensitive REE anomaly (such as Gd, Yb and Lu) (Wyttenbach et al., 1998; Xu et al., 2002), and redox-sensitive REE anomaly (Ce and Eu) (Wyttenbach et al., 1998; Fu et al., 2001; Wei et al., 2001; Ding et al., 2003). Most of the natural chemical fractionations can be attributed to the differences in their ionic radii (with increasing contraction of the 5s and 5p electrons with the increase of atomic number) and variations in valences (Ce³⁺ or Ce⁴⁺, Eu²⁺ or Eu³⁺). However, a radius-independent variation in the chondrite-normalized REE patterns (the REE abundances are normalized to those in chondrite, and then the pattern is achieved by plotting the ratios on a logarithmic scale against the atomic number: the REEs in chondrite are assumed to be no fractionation) called the tetrad effect was also found in plants (Fu et al., 1998, 2001; Fu and Tasuku, 2000). This effect can cause a split of REE patterns into four segments called tetrads [first tetrad, La–Ce–Pr–Nd; second tetrad, (Pm)–Sm–Eu–Gd; third tetrad, Gd–Th–Dy–Hö; and the fourth tetrad, Er–Tm–Yb–Lu] with ‘breaks’ at Gd, between Nd and Pm, and between Ho and Er, corresponding to the half-, quarter-, and three-quarters-filled 4f subshell, respectively (Mclennan, 1994). Fidelis and Siekierski (1966) and Peppard et al. (1969) initially observed the tetrad effect in patterns of liquid–liquid REE distribution coefficients. Since then, the tetrad effect has been recognized in chemistry as affecting REE complexing behaviour, which is assumed to be influenced by variations in the exchange interactions of unpaired 4f-electrons, spin-orbit coupling, or crystal field stabilization (Irber, 1999). There are two types of variations of the tetrad effect, and the overall shapes of the tetrads are either convex or concave and form M-shaped distribution in solid and W-shaped distribution in solution, respectively (Masuda et al., 1987).

A W-type tetrad effect was observed in fern (Matteuccia), implying that the REEs must once have been in a dissolved state (Fu et al., 1998).

Few studies have been carried out on the REE fractionation in the soil–plant system via laboratory experiments. Field investigation may not be suitable for studies on the mechanisms of REE fractionation due to the extremely low concentration ratios of plant/soil, contamination of plant samples with soil and dust particles and the uncertainty of the normalization procedure (Wyttenbach et al., 1994; Mclennan, 1994). In this study, the water culture of wheat with the application of exogenous mixed REEs was adopted. Accumulation and fractionation of REEs among wheat organs or tissues were investigated, and the absorption, distribution, and fractionation mechanisms of REEs were discussed.

Materials and methods

Stock solution of mixed REEs
Stock solution of 2.1 mM mixed REEs used in this study was composed of 14 lanthanides (La to Lu except Pm), with an identical concentration of each element ([Ln³⁺]=0.15 mM, Ln represents any lanthanide). Each REE stock solution of about 10 mM was prepared by dissolving oxide with 1 M HCl, except that Ce was made from CeCl₃. Then the solution of each element was calibrated by an ICP-MS and prepared for the mixed-REE solution. All solutions used in the present study were made using deionized water of 18-mega ohm quality or better.

Plant materials and growth conditions
Seeds of wheat (Triticum aestivum L. cv. Jin-Dong 8) were sterilized for 15 min in a 1% solution of sodium hypochlorite, rinsed with tap water, and soaked in deionized water for 24 h. The seeds were then placed on a nylon net, which was fixed on an aerated solution containing 0.2 mM CaCl₂ in a 7.0 l plastic container. After being kept...
in the dark for 5 d at 25 °C, the seedlings were transplanted to 3.0 l plastic pots (20 seedlings per pot) containing aerated one-fifth strength Hoagland solution. This nutrient solution contained the following macronutrients (mM): KNO₃ (1.0), Ca(NO₃)₂ (1.0), MgSO₄ (0.4), and KH₂PO₄ (0.2); and the micronutrients (µM): Fe-EDTA (4), H₂BO₃ (3.0), MnCl₂ (0.5), CuSO₄ (0.2), ZnSO₄ (0.4), and (NH₄)₆MoO₄₂₋₄ (1.0). The solution was adjusted to pH 5.5 with dilute HCl or NaOH and renewed every other day. Plants were grown under controlled-environment conditions with a 14/10 h 25/20 °C day/night scheme and light intensity of 40 W m⁻² until the appearance of the fifth leaf (about 5 weeks). A part of them were then used for the accumulation and fractionation experiments. The rest of the seedlings continued to grow in high-strength Hoagland solution for another 5 weeks, and were then used for the collection of the xylem sap.

**Accumulation and fractionation of REEs in wheat**

The seedlings with five leaves were exposed to 1 mM CaCl₂ solution (pH 5.5) for 30 min to reduce PO₄³⁻ from adhering to the root surfaces (Ca²⁺ was used to maintain the stability of root tissues) (Marschner, 1995), then to 1 mM CaCl₂ solution (pH 5.5) containing 5 µM mixed REEs for 1 d, and the nutrient solution (one-fifth strength Hoagland solution) for another day. The reason for this intermittent treatment was to avoid the interactions between REEs and other nutrients such as PO₄³⁻. This process was repeated three times, and on the seventh day after treatment, the seedlings were further cultured in 1 mM CaCl₂ solution (pH 5.5) for 30 min to reduce the REEs adhering to root surfaces. Half of the seedlings were then harvested, while the remaining half were transferred to half-strength Hoagland nutrient solution without mixed REEs. When three new leaves (sixth, seventh, and eighth) appeared, the plants were sampled. The plant samples were separated into roots, stems, and leaves and the leaves were separated into the first to the fifth leaf at the fifth-leaf stage and the first to the eighth leaf at the eighth-leaf stage (numbered from the oldest to the youngest). The third leaf at the fifth-leaf stage was cut into three equal parts according to its length, and named as ‘base’, ‘centre’, and ‘top’ from the bottom to the top.

**Collection of the xylem sap**

Prior to collection of the xylem sap, the 10-week-old seedlings were cultivated overnight in a solution containing 1 mM CaCl₂ (pH 5.5), then exposed to 50 µM mixed REEs in 1 mM CaCl₂ (pH 5.5) solution. After 24 h of culture, the stem was severed 2 cm above the root and xylem sap was collected for 8 h with a micropipette during the daytime. The sap in the micropipette was removed into a beaker in the daytime. The sap in the micropipette was removed into a beaker containing 5 ml HNO₃ and transferred into a 10 ml flask, then diluted to the specific volume with deionized water. REEs in solution were determined by ICP-MS (PQ II Turbo, VG). Prior to detection, 0.2 ml of a 1 µg ml⁻¹ indium (In) solution was added in the solution to be used as an internal standard to compensate for matrix suppression and signal drifting. The wheat seeds used in this study were also determined and the REE contents were found to be extremely low (about 0.05 mg kg⁻¹ DW).

Quality control was achieved with a certified reference sample GBW07603 of shrub leaves from the National Research Center for Certified Reference Materials, Beijing, China. Seven duplicates were made. The results for the reference samples were in line with the reference values, with the standard deviations less than 5%, except for Sm (6.7%) and Lu (5.4%).

**Quantification of the tetrad effect**

The proposed method was adopted to quantify the degree of the tetrad effect according to Irber (1999) and Monecke et al. (2002). The method was designed for REE patterns that exhibit a split into four tetrads. To illustrate the four segments of an REE pattern showing the tetrad effect, the segments are consecutively numbered as i = 1, 2, 3, and 4. The size of the tetrad effect in each segment can be given as

\[ t_i = \sqrt{\frac{X_{A_i} \times X_{B_i}}{X_{A_i}\, X_{B_i}}} \]

where X₂, X₃, X₄, and X₅ are the concentration of the first to the fourth element in the tetrad, respectively. The second tetrad (Pm to Gd) cannot be calculated because of the missing Pm in nature.

The values of tₙ determined from three segments can be averaged to produce an overall value of T:

\[ \text{Size of the tetrad effect} = T = (t_1 \times t_2 \times t_4)^{1/3} \]

The tetrad effect can be simply characterized by an M-shape or a W-shape for the T values being larger than one or smaller than one, respectively.

**Results**

After short-term exposure to 1 mM CaCl₂ solution (pH 5.5) containing 5 µM mixed REEs, considerable amounts of REEs were absorbed by wheat and distributed to every organ of the seedling (Fig. 1A). More than 99% of the total
REEs was concentrated in the roots and only a small amount had been transported to the above-ground parts. After further culture in the solution without mixed REEs, REE contents of the roots decreased from 55.662 µg to 50.519 µg, and the contents of the above-ground parts increased from 0.591 µg to 3.404 µg. However, the total REEs in the whole plant did not change (Fig. 2A). REE contents in the leaves varied with leaf position at both growth stages. A decrease in REE contents was observed from the oldest to the youngest leaf. The three youngest leaves at the eighth-leaf stage (the sixth to the eighth leaf), which appeared after the cessation of mixed REEs treatment, contained extremely low REE contents. A decrease in REE contents was also found from the base to the top of the third leaf sampled at the fifth-leaf stage (Table 1). The total concentrations of LREEs decreased about 50% from the base to the top, compared with a slight decrease of HREEs.

As mentioned earlier, the plants were cultivated in a 5 µM mixed REE solution, which is composed of 14 lanthanides with identical concentrations of each element. After intake of REEs into the wheat seedlings, REE patterns should present flat lines if there were no fractionations. However, significant fractionations of REEs occurred in wheat (Figs 1B–D, 2B–D). The roots strongly enriched middle REEs (MREEs, e.g., Sm, Eu, and Gd), and the stems exhibited a slight enrichment of LREEs. Unlike the roots and stems, a significant enrichment of HREEs was found in the leaves. The enrichment of HREEs decreased upwards, associated with the reduction of the total concentrations of REEs (Fig. 3). An inverse feature was found within the third leaf sampled at the fifth-leaf stage, where the total concentrations of REEs slightly decreased from the base to the top, but the HREE enrichment significantly increased upwards (Table 1; Fig. 4).

Besides the radius-dependent fractionation of REEs in wheat, the tetrad effect was also observed in the REE patterns (Figs 1, 2). The size of the tetrad effect in different organs was calculated according to equations (1) and (2). The M-type and W-type tetrad-effect-like patterns appeared in the roots and above-ground parts, respectively (Fig. 5).

After 24 h treatment with 50 µM mixed REEs, REE pattern in the xylem sap was similar to that in the leaves, with obvious HREE enrichment and W-type tetrad effect (T=0.81) (Fig. 6). After 48 h exposure in 5 µM mixed REE solution, the total concentration of REEs in the cell walls of roots was 5.92±0.63 µmol g⁻¹ DW; about 72% of the total (8.17±0.54 µmol g⁻¹ DW) was in the roots. A slight enrichment of MREEs was observed in the cell walls, with...
a slight depletion of HREEs compared with the LREEs. However, the M-type tetrad effect, originally present in the roots ($T=1.09$), disappeared in the cell walls ($T=0.98$) (Fig. 7).

After the reaction of REE ions with phosphate in solution for 10 h at pH 3.7, significant depletions of MREEs, with obvious W-type tetrad effects were observed in the dissolved REEs (Fig. 8A). When citrate was added to the solution, the MREE depletions in the patterns gradually changed to HREE enrichments (Fig. 8B), and the REE patterns were finally close to those observed in wheat leaves (Figs 1B, 2B, 4).

**Discussion**

**Accumulation and mobility of REEs in wheat**

The accumulation of metals from external solution into the shoot (mainly leaves) can be separated into two processes: a non-metabolic, passive movement from the external solution to the roots and a transpiration-dependent translocation from the roots to the shoots via the xylem (Marschner, 1995; Salt *et al.*, 1995). Uptake and translocation may vary considerably depending on plant species and metal types. For example, it is well known that Ca concentrations are higher in old leaves than in young
leaves, and this is related to transpiration rates, phloem mobility, age of leaves, and the low mobility of Ca (Behling et al., 1989). An acropetal decrease in Al concentrations was found in buckwheat (*Fagopyrum esculentum* Moench), caused by the low mobility of Al (Shen and Ma, 2001).

**Fig. 3.** Variations of the total concentration of REEs (ΣREE) and concentration ratio of HREE/LREE in the first to the fifth leaf at the fifth-leaf stage (the curves formed by five data points) and the first to the eighth leaf at the eighth-leaf stage (the curves formed by eight data points). The seedlings at the fifth-leaf stage were exposed to 5 μM mixed REE solution and nutrient solution on alternate days for 6 d. Half of them were further grown in nutrient solution until three new leaves appeared. LREEs refer to La-Eu. HREEs refer to Gd-Lu. Values are means ±SD of three replicates.

**Fig. 4.** REE patterns in different parts of the third leaf sampled at the fifth-leaf stage. The seedlings at the fifth-leaf stage were exposed to 5 μM mixed REE solution and nutrient solution on alternate days for 6 d. The third leaf was separated into three equal parts, as named as ‘base’, ‘centre’ and ‘top’ from the bottom to the top. Values are means ±SD of three replicates.

**Fig. 5.** The tetrad effect (T) in different organs of wheat at the fifth-leaf stage and eighth-leaf stage, respectively. The seedlings at the fifth-leaf stage were exposed to 5 μM mixed REE solution and nutrient solution on alternate days for 6 d. Half of them were further grown in nutrient solution until three new leaves appeared. The T values were calculated according to equations (1) and (2). Values are means ±SD of three replicates.

**Fig. 6.** REE pattern in the xylem sap of wheat. 10-week-old seedlings were exposed to 50 μM mixed REE solution for 24 h, the stem was then severed 2 cm above the root and the xylem sap was collected for 8 h with a micropipette. Values are means ±SD of three replicates.

**Fig. 7.** REE patterns in the roots and cell walls of the roots. 3-week-old seedlings were exposed to 5 μM mixed REE solution for 48 h, the roots were then sampled and parts of them were used for extraction of the cell walls. Values are means ±SD of three replicates.
A similar distribution of REEs was found in wheat (Figs 1A, 2A). It means that the accumulation of REEs in wheat is dominated by their mobility, and the acropetal decrease is caused by the low mobility of REEs. This is mainly attributed to the high valencies of REE ions, leading to high affinity of REEs with plant cell walls, as the cell walls contained 72% of the total REEs in the roots. X-ray absorption spectroscopic techniques verified the binding of La(III) in barley roots via carboxylate groups and hydration of La(III) (Han et al., 2005). Phosphate precipitation of REEs occurring in/on the roots or the transport path is another important factor, which will be explained later.

Redistribution of an element from old to young organs mainly takes place via the phloem (Marschner, 1995). The redistribution of REEs in wheat showed that REE contents were extremely low in the young leaves (Fig. 2A). This means that it is probably hard for REEs to transport in the phloem. The phloem consists of living cells containing substances on which metal ions are easy to bind (Greger, 1999) and thus may prevent REE translocation in it. The accumulation of REEs continued to increase in the older leaves and stems (Fig. 2A), but it seems that they originated from the REEs remaining in the roots due to the constant total contents of REEs in wheat. It is deduced that the acropetal transport via the xylem continues to play an important role in the accumulation of REEs due to the high concentration ratio of root/leaf.

**REE fractionation in the roots**

Conspicuous fractionation of REEs was observed in the roots, characterized by a relative MREE enrichment and a slight M-type tetrad-effect-like feature (Figs 1D, 2D). Laboratory experiments verified that the precipitation of phosphates in the solution system caused the formation of MREE-enriched precipitates, leading to the relative depletions of MREEs and the W-type tetrad effects in the solution (Fig. 8A). Similar results were reported by Byrne et al. (1996). When the REE patterns in the solution (Fig. 8A) were compared with those in the roots (Figs 1D, 2D), inverse fractionation features were observed between them. Based on the fact that, at pH >4.0, phosphate precipitation of REEs can easily take place (Diatloff et al., 1993), it is suggested that the fractionation features of REEs observed in the roots of wheat are partly caused by REE phosphate (LnPO4) precipitation in the roots or on the root surfaces. Ishikawa et al. (1996) reported that the localization of La3+ and Yb3+ in root-tip portions of rice and pea treated with La3+ and Yb3+ was fully consistent with that of phosphate and the phosphate concentration increased both in the apoplast and symplast, suggesting that the distribution of these ions might result from the precipitation of the metal ions with the phosphate ions released in the apoplast. Quiquampoix et al. (1990) confirmed the presence of deposits containing Gd and phosphorus in the extracellular space of root tissue of maize using electron microscopy and X-ray microanalysis. It is concluded that phosphate precipitation is an important factor to control the accumulation and especially the fractionation of REEs in wheat roots.

Selective absorption to cell walls may also play a certain role on REE fractionation in the roots, as the REE pattern in the cell walls exhibited slight MREE enrichment (Fig. 7). However, no tetrad effect was observed in the pattern of cell walls. It means that the tetrad-effect fractionation of REEs in the roots result exclusively from the precipitation of phosphate. Release ofREE ions originally absorbed to the cell walls might occur during the extraction process of the cell walls. Due to the decrease of ionic potential of the
dehydrated REE ions from La to Lu, heavier REEs can form stronger covalent bonds with protoplastic substances (Ishikawa et al., 1996), and the release of these elements might be more serious. It may explain the slight MREE enrichment and the depletion of HREEs in the cell walls compared with those in the roots (Fig. 7). In fact, a more significant enrichment of MREEs was observed when cell walls were extracted from the roots of wheat grown in an REE-free medium followed by short-term absorption in mixed REE solution (data not shown). The mechanisms involved in this remain unclear.

**Tetrad effect in the above-ground parts**

The existence of the W-type tetrad effect in the above-ground parts of wheat suggests that REEs must be in a dissolved state before they are transported to the above-ground parts, as the W-type tetrad effect exists in fluids and the M-type tetrad effect is observed in the corresponding solid materials that react with these fluids (Masuda et al., 1987). The roots, as mentioned above, showed the M-type tetrad effect derived from precipitation of phosphates with REEs (Figs 1D, 2D). As a result, REE patterns in the root solution should exhibit the W-type tetrad effect and REE patterns in the above-ground parts should also show the same feature, as the dissolved REEs in the roots are exclusively the source of REEs in the above-ground parts. This hypothesis is consistent with the results in this study (Figs 1, 2). REE pattern in the xylem sap also showed a W-type tetrad effect (Fig. 7).

**Origin of HREE-enrichment in the leaves**

As mentioned above, the roots exhibited a MREE-enrichment feature due to the phosphate precipitation and cell wall absorption (Figs 1D, 2D), which should result in a relative MREE-depletion feature in REE patterns in the xylem solution, and further lead to the same feature in the above-ground parts after the transfer of REEs into these organs. However, the results observed in this study are far from being the case (Figs 1B, C, 2B, C). Obviously there are other processes different from phosphate precipitation and cell wall absorption causing the changes.

Difference of speciation among individual REEs in the xylem solution might be an important factor to cause the REEs to fractionate from each other. Therefore, REE speciation in simulated xylem solution was calculated initially using an ionic speciation model. The results showed that EDTA complexes were the major species for the dissolved REEs and HREE enrichment existed in the complexes (data not shown). Based on the simulation, it was deduced that the enrichment of HREEs in wheat leaves was caused by EDTA complexation, where more HREEs were complexed by EDTA due to the increase of stability constants with the increase of atomic number across lanthanides (Byrne and Li, 1995), leading to more HREEs moving to the above-ground parts and less was adsorbed to cell walls or precipitated by phosphate. In order to verify the hypothesis, an additional experiment was carried out. The wheat was grown in an EDTA- and REE-free nutrient solution followed by exposure to mixed REE solutions containing different levels of EDTA. The results showed that REE accumulation and HREE enrichment in wheat leaves generally decreased with the increase of EDTA levels (data not shown). Other inorganic ligands (e.g. SO$_4^{2-}$ data not shown) and organic ligands (e.g. citrate; Ding et al., 2005) have similar effects. Like EDTA, stability constants of these ligands with REEs increase with the increase of atomic number across the lanthanides. However, when acetic acid was used as an exogenous ligand, of which the stability constants generally remain constant among individual REEs, the HREE enrichment in wheat leaves remained unchanged (data not shown). The results of the above studies indicated REEs should be absorbed by wheat roots mostly in the form of free ions, and the enrichment of HREEs in wheat leaves do not originate from the complexation of exogenous ligands such as EDTA. This is consistent with researches on other metal ions. It is general accepted that the total dissolved metal concentration of a metal is not a good predictor of its bioavailability for terrestrial higher plants. Accumulation and/or toxicity of dissolved metals correlates best with activities of free, uncomplexed metal ions in solution (Campbell, 1995; Parker et al., 1995, 2001), although a few exceptions to the findings have been reported (White, 2001; Parker et al., 2001).

Exudates from plant transport tissues contain many natural complexing compounds such as small (Cl$^-$, carboxylic acids, amino acids) and macromolecular substances (proteins, DNA, polysaccharides) (White et al., 1981a). Most transition metal ions and some main group metal ions form stable complexes with these ligands present in the xylem of plants, and transport, at least to some extent, in complexed form (Lobinski and Potin-Gautier, 1998; Rouser, 1999). There is also in vitro electrophoretic evidence for stable metal complex formation and quantitative estimates of the equilibrium distribution of metals, ligands, metal complexes, and other solutes in the xylem fluid (White et al., 1981b, c; Mullins et al., 1986). Due to the high ionic potential of the dehydrated REE ions, the REEs in the xylem vessels should mainly be translocated in complexed forms. Such complexations occurring in xylem fluid can prevent the phosphate precipitation, as well as cell wall absorption, of the REEs in solution and, moreover, lead to the formation of a HREE-enriched pattern in solution because of the increase of stability constants of REE complexes with the increase of atomic number of REEs for the most ligands (e.g. citrate, as shown in Fig. 8B) (Wood, 1990; Millero, 1992; Byrne and Li, 1995). As a result, the HREEs become more mobile than the LREEs and cause the HREEs to be enriched in wheat leaves relative to
It is well known that absorption onto negatively-charged sites in cell walls is the major reason for the root-concentrated feature of heavy metals in plants (Hardiman and Jacoby, 1984; Marschner, 1995), and it is also the same for REEs. However, as adsorption onto the cell walls is of limited capacity and has a limited effect on heavy metal activity (Hall, 2002), phosphate precipitation might be another important factor to influence REE accumulation and fractionation in the bioaccumulation processes due to extremely low $K_{sp,LnPO_4}$ [e.g. $K_{sp,GdPO_4} \approx 10^{-24}$] (Byrne et al., 1996). Under natural conditions, concentrations of inorganic phosphates in soil solutions are extremely low (0.6–5 μM) (Baber, 1984), and they are close to that of the total concentrations of REEs in soil solutions (average of 9 μM in 33 types of soils in China) (Xing and Xu, 2003). However, phosphates in plants and xylem solutions can be raised by several hundred times via active absorption by plants (Hocking, 1980; Marschner, 1995), which would largely facilitate precipitation of REEs in plants, and further contribute to the acropetal decrease of REE accumulation and the occurrence of the tetrad effect (Fu et al., 1998; Fu and Tasuku, 2000). The results in this study give valuable information on exploring the mechanisms of REE accumulation and fractionation in bioaccumulation processes.

Slight enrichment of LREEs in the stems

The slight enrichment of LREEs in the stems (Figs 1B, 2B) possibly suggests that there is strong complexing environment present in the transport path before the transfer of REEs into the leaves. It is enough for the environment to keep the HREEs from chemical precipitation or absorption to cell walls in the stems while it is less effective on the LREEs. Fu et al. (2001) pointed out that the degree of fractionation between the LREEs and HREEs might be a reflection of the amount of ligands in the xylem solution.

Implications of this study

It is suggested that soil-normalized REE fractionation patterns might be controlled via a complexation of REEs by root exudates in the rhizosphere (Wytenbach et al., 1998). This is consistent with the results of this study that solution complexation is a major factor affecting REE accumulation and fractionation in plants. However, exogenous and intrinsic ligands may play different (i.e., nearly opposite) roles on them. The fact that high concentrations of low-molecular organic ligands, such as citrate, malate, and oxalate, in rhizosphere solutions (Jones, 1998) may be the reason why LREE enrichment is widely observed in plants when normalized to the host soils (Wytenbach et al., 1998; Fu et al., 1998, 2001; Wei et al., 2001), while strong complexations of intrinsic chelators in the xylem may be responsible for the HREE enrichment (Ding et al., 2003).

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