

INVITED REVIEW

Plant Nutriomics in China: An Overview

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- **Background** Population and environmental pressure have imposed a great challenge on agriculture in China to explore innovative and effective solutions to its pressing plant nutritional problems. Plant nutriomics is a new frontier in plant biology that can provide innovative solutions for improving plant nutrient efficiency, thus increasing crop productivity through genetic and molecular approaches.
- **Scope** This review summarizes current efforts and progress in plant nutriomic research in China with examples from several case studies. It also points out potential obstacles and depicts future perspectives in this emerging frontier of plant nutrition.
- **Conclusions** Although plant nutriomics is still at a conceptual stage, substantial efforts are being made in China aimed at increasing plant nutrient efficiency through a nationwide, co-ordinated project on plant nutriomics. Future studies involving both national and international collaborations are needed to develop nutrient-efficient, stress-tolerant and high-quality crop varieties for both China and elsewhere.

Key words: China, genomics, metabolomics, nutrient efficiency, plant nutriomics, proteomics, transcriptomics.

BACKGROUND

China is the world's largest developing nation, currently having 1.3 billion people with an annual per capita income of less than 1000 US dollars, and its population is projected to continue to increase to a total of 1.6 billion by the year 2030 (FAO, 2004). Clearly, food security in China will be a primary concern in international development over the next few decades. However, options for increased food production to meet this population pressure are limited because most arable land is already under cultivation, and in many areas land use cannot be further intensified without risk to long-term productivity. Agricultural land use has been especially intense in recent years because of rapid urbanization and increasing environmental pollution, which have resulted in an astonishing rate of land loss by about 2 million hectares annually (China Statistical Bureau, 2004). All of this has imposed a great challenge on agriculture in China for exploring innovative and sustainable approaches to increasing crop productivity on the existing arable land.

Traditional cropping systems in China have maintained crop productivity over many centuries through labour-intensive nutrient management until the 'green revolution' in the 1960s, when dwarf wheat and rice varieties were developed to respond to high chemical fertilizer inputs without lodging. This has contributed enormously to food security in China by doubling crop production in the past 40 years. As a result, most modern crop varieties in China

are highly reliant on heavy chemical inputs including fertilizers, making China the largest fertilizer consumer in the world. In 2004, for example, about 30 % of the world's chemical fertilizers were applied in China, including more than 20 million tonnes of nitrogen (N) fertilizer and more than 10 million tonnes of phosphorus (P) fertilizer (China Statistical Bureau, 2004). In addition to generating great environmental impacts, chemical fertilizers impose a major economic burden on Chinese farmers. Chemical fertilizers are the largest direct cost associated with crop production, accounting for more than 50 % of production costs (China Statistical Bureau, 2004). Obviously, great reliance on chemical fertilizers is not a sustainable strategy.

Therefore, a 'second green revolution' is required that does not rely on intensive fertilization. Just as the 'first green revolution' improved crop response to high soil fertility, the 'second green revolution' would aim at improving crop yields in soils with reduced fertilizer applications. This would be possible if new crop varieties could be developed with enhanced adaptation to low-fertility soils. This approach is valid both for soils with over-fertilization in high-input areas and soils of low fertility in low-input areas that are deficient in a number of major nutrients such as nitrogen, phosphorus, potassium and other essential elements, as well as having toxic levels of aluminium, manganese and salt (Li, 1985).

Numerous studies have indicated substantial genetic variation in nutrient efficiency in plants, and attempts have been made to breed crops adapted to low-fertility soils.

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However, these breeding efforts were mainly implemented through simple selection of biomass or yield in the field. Biomass or yield selections in the field are not only costly but also subject to confounding environmental interactions and spatial heterogeneity. Therefore, it would be preferable to identify and select specific traits that are directly related to a specific nutrient efficiency. Once clearly identified, these traits could be used for more efficient screening in controlled environments, or tagged with molecular markers and then improved through marker-assisted selection or gene transformation.

Useful traits for nutrient efficiency may be associated with altered physiological and biochemical pathways in adaptation to nutrient stress. Specific nutrient-signalling pathways, such as Pi-signalling, and their regulatory systems in plants have been revealed, making it feasible to modify some key regulator(s) to enhance the uptake and use efficiency of the nutrient through genetic engineering. However, systemic mechanisms might be involved in adaptation to nutrient stress at the whole plant level. The fact that many of the molecular and biochemical changes in response to nutrient deficiency occur in synchrony suggests that the genes involved are co-ordinately expressed and share a common regulatory system. Therefore, systematic studies are needed to understand the genomic, transcriptomic, proteomic and metabolomic aspects of nutrient efficiency. This area of studies is termed 'plant nutriomics', a new frontier of plant biology that is attracting more and more attention by scientists all over the world.

PLANT NUTRIOMICS: PRINCIPLES AND CONCEPTS

Seventeen nutrient elements are known to be essential for higher plants, among which 14 are mineral elements that plants mainly acquire from the soil (Epstein and Bloom, 2004). Plant nutrition as a science is the study of the acquisition of nutrient elements by plants and the functions of these elements in the life of plants (Epstein and Bloom, 2004). In the agronomic context, plant nutrition deals mainly with the mobilization, uptake and utilization of the most limiting nutrient elements (N, P, K, Ca, Fe, Zn, B, etc.) as well as their effects on plant growth, development, reproduction and adaptability to adverse environments. Although specific processes involved in nutrient mobilization, uptake and utilization are all important, it is the integrated function of all these processes that determines the overall nutritional performance (efficiency) of the nutrient elements (Fig. 1).

Traditional plant nutrition studies look at nutrient efficiency mainly at the whole-plant level. Although useful, studies with whole plants cannot provide sufficient insight into the genetic nature and its specific modification of the nutritional processes. Recent progress in plant molecular biology has provided the means to tackle complex plant nutritional problems through genomic, transcriptomic, proteomic and metabolomic approaches. All these approaches, together with phenotypic analyses,

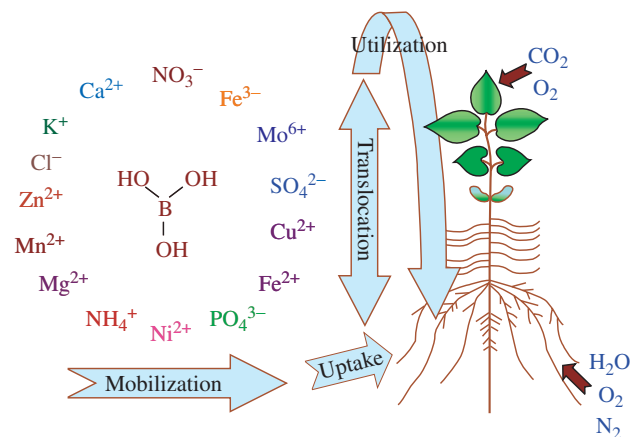


FIG. 1. The nutritional processes in higher plants, indicating their need of 17 essential elements that may be acquired from the soil or the air in various forms and the processes of mobilization, uptake and utilization.

will enable us to elucidate the functions and interactions of plant nutrients at the molecular, cellular, organ and whole-plant levels. The concept of plant nutriomics, therefore, is to integrate nutritional functions at various levels (molecular, cellular, organ and whole-plant) with different tools (genomics, transcriptomics, proteomics and metabolomics), as schematically presented in Fig. 2.

PLANT NUTRIOMICS IN CHINA: CASE STUDIES

Nitrogen efficiency

In the last 50 years, the global use of N fertilizer has increased approximately ten-fold in order to raise crop productivity (UNEP, 1999), as a consequence of the fact that most of the high-yielding modern crop varieties have high demands for N and other nutrients. The total N input of chemical nitrogen fertilizers reached 24 million tonnes in China in 2003 and is still increasing (China Statistical Bureau, 2004). Taking rice as an example, China accounts for 20 % of the total area of rice production but accounts for 37 % of the total N fertilizer consumption in rice production in the world (China Statistical Bureau, 2004). In Jiangsu Province, for example, the average N application rate for rice is about 260–290 kg ha⁻¹, which is much higher than the world average of 103 kg ha⁻¹ (Peng *et al.*, 2002).

Overloading farmlands with N fertilizers has led to the deterioration of water quality of lakes and rivers in many regions of China (Zhu *et al.*, 2005). It has been forecast that the N surplus (total N from fertilizers applied, biologically fixed and plant residues minus the amount of crop uptake) in crop production will increase from about 137 kg ha⁻¹ in 2002 to 175 kg ha⁻¹ in 2010 in China (Zhu *et al.*, 2005). Although the current high-risk area resulting from over-fertilization is mainly in the coastal provinces in the south-east, it will extend to 13 provinces in the middle and south-west of China by 2010 if current policies and trends continue (Table 1). China therefore has

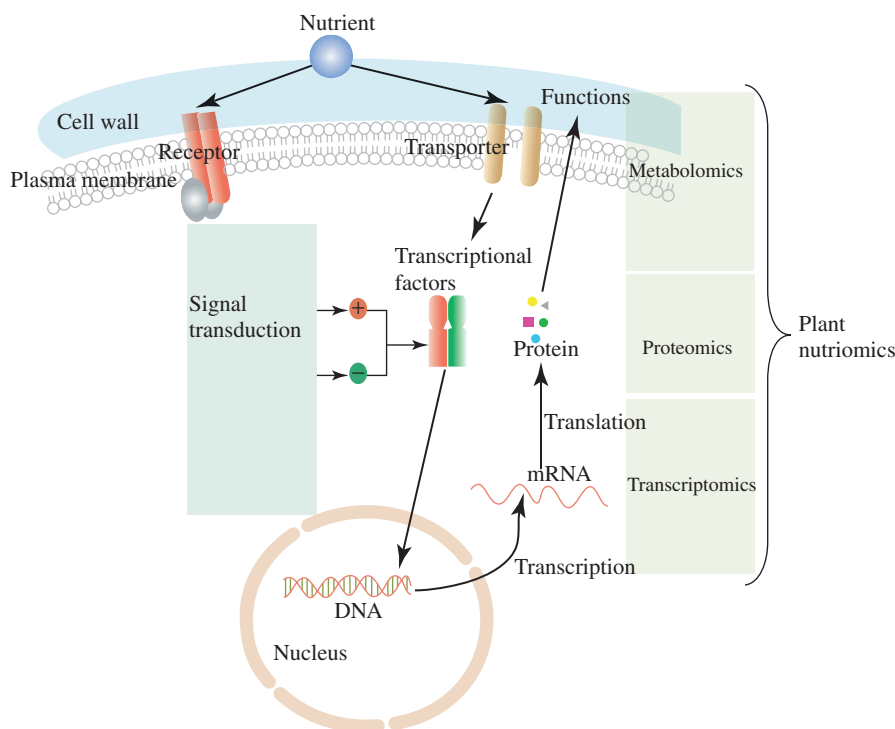


FIG. 2. Schematic representation of the basic concepts of plant nutriomics. The nutrient either as a signal or a substrate may stimulate a series of biochemical processes in the cell that can be regulated at transcriptional, translational or metabolic levels. Plant nutriomics is a new frontier of study that integrates nutritional functions at various levels with genomic, transcriptomic, proteomic and metabolomic tools.

to control excessive inputs of chemical N fertilizers into the field by improving the N use efficiency of the crops.

Tolerance of a crop to low N conditions is a highly desired characteristic for sustainable crop production. Progress has been made in China in genetic and molecular analysis of low N tolerance and breeding crops for low N conditions. Lian *et al.* (2005) analysed the genetic components associated with low N tolerance in rice at the seedling stage, including main effects, epistatic effects of the QTL (quantitative trait locus or loci), and QTL-by-environment interactions, using a population of 239 recombinant inbred lines (RILs) from a cross between 'Zhenshan 97' and 'Minghui 63', the parents of an elite hybrid. A genetic linkage map with 253 DNA marker loci was constructed. Seedlings of RILs were cultivated in low and normal N solutions. Root, shoot and plant weight in the two N treatments were measured and the relative weight of the two treatments for each trait was considered as a measure of low N tolerance. Four to eight QTL with main effects were detected for each of the nine traits. Very few QTL were detected in both low and normal N conditions, and most QTL for the relative measurements were different from those for traits under the two N treatments, indicating very little commonality in the genetic basis of the traits and their relative performance under low and normal N conditions. A total of 103 digenic interactions were detected for the nine traits, collectively accounting for large proportions of the variation for several traits. It was concluded that low N tolerance of rice seedlings has a complex genetic basis.

Combining genetic analysis with gene expression profiles may provide an effective strategy for identifying genes and pathways involved in specific physiological processes. To characterize genes involved in response to low-N stress, the expression profiles of an *indica* rice cultivar, 'Minghui 63', were examined after short-term low-N stress (0.5–2 h) with the objective to identify genes involved in early response to low-N stress (Lian *et al.*, 2006). The study was conducted using a microarray of 11 494 rice expressed sequence tags (ESTs) representing 10 422 genes. Although no significant difference was detected in the leaf tissue, 471 ESTs were detected as responsive to low-N stress in the root tissue, 115 ESTs showing up-regulation and 358 ESTs down-regulation. Analysis of expression profiles under low-N stress identified the following patterns: (1) the genes involved in photosynthesis and energy metabolism were down-regulated rapidly; (2) many of the genes involved in early responses to biotic and abiotic stresses were up-regulated whereas many other stress responsive genes were down-regulated; (3) regulatory genes including transcription factors and those involved in signal transduction were both up- and down-regulated; and (4) the genes known to be involved in N uptake and assimilation showed little response to the low-N stress. In a separate study with a *japonica* cultivar, 'Wuyunjing 7', it was revealed that genes known to be involved in N uptake and assimilation were responsive to relatively long-term (4 d) N starvation and change in N forms (G. Xu *et al.*, unpubl. data). The challenges for future studies are to characterize the

TABLE 1. The risk of nitrogen application in mainland China estimated for the year 2002 and projected for 2010 (based on Zhu et al., 2005)

| Risk class of N application | Number of provinces subject to risk | |
|-----------------------------|-------------------------------------|------|
| | 2002 | 2010 |
| High | 7 | 13 |
| Middle | 10 | 7 |
| Low | 12 | 9 |

functional roles of the low-N stress-responsive genes in N metabolism, including the large number of genes with presently unknown functions.

Nitrate is the primary N source for many plants and its cellular uptake process is of fundamental importance for plant N nutrition. Two families of nitrate membrane transporters (NRT1 and NRT2) are primarily responsible for uptake and translocation of nitrate in plants (Crawford and Glass, 1998; Forde, 2000). It was reported that a high-affinity nitrate transport system from the unicellular green algae *Chlamydomonas reinhardtii* requires two gene products for functioning. One gene, *NRT2*, encodes a typical carrier-type structure with 12 putative transmembrane (TM) domains, and the other gene, *Nar2*, encodes a much smaller protein that has only one TM domain (Zhou et al., 2000). A collaborative research between a Chinese group and a group from the Rothamsted Experiment Station, UK, showed that this two-transport model could be applied to some NRT2-type transporters in higher plants as they also have a highly specific two-gene requirement for functioning (Tong et al., 2005). The functional activity of a barley nitrate transporter, HvNRT2-1, in oocytes required co-injection of a second mRNA. Although three very closely related *Nar2*-like genes were cloned from barley, only one of these was able to give functional nitrate transport when co-injected into oocytes (Tong et al., 2005).

Rice and other wetland plants growing in flooded soil may also absorb significant amounts of nitrate produced by nitrification of ammonium in the rhizosphere (Kirk and Kronzucker, 2005). Nitrate uptake by wetland plants may therefore be far more important than hitherto recognized. Rice not only takes up nitrate as a N source, but also differs greatly in nitrate use efficiency among different cultivars. Fan et al. (2005) investigated the effects of N sources on rice plants using four cultivars commonly grown in Jiangsu Province, China. These cultivars produced similar shoot biomass with ammonium as the only N source, but the *indica* cultivars had much greater biomass than the *japonica* cultivars when nitrate was supplied as the only N source (Fan et al., 2005). Electrophysiological measurements on root rhizodermal cells showed that the root cell membrane potential of *indica* cultivars was more responsive to low external nitrate (<1 mM) than that of *japonica* cultivars. The expression pattern of 12 different NO₃⁻ transporter genes was tested by RT-PCR using specific primers, but only *OsNRT1.1* and *OsNRT2.1* expression could be detected

that showed significant differences among the four cultivars (Fan et al., 2005). These results have important implications for genetic manipulation of N nutrition in rice plants.

Phosphorus efficiency

Among the fertility constraints to crop production in China, low P availability is the primary limiting factor for two major reasons. (1) The overwhelming majority of soils in China are P deficient with a high P-fixing capacity. Most of the arable soils are either acidic (South China) or calcareous (North China). In South China, with a humid tropical and subtropical climate, warm, moist conditions result in weathered acid soils (mostly ultisols and oxisols), in which free iron and aluminium oxides bind native and applied P into forms unavailable to plants (Li, 1985). In North China, by contrast, the amounts of calcium and magnesium compounds are usually high in the calcareous soils (mostly aridisols), binding inorganic phosphates into forms highly unavailable to plants. The high P-fixing capacity of the soils results in very low P uptake rate (defined as percentage of P in a plant over that in soil and fertilizer), usually below 10 % for many annual upland crops, although the total P content in the soils ranges from 0.04 to 0.25 %, sometimes even up to 0.4 % due to continuous P fertilization (Li, 1985). (2) China lacks high-quality P mines, and existing P resources are estimated to be depleted by the end of this century. It is also alarming that global P resources will be exhausted no later than the end of this century (Vance et al., 2003).

It is therefore necessary to address this problem through a new approach, namely by developing improved crop germplasms that are better adapted to low-P conditions, with more efficient uptake and utilization of P fertilizers. Attempts are being made in China towards selecting and breeding crop varieties for P efficiency in wheat, rice, soybean, oilseed rape and other crops, and promising results have been obtained. Zhensheng Li's group in the Institute of Genetics, Chinese Academy of Sciences, pioneered the work on breeding wheat with improved P use efficiency in China (Li, 1990). In early 1990s, they identified a number of P-efficient wheat genotypes in germplasms collected from different areas of China, and found that P-efficient genotypes secreted more organic acids (e.g. malic acid and citric acid) into the rhizosphere than P-inefficient genotypes (Li et al., 1995). The results demonstrated the feasibility of developing wheat varieties with improved P use efficiency by engineering more efficient organic acid secretion under P deprivation. They also analysed genetic differences in P use efficiency among wheat varieties and their wild relatives (Liu et al., 2001; Davies et al., 2002; Su et al., 2006). Through a combination of physiological and genetic approaches, they developed a P-efficient wheat cultivar, Xiaoyan 54 (Fig. 3A). Molecular analysis showed that a phosphate transporter is highly transcribed in this variety under both normal and phosphate-deprived conditions (Davies et al., 2002). This indicates that the P uptake and/or metabolic systems in this variety may possess unique features that deserve further study.

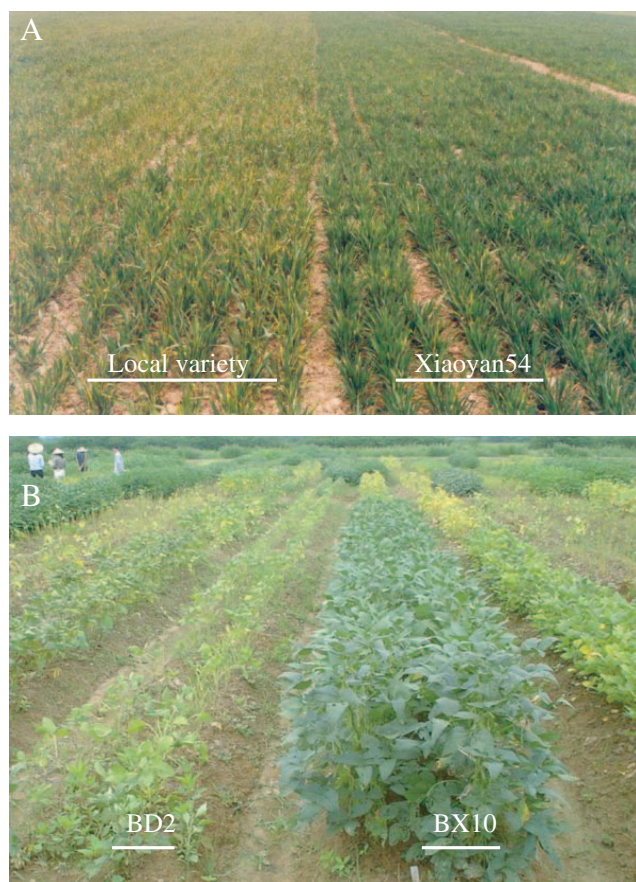


FIG. 3. Differences in P efficiency of wheat and soybean varieties grown on low-P soils. (A) The P-efficient wheat variety Xiaoyan 54 grew significantly better than the local wheat variety in a low-P calcareous soil in North China (photo courtesy of Prof. Zhensheng Li). (B) The P-efficient soybean breeding line BX10 performed much better both in vegetative growth and in grain yield than a local conventional variety BD2 in an acid low-P soil in South China (photo by X. Yan).

Chinese researchers are also active in studying other crops for physiological mechanisms of adaptation to low-P soil conditions, including root morphology, root architecture, root exudates, P transport and key enzymes. Among these useful traits, root architecture, or the spatial configuration of the root system in the soil profile, appeared to be critically important for P uptake efficiency in many crop species studied, particularly in legumes such as common bean and soybean (Liao *et al.*, 2001; Dong *et al.*, 2004; Zhao *et al.*, 2004). Genetic studies indicated that many root traits are closely associated with several major QTL that can be used to facilitate selection and breeding for higher P efficiency in crops (Liao *et al.*, 2004; Yan *et al.*, 2004). This has resulted in plant molecular breeding programmes aimed at genetic improvements of root traits. One of the promising examples of so-called ‘root breeding’ is that a number soybean breeding lines with superior root characteristics have been developed in South China that enable better adaptation to low-P conditions and more efficient utilization of applied fertilizers (Fig. 3B).

In recent years, significant progress has been made towards understanding the molecular basis of the morphological and biochemical processes associated with phosphate (Pi) deficiency, including some of the work done by Chinese scientists. Wu *et al.* (2003) was among the first to employ genomic tools to study gene expression profiles of plant adaptation to Pi deficiency. They found that thousands of genes, including many transcription factors, were simultaneously regulated by Pi deficiency in *Arabidopsis*, indicating that plant adaptation to Pi deficiency might be a systemic process with co-ordinated induction or suppression of many genes in the genome. These findings were later confirmed by a comprehensive survey of global gene expression in response to Pi deprivation, using the *Arabidopsis* whole-genome Affymetrix gene chip with 22810 features (Misson *et al.*, 2005). These adaptation systems may include many features: enhanced uptake ability through activation of high-affinity transporters and adaptive root development leading to altered root morphology and root architecture, induction of phosphate scavenging and recycling enzymes, induction of alternative pathways of cytosolic glycolysis, induction of tonoplast H⁺-pumping pyrophosphatase, alternative pathways of respiratory electron transport, and other metabolic pathways associated with signal transduction and transcription regulations (Wu *et al.*, 2003; Misson *et al.*, 2005). By contrast, specific Pi-signalling pathways and regulation systems may be involved in the adaptation of plants to P deficiency. In fact, a Pi-specific signalling pathway and a regulation system have recently been revealed in rice (Hou *et al.*, 2005), making it feasible genetically to modify some key regulator(s) through Pi-specific signalling pathways for better P efficiency.

From a cDNA library constructed by the suppression subtractive hybridization (SSH) method, a transcription factor with the basic helix–loop–helix (bHLH) domain corresponding to a QTL for P efficiency was identified in rice roots and found to be responsive to Pi deficiency (Yi *et al.*, 2005). The gene was cloned from a P-efficient *indica* landrace, Kasalath, and designated as *OsPTF1* (*Oryza sativa* L. phosphate transcription factor). When the transcription factor was introduced into a low-P-sensitive rice variety ‘Nipponbare’ by *Agrobacterium*-mediated transformation, the transgenic rice overexpressing *OsPTF1* showed enhanced Pi efficiency in both solution culture and soil experiments. Tillering ability, root and shoot biomass, and P content of transgenic rice plants were about 30 % higher than those of the wild-type plants in Pi-deficient conditions in hydroponic experiments. In soil pot and field experiments, a more than 20 % increase in tiller number, panicle weight and P content was observed in transgenic plants compared with wild-type plants at low Pi levels (Yi *et al.*, 2005).

To investigate further the downstream genes regulated by *OsPTF1*, a microarray analysis was performed using rice whole-genome oligo chips. The microarray data showed that expression of 158 genes was up-regulated more than two-fold in roots, shoots or both in the transgenic plants. These genes could be arranged into a

number of functional classes such as nutrient transport and metabolism, carbon metabolism, transcription factors, ATP-binding protein, oxidoreductase, protease, disease resistance protein, RNase, H⁺-transporting ATPase, vacuolar H⁺-pyrophosphatase, senescence-associated protein, receptor-like kinase, and cytochrome P450 genes. In addition, many 'function unknown' or putative genes were strongly up- or down-regulated by the overexpression of *OsPTF1*, although some of them did not respond to Pi starvation. The marked induction of the *PHO* genes, such as *RNS1* and H⁺-transporting ATPase, in the transgenic rice plants under Pi-supplied conditions strongly suggests that overexpression of *OsPTF1* triggered a rescue system in response to Pi starvation and played an important role in the increased tolerance to Pi deficiency (Yi *et al.*, 2005). Moreover, the identification of the effect of *OsPTF1* on P nutrition may speed up molecular breeding programmes for P efficiency in rice. This is because *OsPTF1* was derived from rice rather than a different plant species, so that new rice varieties containing the modified gene could be developed by combining traditional breeding with molecular techniques. This study provides evidence that genetic modification of a key regulator involved in the Pi-signalling pathway may greatly facilitate P uptake and utilization in plants.

The involvement of cytokinin (CTK) in signalling the status of nutrients has been highlighted in some recent studies on phosphorus in *Arabidopsis* (Martín *et al.*, 2000; Franco-Zorrilla *et al.*, 2005) and nitrogen in maize (Takei *et al.*, 2002). To understand this aspect of CTK action in rice further, a global analysis of Pi-starvation-induced gene expression was conducted with exogenous application of 6-benzyladenine (6BA) to rice seedlings under both Pi-starvation and Pi-sufficiency conditions, using a rice oligo microarray containing 60 727 oligo spots on two slides (http://omad.qiagen.com/download/storage/rice_V1-0.2_genelist_s.xls.gz). Our data revealed that physiological and biochemical changes were detected 10 d after Pi-starvation treatments in the rice seedlings, and Pi-starvation-induced global gene expressions were depressed by an exogenous CTK application. A remarkable increase in cellular Pi concentration driven by exogenous 6BA was found, which may be attributed to the CTK repression of systemic Pi-starvation signalling (P. Wu, unpubl. res.). To investigate further the checkpoints of CTK signalling and Pi signalling in rice, a screening experiment was conducted to isolate rice mutants insensitive to exogenous CTK. It was observed that Pi-starvation signalling was blocked only in some rice mutants but not in others. The results suggest that specific checkpoints for the interaction between CTK signalling and Pi signalling may exist. However, further studies with a plant nutriomic approach are needed to elucidate the genes and functions involved in the integration between Pi signalling and CTK signalling.

Iron efficiency

Iron deficiency is a common nutritional disorder in crop plants in China, particularly in northern China where

aerobic and calcareous soils are widespread. In China, several research groups are working on plant iron efficiency. Most activity is focused on illustration of molecular regulation mechanisms of the effective uptake system of so-called strategy I plants, which include all dicots and non-graminaceous monocots and use the efficient iron acquisition system of strategy I as described by Römheld (1987), using *Arabidopsis thaliana* and tomato as model plants. Considerable progress has been made.

One important research area of plant iron nutrition in China is the functional characterization of iron chelate reductases in *Arabidopsis* and tomato. Iron is a transition metal involved in electron transfer reactions of many fundamental biological processes. In strategy I plants, iron is first reduced on the root surface from ferric to ferrous iron by a plasma membrane-bound Fe(III)-chelate reductase and subsequently transferred across the rhizodermal plasma membrane barrier by a high-affinity Fe(II) transporter such as IRT1 into roots. It is then oxidized and transported as an Fe(III)-citrate complex for long-distance transport in xylem to the shoot (Hell and Stephan, 2003). For assimilation in leaves and other tissues, iron is again reduced to ferrous iron. *AtFRO2* encodes an Fe(III)-chelate reductase mainly functioning in reduction of ferric to ferrous iron on the root surface of *A. thaliana* (Robinson *et al.*, 1999). In addition to *AtFRO2*, there are seven other iron chelate reductases in the genome of *A. thaliana*. *AtFRO2*, *AtFRO3*, *AtFRO4*, *AtFRO5*, *AtFRO7* and *AtFRO8* displayed significantly increased iron reduction activity when expressed in yeast cells (Wu *et al.*, 2005). RT-PCR analysis and monitoring with the promoter-GUS method revealed the tissue specificity of gene expression among the eight *AtFROs*. *AtFRO2* and *AtFRO3* are mainly expressed in roots of *Arabidopsis*, *AtFRO5* and *AtFRO6* in shoots and flowers, and *AtFRO7* in cotyledons and trichomes, whereas *AtFRO8* transcription was specific to leaf veins (Wu *et al.*, 2005). These results indicate that the eight *AtFROs* might possess tissue-specific functions involving iron homeostasis in *Arabidopsis*. Based on the expression profiles of the eight *AtFROs*, Wu *et al.* (2005) suggested that *AtFRO2* and *AtFRO3* might be two ferric-chelate reductase genes mainly functioning in iron uptake and metabolism in roots, whereas *AtFRO5*, *AtFRO6*, *AtFRO7* and *AtFRO8* are involved in iron homeostasis in different tissues of shoots.

Using PCR-based homologue cloning, a major Fe(III)-chelate reductase gene, *LeFRO1*, of tomato has been isolated and characterized (Li *et al.*, 2004). Exogenous expression of *LeFRO1* in yeast cells resulted in a significant increase of Fe(III)-chelate reductase activity in transformed yeast cells. The transcription of *LeFRO1* in roots was induced under iron-deficiency stress whereas it was constitutively expressed in leaves, indicating that the transcription of *LeFRO1* in roots and shoots is regulated by different control mechanisms. The transcript of *LeFRO1* was also detected in cotyledons, flowers and fruits, suggesting that *LeFRO1* is not only required for iron uptake in roots, but is also involved in iron homeostasis in other organs.

Another important research area of plant iron nutrition in China is the molecular regulation mechanism of iron acquisition in tomato. Tomato was used as a model plant for studying the molecular regulation mechanism of iron uptake in strategy I plants. Seven genes (*LeFRO1*, *LeIRT1*, *LeIRT2*, *LeNRAMP1*, *LeNRAMP3*, *FER* and *CHLN*) involved in iron homeostasis in tomato have been isolated (Ling *et al.*, 1999, 2002; Eckhardt *et al.*, 2001; Bereczky *et al.*, 2003; Li *et al.*, 2004). *FER* and *CHLN* are two genes directly or indirectly functioning in the regulation of iron deficiency responses in tomato. *FER*, located on chromosome 6 of tomato (Ling *et al.*, 1996), was isolated using a map-based cloning approach (Ling *et al.*, 2002). It encodes a bHLH protein involved in the control of iron deficiency responses and iron uptake in roots. When loss of *FER* function is achieved by an insertion mutation in T3238fer of tomato, the mutant plants are not able to turn on the whole iron deficiency responses under iron-limiting stress and show strong symptoms of iron deficiency (chlorosis) and die off at an early stage. The mutants can be rescued by grafting on a wild-type or by constant fertilization with iron. *FER* is expressed in tomato roots and its transcription is pH-dependent (T. Zhao and H. Ling, unpubl. res.). The mRNA abundance of *FER* was much higher at pH 5 than at a higher pH (7.0). Molecular characterization of the T3238fer mutant indicated that ferric-chelate reductase *LeFRO1* (Li *et al.*, 2004) and the metal transporters *LeIRT1* and *LeNRAMP1* (Eckhardt *et al.*, 2001; Bereczky *et al.*, 2003) are directly or indirectly controlled by the *FER* protein at transcriptional level in tomato roots. The knockout mutant of *FER* (T3238fer) exhibited loss of induction capability of the genes. Overexpression of *FER* in the tomato cultivar Ailsa Craig resulted in increased transcription of *LeFRO1* and *LeIRT1* under iron-limiting conditions and the transgenic plants were more tolerant of iron deficiency than their wild-type under iron-limiting conditions (L. Li and H. Ling, unpubl. res.).

FER is the first regulator controlling iron homeostasis in tomato to be isolated. To determine if *FER* homologues exist in the genome of other strategy I plants, we analysed the genome sequence of *A. thaliana* by use of BlastX. The AtbHLH029 predicted on chromosome 2 of *Arabidopsis* shows high similarity (>60%) to *FER* at the protein level. Further analysis by phenotypic complementation demonstrated that *AtbHLH029* is an *FER* homologue in the genome of *A. thaliana*. It could completely complement the defect functions of the *FER* gene when expressing AtbHLH029 in the mutant T3238fer (Yuan *et al.*, 2005). The T-DNA insertion mutant of *AtbHLH029* showed typical iron-deficiency symptoms as with T3238fer and strong growth impairment. Like *FER*, the AtbHLH029 protein is required in controlling the iron chelate reductase AtFRO2 and the ferrous transporter AtIRT1 (Colangelo and Guerinot, 2004; Jakoby *et al.*, 2004) in *Arabidopsis*. Such similar biological functions of *FER* and AtbHLH029 (FIT1 or FRU), which are isolated from two highly divergent 'strategy I' plants, suggest that *FER* might be present in all strategy I plants in controlling iron acquisition in roots.

CHLN, a gene encoding a nicotianamine synthase responsible for synthesis of nicotianamine (Ling *et al.*, 1999), is located on chromosome 1 of tomato (Ling *et al.*, 1996). A single transition (T to C) at amino acid position 238 of *CHLN* led to the abolishment of the synthesis function of nicotianamine in mutant plants. Nicotianamine is a polyamine and plays a central role in iron homeostasis of all higher plants. In opposition to *FER*, the *CHLN* mutant (*chloronerva*) is not able to turn off the iron-deficiency responses under iron-replete conditions, and thus takes up more iron from soil and accumulates it in shoots. Meanwhile, the mutant plants display morphological and physiological symptoms of iron deficiency due to lack of nicotianamine. RT-PCR analysis showed that *LeFRO1* and *LeIRT1* lost their down-regulation capability in roots of *chloronerva* under iron-sufficient conditions and maintained iron at a high level. This indicates that *CHLN* is required for down-regulation of the two genes.

Biofortification of iron content in plants is an emerging research area of plant iron nutrition in China. Anaemia as a result of iron deficiency afflicts more than two billion people worldwide, especially in developing countries including China (<http://www.harvestPlus.org/iron.html>). Biofortification of iron content and availability in plant foods could be an economic solution to this problem. Recently, a nationwide programme has been initiated in China to tackle this problem through genomic and breeding approaches to increase iron content and availability in grains of wheat, rice, maize and other cereal crops (<http://www.harvestPlus-China.org>).

Boron efficiency

Boron deficiency is a widespread plant nutrition disorder found throughout the world, including China, the USA and Brazil, and B-deficient soils make up over 50% of the arable land in Southeast Asia (Shorrocks, 1997). In China alone, more than 33 million hectares of cultivated land are B-deficient, with hot-water-soluble B of <0.50 mg kg⁻¹ (Liu, 1996, pp. 63–88). Application of B fertilizer is a common practice for production of many crops, such as oilseed rape, cotton and sugar beet.

Oilseed rape (*Brassica napus*) is one of the major oil crops in China, with a total planting area of greater than 7.3 million hectares (Wang and Lan, 1995). Oilseed rape has a high demand for boron and is thus one of the crops most sensitive to B deficiency. However, there are significant differences in the response to B deficiency among *B. napus* cultivars (Hu *et al.*, 1991; Xue and Yang, 1994; Xue *et al.*, 1995). Thus, screening and genetic improvement for B-efficient germplasm of *B. napus* may improve B nutrition on soils with low B availability. In the past decade, significant progress has been made in China in screening and utilization of B-efficient germplasm of *B. napus* (Wang and Lan, 1995; Xu *et al.*, 2002).

Extensive research has been conducted in revealing the physiological mechanisms of B efficiency in oilseed rape. It was found that B deficiency affected root exudates and the composition of bleeding sap on oilseed rape cultivars with contrasting B efficiency. Under conditions of B

deficiency, secretion of soluble sugar in some cultivars decreased significantly, but no difference existed in others. The total content of amino acids in the root exudates was not affected by boron levels among various cultivars, whereas significant genotypic differences existed in the composition of amino acids. B deficiency resulted in a decrease of bleeding sap, soluble sugar and amino acid content in B-inefficient cultivars (Cao *et al.*, 1997). The differences in root exudates and composition of bleeding sap between the two types of cultivars resulted in a significant decrease of carbohydrates and free amino acids in B-inefficient cultivars under B-deficient conditions, and the plants showed typical B-deficiency symptoms (Du *et al.*, 2002).

It was long considered that B absorption by plants was a passive uptake mechanism, and its transport in plants was dependent on the transpiration rate (Bowen, 1972; Raven, 1980). However, it was found that B is partly retranslocated in phloem in radish and cauliflower (Shelp *et al.*, 1987) and in *B. napus* (Shen and Shen, 1991). Recently, a B transporter, *BOR1*, from *Arabidopsis* was isolated by a Japanese group (Takano *et al.*, 2002).

Xu *et al.* (2002) studied B distribution and re-use of various B-efficient cultivars at the seedling and bolting stages in pot culture, and found that under B-deficient conditions, the B concentration in the oldest leaf of B-inefficient cultivars was higher than that of B-efficient cultivars at the two stages, but that the reverse was the case in the youngest leaf. These results were identical with those of Xiong *et al.* (1995), who found that the B concentration of the first base leaf of B-inefficient cultivars was significantly higher than that of B-efficient cultivars at the flowering stage under B-deficient conditions. By contrast, the B concentration was lowest in the youngest leaf, and the B concentration in the flower and bud of the B-efficient cultivars was 2–4 times higher than that of B-inefficient cultivars.

Yu *et al.* (1999) reported that B accumulation and accumulation ratios in different organs of *B. napus* were different at various developmental stages. During vegetative growth, B was mainly accumulated in the vegetative organs. In reproductive growth, B accumulation gradually increased in reproductive organs, and reached the highest level at the budding stage. However, different genotypes differ in the pattern of B accumulation. For example, the B accumulation ratio of reproductive to vegetative organs in B-efficient cultivars was significantly higher than that in B-inefficient cultivars at the flowering and budding stages under severe and mild B deficiency. B-efficient cultivars had a stronger ability to transfer B from old to young leaves (Yu *et al.*, 1999). This might be one of the major physiological mechanisms of B efficiency in *B. napus*.

Xu *et al.* (2002) selected one B-inefficient cultivar and crossed it with two B-efficient cultivars to obtain two hybrids. Under B deficiency, the response of the hybrid F₁s to B deficiency was similar to that of their respective B-efficient cultivars, indicating that B efficiency is genetically dominant. Further studies on the segregation of the B nutrition trait in F₂ plants and mapping of

B-efficient gene(s) using the molecular markers showed that the ratio of B efficiency to B inefficiency individuals fitted the expected 3:1 ratio, indicating a major gene controlling the B efficiency trait. A genome-wide QTL analysis detected one large QTL in the ninth linkage group and three smaller QTL in three linkage groups (Xu *et al.*, 2001, 2002). These results demonstrated that the B efficiency gene(s) could easily be manipulated using marker-assisted selection in breeding programmes.

CONCLUSIONS

This review indicates that although plant nutriomics is still at a conceptual stage, substantial efforts are being made in China aimed at increasing plant nutrient efficiency through genetic and molecular approaches. Although we have focused here largely on N, P, Fe and B, with which we have been working on along molecular biology lines, there are other plant nutritional problems in China that also require urgent attention, including deficiencies of K, Ca, Mg, S and other essential elements, toxic levels of Al, Mn and Fe, and other abiotic stresses such as salinity and drought. All these nutritional constraints need to be tackled by intensified efforts through a nationwide, co-ordinated project on plant nutriomics. Future studies involving both national and international collaborations are needed to develop nutrient-efficient, stress-tolerant and high-quality crop varieties that will contribute to agricultural sustainability, food security and environmental safety in China and elsewhere.

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