Plant nutrition research: Priorities to meet human needs for food in sustainable ways

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Abstract

The world population is expanding rapidly and will likely be 10 billion by the year 2050. Limited availability of additional arable land and water resources, and the declining trend in crop yields globally make food security a major challenge in the 21st century. According to the projections, food production on presently used land must be doubled in the next two decades to meet food demand of the growing world population. To achieve the required massive increase in food production, large enhancements in application of fertilizers and improvements of soil fertility are indispensable approaches. Presently, in many developing countries, poor soil fertility, low levels of available mineral nutrients in soil, improper nutrient management, along with the lack of plant genotypes having high tolerance to nutrient deficiencies or toxicities are major constraints contributing to food insecurity, malnutrition (i.e., micronutrient deficiencies) and ecosystem degradation. Plant nutrition research provides invaluable information highly useful in elimination of these constraints, and thus, sustaining food security and well-being of humans without harming the environment. The fact that at least 60% of cultivated soils have growth-limiting problems with mineral-nutrient deficiencies and toxicities, and about 50% of the world population suffers from micronutrient deficiencies make plant nutrition research a major promising area in meeting the global demand for sufficient food production with enhanced nutritional value in this millennium. Integration of plant nutrition research with plant genetics and molecular biology is indispensable in developing plant genotypes with high genetic ability to adapt to nutrient deficient and toxic soil conditions and to allocate more micronutrients into edible plant products such as cereal grains.

Introduction

The world's population is expected to grow from 6 billion to around 10 billion by 2050. This is a median projection representing the addition of an extra 4 billion people onto the present population (Byrnes and Bumb, 1998). Virtually all of the anticipated increase in world population will occur in Africa, Latin America and Asia. Presently, these regions have already serious problems concerning food production, access to food, water scarcity and nutritional disorders/malnutrition. Pinstrup-Andersen et al. (1999) reported that more than 800 million people living in the developing countries are undernourished caused by inadequate food availability. The problem is par-

ticularly widespread in Asia; about 70% of the undernourished people globally live in Asia, predominantly in India and China. Sub-Saharan Africa with about 200 million undernourished people is another critical region of the world suffering from malnutrition. According to the FAO projections, the number of undernourished people will decline to only 680 million by 2010, and this decrease will mainly take place in Asia, while in Sub-Saharan Africa food insecurity will likely continue with increasing numbers of people affected (FAO, 1996, 1999; Rosegrant et al., 2001).

Among the undernourished people children under 5 years of age are particularly at risk. Globally, 30% of children are underweight, and low birth weight becomes an important factor in child malnutrition and premature death (Pinstrup-Andersen, 1999; UNDP, 1999). Micronutrient deficiencies such as iron (Fe),

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zinc (Zn), iodine (I) and vitamin A deficiencies are critical issues in the developing countries, and result in severe impairments of human health and development such as impairments of physical growth, immune system, cognitive development and enhancements in anemia and maternal mortality (Pinstrup-Andersen, 1999; Welch and Graham, 2000). Micronutrient deficiencies are also widespread in well-developed industrialized countries. More than 3 billion people globally suffer from Fe and Zn deficiencies (Graham et al., 2001). Heavy and monotonous consumption of cereal-based foods with low concentrations and reduced bioavailability of Fe and Zn has been considered a major reason for the widespread deficiencies of Zn and Fe in developing countries (Graham et al., 2001; Welch and Graham, 1999).

To feed a world with huge increases in population and to sustain the well-being of humans, a large increase in food production must be achieved. The expected increases in world population will result in a serious pressure on the existing agricultural land via urbanization and intensification of crop production (Alexandratos, 1995). The global food projections model (The International Model for Policy Analysis of Commodities, and Trade, IMPACT) developed by the International Food Policy Research Institute (IFPRI) indicates that to meet the food demand of the world population in 2020, annual cereal production needs to increase by 40%, from 1773 billion tonnes in 1993 to nearly 2500 billion tonnes in 2020 (Rosegrant et al., 1999, 2001). Of this increase in total cereal demand, 85% will come from the developing countries. Others estimates that the total world cereal production must rise to 2.7 billion tons (Dyson, 1999) or to nearly 4 billion tones (Borlaug and Dowswell, 1993) by 2025 to match food requirements of the world population.

The projected increase in food production must be accomplished on the existing cultivated areas because the expansion of new land is limited due to environmental concerns, urbanization and increasing water scarcity. Evenson (1999) reported that global plant-based food production will increase by more than 60% by 2020 while the area used for crop production will expand by only around 10% (Table 1). However, recent trends indicate that the growth rate (based on ton ha^{-1}) in crop production has begun to decline in the last 10 years, and possibly, cannot keep pace with the projected increase in global food demand in the following decades (Fig. 1; Mifflin, 2000; Rosegrant et al., 2001). This situation is especially dramatic in many developing countries in Africa and Asia where pop-
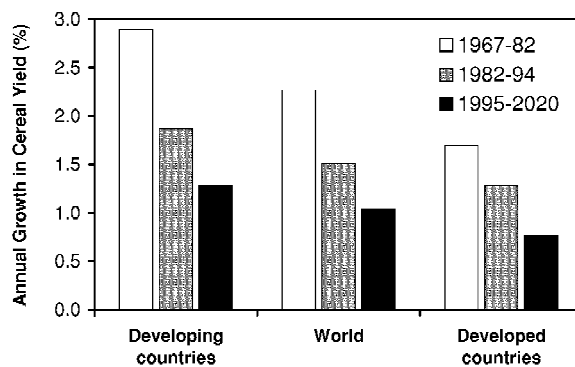

Figure 1. Annual growth rates in cereal yields during 1967–82, 1982–94, and 1995–2020 (redrawn from Pinstrup-Andersen et al., 1999).

Table 1. Expected ratios of crop production and cultivated land in 2020 relative to those in 1990 (from Evenson, 1999)

Crop	Production	Land cultivated
Wheat	1.58	1.06
Maize	1.56	1.13
Rice	1.66	1.07
Other grains	1.48	1.09
Soybeans	1.77	1.14
Roots/tubers	3.28	1.15
Mean	1.89	1.11

ulation pressure on agriculture is already very high. For example, in Sub-Saharan Africa the annual growth of total cereal production dropped from 1.9% in the 1970s to 0.66% in the 1990s. These decreases were attributed to falling cereal prices, decreases in use of fertilizers, growing water shortages and impairments in soil fertility and management practices (Gruhn et al., 2000; Pinstrup-Andersen et al., 1999; Rosegrant et al., 2001). The decreasing trend in cereal production must be reversed; otherwise very serious food deficits will occur resulting in a significant threat to human nutrition and health.

Soil productivity is decreasing globally due to enhanced soil degradation in the form of erosion, nutrient depletion, water scarcity, acidity, salinisation, depletion of organic matter and poor drainage. Nearly 40% of the agricultural land has been affected by soil degradation, particularly in Sub-Saharan Africa and Central America (Table 2; Scherr, 1999). Enhanced soil degradation along with slowdown in cereal production contributes to food insecurity in developing

Table 2. Global estimates of soil degradation in agricultural land (from Scherr, 1999)

Region	Agricultural land		
	Total	Degraded	Percent
	(million hectares)		
Africa	187	121	65
Asia	536	206	38
South America	142	64	45
Central America	38	28	74
North America	236	63	26
Europe	287	72	25
Oceania	49	8	16
World	1475	562	38

Figure 2. Growth in fertilizer use and cereal production in developing countries during 1961–1995 (redrawn from FAO, 1996).

countries. To improve food security for developing countries, investments (including education, clean water, irrigation, rural roads and agricultural research) are projected to cost 579 billion US dollars between 1997 to 2020. Among the investments agricultural research activities will account for 120 billion US dollars (Rosegrant et al., 2001). According to Rosegrant et al. (2001), the projected investments for improving food security between 1997 and 2020 are feasible and represent less than 5% of the government expenditures in the developing world over the next 20 years.

Fertilizer roles in meeting global food demand

To meet the challenge of 'food security', agricultural production must increase on the existing land, and therefore crop production must be intensified per unit of agricultural land. Mineral nutrients are the major contributor to enhancing crop production, and in maintaining soil productivity and preventing soil degradation. Generally, improving the nutritional status of plants by applying fertilizers and maintaining soil fertility has been the critical step in the doubling of food production both in developed and developing countries since the beginning of 'Green Revolution' (Loneragan, 1997). As presented in Figure 2, increases in cereal production in the past 40 years were well associated with corresponding increases in fertilizer consumption in developing countries (FAO, 2000). According to Tillman (1999) the doubling of food production during the past 40 years has been associated with about 6.9-fold increase in N fertilization, 3.5-fold increase in P fertilization and only 1.1-fold increase in cultivated land area. In Asia, there was a 2-fold
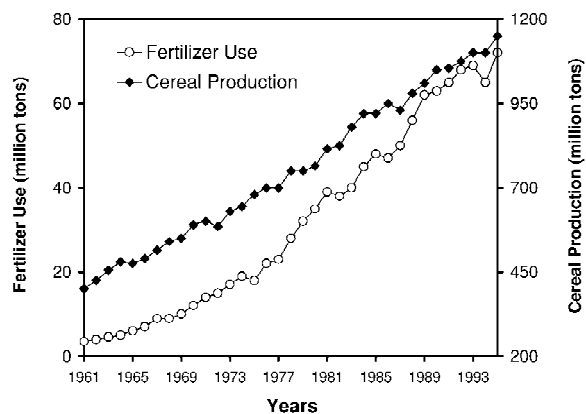
increase in rice production since 1966 (from 240 to 483 million tons), while the area used for rice production increased by only 13%, and fertilizer consumption per hectare of land has increased from 20 to 145 kg (Hossain and Singh, 2000). According to Hossain and Singh (2000), this increase in fertilizer consumption is not enough to sustain food security in the Asian countries, and should be increased to over 250 kg ha^{-1} of NPK.

Nearly all increases in projected food requirements in the next decades will the result of enhancements in yield per unit area and intensive use of agricultural land. To increase yield capacity of crop plants and to ensure global food demand in 2020, fertilizer use should increase from 144 million tons in 1990 to 208 million tons in 2020 (Bumb and Baanante, 1996; FAO, 2000). Possibly, this projected increase in fertilizer consumption by 2020 will not be adequate to meet both food production requirements and nutrient depletion from soil resulting from nutrient removal by harvesting crops. Byrnes and Bumb (1998) estimated that fertilizer consumption should increase up to 300 million tons to match required demands of food production and nutrient removal from soils by 2020. In view of this estimate, in future, developing countries should develop new policies and take new measures to provide more support to farmers regarding the supply of fertilizers.

Environmental concerns

Enhanced use of fertilizers raises concerns due to adverse effects on the environment in terms of eutrophication of surface waters, pollution of drinking water and gaseous emission causing global warming.

Therefore, nutrient use efficiency and improved soil management become an important challenge, particularly for N and P fertilizers. When the application of N fertilizers is not properly managed and is realized at excessive levels, losses of N from agricultural lands can occur by NO_3 leaching, NH_3 volatilisation or by N oxides emission. Leaching and runoff of NO_3 into ground water and surface waters is a major environmental problem in developed countries, particularly in Europe (Howarth, 1998; Kroeze and Seitzinger, 1998). Pollution of groundwater with NO_3 impairs the quality of drinking water and causes various harmful effects on human health. Contamination of lakes and rivers with NO_3 stimulates algal growth and depletion of O_2 resulting in an increasing risk of fish deaths on a large scale, especially in coastal areas (Maene, 2000; Mosier et al., 2001). Large areas on the North Atlantic Ocean and North Sea in Europe have been reported to suffer from eutrophication caused by excessive use of N on agricultural land (Howarth, 1998).

There is a very close relationship between application rates of N fertilizers and emission of nitrous oxide (N_2O) (Erickson et al., 2000). Nitrous oxide is one of the most important greenhouse gases affecting global warming and increasing ozone destruction. About 0.5% (Veldkamp and Keller, 1997) or 1.5% (Smith et al., 1997) of fertilizer N applied is lost from soil as gaseous emission. Several management strategies have been developed to control and minimize N losses such as use of N fertilizers with enzyme inhibitors (urease and nitrification inhibitors) and controlled-release N fertilizers, timing and placement of fertilization, and soil and plant analysis to define rates of N application. For example, use of nitrification inhibitors is effective to reduce N_2O emission under field conditions (McTaggart et al., 1997; Mosier et al., 1994) and the timing of N application plays an important role in controlling N losses as shown in flooded rice (Humphreys, 1988).

Like NO_3, P is also involved in eutrophication of water through its runoff from soils into rivers and lakes. Solubility of P in soils is very low, and therefore P cannot be leached as easily as NO_3. Due to repeated applications of P-containing fertilizers together with manure, P concentrations in topsoil increases, leading to saturation of soil binding sites with P and thus facilitating movement of P into surface waters. Holford et al. (1997) showed that P could be leached when 17–38% of the sorption capacity of soils was saturated. There is increasing evidence showing that the major source of P in surface water showing eutrophication is the application of compost, manure and sewage (Eghball and Gilley, 1999; Reynolds and Davies, 2001). Phosphorus from manure application moves much deeper in soil than the P from fertilizer (Eghball et al., 1996). Higher movement of P from manure is attributed to existence of greater amount of organic compounds in manure, facilitating P solubility and movement in the soil profile.

Generally fertilizer-dependent environmental problems generally take place under specific soil conditions or in poorly managed agricultural areas, in particular in developed countries that employ high fertilizer application rates. In contrast, in many developing countries with tremendous requirement for food, continuous nutrient depletion and low usage of mineral fertilizers are the concerns, not the environmental pollution (Gruhn et al., 2000). Nearly 40% of all globally cropped land shows degradation (Table 2), with nutrient depletion being a particular form of soil degradation (Scherr, 1999). Nutrient depletion occurs in many developing countries (e.g., in Sub-Saharan Africa). In Sub-Saharan Africa, due to increasing pressure on the cultivable land, farmers use the existing land extensively without addition of adequate amounts of fertilizers or consideration of proper soil management practices. Annual use of mineral nutrients per ha of arable land in 1996 has averaged around 9 kg in Sub-Saharan Africa and 98 kg in the world (Gruhn et al., 2000). When the nutrients removed by plants at harvest are not replenished by fertilization or by proper soil management practices (i.e., by crop rotation, use of plant residues and manure), soils become depleted of mineral nutrients. With the cumulative removal of nutrients marked decreases occur in soil productivity. Bumb and Baanante (1996) reported that nutrient removal exceeds nutrient replenishment by a factor 3–4 in Sub-Saharan Africa. About 86% of the countries in Africa show a net annual nutrient depletion greater than 30 kg of NPK per hectare (Henao and Baanante, 1999). In many developing countries net nutrient depletion varies between 60 and 100 kg ha^{-1} per year, resulting in serious decline in soil fertility. It is estimated that in Africa, 1.5 billion US dollars per year is needed to minimize nutrient depletion and related decreases in soil productivity by applying mineral fertilizers (Henao and Baananate, 1999).

Obviously, enhancing and maintaining soil fertility are very critical issues in meeting food security in developing countries. Impaired soil fertility by continuous cropping with low supply of mineral nutrients is considered a major threat not only to food production but also to ecosystem viability (Pinstrup-

Andersen, 1999; Tillman, 1999). Reduced soil fertility and crop production results in increased pressure to bring more land into crop production at the expense of forests and marginal lands. Such areas are generally poor in fertility and sensitive to rapid degradation when cultivated. Conversion of forests into agricultural uses also contributes to global warming. Mosier (1998) reported that conversion of forests to agricultural use promotes the turnover of soil-C and soil-N, and thus increases in C loss and N_2O emission from soil. These consequences suggest that in developing countries there is an urgent need for application of adequate amounts of mineral nutrients and proper nutrient management systems, such a combination of mineral fertilizers with organic materials, including legumes in the rotation systems, timing and placement of nutrient applications and balanced supply of mineral nutrients. The contributions of these management systems to improvements in soil productivity are discussed below.

Nutrient management

Manure applications

Improvements of soil fertility and efficient use of mineral nutrients are of high ecological and economic importance. Use of basic and practical research information provided through plant nutrition studies will play a decisive role in establishing efficient and ecologically based nutrient management systems. Combination of mineral fertilizers with different types of organic materials, including legumes in rotational cropping systems and recycling of crop residues are widely recommended strategies to improve soil fertility and enhance nutrient use efficiency.

Applying and promoting these strategies is of particularly importance in long-term continuous cropping systems. The rice–wheat cropping system is the most widespread cropping system in Southern and Eastern Asia. Around 12.5 million hectares of land in Pakistan, India, Nepal and Bangladesh and 10 million hectares in China are under the rice–wheat cropping system (Yadav, 1998; Yadav et al., 2000a,b). In India, 33% of the rice and 42% of the wheat production occurs under this cropping system. Possibly, as a result of long-term continuous rice–wheat cropping, yields of both rice and wheat began to decline. Application of green and farmyard manures has been shown to be an important management strategy to reverse and sustain

Table 3. Grain yields averaged over years and locations in long-term rice–wheat system fertilized with NPK alone or with different organic materials in India (from Yadav et al., 2000b)

Treatment	Yield (t ha^{-1})	
	Rice	Wheat
Control	1967	1065
50% NPK	3180	2321
100% NPK	4551	3506
50% NPK + farmyard	4242	3629
50% NPK + crop residues	4007	3468
50% NPK + green manures	4465	3532

high crop productivity in rice–wheat cropping systems in Asia. This effect of manures becomes more distinct when they are applied together with mineral fertilizers. In a long-term field experiment at six different locations using a rice–wheat rotation system, Yadav et al. (2000a) demonstrated that incorporation of green manure resulted in additional increases in grain yield of rice and prevented yield decline in wheat. Green manures or farmyard manure applied along with a reduced rate of NPK applications were able to reduce the mineral fertilizers used as much as 50% (Table 3). Furthermore, also in continuous sorghum cropping systems without rotation, grain yield decreased steadily, and increases in yield and its maintenance could be achieved when mineral fertilizers were combined with manure applications (Bekunda et al., 1997).

Green and farmyard manures have been shown to improve the solubility and uptake of P from sparingly soluble P compounds in soil and enhance the utilisation of P from fertilizers. Organic compounds released during the decomposition of manures increases the availability of P from soil or fertilizers (Iyamuremye and Dick, 1996). In India, the integrated supply of P fertilizers along with cattle manure under a wheat–soybean cropping system over 5 years greatly improved wheat and soybean yield (Reddy et al., 1999). At an identical rate of P supply from fertilizer or manure, yield increases were much greater with manure-P than with the fertilizer-P. This positive effect of manure was ascribed to mobilization of native soil P and improved physico-chemical properties of the soil by manure use (Reddy et al., 1999). Organic manures are also highly beneficial in acid soils and contribute to alleviation of adverse effects of Al toxicity on crop production by reducing levels of exchangeable Al and increasing concentrations of soluble P (see below).

Crop residues

Soils under continuous cultivation are depleted of nutrients, and soil physical properties are impaired because organic matter is exhausted. In many countries in Africa and Asia very little or no crop residue is left in the field, much being used for feeding animals or for fuel. Crop residues represent a good substrate providing C and N for the activity of microorganisms in the soil. Many of these organisms are involved in the formation and stabilisation of soil aggregates. As plant residues contain relatively large amounts of mineral nutrients, their retention in the field also contributes to the mineral content of soils and leads to savings in the need to apply mineral fertilizers (Timsina and Connor, 2001). In West Africa Sahelian region, use of crop residues in the field resulted in marked increases (up to 73%) in total dry matter production of different cereals. The enhancing effect of crop residues on cereal growth was ascribed to improved P availability and by the protection of seedlings against wind erosion provided by the crop residues (Buerkert et al., 2000). In a recent field experiment in West Africa, the effect of a composted material, prepared from crop residues, animal manure and household refuses, was studied with respect to soil properties and grain yield of sorghum (Quedraogo et al., 2001). Application of 10 tons of compost per hectare resulted in up to 3-fold increases in grain yield depending on the location. The compost application was also effective in overcoming the marked decreases resulting from delayed sowing. The results reported by Buerkert et al. (2000) and Quedraogo et al. (2001) indicate the importance of crop residues and compost applications in preventing soil degradation and maintaining crop productivity. Such applications, when adopted on large scale areas in Africa, could increase yields and maintain soil productivity.

Legumes

In view of their well-documented role in reducing the need for fertilizer N and in their ability to improve soil fertility, there is an increasing trend to include legumes in the crop rotation systems. Inclusion of legumes in intercropping and crop rotation system provides a number of valuable benefits including enhanced soil physical structure, improved soil fertility, reduced need for mineral N fertilizers, and improved use efficiency of N and other mineral nutrients. Such crop rotations also reduce the risk to crops from crop pests and suppresses weed growth (Becker and Johnson, 1999; Van Kessel and Hartley, 2000). The combination of relatively low amount of fertilizers with crop rotations including legume crops significantly enhances crop production (Boddey et al., 1997; Dakora and Keya, 1997). Continuous cropping systems without rotation with legumes were seen as a key factor involved in declining crop production and soil fertility in developing world, particularly in Africa.

In southern Africa, groundnut is increasingly considered an important grain legume in improving soil fertility and sustaining crop production in the maize-based cropping system. In field conditions in Zimbabwe, grain yield of maize was studied under continuous maize and groundnut–maize rotation systems with and without NPK supply (Waddington and Karigwindi, 2001). The inclusion of groundnut in the rotation system almost doubled the grain yield of maize in plots without application of inorganic fertilizers. When inorganic fertilizers were applied, groundnut rotation was still effective in increasing the grain yield. In the northern Guinea Savanna of Ghana, the beneficial effects on maize yield of rotating maize with legumes could be demonstrated in spite of adequate N fertilization and optimization of soil physical properties. Therefore, the positive effects of legumes were attributed to elimination of allelopathic effects (Horst and Hardter, 1994). Obviously, there are several causal factors other than N nutrition and soil physical properties contributing to the yield improvement of crops succeeding legumes. This area is a further potential area of investigation in future.

The beneficial effects of legumes on crop productivity vary between different types of cropping systems. The most widespread cropping systems used in Africa include the single cropping system, the crop rotations and the intercropping of legumes and cereals. The contributions of legumes in these cropping systems with maize were studied in the West African Savanna. The results demonstrated that maize yields following the sole-cropped legumes were 2-fold higher compared to the 'maize after maize' system (Dakora and Keya, 1997). Maize yields were also less after maize-legume intercropped system when compared to the sole-cropped legume. Based on these results, possibly, the intercropping of legumes and cereals is less effective in increasing yield than the crop rotation system involving cereal production after the sole-cropped legumes system (Dakora and Kaya, 1997).

Inclusion of legumes in cropping systems enhances soil N levels and reduces decline in soil fertility associated with intensive cultivation. In field experiments in Australia, cotton grown following non-legume rotation crops (i.e., wheat) required an optimum N fertilizer application of 179 kg N ha^{-1}, while following the grain or green manure legumes only 90 and 52 kg N ha^{-1} were sufficient, respectively (Rochester et al., 2001). Rochester et al. (2000) also showed that including legumes in rotation system positively affected soil quality by improving soil physical properties. When compared to continuous sorghum cropping system, a legume rotation system was effective in increasing the pH of the rhizosphere (Alvey et al., 2001). The increase in pH was considered an important change for plants allowing them to cope with acidity-induced nutrient deficiency and Al-toxicity.

The application of rotation systems including legumes in developing countries appears to be a fundamental strategy to improve soil fertility and crop production. However, the soils in developing countries generally also suffer from acidity (Al toxicity), P deficiency or water stress, which are major constraints to nodulation and N_2 fixation (Hungria and Vargas, 2000). Therefore, future research activities should focus on development of new legume genotypes having a high tolerance to the mentioned edaphic stress factors.

Balanced nutrient supply

Cultivation of high yielding cultivars under continuous monoculture or via intensive cropping systems without sufficient fertilization leads to depletion of nutrients in soils. Consequently, over time high yields are no longer sustainable and make these cropping systems uneconomical. Long-term intensive cropping systems also disturb the balance of mineral nutrients that exist in soils. In India, the deficit between removed and added nutrients has been estimated to range between 4 and 5 million tons in wheat and rice cultivated areas only (Singh, 1998). The nutrients depleted should be replenished to sustain high yields under continuous cropping systems. Currently, the mineral nutritional problems occurring in this cropping system are not confined to only N, P or K. This is a possible reason for declining yields of rice and wheat in the last years in several Asian countries, despite application of high level of NPK (Tandon, 1995, 1998; Hossain and Singh, 2000). Deficiencies of Zn and S are particular examples occurring in the rice-cropping systems. Un-
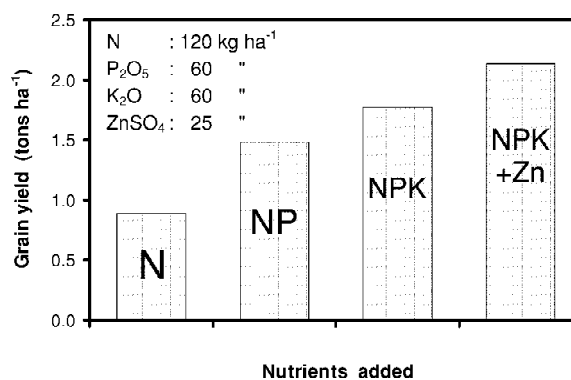

Figure 3. Wheat grain yield based on long-term multi location experiments (based on Tandon, 1995).

less these mineral deficiencies are not corrected, high yields cannot be ensured, and responses to increasing supplies of N, P and K will become very small or zero depending on the soil type. Based on a large number of long-term field experiments at many locations, Tandon (1995) summarized the effects of balanced nutrient supply on grain yield of wheat (Figure 3). Each added nutrient other than N greatly increased grain yield over the N application alone. Obviously, continuous use of N alone leads to severe depletion of other nutrients, with a corresponding decrease in the grain yield. The substantial increases in grain yield by Zn application in addition to the NPK application indicate critical importance of these nutrients in crop production in Asian countries. Likewise, S deficiency is also a common nutritional disorder under intensive cropping system. Long-term field experiments in Bangladesh demonstrated high requirement for S in maintaining high yields in rice production (Tandon, 1995). Applications of S fertilizers markedly improved grain yield: depending on the season increases in rice grain yield by S fertilization varied between 19 and 40%.

An adequate and balanced supply of mineral nutrients in acid soils can be ineffective in maintaining high yield when the soil pH is not elevated to a certain level by addition of lime. In acid soils, crop production can be markedly decreased to very low levels or even to zero levels under long-term supply of N only or only NPK. However, application of NPK together with lime in acid soils can result in sustainable high yield levels (Gruhn et al., 2000; Tandon, 1995).

Aluminium toxicity

Acidity is a critical yield-limiting problem in many soils. About 40% of cultivated soils globally have

acidity problem leading to significant decreases in crop production despite adequate supply of mineral nutrients such as N, P and K (Herrera-Estrella, 1999; von Uexküll and Mutuert, 1995). In acid soils major constraints to plant growth are toxicities of hydrogen (H^+), aluminium (Al) and manganese (Mn) and deficiencies of P, calcium (Ca) and magnesium (Mg). Among these constraints Al toxicity is the most important yield-limiting factor (Marschner, 1991). As mentioned above, liming represents an effective management strategy in overcoming or minimizing soil acidity and related Al toxicity. A continuous acidification without liming may result in deterioration of soils that can make even the acid-tolerant genotypes useless.

By raising pH up to above 5.5 through lime (i.e., $CaCO_3$) applications soluble and exchangeable Al are precipitated as hydroxy-Al species. Generally, lime is added at 1.65 ton ha^{-1} of $CaCO_3$-equivalent per milliequivalent of exchangeable Al per 15 cm soil depth (De Pauw, 1994). There is, however, a risk of overliming in soils by causing occurrence of deficiencies of P and micronutrients. For amelioration of Al toxicity and related P deficiency in acid soils application of organic materials in form of crop residues, compost and green manure have been considered a practical and potentially cheaper practice over lime applications (Haynes and Mokolobate, 2001). Incorporation of organic materials can also lower the requirement for lime application. During the decomposition of organic materials in soils several organic compounds are released which complex phytotoxic monomeric Al species in soil solution and render them into non-toxic forms. The most important organic compounds involved in Al detoxification in soil solution are low molecular weight organic acids and humic and fulvic acids (Haynes and Mokolobate, 2001; Hue and Amien, 1989). The organic compounds adsorb onto Al and Fe oxides and thereby prevent sorption of P on these oxides, a process which improves the availability of P to plant roots (Haynes and Mokolobate, 2001; Hue et al., 1994). Alleviation of phytotoxic effects of Al by organic materials has also been attributed to increases in soil pH (Noble et al., 1996, Wong et al., 1999). Apparently, incorporation of organic matter into acid soils should be widely adopted at the farm level. However, there are some concerns about whether adequate amounts of animal manures are available to improve soil fertility on all affected cultivated lands due to insufficient numbers of animals (e.g., in West Africa; Williams et al., 1995).

Development of new genotypes with high Al tolerance might be an important and sustainable strategy to cope with soil acidity related constraints in soils. As both liming and incorporation of organic materials have some limitations to affect subsoil acidity, a combination of genotypes having high Al tolerance with liming and organic matter addition would be the ideal solution to the Al-toxicity problem in soils. Plant species and genotypes of a given species greatly differ in their tolerance to Al toxicity indicating the existence of a high genetic potential to develop Al-tolerant genotypes (Aniol, 1991; Carver and Ownby, 1995; de la Fuente and Herrera-Estrella, 1999). Two major plant mechanisms have been described as being involved in tolerance to Al toxicity: exclusion of Al from root apex and internal detoxification of Al (Horst, 1995; Kochian, 1995; Ma et al., 2001). Recent evidence suggests that organic acids with their high chelating ability play a fundamental role in Al tolerance both externally and internally. Root exudation of certain organic acids is closely correlated with the level of Al tolerance, for example malate in wheat (Ryan et al., 1995), citrate in maize (Pellet et al., 1995), oxalate in buckwheat (Ma et al., 1997) and citrate in soybean (Figure 4; Yang et al., 2000). The exudation of organic acids from roots in response to Al toxicity appears to be a highly promising adaptive mechanism to allow plants to tolerate acid soils. Therefore, development of novel plant genotypes with a high genetic ability to exude organic acids from their roots when grown on Al-toxic soils is a high priority for research. Exudation of organic acids from roots under acidic soil conditions can also be helpful in solubilisation of P from sparingly soluble hydrated oxides of Al and Fe (Marschner, 1995). Supporting these suggestions, very recently, excellent molecular evidence has been presented showing that the expression of a citrate synthase gene, isolated from *Pseudomonas aeruginosa*, in tobacco resulted in a large increase in citrate efflux and, accordingly, in a high tolerance to both Al toxicity (de la Fuente et al., 1997) and P-deficiency (Lopez-Bucio et al., 2000) (see below for further details).

Phosphorus deficiency

Phosphorus deficiency is also a widespread nutritional problem greatly affecting crop production. It is estimated that 5.7 billion hectares of land (equivalent to about 67% of the total farmland used worldwide) con-
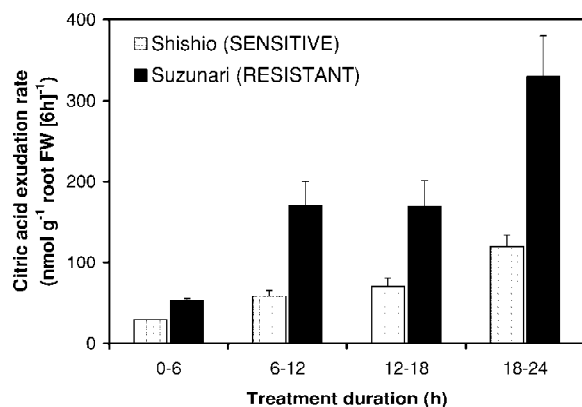
Figure 4. Effect of aluminum (50 μmol) treatment on citrate exudation over a 24-h period at 6-h intervals in soybean cultivars differing in their resistance to aluminum toxicity (redrawn from Yang et al., 2000).

tain too low levels of plant available P, which is limiting crop production in many world regions (Batjes, 1997). Availability of P to plant roots is limited both in acidic and alkaline soils, mainly, due to formation of sparingly soluble phosphate compounds with Al and Fe in acidic and Ca in alkaline soils (Marschner, 1995). Every year large amounts of P fertilizers, up to 35 million tons of P_2O_5 (Isherwood, 2000) are applied to soils for crop production globally, and only 10–20% of the applied P fertilizers can be absorbed by plants (Holford, 1997). The remaining is rapidly transformed into unavailable P forms, which are not readily absorbed by plant roots. Development of plant genotypes having a high capacity to use of both native soil-P and added fertilizer-P is, therefore, very important. Some plant species have evolved several adaptive mechanisms to improve their ability to cope with soils having low levels of available P. Enhancements of root system to better exploration the soil for P is a typical response of plants to P deficiency, particularly by increasing the production and elongation of root hairs (Gahoonia and Nielsen, 1998) and by the formation of mycorrhizal association (Dodd, 2000; Marschner, 1995).

Root hairs greatly contribute to the ability of roots to take up mineral nutrients from the soil. Plants possessing a high root hair response to limited available soil-P conditions are able to acquire/use P in larger amounts than plants that have a poorer ability to proliferate root hairs (Bates and Lynch, 2000). In a study with [32]-P labelled soil, Gahoonia and Nielsen (1998) demonstrated that 70% of root hairs grown into the labelled soil contributed up to 63% of the total P uptake by barley. This is convincing evidence of the

particular role of root hairs in P acquisition from soils. Therefore, increasing attention should be paid to the research aiming at developing genotypes that have a high genetic ability to produce extensive root hairs under P-deficient soil conditions.

Mycorrhizal colonization of roots enhances the ability of roots to explore the soil for P. This occurs through the action of the symbiotic soil fungal mycelliums (hyphaes) infecting roots. This association results in greater exploration of the soil for available nutrients and delivers more mineral nutrients, particularly P, to plant roots (Dodd, 2000; George and Marschner, 1996; Marschner, 1998). The most widely distributed type of mycorrhizal fungi is the arbuscular mycorrhizae (AM). It is estimated that the extent of fungus mycelium may be in the range of 10–100 m per cm root or per gram of soil under field conditions in P-poor soil (McGonigle and Miller, 1999). Therefore, the contribution of mycorrhizal association to mineral nutrition of host plants is much higher in soils with poor availability of mineral nutrients than in soils rich in nutrients. In pot experiments, mycorrhizal colonization contributed between 70 and 80% of the total P uptake and 50 and 60% of the total Zn and Cu uptake in white clover plants (Li et al., 1991). Benefits of AM are not only confined to mineral nutrition of host plants. Mycorrhizal fungi also increase plant tolerance to root pathogens, water stress and heavy metals, and are involved in improvement of N-fixation by *rhizobia* and in physical structural stability of soils (Dodd, 2000; George and Marschner, 1996; Marschner, 1995). Due to such diverse beneficial effects mycorrhizas have a high potential in sustaining soil fertility and restoration of agricultural lands that are both physically and chemically degraded. Soils adversely affected by physical and chemical factors are widespread in developing countries (Table 2), and can be improved through inoculation with selected AM populations. There are a few examples showing the importance of AM inoculation in low-input crop production systems. On acid soils in Latin America, several field experiments were performed to demonstrate the role of AM inoculation on the growth of cassava. The results showed that AM improved cassava yields by 20–25% on the average (Sieverding, 1991, cited in Dodd, 2000).

A further particular adaptive response of plants to P deficiency is the release of organic acids and enzymes from roots into the rhizosphere. Root exudation of organic acids (i.e., citrate and malate) when cultivated under P deficiency has been shown for rape

(Hoffland et al., 1989) and for white lupin (Dinkelaker et al., 1989, 1992; Johnson et al., 1995). Organic acids released from roots of rape plants were able to solubilize P from Ca-phosphates (Dinkelaker et al., 1989; Hoffland et al., 1989) and contribute to P nutrition of plants. Calcicoles plants adapted to alkaline soils released larger amounts of organic acids from roots than the species with poor adaptation ability to alkaline soils (calcifuges) (Tyler and Strom, 1995). Studies dealing with genotypic variation in release of organic acids within a species and its relation to P deficiency tolerance are very few (Gahoonia et al., 2000; Gaume et al. 2001), and therefore, more extensive research is needed in this area. Recently, transgenic tobacco plants have been developed with high citrate-overproducing capacity, and these plants had a higher tolerance to P deficiency under alkaline soil conditions compared to their wild-type counterparts (Lopez-Bucio et al., 2000). This work emphasizes the importance of organic acids in the adaptation of plants to P-limited soils.

Iron and zinc deficiencies in plants

Micronutrient deficiencies in plants are becoming increasingly important globally. Intensive cultivation of high yielding cultivars with heavy applications of N, P and K fertilizers leads to the occurrence of micronutrient deficiencies in many countries. Among the micronutrient deficiencies reported to occur worldwide, Zn and Fe deficiencies are particularly good examples. In plants, Fe deficiency often occurs in calcareous soils where chemical availability of Fe to plant roots is extremely low. It is estimated that Fe deficiency is widespread occurring in about 30% of the cultivated soils on the world, and results in large decreases in crop production and quality (Chen and Barak, 1982; Vose, 1982). Correction of Fe deficiency is not always easy through the use of Fe fertilizers because of their extremely poor solubility and the requirement that repeated applications during the cropping season are needed to correct the deficiency. Therefore, remediation of Fe deficiency chlorosis by fertilizers is a costly and time-consuming management. Plants evolved on Fe-deficient calcareous soils have developed adaptive mechanisms to overcome or minimize the effects of Fe deficiency stress. Marschner and his colleagues have identified two different types of adaptive root responses to Fe deficiency. The first strategy exists in all plant families other than graminaceous family, and is characterized by the mechanisms involving acidification of rhizosphere, activation of a membrane-bound ferric reductase enzyme and the release of reducing substances from roots (Marschner, 1995; Marschner and Romheld, 1994; Marschner et al., 1986). These mechanisms are highly inducible in response to Fe deficiency; they improve solubilisation and uptake of Fe from sparingly soluble Fe compounds in soil.

The other strategy (Strategy II) is confined only to graminaceous species, and characterized by the release of the mugineic acid family phytosiderophores (MAs) to chelate Fe in rhizosphere. The resulting Fe(III)-MAs are taken up into root cells by an inducible specific transporter in the root cell plasma membrane (Marschner and Romheld, 1994; Romheld and Marschner, 1986). The efficiency of the mechanisms occurring in response to Fe deficiency greatly differ among and within plant species, and this explains the reason why a large genotypic variation exists within and among plant species in their sensitivity to Fe deficiency (Kawai et al., 1988; Marschner et al., 1986; Rengel, 2001; Wei et al., 1997). For example, among cereal species rice has high sensitivity to Fe deficiency and very poor ability to release MAs (Kanazawa et al., 1994; Marschner and Romheld, 1990). As discussed in detail below, very recently it has been demonstrated that genetically engineered rice plants to release more MAs from their roots was associated with enhanced growth and increased grain yield under Fe-deficient soil conditions (Takahashi et al., 2001).

Currently, the genetic variation for tolerance to Fe deficiency within Strategy I and II plants is being exploited by using classical and modern breeding tools to develop novel genotypes with high genetic ability to induce adaptive root responses to Fe deficiency. Therefore, in screening of genotypes for tolerance to Fe deficiency, root mechanisms are increasingly considered to be a reliable selection parameters (Ellsworth et al., 1998; Jolley et al., 1996; Romera et al., 1991). Studies concerning breeding for tolerance to Fe deficiency are becoming numerous with the recent developments in molecular marker technology (Fairbanks, 2000; Lin et al., 2000). The past studies concerning the characterization of Fe deficiency tolerance in plants mostly focussed on physiological and biochemical characterization of root mechanisms. In recent years there is an increasing trend to characterize localisation, identification and isolation of genes determining tolerance to Fe deficiency (see below).

Like Fe deficiency, Zn deficiency is widespread throughout the world and occurs in nearly all coun-
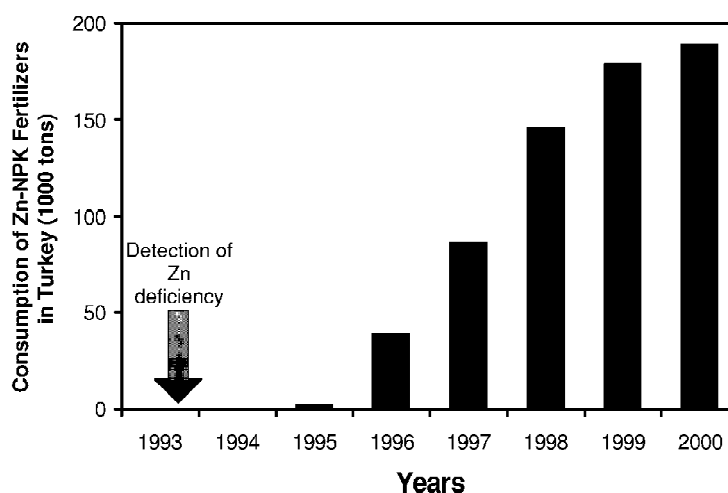
Figure 5. Consumption of Zn-containing NP and NPK fertilizers in Turkey (Cakmak et al., 1999a; and unpublished results of the Turkish Fertilizer Producer Association, 2001).

tries. Based on analysis of 298 soil samples from different countries Zn deficiency has been found to be the most widespread micronutrient deficiency world-wide (Sillanpää, 1990; Sillanpää and Vlek, 1985). According to Graham and Welch (1996), nearly 50% of the soils cultivated for cereal production globally have low levels of plant available Zn. In Turkey, Zn deficiency is the most widespread micronutrient deficiency: the half of all cultivated soils (28 million hectares) has Zn deficiency (Eyupoglu et al., 1993). In Central Anatolia, the major wheat growing area of Turkey, increases in grain yield by soil application of Zn varied between 5 and 554% depending on location and plant available concentration of Zn in soils (Cakmak et al., 1996a). Large increases in grain yield by Zn applications were also demonstrated in Australia (Graham et al., 1992) and India (Tandon, 1995, 1998).

The critical importance of Zn deficiency for wheat production in Turkey has been shown first in 1994 in the framework of a large-scale project supported by NATO-Science for Stability Program (Cakmak et al., 1999a; Kalayci et al., 1999). Before 1994 there was no Zn-containing mix (NPK) fertilizer in Turkey. The spectacular increases in grain yield by Zn fertilization evoked a growing interest in the project results among farmers and fertilizer companies. In 1995 the first Zn-containing NPK fertilizer was produced in the amount of 2000 tons, and this amount has increased progressively and reached 189 000 tons in 2000 (Figure 5; Cakmak et al. 1999a, and unpublished results of the Turkish Fertilizer Producer Association). This is an excellent example showing the importance of

diagnosis of micronutrient status of plants and soils in sustaining high yields in crops. In the future, special attention should be paid to the micronutrient status of plants and soils to ensure further increases in crop production and sustained soil fertility, especially in developing countries.

Cereal species and genotypes of a given cereal species differ greatly in their response to Zn deficiency and Zn fertilization. Tolerance to Zn deficiency was found to decline in the order rye>triticale>barley>bread wheat>oat >durum wheat (Cakmak et al., 1998; Ekiz et al., 1998). Among wheat species, durum wheats are particularly sensitive to Zn deficiency, and this high sensitivity is possibly related to a poor capacity of durum wheat to synthesis and release MAs from roots (Cakmak et al., 1996b, 1998; Rengel et al., 1998). Under Zn-deficient conditions, concentrations of MAs in both root extracts and exudates were much lower in durum than in bread wheat cultivars (Table 4).

Differences in tolerance to Zn deficiency between sorghum, wheat and corn correlated well with the amounts of MAs released from roots under Zn deficiency (Hopkins et al., 1998). In the case of bread wheat cultivars differing markedly in tolerance to Zn deficiency, the release rate of MAs from roots was, however, not related well to the differences in tolerance to Zn deficiency (Cakmak et al., 1998; Erenoglu et al., 1996). Apparently, besides release of MAs, other mechanisms are also involved in tolerance to Zn deficiency tolerance, such as root uptake and root-to-

Table 4. Effect of Zn supply (+Zn = 1 μM, −Zn = 0) on the rate of phytosiderophore (PS) release from roots and DMA (2′-deoxymugineic acid) concentration in roots of Zn-efficient bread wheat cultivar Kirac and Zn-inefficient durum wheat cultivar Kiziltan grown for 14 days in nutrient solution (from Cakmak et al., 1996b)

Cultivars	PS release from roots (μmol g^{-1} root dry wt)		PS concentration in root (μmol DMA g^{-1} root fresh wt)	
	−Zn	+Zn	−Zn	+Zn
Kirac (Zn-efficient)	8.7	0.8	2.76	0.15
Kiziltan (Zn-inefficient)	0.8	0.9	0.65	0.31

shoot transport rates of Zn and internal Zn utilisation (Cakmak et al., 1999a; Rengel, 1999, 2001).

In view of global widespread occurrence of Zn deficiency, development and release of Zn deficiency tolerant genotypes is a relevant topic. Existence of substantial genotypic variation in tolerance to Zn deficiency can be exploited to enhance tolerance. Little information is available on how tolerance is inherited. In future, an intensive research activity should be carried out on this subject. Identification of molecular markers for the genes affecting Zn deficiency tolerance and their mapping within the chromosomes would greatly stimulate breeding activities for Zn deficiency tolerance. By using wheat–rye translocation, substitution and addition lines in pot experiments it has been shown that the rye chromosomes 1R, 2R and 7R most likely carry genes enhancing tolerance to Zn deficiency (Cakmak et al., 1997; Schlegel et al., 1998), with genes on 1RS and 7RS being most effective (Schlegel and Cakmak, 1997).

Recently, differences in tolerance to Zn deficiency were studied in wild and primitive wheats, as these species are increasingly used as a genetic material for improving modern wheats for different traits. For example, *Aegilops tauschii* (DD), the source of the D genome in bread wheat (BBAADD), and *Triticum monococcum* (AA) are being used in such programs. Interestingly, many of the wild wheats and *Aegilops* species that exhibited very high tolerance to Zn deficiency originated from Turkey (Harlan, 1981; Nesbitt and Samuel, 1998). Since Turkey is among the countries having the most severe Zn-deficient soils in the world (Cakmak et., 1996a, 1999a; Sillanpää, 1990), it is mostly likely that these wild wheats and wild relatives of wheat possess an exceptional ability to tolerate Zn deficiency in soils. They can be exploited in breeding wheats for high tolerance to Zn deficiency. This suggestion is supported by our recent results:

addition of the whole D genome from *Ae. tauschii* (DD) or whole A genome from *Tr. monococcum* (AA) to durum wheat (BBAA) very significantly enhanced the tolerance of the synthetic wheats to Zn deficiency (Cakmak et al., 1999b). It seems likely that the genes contributing to high tolerance to Zn deficiency tolerance are located on the A and D genomes. Future research should focus on exploitation of the selected wild and primitive wheats and *Aegilops* species to improve modern wheats in terms of increased tolerance to Zn deficiency.

Enhancing iron and zinc concentrations in seeds

As is the case with plants and soils, Fe and Zn deficiencies are also the most widespread micronutrient deficiencies in humans. These deficiencies affect more than 3 billion people worldwide causing serious health and productivity problems for various population groups, especially among resource-poor women, infants and children (Graham et al., 2001; Welch and Graham, 1999). These deficiencies are particularly widespread in developing countries where diets are rich in cereal-based foods with low concentration of bioavailabable Zn and Fe. Major health consequences of Zn and Fe deficiencies include retardation of growth, anemia and impaired immune functions, diminished intellectual development and retarded sexual maturation (Shrimpton, 1993; Yip, 1994). The problem with Zn and Fe deficiency has been intensified with the increased cultivation of high-yielding cultivars of the 'Green Revolution' cereals whose grains contain low concentrations of Zn and Fe, that are rich in compounds which limit the bioavailability of Zn and Fe to humans (Welch and Graham, 1999). Increasing the concentrations of Zn and Fe in cereal grains is a high priority research task, and will greatly contribute to the alleviation of micronutrient deficiencies in

human populations worldwide. Enhancements in concentration of micronutrients, especially Zn, also result in several positive consequences for crop production, such as improvements in seedling vigour, pathogen resistance, competition against weeds and, finally, an enhanced yields (Graham and Rengel, 1993; Rengel and Graham, 1995).

One important strategy to increase micronutrient concentrations in grains is fertilization of plants via soils or foliar applications. Depending on the plant species, soil application of Zn to soil can increase Zn concentration of plants by as much as 2–3-fold (Rengel et al., 1999). However, even with very high Zn fertilization rates, the Zn concentration in wheat grain does not show correspondingly high increases from Zn fertilization. Furthermore, the same applies to Fe as well. Possibly, because of highly limited phloem mobility of Fe, soil or foliar applications of Fe remain ineffective in increasing Fe concentrations in grains (Gupta, 1991). Interestingly, as has been shown in field experiments in Central Anatolia and Australia, Zn deficiency in wheat can easily be corrected, and yield maximized by broadcast application of Zn fertilizers; however, broadcast application of Zn is not very effective in increasing Zn concentrations in grains up to desired levels to meet human requirements (Graham et al., 1992; Yilmaz et al., 1997). Under Zn-deficient soil conditions, the highest concentrations of Zn in grain could be obtained by a combined soil and foliar application of Zn (Table 5). In most cases, Zn and Fe concentrations in grain do not increase to the desired levels, even by using high rates of fertilizer applications (Rengel et al., 1999). There is also no direct economic motivation for farmers to improve the nutritional quality of grains alone by fertilization. However, as indicated above, if this type of fertilizer improves crop yields as well as nutritional quality, farmers would be likely to adopt such practices. In addition, most of the research conducted on the micronutrient nutrition of plants deals with correcting deficiencies and with improving grain yield. By contrast, research on enhancing micronutrient concentrations in grain or other edible parts of plants is very limited. More research activities are, therefore, needed aiming at improving the bioavailable levels of micronutrients in cereal grains.

An alternative approach to increasing Fe and Zn concentrations in grain is to exploit the genetic variation in grain concentrations of these micronutrients within plant genome. Highly significant genetic differences have been reported for both Fe and Zn concen-

Table 5. Effects of different Zn application methods on Zn concentrations in whole shoots sampled at the beginning of stem elongation stage and in mature grains of the bread wheat cultivar Gerek-79 and the durum wheat cultivar Kunduru-1149 grown on a Zn-deficient calcareous soil in Central Anatolia (from Yilmaz et al., 1997)

Zinc application methods	Zinc concentration (mg Zn kg^{-1} dry weight)	
	Gerek-79	Kunduru-1149
Whole shoots		
Control	11	10
Soil	21	20
Seed	14	12
Leaf	68	47
Soil+leaf	82	52
Seed+leaf	93	59
LSD (5%)	15	16
Grain		
Control	9	12
Soil	17	19
Seed	11	10
Leaf	30	20
Soil+leaf	34	35
Seed+leaf	34	25
LSD (5%)	6	6

trations in cereal grains. For example, Peterson et al. (1986) analyzed grains of 27 wheat cultivars from 24 countries grown at six different locations, and showed that grain concentrations of Fe ranged from 26 to 69 and that of Zn from 20 to 53 mg kg^{-1}. However, in that study the environmental factors exerted greater influence on the genotypic variation found for micronutrient concentrations than the genetic factors. In the framework of the Consultative Group on International Agricultural Research (CGIAR) Micronutrient Project (Bouis et al., 2000), using large number of genotypes, a significant variation was demonstrated in grain concentrations of Zn and Fe as reported by Gregorio et al. (2000) for rice, Beebe et al. (2000) for bean, Monasterio and Graham (2000) for wheat and Bänziger and Long (2000) for maize. Presently, the variation in micronutrient concentration found is being exploited in the breeding programs conducted at several CGIAR centres.

Table 6. Concentration and content (total amount) of zinc in seeds of wild diploid (*T. boeoticum*) tetraploid (*T. dicoccoides*) and modern tetraploid (*T. durum*) and hexaploid (*T. aestivum*) wheats (from Cakmak et al., 2000)

Species	n	Concentration (mg kg^{-1})		Content (μg seed^{-1})	
		Mean	Range	Mean	Range
Wild wheats					
T. boeoticum	12	89	45–177	1.3	0.7–3.0
T. dicoccoides	19	91	20–159	3.1	0.5–5.3
Modern wheats					
T. durum	11	31	18–50	1.6	0.6–2.7
T. aestivum	16	27	15–61	1.2	0.6–3.1

n – number of accessions analysed.

Despite the important genetic variations among crop genotypes, average values for Zn and Fe concentrations in cereal grains are still low, especially when compared with legume grains which show higher concentrations of Zn and Fe as well as a wider range of concentrations (Welch and Graham, 1999). As an alternative to cultivated wheats, wild wheats have been recommended as an important source of genetic material for enhancing micronutrient concentrations in grains (Cakmak et al., 2000). In screening of several wild diploid (*Triticum boeoticum*) and wild tetraploid (*Triticum dicoccoides*) wheats, an impressive variation was found in Zn concentrations (Table 6). The variation found for Zn within wild wheats was much greater than the variation within the cultivated wheats. Certain accessions of *T. dicoccoides* also had substantial amounts of Zn per seed, and this was not related to seed size or seed weight (no concentration effects), and could not be shown for other mineral nutrients. These results suggest that the wild wheats, in particular *T. dicoccoides,* represents as a valuable source of genetic diversity for increasing the Zn concentration in grains of cultivated wheats. The studies with wheat–*dicoccoides* substution lines revealed that the genes determining high density of Zn in grains are located on the T. *dicoccoides* chromosomes 6A and 6B (Cakmak et al., 2000). Little is, however, known about the mechanisms responsible for the high accumulation of Zn in *T. dicoccoides* grain. Research investigating the mechanisms of Zn accumulation in the grain of *T. dicoccoides* is particularly important and urgently needed.

Perspectives on the molecular biology of plant nutrition

Contribution of plant nutrition to food security is not confined only to the maintenance of soil fertility and identification and correction of nutrient deficiencies or toxicities by applying mineral fertilizers and organic materials to soils. Selection and characterisation of plant genotypes with enhanced genetic ability to tolerate deficient or excess levels of mineral nutrients and adapt to adverse soil physical and chemical conditions, including soil acidity, salinity and water deficiency, are important research areas for plant nutritionists. The information obtained from this area during the last 20 years has substantially contributed to increased world food production (Gruhn et al., 2000; Loneragan, 1997; Marschner, 1995). Linking the knowledge obtained from plant-nutrition research to molecular biology-based research will result in major progress in the development of genotypes with elevated capacity to adapt to adverse soil conditions. Presently, a number of genes have been isolated and cloned which are involved in root exudation of nutrient-mobilizing or ion-detoxifying organic compounds (i.e., organic acids and phytosiderophores) (Lopez-Bucio et al., 2001; Ma et al., 2001; Richardson et al., 2001; Takahashi et al., 2001) and uptake, transport and accumulation of mineral nutrients such as, NO_3^-, NH_4^+, $H_2PO_4^-$, K^+ and some micronutrients (Crawford and Glass, 1998; Goto et al., 1999; Hirsch and Susman, 1999; Raghothama, 1999, 2000; von Wiren et al., 2000a).

Aluminium toxicity and phosphorus deficiency

Several examples are given below showing the importance of molecular approaches to improving plant growth in soils having mineral nutrient problems. Successful attempts have been made in the past 5 years to develop transgenic plants that produce and release large amounts of organic acids. Organic acids are key compounds involved in the adaptive mechanisms used by plants to tolerate Al-toxic and P-deficient soil conditions (Kochian, 1995; Lopez-Bucio et al., 2000; Marschner, 1995). To test whether or not increased biosynthesis and root exudation of citrate improves tolerance of plants to Al toxicity and P deficiency, Luis Herrera-Estrella's research group developed transgenic tobacco plants overexpressing a citrate synthase gene from *Pseudomonas aeruginosa* (de la Fuente et al., 1997; Lopez-Bucio et al., 2000). Overexpression of citrate synthase enzyme in tobacco

plants enhanced citrate concentration in root tissue by as much as 10-fold greater than the control plants. The transgenic plants also increased citrate efflux from roots by as much as 4-fold over the control plants. These increases are associated with elevated tolerance to Al toxicity demonstrated by less Al inhibition of both root growth and root hair formation (de la Fuente et al., 1997). Recently, the same research group demonstrated that the transgenic tobacco plants were also highly tolerant to low P supply in an alkaline soil. The increased tolerance of these transgenic plants to P-deficient soil conditions was attributed to enhanced solubilisation of sparingly soluble calcium phosphates in soil by the increased citrate efflux from roots (Lopez-Bucio et al., 2000). Similar results were reported by Koyama et al. (2000) using transgenic *Arabidopsis thaliana* overexpressing a mitochondrial citrate synthase enzyme from carrot (Koyama et al., 2000). In the latter experiment the transgenic *Arabidopsis* plants having an elevated citrate efflux from roots had a greater capacity to absorb P from soil containing Al-phosphates. The above studies convincingly demonstrate that enhanced synthesis and root exudation of organic acids is a decisive step in developing high tolerance to both P deficiency and Al toxicity in soils. However, in a very recent study, Delhaize et al. (2001) using the same transgenic tobacco lines as well as additional transgenic lines, with even with much greater expression of citrate synthetase protein than found by Lopez-Bucio et al. (2000) (up to 100-fold greater level) did not have either increased citrate concentrations in their roots or increased citrate efflux from their roots when compared with the control plants. Consequently, increased Al tolerance reported by de la Fuente et al. (1997) was not confirmed by Delhaize et al. (2001). These controversial results were attributed to a possible sensitivity of the *Pseudomonas aeruginosa* gene to different environmental conditions. Irrespective of such controversial results, manipulation of both biosynthesis and efflux of organic acids to the external solution by roots would be a key genetic engineering approach in developing novel genotypes for sustainable crop production on soils rich in Ca and Al phosphates.

In addition to the secretion of organic acids by roots, the secretion of the enzyme phytase represents an important target for engineering plants for growth on P-limited soils. In cultivated soils most of the total P exists in the form of organic P (Marschner, 1995). Phytate represents an important proportion of the total organic P in soils (Delal, 1977). To use this source of P, some plants are able to activate extracellular enzymes including both acid phosphatases and phytases. It is believed that plants are not able to efficiently use P from phytate, due to limited ability of plant roots to hydrolyze phytate in the rhizosphere (Hayes et al., 1999). Recently, Richardson et al. (2001) generated transgenic *Arabidopsis* plants expressing a phytase gene from *Aspergillus niger*, and these plants could secrete a substantial amount of this phytase enzyme into their growing medium. Consequently, the transgenic plants showed a greater ability to use P from phytate and grew much better over the control plants. Richardson et al. (2001) suggested that the phytase activity around plant roots is a significant factor in utilizing of P from phytate in soils. Therefore, developing plants that overexpress extracellular phytases and other phosphatases is an important challenge in future.

In improvement of uptake and use efficiency of P in plants, Pi transporters might play a critical role. With the development of P deficiency stress an extensive expression of Pi transporter proteins occurs in root cells, particularly in cells staying in close contact with soil solution, i.e., epidermal cells and root hair cells (Daram et al., 1998; Raghothama, 2000; Smith, 2001). Such preferential expression and localisation of Pi transporter proteins in roots indicate a direct role of Pi transporters in utilisation of soil and fertilizer P. Studies are needed to demonstrate the role of Pi transporters in growth and P uptake of crop plants when grown in soils limited in P supply. In cell culture experiments, expression of a high-affinity Pi transporter gene of *Arabidopsis thaliana* in tobacco cultured cells enhanced biomass production and P uptake capacity of transgenic cells under P-limited conditions (Mitsukawa et al., 1997). Like Pi transporters, many other mineral nutrient transporters have been isolated and functionally characterized in plants, such as nitrate, ammonium and sulphate transporters. Also these transporters are preferentially expressed in root hairs under deficiency of the corresponding nutrients (Hell and Hillebrand, 2001; Smith, 2001; Von Wiren et al., 2000a,b; Williams and Miller, 2001). A major future challenge is to improve crop plants with elevated levels of nutrient transporters to contribute to the nutrient uptake of plants when grown under soil conditions with limited supply of mineral nutrients.

Micronutrient deficiencies

In view of the fact that over 3 billion people suffer

from micronutrient deficiencies (Graham et al., 2001), a considerable amount of research in the 21st century should be devoted to the development of genotypes for enhanced uptake and accumulation of micronutrients in edible plant parts. In this regard, genetic engineering provides excellent possibilities to enhance plant capacity for acquisition of micronutrients from soils. An increased uptake of Zn or Fe might be related to the level of expression of the corresponding transporter proteins located at the plasma membranes of root cells. Iron deficiency-induced expression of transporter proteins are known and characterized in yeast (Stearman et al., 1996) and *Arabidopsis thaliana* (Eide et al., 1996). The transporter protein encoded by the gene *IRTI* is expressed in roots in response to Fe deficiency and is involved in mediating Fe uptake in *Arabidopsis*. Also the uptake of Zn is affected by the expression of transporter proteins. In yeast and *Arabidopsis thaliana* three genes, *ZIP1, ZIP2* and *ZIP3*, encoding Zn transporters, were isolated and functionally characterized (Grotz et al., 1998; Zhao and Eide, 1996a,b). These Zn transporter proteins are expressed in roots under Zn-limited conditions, and involved in high- and low-affinity Zn uptake system. The Fe and Zn transporter genes can be engineered in crop plants to enhance their capacity for uptake and accumulation of Zn and Fe. Such genetically modified plants might contribute greatly to improving both plant and human nutrition on a global scale. However, the present progress in this area is limited and, therefore, an extensive research is needed in the future, particularly on the roles of such transporters in translocation of micronutrients to edible parts of plants, e.g., grains and seeds.

In Strategy I plants (i.e., dicots and non-graminaceous monocots) reduction of Fe(III) to Fe(II) by a ferric reductase enzyme is an obligatory step in uptake of Fe. Therefore, genetic manipulation of this enzyme can be an efficient way to improve Fe nutrition of plants. There is a little work on the molecular characterisation of the inducible plasma membrane ferric chelate reductase enzyme in crop plants. Robinson et al. (1997, 1999) isolated the *FRO2* gene from *Arabidopsis thaliana*. This gene is induced in Fe-deficient roots in response to Fe deficiency, and encodes for the expression of the ferric chelate reductase protein within the plasma membrane. In yeast, two Fe(III) reductase genes, *FRE1* and *FRE2*, have been isolated and cloned (Georgatsou and Alexandraki, 1994; Oki et al. 1999). Samuelsen et al. (1998) studied the role of these *FRE* genes in the Fe nutrition of plants by generating transgenic tobacco plants containing the *FRE1*,

the *FRE2* or both genes. The transgenic lines having the *FRE2* gene, but not the *FRE1* gene, showed higher tolerance to Fe deficiency as a result of greater root reductase activity that caused higher chlorophyll and leaf Fe concentrations in these transformants. These results together with the results of Robinson et al. (1997, 1999) suggested that the *FRE2* and *FR02* genes represent promising candidates to manipulate Strategy I plants to enhance their tolerance to Fe deficiency.

In Strategy II plants ferric reduction is not an obligatory step; these plants take up Fe in the form of Fe(III)–MAs complex (see above). Among the graminaceous species, barley is the most tolerant to Fe deficiency and releases larger amount of MAs from roots, while rice is the most susceptible one and accordingly secretes little MAs (Römheld and Marschner, 1990; Takagi et al., 1984). During the past 5 years, Mori and his research group have published extensively in this area. They have isolated, cloned and characterized some of the crucial genes involved in the biosynthesis of MAs, e.g., nicotianamine synthase (NAS) and nicotianamine aminotransferase (NAAT) (Higuchi et al., 1999, 2001; Takahashi et al., 1999). Kanazawa et al. (1994) demonstrated that the activity of NAAT very closely correlated with the differences between barley and rice in Fe deficiency chlorosis and the release rate of MAs from roots. Recently, Takahashi et al. (2001) generated transgenic rice plants by transferring the NAAT genes from barley into rice. They demonstrated that the transgenic rice plants with overexpression of NAAT genes developed a higher tolerance to Fe deficiency in an alkaline soil than the control (nontransformed) rice plants. The higher tolerance of transgenic rice plants to Fe deficiency was closely associated with the greater levels of NAAT activity, root secretion of MAs and shoot and grain yield. This excellent work by Mori's research group represents a breakthrough in the plant nutrition research, and will result in profound impacts on developing Fe deficiency-tolerant cereals for large areas of Fe-deficient calcareous soils, and may greatly contributing to food security in the world.

The Fe concentration in grain of the transgenic rice developed by Takahashi et al. (2001) was not studied, and it would be interesting to know the levels of bioavailable Fe in the grain of the transgenic rice with respect to their possible contribution to improving the Fe nutrition of people. High Fe concentrations in grains can be achieved by manipulating or adding the genes responsible for the synthesis of the Fe storage proteins (i.e., ferritin), as has been successfully

demonstrated by Goto et al. (1999). Goto et al. (1999) were able to transform rice plants using soybean ferritin genes. Ferritin is a major Fe storage protein in plants. The ferritin genes were expressed in the endosperm of rice by including an endosperm promoter gene. This resulted in about a 3-fold increase in Fe concentration in the grain when compared to the untransformed rice. They speculated that the Fe content in a meal-size portion of the transgenic rice would be sufficient to provide 30–50% of the daily adult Fe requirement. However, the proposed contribution of the Fe present in transgenic rice grain to human nutrition is questioned, because the bioavailability of ferritin-Fe from plant sources to humans is possibly very low (Graham et al., 2001). Nevertheless, the work of Goto et al. (1999) opens an exciting research area to transform various food crops using the same or similar molecular approach and other potential Fe storage proteins or metabolites.

At present, there is very little adaptive research performed under field conditions such as studying the role of the genes affecting synthesis of MAs, organic acids, and storage proteins, in agricultural soils having limited availability or excessive levels of mineral nutrients. This will be an important research priority of plant nutrition in the future. In a recent study, it was shown that the transgenic plants overexpressing ferritin and accumulating high levels of Fe in tissue under controlled environmental conditions were not superior to the non-transformed plants regarding the accumulation of Fe in tissue, when grown in different soil conditions in greenhouse (Vansuyt et al., 2000). This result indicates that integrating molecular biology-based research with the knowledge provided by plant nutrition research is essential to better understand and characterize nutrient uptake and transport in transgenic plants.

Therefore, integrated research between molecular biology and plant nutrition will advance research activities concerning the development of new plant genotypes with (i) greater efficiency for nutrient acquisition from soils or efficient utilization of nutrients within plants, (ii) enhanced adaptation ability to acid or metal-contaminated soils, and (iii) increased seed density of micronutrients and proteins. Progress in these research areas will greatly contribute to the alleviation of food needs and will improve the nutritionally well-being and health of humans.

Conclusions

Plant nutrition-based research activities are indispensable in meeting food security needs in the 21st century (Gruhn et al., 2000; Loneragen, 1997; Marschner, 1995). The fact that at least 60% of the presently cultivated soils globally have several mineral problems, like toxicities of Al, Mn and Na, and deficiencies of N, P, K, S, Fe and Zn, makes plant nutrition-based research a major promising research area needed to meet the demand for massive increases in food production required for the growing world population. One of the high priority objectives of plant-nutrition research will be ensuring a long-term sustainable nutrient management system for crop production, and developing more efficient mineral nutrient uptake by crop plants and improving intra and intercellular use of nutrients without detrimentally affecting the environment. Creation of a synergy between plant nutrition and plant molecular genetics is required to assure a rapid progress towards alleviating food insecurity issues in the 21st century.

Acknowledgements

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