Agronomy **2015**, *5*, 374-404; doi:10.3390/agronomy5030374

OPEN ACCESS

agronomy

ISSN 2073-4395

www.mdpi.com/journal/agronomy

Review

Trace Element Management in Rice

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Academic Editor: Gareth J. Norton

Received: 31 March 2015 / Accepted: 12 August 2015 / Published: 18 August 2015

Abstract: Trace elements (TEs) are vital for the operation of metabolic pathways that promote growth and structural integrity. Paddy soils are often prone to TE limitation due to intensive cultivation and irrigation practices. Apart from this, rice paddies are potentially contaminated with transition metals such as Cd, which are often referred to as toxic TEs. Deficiency of TEs in the soil not only delays plant growth but also causes exposure of plant roots to toxic TEs. Fine-tuning of nutrient cycling in the rice field is a practical solution to cope with TEs deficiency. Adjustment of soil physicochemical properties, biological process such as microbial activities, and fertilization helps to control TEs mobilization in soil. Modifications in root architecture, metal transporters activity, and physiological processes are also promising approaches to enhance TEs accumulation in grains. Through genetic manipulation, these modifications help to increase TE mining capacity of rice plants as well as transport and trafficking of TEs into the grains. The present review summarizes that regulation of TE mobilization in soil, and the genetic improvement of TE acquisition traits help to boost essential TE content in rice grain.

Keywords: trace elements; bioavailability; mobilization; fertilizer; bio-fortification; root phenology; molecular physiology; root biology; metal transporters; photosynthesis

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1. Introduction

Rice is the main dietary source of trace elements (TEs) in Asian populations. However, rice grain possesses relatively low TE content compared to other cereal grains [1–3]. Hence enhancement of TEs in rice could be a practical solution to avoid TE deficiency disorders prevalent among populations which consume rice as staple food (Table 1). Apart from this, rice grain contaminated with toxic TEs such as Cd evokes the need of food safety measures that help to avoid toxic TE accumulation in rice. It is well known that nutrient cycles influence the mineral composition of the crop produce [4,5]. More than 90% of rice is cultivated in low land conditions where the soils are subjected to intermittent wet and dry periods. The field is subjected to water either by rain or irrigation. Occurrence of intermittent flooding especially in low land rain fed fields causes temporal changes in mobility of TEs [6–8]. However, irrigation is favorable to control the flooding periods and opens ways to manage TEs in the field through fertilization. The low land fields are also characterized by adequate water supply at the time of flowering, buffering of soil temperature and low risks of weed competition. All these features of low land fields help to prevent crop failure, and hence low land cultivation of rice is the most common cultivation practice.

Table 1. Trace elements (TEs) and human health.

TEs	Health Disorder
Se	Keshena (China), bone, arthritis, cardio vascular and Cancer
Zn	Dwarf ness (Iran & Egypt) infertility, impaired taste and smell
Cu	Anemia, Skeletal defects
Mn	Tremors, Stiff muscles
I	Goiter
Fe	Anemia
Mg	Depression, Nervous system disorders
Mo	Mouth/esophageal cancer, Neurologic damage
Co	Anamia
Na	Coma
Toxic TEs	Toxicity
Hg	Mina-Mata, Neurological disorders
Cd	Itai-Itai, Bone crippling, Cancer, Heart problems
F	Yellowing of teeth, Skeletal deformities, Dental caries
Pb	Lung cancer, Neurological disorders
As	Skin, Lung cancer
Cr	Lung cancer, Kidney dysfunction, Skin ulcer

Adaptability of rice plants to varying fertilization strategies promises TE management in low land rice paddies. Most often the rice paddies are fertilized with elements such as nitrogen, phosphorous, and potassium that enhance productivity. Zn, Fe, B, Mn, Cu, Mo and Si are the critical TEs for rice growth. Wetland rice fields are prone to Zn deficiency because of increased availability of macronutrient cations during the course of submergence [9]. But Fe and Mn deficiency is common in upland paddy fields due to formation of metal oxides as a consequence of higher aeration [10]. Moisture stress is well known to cause B deficiency in rice paddies whereas deficiency of Si is often related to mineral reserve deficits in soil rich in organic matter. Paddy soils also face Cu deficiency because of the application of lime to correct the acidic soil pH. TEs not only serve as enzyme cofactors but also play an important role in inhibition of plant uptake of toxic TEs such as Cd [11]. Thus one of the major drawbacks of micronutrient deficiency is the increase in availability of toxic TEs to crops.

Rice paddies can also be contaminated with toxic TEs such as Cd because of the usage of Cd contaminated phosphate fertilizer [12–14]. Apart from this, irrigation using mine water leachate also contaminates paddy soils with toxic TEs [15]. Thus rice plants are prone to toxic TE accumulation, which ultimately leads to a diet rich in toxic TEs. Since toxic TEs are detrimental to human health, accumulation of these elements needs to be reduced in rice grain. The present review focuses on the role of soil factors and molecular physiological processes on TE management in rice grain. The regulatory roles of nutrient cycles deserve attention as these cycles determine the TE availability to plants [16].The importance of soil physiochemical characteristics and biological activities responsible for TE mobilization in paddy soils is also discussed. It is well known that nutrient acquisition is the key physiological process, which has a direct role in plant uptake of TEs. This process depends on multiple factors such as root architecture, photosynthetic efficiency and activity of metal transporters. Hence the scope of manipulation of these factors is also explored. Advent of fertilization strategies, availability of rice germplasm and knowledge about genes responsible for TE accumulation can help to formulate approaches for TE fortification in rice.

2. Nutrient Cycle and Plant Uptake of TEs

Plant uptake of both toxic and non-toxic TEs depends on nutrient cycling in the soil [17,18]. Hence keeping balance of nutrient cycling is important to ensure crop yield together with restriction of toxic TE accumulation in rice grain. However, nutrient cycling in rice paddy agro ecosystems is often unbalanced due to anthropogenic intervention (Figure 1). Seasonal variations in soil physicochemical processes, especially in lowland rain fed fields where intermittent wet and dry periods occur, cause fluctuations in nutrient cycling. The flooding periods in rice fields cause leaching of cations [19]. Flooding also causes shifts in soil physicochemistry and results in precipitation or co-precipitation reactions of TEs. Soil microbial activity and incorporation of organic matter also affects solubility as well as plant uptake of TEs [20,21]. The difference between nutrient input into the soil through fertilization and nutrient removal during harvest also causes disturbances of nutrient cycles in paddy soils. Most often the fields receive fertilizer input in significantly higher amounts than needed by the rice plants. This could lead to an accumulation of mineral nutrients, especially ammonium, in the field that will reduce soil pH, and hence increase the mobility of soil TEs.

A nutrient cycle comprises the in and out flow of nutrients of the paddy field. Crop harvest is the major route of nutrients outflow in a paddy field. However, fertilizer application often replenishes the nutrient pool. It has been reported that TE availability in the soil depends on the parent material from which the soil is formed [22]. Even though paddy soils around the world are different because of variation in parent material from which they are derived, repeated agricultural practices such fertilization have rendered these soils uniform, especially with regard to macronutrient content.

Figure 1. Field management practices that influence TE availability in rice fields (**a**); Irrigation (**b**); Puddling (**c**); Dry land cultivation (**d**);Mixed or co-cropping (**e**); *Azolla*cultivation (**f**); System of rice intensification (**g**); Fertilizer application (**h**); Burning of crop residues (**i**); Rice cultivation near mine tailings.

TE concentrations in most paddy soils are higher than in non-paddy soils because of anthropogenic input. Decay of organic matter also contributes to the TE pool. Crop residue, especially roots and culms account for a major portion of the organic matter in paddy soil. Since the concentration of many of the TEs in rice plants are—in the decreasing order—root, shoot and grain, retention and decay of crop residues in the soil significantly contribute to replenishment of the TEs pool in the paddy soil. Occurrence of TEs in the soil solution is limited as they bind with organic matter or parent material [23]. Thus a majority of TEs are associated with the solid phase of soil where they occur as precipitate or co-precipitate of mineral salts. Co-precipitation usually occurs with newly formed chemical species such as carbonates, phosphates, oxides and hydroxides [24,25]. These kinds of co-precipitation help to limit exposure of toxic TEs to biota, and also slow down the release of TEs into the soil solution. As seen among soil types of the world, when the concentration of a particular mineral nutrient exceeds a certain threshold value, mineral ions precipitate in the paddy soil. Similarly when the concentration of the nutrient ion is lower than the chemical solubility equilibrium, dissolution of that particular element occurs. Nutrient ions in the paddy soil solution are found in association with organic acids, inorganic ion pairs and free ions [26]. All these contribute to the dynamics of nutrient cycling in paddy soils that influence plant availability of TEs (Figure 2).

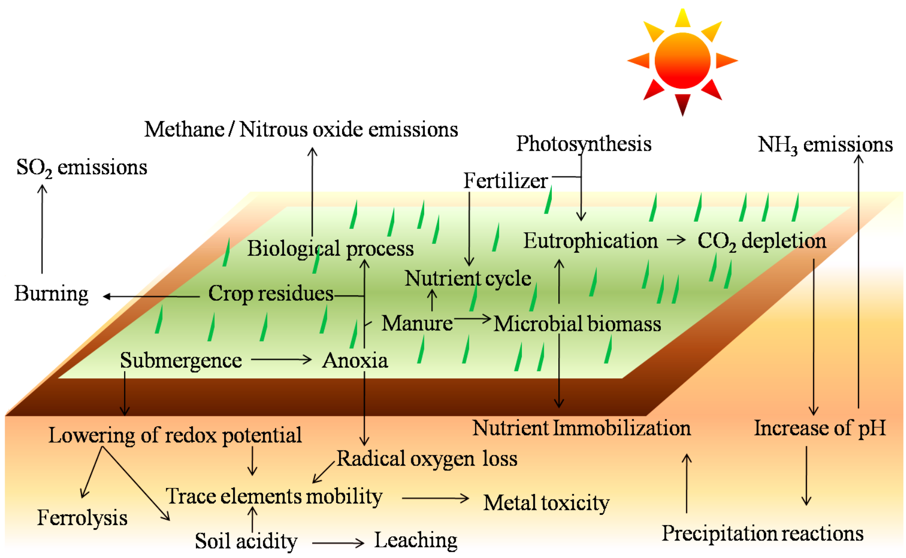

Figure 2. Nutrient cycling and mobilization of TEs in rice paddies.

2.1. Regulatory Role of Macronutrients

Macronutrients are the primary nutrients required for plant growth. Macronutrients affect TEs availability by shifting soil pH and precipitation reactions. Application of ammonium-based fertilizers is common in paddy fields for replenishing soil nitrogen. The applied fertilizer stimulates algal growth in paddy fields with enhanced utilization of CO_2. This reduces the carbonic acid content in submerged paddy fields with a subsequent rise of soil pH (even up to a pH of 9). But increase of pH leads ammonia evolution as well as precipitation of TEs. Apart from this, nitrogen loss occurs through leaching as well as denitrification [27]. Ammonia is also produced during the breakdown of organic materials such as manure, and volatilization of this gas further increases soil pH, which causes precipitation of TEs. It must be noted that a significant quantity of ammonia can be adsorbed into soil colloids, and hence plants growing in soils with a lower colloid contents, such as clay and humus, are more prone to suffer from nitrogen deficiency [28]. SO_2 held with soil particles causes a lowering of soil pH during submergence, and hence increases mobility of TEs. Microbial degradation of S-containing compounds also releases various S-containing gases, such as hydrogen sulfide (H_2S), carbon sulfide (CS_2), carbonyl sulfide (COS) and methyl mercaptan (CH_3SH) in submerged paddy soils which can react with TEs, which leads to precipitation of TEs [29,30].

Phosphorous (P) in paddy fields often meets the crop's demand. However, tillage practices often remove particulate P in the paddy soil through erosion. A significant amount of P loss also occurs during flooding related runoff in submerged rice paddies. In strongly acidic soils, P undergoes a precipitation reaction with elements such as Al, Mn and Fe. These precipitates are sparingly soluble, and hence rice plants growing in such soils face P deficiency as well as a deficit of Mn or Fe [31]. On the other hand, when the field is submerged, TEs undergo a reduction reaction that enables the release of P as well as TEs into the soil solution. Potassium (K) is prone to leaching, and this leads to a shift of soil pH towards the acidic phase, which mobilizes both TEs and toxic TEs. Apart from this, acidic soils tend to mask plant available K, and hence result in K deficiency, which accelerates TE toxicity [32]. It is noteworthy that poultry manure and wood ashes are good sources of K and have the capacity to immobilize toxic TEs [33]. Temporally sequential wetting and drying patterns in the rice field may also lead to the release of fixed K together with mobilization of TEs in the solid phase. Both Ca and Mg are also prone to leaching, especially in acidic paddy soils that cause more availability of toxic TEs for plant uptake. However, application of lime and dolomite in acidic paddy soils not only increases soil pH but also acts as potential source of Ca and Mg that can decrease plant uptake of toxic TEs.

2.2. Regulatory Role of Micronutrients

TEs act on each other with regard to plant availability by co-precipitation and competition for metal binding sites or transporters. Solubility of TEs increases during the course of submergence [34]. Mass flow of elements into roots especially that of Fe and Mn, accelerates at this stage. Similarly, movement of TEs towards the subsoil also increases in speed. Plaque formation in rice roots with Fe and Mn compounds is a noticeable feature of paddy soils during periods of submergence. This process occurs due to the development of an oxygen rich network of aerenchyma tissue in rice roots. When reduced forms of Fe/Mn reach the surface of this oxygen rich zone, these elements undergo oxidation and precipitate with silica or phosphate. This kind of precipitation also occurs in oxygen rich plow sole and surface soil. Thus, during a course of submergence, the movement and precipitation of Fe or Mn causes characteristic paddy soil development where a zone of reddish-brown streaks of the root channels is sandwiched between oxygenated surface layers and the Fe or Mn illuviated zone [35]. It is noteworthy that the downward movement of soluble Fe or Mn towards comparatively oxygen rich zones followed by precipitation reactions cause depletion of these elements from the top soil. The cycling of TEs in the solid phase of soil to the solution phase generally occurs either by adsorption-desorption reactions or precipitation-dissolution processes [36]. TEs in the solid phase often form stable bonds with surface functional groups, and hence the availability of the element to the biota depends on the strength of the bond formed between the particular nutrient element and the functional groups present in the solid phase [37]. Soil microbial processes play a significant role in the breakage of these bonds, and thus microbial action controls TEs availability. The biogeochemical transformation of Fe or Mn not only affects solubility and speciation of Fe or Mn but also the availability of toxic TEs such as Cd. Ability of Fe to form a complex with S reduces the chance of precipitation of Cd in the form of CdS in submerged soils. This situation also decreases the chance of adsorption of Cd to Fe or Mn oxides. Thus reductive dissolutions of Fe or Mn oxides in submerged soils lead to mobilization of Cd from the solid phase of the soil. However, the higher reactivity of FeS_2 may favor sorption of some parts of Cd in the solution. Similarly, Fe in the soil protects the rice plants from other toxic TEs.

Periodical changes of submergence and drainage sequences also cause variation in occurrence of forms of TEs in paddy soils, which influence TEs availability to plants (Table 2). For example Zn occurs in at least seven kinds of ionic forms or mineral salts in paddy soils (Zn^{2+}, $ZnCl^{+}$, ZnSO4, $ZnOH^{+}$, $ZnCO_3$, ZnS, $ZnSiO_3$). It is well known that availability of nutrient ion to biota depends on the chemical speciation. Most often TEs undergo speciation with OH^{-}, Cl^{-}, SO_4^{2-}, HCO_3^{-} and F^{-}. Humic substances and fulvic acids rich in poly carboxylic acids are also potential speciation agents of TEs in paddy soil [38]. Amino acids and tricarboxylic acids also play a significant role in the speciation of mineral nutrient ions [39,40].TEs in the solid phase are mobilized during suspension of organic matter or soil particles. Apart from these, puddling of rice paddies also contributes to mobilization of TEs in the soil sediments. However, flood related anoxia in the field in turn favors formation of hydroxide salts of TEs that make them unavailable for plant uptake [41,42]. Irrigation with chlorinated water also contributes to mobilization of TEs through the formation of water-soluble TEs salts with chlorine. Environmental factors are also involved in TE cycling in paddy soils [43,44]. Small quantities of TEs are incorporated into the field during rain fall and are deposited by the wind. TEs undergo leaching in the presence of runoff water, and hence rain fed rice paddies often face threat of macronutrient leaching [45]. Leaching of macronutrient cations not only causes nutrient deficiency in the soil but also contributes to soil acidification. On the other hand, acidification of soil accelerates the weathering process which in turn increases the TE pool in the paddy soil. However, this event often favors an increase of Al/Fe/Mn ions and causes Al/Fe/Mn toxicity in rice plants rather than promoting plant growth.

Table 2. Ionic forms of major TEs and toxic TEs in the soil solution.

TEs	**Occurrence**
Mn	Mn^{2+}
Fe	Fe^{2+}, $Fe(OH)_2{}^{+}$, $Fe(OH)^{2+}$, Fe^{3+}
Zn	Zn^{2+}, $Zn(OH)^{+}$
Mo	$MoO_4{}^{2-}$, $HMoO_4{}^{-}$
B	$H_3BO_3{}^{-}$
Cu	Cu^{2+},$Cu(OH)^{+}$
Co	Co^{2+}
Toxic TEs	Occurence
Pb	Pb^{2+}
Hg	Hg^{2+}
Cd	Cd^{2+},Cd^{1+}
As	As(III), As(V)
Cr	Cr^{3+},Cr^{6+}

3. Soil Dynamics Act on Mobilization of TEs

The dynamics of the paddy soil properties occurs mainly due to changes in physiochemical properties, biological activities and fertilization. Physiochemical characters of soils such as redox potential, pH, alkalinity, cation exchange capacity and texture significantly influence TEs mobilization in paddy soil [46–48]. An important feature of paddy fields consists of the progressive changes in oxidation and reduction states upon irrigation. Management of water supply of rice in the field leads to temporal changes in the water table called hydroperiods as well as changes in redox status of paddy soils. Submerged rice fields are also characterized by a low reduction potential because of poor aeration. In submerged conditions, TEs in the paddy soil undergo reduction reactions that reduce ionic forms of these elements in the soil solution. Biological processes and fertilization also play a crucial role in mobilization of TEs and hence help to control the mobility of TEs in paddy soils.

3.1. Redox Process

Reducing periods in the paddy soil causes redox depletion of elements such as Fe [49]. Reducing events cause formation of more $Fe^{2+}$from the Fe^{3+} pool, which migrate away from reduced zones. The migrated Fe^{2+} later precipitates as Fe^{3+} in oxygen rich zones. The process causes depletion of iron coatings from soil mineral grains. This causes the redoximorphic appearance of paddy soils with contrasting colors of redox depletions (gray color) and zones of oxidized Fe (blue-green). TEs posses multiple oxidation states, and the reducing surroundings favor solubility of TEs [50,51]. Most often metal ions such as Cu and Zn occur in association with oxides of other elements such as Mn. Hence the solubility of the oxides of TEs also favors the solubility of other TEs by releasing adsorbed TE ions. Submergence induced anoxia in the field favors the formation of TE sulfides and this makes many of TE cations immobile in soil. The organic matter of soil as well as carbonates also contribute to the above-mentioned anoxia induced TE immobilization in paddy soils.

Paddy soils differ in their degree of depletion of oxygen because of differences in soil texture, bulk density, aggregate stability, organic matter content, bio-pore formation, thermal properties and seasonal variability. The puddled paddy soil, which is saturated with water, undergoes alternate frost action during the winter season that breaks up the large soil masses and hence increases aeration events [52]. When the soil oxygen is depleted (Eh ~ 0.34), N in the NO_3^- undergoes reduction reactions resulting in the formation of NO_2^-, N_2, *etc.*, and causesa drop in redox potential (Eh ~ 0.24). At this point, Mn reduction followed by a sequence of reduction reactions of other TEs occurs with a drop of the reduction potential of the soil. This indicates transformation of macronutrients and TEs in sequential order. The reduction reactions during submergence follow the sequence N > Mn > Fe > S > C. Many of the TEs other than Mn and Fe undergo reduction reactions preferentially in the order between Fe and S. When the soil is drained, oxidation reactions occur in the reverse sequence. Another characteristic feature of paddy soils is the anoxia mediated methane production [53]. This occurs at E_h below −0.2 V. Thus monitoring of nitrous oxide and methane helps to determine the reducing conditions in paddy soils. It can be concluded that dissolution or precipitation of TEsin paddy soils depend to a large extent on the dynamics in the anoxia mediated shifts in redox potential. Hence control of irrigation, which helps to regulate anoxic conditions in the field, could be a practical solution for the management of TE availability to rice plants.

3.2. Soil pH

Soil pH influences TE solubility and hence TE mobility [54,55]. Lowering of soil pH increases the solubility of TEs. Changes in redox potential are associated with shifts in soil pH. Decrease in reduction potential of soil causes a phenomenon called ferrolysis that accelerates soil acidity mediated weathering of the parent rock material [56,57]. When paddy soils are submerged, cations displaced by Fe^{2+} migrate out ofthe reduced zone resulting in a deficiency of cations. When such a paddy soil is drained, the Fe^{2+} in the soil becomes Fe^{3+}, and results in the precipitation of ferric oxide, which adsorbs TEs [58].This often results in the occurrence of a H^+ ion as the major cation in the paddy soil and causes lowering of the soil pH. Thus even though solubility of TEs could be higher in paddy soil, plant available TEs are limited.

Rice plants growing in submerged acidic soils often experience TEs or toxic TEs toxicity [59,60]. Al, Mn and Fe are the chief elements that cause metal toxicity in acidic paddy soils. The type of metal toxicity depends on the composition of the parent material from which the paddy soils are formed. For example, rice plants grown in paddy soils derived from Mn rich parental materials experience Mn toxicity during soil acidification. Generally non-acid forming cations such as Ca^{2+} and Mg^{2+} form soluble complexes with organic ligands and undergo leaching in acidic paddy soils [61]. Apart from this, organic matter contributes to soil acidity as it contains many functional groups that dissociate into H^+ ions. However, many of the acidic functional groups of organic matter dissociate at a higher soil pH. Oxidation of the sulfhydryl group (-SH) results in the formation of H_2SO_4 and accelerates the lowering of the paddy soil pH. Wet precipitation containing acids is also an agent of soil acidity. Thus rice plants grown in acid soil are confronted with a deficiency of macronutrients (Ca, Mg, K, P, N, and S) and micronutrients (Mo and B) due to leaching with runoff water as well as soil acidity. This situation leads to more availability of elements such as Al, Fe, Zn, Mn, Cu, Cd, Pb and Co in acidic paddy soils. Apart from all the above factors, intensive uptake of non-acidic mineral nutrients by rice plants as well as nitrogen fertilization also contributes to soil acidification in paddy soils that end up in TE toxicity. Hence corrective measures of soil pH play a critical role in the management of plant uptake of both essential and toxic TEs.

3.3. Alkalinity

The alkalinity of the soil often reflects the buffering capacity of the soil. Buffering activity is prominent in alkaline soils. Buffer reactions in paddy soils are mediated by carbonates, bicarbonates, carbonic acid and water [62,63]. Dissolution of carbonate minerals releases bicarbonate ions, which upon hydrolysis produce carbonic acid and hydroxyl ions as follows:

$$CaCO_3 + H_2O + H^+ \text{<---->} Ca^{2+} + H_3CO_3 + OH^- \quad (1)$$

When acid influx into bicarbonate buffered soil (usually alkaline soil) occurs, the reaction shifts to the right. On the other hand, the reaction will shift to left when the base content prevails in the soil. Both these reactions help in the maintenance of soil pH. Thus variations in soil pH seldom occur in alkaline paddy soil. Hence soil acidification due to nitrogen fertilization is often limited in alkaline paddy soils. In fact, applications of ammonium in alkaline soil help to mobilize TEs. On the other hand, buffering in acidic soil is usually maintained by reversible conversion of aluminum hydroxide ($AlOH_2^+$) to gibbsite (Al $(OH)_3$).Thus acidification of soil during fertilizer application is limited to a certain extent in acidic paddy soils due to the presence of hydroxyl groups containing Al compounds. Organic matter in the soil also assists in the maintenance of the buffering activity by protonation and deprotonation, which help to control TE chelation in the soil.

3.4. Cation Exchange Capacity

The cation exchange capacity playsa crucial role in TE mobility in soil solution [64,65]. Hydrogen ions will be attracted to cation exchange complexes where exchange of cations such as Ca^{2+} occurs. Thus paddy soils having a higher cation exchange capacity tend to resist a decrease in soil pH (especially when pH > 6).The higher cation exchange capacity also indicates higher concentrations of cations, which can be adsorbed to soil. Thus paddy soils with a higher cation exchange capacity favor rice growth by providing more TE cations. Paddy soils with higher clay content were found to have more available TEs [66]. Clay hydroxides assist mineral nutrient retention in soil. However the adsorption of TEs in clay acts as a barrier for cation exchange. This accounts for the relatively lower mobility of TEs ions in alfisols that have more clay content. Hence alfisols retain more toxic TEs such as Cd in the soil solution because of the saturation of clay with TEs compared to vertisols that have lower clay content [67].

3.5. Biological Processes

Soil flora and fauna contribute to biological processes mediating TE accumulation in rice [55,68,69]. Biological activities bring changes in nutrient mobility, and most often these changes increase TE savailable to the plant. Many of the biological activities in paddy soil are found in the plow layer where root biomass is dense. Rhizosphere secretions such as organic acids act as energy sources for soil microbes. This situation increases soil microbial activity and stimulates redox reactions in the soil. These changes not only bring changes in chemical speciation of TEs but also affect solubility. Microbe mediated redox changes often influence solubility of multivalent elements such as As, Se and Fe. Arsenic, which is a toxic metalloid, occurs as As (III) and As (V) forms. Transformation of this metalloid in paddy soil due to oxidation, reduction and methylation is driven by microbes [70]. The addition of organic matter causes an increase in As methylation in paddy soils through microbial activity, and hence enhanced volatilization of As due to microbial activity, as may occur in the form of trimethylarsine, monomethylarsine, dimethylarsinic acid, and arsine. The form of occurrence depends on the concentration of As in the soil as well as the nature of microbes found in the soil.

Soil microbes also secrete compounds having metal binding ligands such as siderophores, which enhance plant uptake of TEs [71,72]. Plant growth promoting bacteria also promote both mobilization of TEs from paddy soils and root growth by hormonal activities [73–75]. Aerobic paddy fields are also benefitted by burrowers that enable air and water movement [76]. It is well known that the activity of earth worms incorporates organic matter such as plant residues into soil. Beetles also play a significant role in the dispersal and burial of organic matter that helps to prevent TE loss from green manures. Thus activities of soil fauna bring both temporal immobilizations of nutrients, especially TEs, and favor mineralization of organic matter. Soil microbes assist oxidation reactions that accelerate the formation of the ionic forms of nitrate, sulfates and phosphates in paddy soil which in turn release bound TEs into the soil solution [77]. Secretion of reducing agents such as NADPH, caffeic acids *etc.* by soil microbes also enhance solubility of TEs in the paddy soil. A characteristic feature of submerged paddy soil is the emission of greenhouse gases such as methane and nitrous oxide [78]. This phenomenon occurs due to anoxygenic degradation of organic matter. The periodic draining of paddy soils prevents anoxia, and substantially reduces greenhouse gases, for example methane. Apart from the microbial activities, cellulose degradation by termites also contributes to methane production in well-aerated paddy soils. The degradation of organic matter leads to a decrease of holding capacity of TEs especially that of K, Ca and Mg. Rice plants acclimatize to nutrient deficiency especially that of Fe, by secreting muginenic acid derivatives called phytosiderophores [79]. These compounds lead to dissolution of Fe in the soil. Phenolic secretions are also an adaptive strategy found in rice plants that mobilize Fe into the soil. Similarly, rice plants growing in acidic soil respond to aluminum toxicity by secretion of low molecular weight carboxylic acids such as citrate, malate and oxalate [80]. Thus regulations of biological activities in paddy soils help to control TE availability in rice plants.

3.6. Fertilization

Paddy soils are often spiked with fertilizer to replenish nutrients [81–83]. Many of the fertilizers are inorganic salts containing plant available mineral nutrients. Site-specific nutrient management is also carried out in paddy soils [84]. This kind of nutrient management practices incorporate nutrient elements that are deficient in a particular paddy soil. This also helps to avoid the application of wrong fertilizers which otherwise retard crop yield. For example, application of nitrogen in a field deficient in phosphorous delays plant growth more severely. The added nutrients enter complex nutrient cycles in the soil. Fertilizer application often increases cycling of TEs. This is due to the fact that plant uptake of TEs is from the TEs pool in the soil rather than from the fertilizer. Apart from this, the nutrients added in the form of fertilizer are not converted into biomass, and relatively little nutrients (10%–60%) are taken up by plants from fertilizer. This leads to the building up of nutrients in the soil, which stimulate cycling of nutrients including that of TEs.

Fertilizer application in the form of inorganic salts often possesses side effects. For example, nitrogen fertilization in the form of ammonium leads to accumulation of H^+ ions in the soil and promotes soil acidity [85]. Agricultural practices that increase crop yield through fertilization are often performed with the application of N, P and K in the field. Application of such a fertilizer mix not only enhances biomass but also depletes TEs. The reason for this effect is that more TEs are utilized to cope with macronutrient stimulated biomass production [86]. To avoid TE deficiency, organic farming or application of micronutrient fertilizers can be a practical solution [87,88]. However, the organic matter treatment must be monitored because of submergence mediated anoxia related changes in the paddy soil, which alter TE cycling. Application of vermicompost enhances soil porosity in aerobic paddy soils and hence supports root growth [89]. Green manures are a more reliable source of plant nutrients compared to composts. This is because about 70%–90% of nutrients taken up by animals appear in manure.

Organic matter as well as organic residue influence the availability of TEs in paddy soils [90,91]. Some organic matter form insoluble TE complexes and many of these complexes release TE cations slowly. Hence it can be predicted that addition of organic matter increases the chance of immobilization of TEs. Submerged paddy soils rich in organic matter often face deficiencies of Cu, Zn and Mn because of the immobilization of these elements by organic matter. TE deficiencies are commonly seen in aerated paddy soils due to high pH, high carbonates and low redox potential due to short-term submergence [92].

4. Nutrient Acquisition Directs TE Accumulation

Nutrient uptake in plants depends on nutrient availability, root architecture, presence of transition metal transporters in the roots and physiological processes such as transpiration [93–95]. The radial structure of rice root with aerenchyma in the cortex indicates the flexibility of the rice root to grow under both aerobic as well as anaerobic conditions [96]. It is well known that an extensive root system promotes the uptake of water and nutrients from the soil. Hence rice cultivars with a deeper and extensively branched root system pump more nutrients to aerial parts of the plant. Rice plants grown in upland conditions will have deeper roots than those grown in irrigated conditions. Thus the chance of accumulation of TEs is higher in upland rice compare with lowland rice. Rice plants pose a maximum root density at the time of heading, and the root density tends to decrease at the time of flowering. Hence TEs as fertilizers must be applied during the heading period. However, fertilizer application must be carried out under strict control to avoid limiting of plant growth due to excess mineral salts and resulting saline soils.

4.1. Root Phenology

The phenotypic characterization of rice germplasm indicates the existence of a variation in surface area, number of lateral roots, length, diameter, root hairs *etc.* among rice cultivars [97–100]. Rice cultivars with abundant root hairs require attention because root hairs not only help with water absorption but also with TE acquisition. Apart from this, root tip integrity is a critical factor for rice plant growth, as this is where the major quantity of ammonium is absorbed. Ammonium supports cell division in meristematic tissues in roots where carbohydrate is often limited. However, elements such as K, P and Fe are generally taken up by plants though any active locations in the root. Since root growth is an underground process, breeding approaches for utilization of difference in root architecture for better nutrient uptake are restricted, and the research on the development of better root phenotype are focused on quantitative trait loci (QTL) mapping [97]. QTLs clusters of rice root development have been observed in Chromosomes 1 (30–40 Mb), 2 (25–35 Mb), 3 (0–5Mb), 4(30–35 Mb), and 9 (15–20 Mb). Some of the major outcomes of QTLs studies on the control of root growth ctivity are presented in Table 3.Among the QTLs, over-expression of the *PSTOL1* (PHOSPHORUS-STARVATION TOLERANCE 1) gene was found to enhance root growth and resulted in a 60% increase of crop yield. Expression of this gene was also found to increase the content of P, N and K in rice plants. The increase of P uptake is well suited to inhibit toxic TE allocation to the rice grain because of the ability of P to form insoluble complexes with heavy metals. Expression of another candidate gene *DRO1* (DEEPER ROOTING 1) associated with the control of the root angle and deep rooting helps to increase the surface area for TE uptake. It is clear that genetic approaches that enhance root growth activities are highly promising to enhance nutrient fortification of rice grains.

Table 3. Rice root growth and development related genes.

Gene	Function	Refs.
OsGNOM1	PIN protein expression and auxin response	[101]
OsWOX3A	Inhibition of PIN protein expression	[102]
OsHO1	Latteral root initiation	[103]
PSTOL1	Crown root initiation	[104]
ARL1	Adventitous root initiation	[105]
OsABF2/OsTIR1/OsCYP2	Inhibition of lateral roots emergence	[106–108]
OsIAA11/OsIAA13	Lateral root initiation	[109,110]
OsLBD3-2	Crown and latteral root initiation	[106,111]
OsIAA23	Quicent center inhibition	[112]
OsSCR1	Quicent center activation	[113]
OsSHR1	Endoderm diffewrentiation	[114]
OsCAND1/OsPIN1	Crown root emergence	[115]
OsRPK1	Inhibition of root growth	[116]
DRO1	Root growth	[117]
OSGLU3/OSDGL1/OsEXPA8	Cell elogation related root growth	[118–120]
OsORC3	Lateral root growth	[121]
DES	Root thickness	[122]
OsRR1	Crown root initiation	[123]
RAL1,RAL2,RAL3	Radicle initiation	[124,125]

4.2. Molecular Physiological Process

Mineral nutrient uptake in plants depends on physiological processes [126–129]. Transpiration pull accounts for a mass flow of nutrients from the soil solution to the root. Transpiration is also believed to support upward movement of nutrient ions even though metabolic incorporation of ions occurs independently. Recent studies have shown that convective water transport in the xylem, brought about by root pressure, and the resultant guttation and Munch's phloem counter flow are in itself sufficient for long-distance mineral supply [130]. Negative charges on the root surface attracting cationic TEs also represent a critical factor that regulates TE uptake in plants [131,132]. The polarity of the root surface is maintained by H^+-ATPase. This proton pump takes part in pumping H^+ ions out of the root, which leads to changes in the rhizosphere pH, and an electrochemical potential difference that affects the movement of ions and solutes across the plasma membrane of the root. Activity of this pump requires ATP, and hence physiological and biochemical events in the root indirectly control plant uptake of TEs. Even though respiration brings ATP for H^+-ATPase activity, the process depends on photosynthesis, which produces substrates for respiration. Apart from this, sequestration of metals in the vacuole is mainly assisted with metal chelators generated through photosynthesis [133,134]. Hence maintenance of physiological processes such as photosynthesis is critical for limiting the transport of toxic TEs from the root to the grain. Regulation of action of transition metal transporters, which control the movement of TEs in the plants, is also critical for the enhancement of TE accumulation.

4.2.1. Photosynthesis

Among the physiological processes, photosynthesis plays an important role in the allocation and reallocation of TEs in the plant. The reason for this is that photosynthesis primarily accounts for the production of metabolites that are essential for the synthesis of metal chelators. Metal chelators in rice plants take part in extracellular complexation, rhizo-complexation and vacuolar complexation of metals. Secretion of tricarboxylic acids in response to metal toxicity is well studied among rice plants. Oxalate, citrate and malate are the tricarboxylic acids that are found to secrete into the rhizosphere and decrease aluminum toxicity in rice plants [135]. These acids tend to bind extracellular aluminum and prevent plant uptake of this metal. Organic acids involved in the above process are intermediates of primary metabolisms, especially respiration. Photosynthesis also influences root respiration by providing sugar skeletons, which act as substrates for respiration [136]. Thus rice cultivars that have higher photosynthetic efficiency and biomass productivity are able to avoid toxicity of metals because of their superior capacity of metal chelation. Extracellular chelation of metals is also noticed in rice plants with an excess of Cd, Cr, Fe, Pb and Mn in the rhizosphere [137–139]. Muginenic acids derivatives and phytosiderophores make up another class of extracellular chelators that is responsive to mineral nutrient deficiency and enhances plant uptake of TEs, especially Fe [140].

Apart from extracellular chelation of metals, plants have the ability to chelate metals within their roots that prevents the transport of the metals to the aerial parts. This is achieved by the transport and storage of metals in vacuoles of the root cells with help of metal chelators. Chelators involved in vacuolar sequestration of metals can be organic acids or phytochelatins [141,142]. Even though phytochelatins belong to the peptides, the dependency of the aminoacid metabolism on photosynthetic products points at the importance of photosynthesis in the maintenance of metal chelation activity in roots. Photosynthetic activity influenced Cd rhizo-complexation has been reported in rice plants [143]. Transport of TEs from root to leaf, and flag leaf to rice grain also occurs with help of metal chelators especially citrate and nicotianamine derivatives, respectively. Existence of citrate and nicotianamine transporters in plants is well characterized [144–146]. Hence the presence of metal chelators and corresponding transporters involved in cellular and vacuolar uptake of TEs complexes chiefly determines accumulation of TEs in rice grain. Site-specific over-expression of transition metal transporters helps to mobilize TEs into rice grains. It is also clear that any alteration in photosynthetic productivity affects production of metal chelators. Hence loss of photosynthetic activity restricts the trafficking of TEs in the plants, which ultimately disturbs the micronutrient composition of the grain.

4.2.2. Transition Metal Transporters

Plant uptake and transport of TEs is mediated by plasma membrane transporters. Transition metal transporter families such as ZIP, CDF, P1B-type ATPases, NRAMP, YSL, IREG1 and CCC1 are involved in the transition TE transport in rice [147] (Figure 3). Regulation of these transporters helps to control TE accumulation in rice plants. Most often deficiency of any of the TEs leads to strategic responses within plants. For example, rice plants secrete muginenic acids derivatives when they are subjected to Fe deficiency. But this will also promote the uptake of toxic TEs such as Cd because of the similarity of chemical properties that enables Cd to bind with these chelators. It is also reported that the OsNramp5 transporter assists with the uptake of both Mn and Cd in rice plants [148]. These reports suggest that the uptake and transport of both essential and toxic TEs are interrelated. This relationship can be used to manipulate plant uptake of specific TEs while limiting availability of toxic TEs. A transition metal ion is able to displace another metal ion from its specific binding site downstream in the Irving-Williams series in the following order: $Zn^{2+} < Cu^{+} > Cu^{2+} > Ni^{2+} > Co^{2+} > Fe^{2+} > Mn^{2+} > Mg^{2+} > Ca^{2+}$. Hence, there is a chance of competitive binding of TEs at metal binding sites where excess of a TEs ion disrupt cellular functions due to displacement of cofactor often occurs.

Over-expression and tissue specific expression of transition metal transporters are potential solutions for both enhancement of TEs and restriction of toxic TEs content in rice plants [11]. A node-based switch in rice plants has been found that regulates Mn allocation in rice shoot [149]. The switch operates through expression and degradation of a Mn transporter, OsNramp3. When there is low Mn in the external medium, *OsNramp3* gene expression occurs in the nodes and allocates Mn to young leaves and the panicle. However, in the presence of excess Mn, the transporter degrades, which causes Mn to be allocated to older leaves. This study points to the existence of a feedback regulation of TE trafficking at the molecular level in rice plants. Up regulation of magnesium transporter OsMGT1 was found to enhance Mg accumulation and aluminum tolerance in rice plants [150]. This finding supports that up regulation of specific proteins helps to avoid metal toxicity and to increase the accumulation of beneficial TEs ions. Macronutrient uptake such as that of K also plays a critical role in TE uptake [151]. It has been reported that there is a synergetic relationship between K uptake and Fe and Mn uptake. On the other hand, K is reported to negatively influence plant uptake of Mo. Thus it is clear that uptake of macronutrients influences TE accumulation in rice. However, the advent of studies with transition metal transporters points out that the uptake and distribution of TEs can be regulated by manipulation of transition metal transporters.

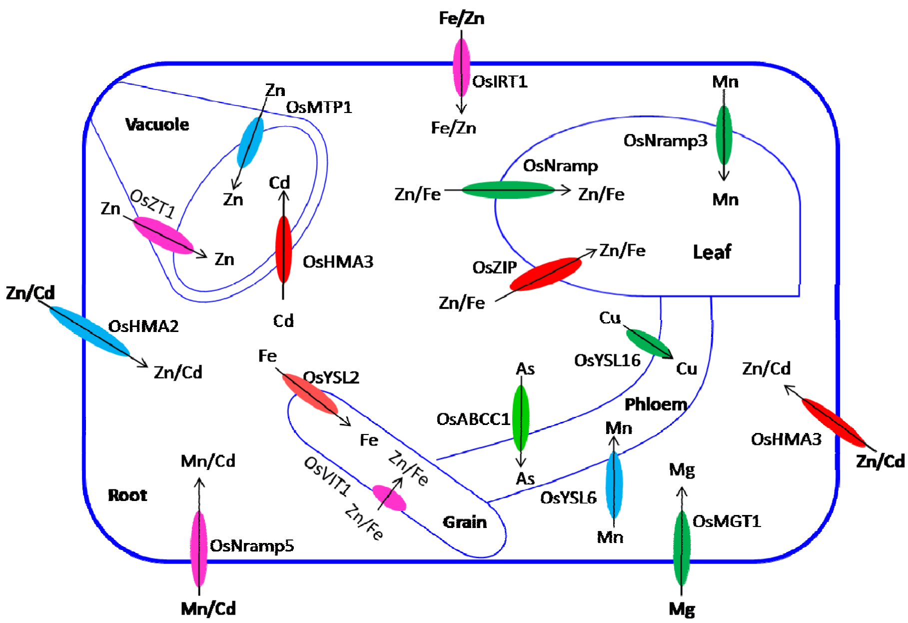

Figure 3. Transition metal transporters implicated for TEs management in rice.

Zinc is essential for rice plant growth. The influence of Zn on plant growth mainly relies on the functioning of redox-mediated processes. It has been reported that *OsHMA2* gene expression preferentially loads Zn to developing tissues of rice plants [152]. This transporter was also implicated in Cd transport. *OsIRT1* is another candidate gene reported to have a role in excess accumulation of both Zn and Fe in rice plants [153]. Over-expression of *OsHMA3* in rice was also found to have a dual role, i.e. enhancement of Zn uptake in the root and Cd tolerance [154]. This gene was found to enhance Zn uptake by over-expressing the ZIP family of metal transporters in the root. Decreased Cd accumulation in rice grain during *OsHMA3* over-expression was due to the vacuolar sequestration of Cd in the root. *OsYSL16* is a metal transporter involved in Cu transport in rice [155]. This transporter was found to enhance Cu allocation to younger leaves from older leaves and flag leaf to panicle with help of nicotianamine. The transport of Cu from flag leaf to panicle is important to form fertile flowers, and hence this transporter plays a crucial role in ensuring the crop yield. Arsenic accumulation in rice grain reduced during manipulation of the gene *OsABCC1* [156]. This gene was found to enhance vacuolar sequestration of As in companion cells of phloem of the upper nodes. This study also indicates the importance of site-specific localization of toxic TEs in rice plants which reduces the accumulation of toxic TEs in the grain.

Polished rice often contains limited concentrations of TEs. However, expression driven by insertion of promoter of *OsYSL2*, which causes over-expression of *OsYSL2*, has resulted in a 4.4-fold increase of Fe in rice grain. This transporter was also found to play a crucial role in long-distance transport of Fe and Mn in rice plants [157]. OsYSL6 is a transporter found in rice, which is involved in Mn detoxification [158]. This transporter mediates Mn transport with help of nicotianamine and is functional in the presence of excess Mn. Available reports with transition metal transporters indicate promising role of metal transporters in the restriction of toxic metals in rice grains while enhancing loading of TEs.

5. Trace Element Fortification

TEs such as Fe and Zn are the major TEs that are found to be limited in rice based diets [1,159]. Hence micronutrient fortifications of rice grain are mainly focused on Fe and Zn. Bio fortified rice grains with increased Zn and Fe amounts have been released in India and Bangladesh [160,161]. Bio fortification approaches are mainly focused on plant breeding and transgenic approaches (Figure 4). However, TEs nutrition through fertilization also requires a successful fortification program that provides the ambient availability of TEs for plant uptake.

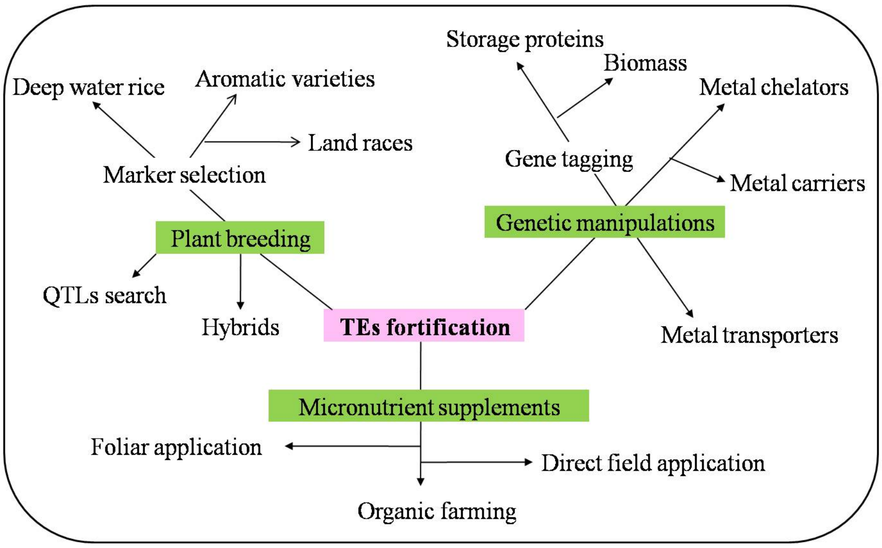

Figure 4. TEs fortification strategies for rice.

5.1. TEs Supplements

TE availability in the soil is essential for successful bio fortification programs. Some of the practical methods of application of TEs to rice plants are given in Table 4. It must be noted that toxic TEs also potentially enter into plants through transition metal transporters that are meant for TEs [162,163]. Hence more availability of TEs in the rhizosphere will have beneficial effects in the sense that these elements delay plant uptake of toxic TEs by competitive inhibition. Application of redox active TEs such as Mn and Fe in the soil requires attention because they are known to create metal toxicity. Rice plant grown in acidic soils are especially prone to toxicity of these elements [164,165]. In acidic soils, the process of submergence causes depletion of oxygen that results in the formation of reduced forms of Fe and Mn that are more toxic to plants compared to their oxidized form. It is recommended that TE application must be carried out with the help of metal chelators to prevent toxicity of metals. Foliar application of TEs is a widely accepted method. This method helps to avoid accumulation of TEs in the soil that can create metal toxicity. Thus along with prophylactic and a curative benefits, foliar application could be a practical solution for the enrichment of TEs in rice grain. Mineral salts of TEs in the form of sulfate, oxysulfate, oxide and chelate are the commonly applied forms of TEs. Metal chelates are well-suited for the application in soil because the presence of chelators slows down the reactivity of TEs with soil minerals. Application of organic manures is also a potential practical solution for ensuring ambient availability of TEs, especially, Zn and Cu. Silicon is an essential element of rice growth, and the application of silica based gels not only protects rice plants from abiotic stress but also helps to enhance crop productivity [166].

Table 4.TEs supplementpracticesapplicable to rice paddies.

Nutrient	Mode of Application
Zn	Addition of compost, Zincated urea and Gypsum (20 Kg Zn/ha), Periodic draining, Foliar spraying or seed treatment of 0.2 to 0.5% $ZnSO_4$
Fe	Addition of farm yard manure, FeSO4, Fe-EDTA, and Fe-EDDHA (25 Kg Fe/ha). Foliar spraying or seed treatment of 1%–2% $FeNH_4SO_4$ (pH5.2).
B	Application of Borax andH_3BO_3 (1Kg/ha), Foliar spray of 0.5% H_3BO_3
Mn	Application of $MnSO_4$ (2–5 Kg/ha)or Farm yard manure (10 ton/hectare), Foliar Spray of 1% $MnSO_4$ at tillering
Mo	Liming of acid soils to a pH 6.2–6.5, Dustingof $(NH_4)_2MoO_4$ at 100–500 g/ha, Foliar spray of 0.1% $(NH_4)_2MoO_4$
Cu	Addition of $CuSO_4$ (5 Kg/ha at an interval of 5 years), Root dipping in 1% $CuSO_4$ solution
Si	Irrigation with Si rich water, Application of Ca or K silicate (60 Kg/ha), Foliar spray of 0.1% Na_2O_3Si

5.2. Plant Breeding

Marker assisted selection in breeding programs with help of loci associated with nutrient accumulation in the grain allows the development of TE bio fortified rice as well as restriction of accumulation of toxic TEs. Among the TEs, increase in accumulation of Fe and Zn has gained attention because of the direct human health benefits. The existence of four fold differences in Fe and Zn concentrations among rice germplasm has been identified [167,168]. This indicates vast opportunities for plant breeding that can significantly increase the content of Fe and Zn in the grain. Studies with Indian rice varieties have shown the highest Fe contents to be found in the varieties Varsha (37.5 mg/kg) and Phou Dum (37.2 mg/kg) [160]. It was also found that basmati genotypes, deep-water rice and landraces havehigher iron and zinc contents in the grains compared with Swarna, Jaya, Uma *etc*. that are widely cultivated. Poornima, Ranbir Basmati, ADT 43 and Chittimutyalu were identified as the Indian rice varieties with the highest Zn content. The trait for high Fe content in grain was located on chromosomes 3, 4 and 8 in the Chittimutyalu variety. QTL mapping indicated four loci of high Fe content on chromosomes 3, 4, 6 and 12 in Ranbir Basmati.

Aromatic rice varieties such as Jalmagna, Zuchem, and Xua Bue Nuo are reported to be rich in both Fe and Zn [161]. The aromatic trait was not pleiotropic for grain-Fe or grain-Zn concentrations even though the linkage was broken at a low frequency. Thus the trait can be used to screen for high Fe and Zn levels in rice grain. Crossing of rice varieties such as BPT 5204 and Chittimutyalu was success to produce rice with higher Zn and Fe content [160]. Results of crossing between IR72 and Zawa Bonday resulted in a higher Fe content as well as an enhanced yield. The progeny of the cross possessed favorable characteristics such as tolerance of rice tungro virus, and P, Zn and Fe deficiency. Breeding of rice varieties is beneficial to prevent accumulation of toxic TEs such as Cd. Natural variation of a 13- to 23-fold difference in grain Cd concentration in diverse japonica rice germplasm points to plant breeding opportunities in the context of Cd minimization. Cd accumulation related QTLs in rice have been found on Chromosomes 2, 3, 4, 5, 6, 7, 8, 10 and 11 [169–171]. Cd minimization related alleles are also found to relate with crop yield. Thus approaches that are aimed to reduce Cd accumulation help to enhance crop yield too.

5.3. Genetic Manipulations

Gene based regulation of micronutrient transporters and carriers in the plant promise controlled accumulation of TEs in rice while restricting entry of toxic TEs. Nutrient content in the grain can be enhanced by expression of specific genes in the endosperm or expression of genes that take part in the transport of TEs to the grain. For example, rice endosperm expressing the soybean ferrtin gene, *SoyferH1*, was found to have a three-fold increase of Fe in seeds [172]. However, when the gene was expressed under endosperm specific multiple promoters such as*OsGlb1* and *OsGluB1*, there was no increase of Fe content in the seeds [173]. This study indicates that the enhancement of nutrient storage capacity alone cannot fulfill the requirement of more accumulation of TEs. Basmati rice (Pusasugandh II) plants over-expressing rice ferritin (*OsFer2*) under the control of the endosperm-specificGlutelinA2 (*OsGluA2*) promoter were found to accumulate more Fe and Zn [174]. Simultaneous over-expression of ferritin under the control of the endosperm-specific promotersglobulinb1(*OsGlb1*) and glutelin B1 (*OsGluB1*), NAS under the control of the *OsActin1*promoter, and *OsYSL2* under the control of *OsGlb1* promoter, and the *OsSUT1* transporter promoter significantly increased the Fe, Zn, Mn and Cu concentrations in polished rice [175]. These studies indicate that over-expression of ferritin and metallothionein in grain could enhance TE accumulation in grains because of the ability of these proteins to chelate metals. Over-expression of phytase, which degrades phytic acid, also helps to enhance availability of TE fortification in rice [176].

The nicotianamine synthesis related metabolic pathway plays a critical role in the regulation of metal homeostasis, especially Fe in plants [177]. Activation tagged line of *OsNAS3* showed a 3-fold increase of Fe content in polished rice grain [178]. Constitutive over-expression of the *OsNAS2* gene also resulted in Fe and Zn-bio fortification of rice endosperm [179]. It has been also demonstrated that over-expression of Fe homeostasis genes in rice such as *OsIRO2* increases Fe content in rice plants grown in calcareous soils [180]. Over-expression of deoxymugineic acid synthase gene *OsDMAS1* involved in synthesis of muginenic acid also could be a practical solution for enhancement of Fe^{3+} uptake [181]. Thus it is clear that approaches with over-expression of carriers involved in metal translocation as well as metal homeostasis promotes accumulation of TEs in the grain. Metal transporters are also promising to enhance accumulation of TEs in plants. Hence focus should be placed on transgenic rice plants, which pose the capacity to express transition metal transporters and TEs carriers. *OsVIT1* and *OsNRAMP7* are potential candidate genes that can be targeted for Zn fortification in rice grain [182]. Over-expression of Fe transporters *OsIRT1* and *OsYSL15* were found to promote Fe content in grains of rice plants [183]. Expression of *OsNRAMP1*, *OsNRAMP7* and *OsNRAMP8* in flag leaves also was also shown to correlate with grain Fe and Zn content [181]. Up regulation of *OsZIP1*, *OsZIP4*, *OsZIP6* and *OsZIP8* was also reported in the above-mentioned study during Fe deficiency. Thus it can be assumed that over-expression of the Nramp and ZIP metal transporter family in the flag leaves of rice plants has the potential to enhance accumulation of Fe and Zn in the grain. However, studies with over expression of *OsZIP4*, *OsZIP5* and *OsZIP8* where a decrease of root-to-shoot translocation of Zn and of Zn concentrations in the seed were observed indicate that up regulation of proteins that are observed under nutrient deficiency does not always help to enhance plant uptake of TEs when plants grow with sufficient availability of ambient nutrients [184,185]. Non-specificity of transition metal transporters may lead to the accumulation of non-specified elements during a transgenic approach [186]. For example, over-expression of nicotianamine synthase and ferritin using CaMV 35S not only enhanced accumulation of Fe and Zn in rice but also enhanced Mn accumulation. Entry of toxic TEs can also be blocked in rice plants using transgenic approaches [11]. Cd excluder phenotypes screened in rice cultivars indicate potential Cd excluding traits in the rice genome that help to develop Cd excluder rice [187]. It has been found that transgenic tobacco carrying cDNALTC1, a nonspecific transporter, accumulated low concentrations of Cd [188].

6. Outlook

Rice-based diets often cause TE deficiency in humans. Approaches for enhancing TE content in rice grains can be either implemented on the field level or plant based. Nutrient cycling in paddy soils must be characterized with respect to TE content for ensuring TE availability for plant uptake. Optimal soil physiochemical characteristics for TE accumulation in grains need to be explored under varying environmental conditions especially during dry and wet periods in paddy soils. TE fertilization along with NPK fertilization is recommended. Fertilization often activates nutrient cycling in rice paddies, and hence the dynamics of TE availability to plants under fertilization must be monitored. Adequate management of organic matter in the soil and the soil's influence on TE accumulation in rice grains must be screened during biodynamic farming. Available data on rice root architecture must be screened for TE mining capacity. Genetic manipulation, especially with genes that control metal chelator production, and transition metal transporters are promising possibilities to enhance accumulation of TEs. Endosperm, representing the major part of the TE reserve in the grain, site-specific expression of metal chelators and transporters that load metal to endosperm are practical solutions for loading TEs into rice grains.

Acknowledgements

The authors gratefully acknowledge the receipt of financial support ref. DST/INT/THAI/P-02/2012 dated 31-1-13.

Conflict of Interest

The authors declare no conflict of interest.

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