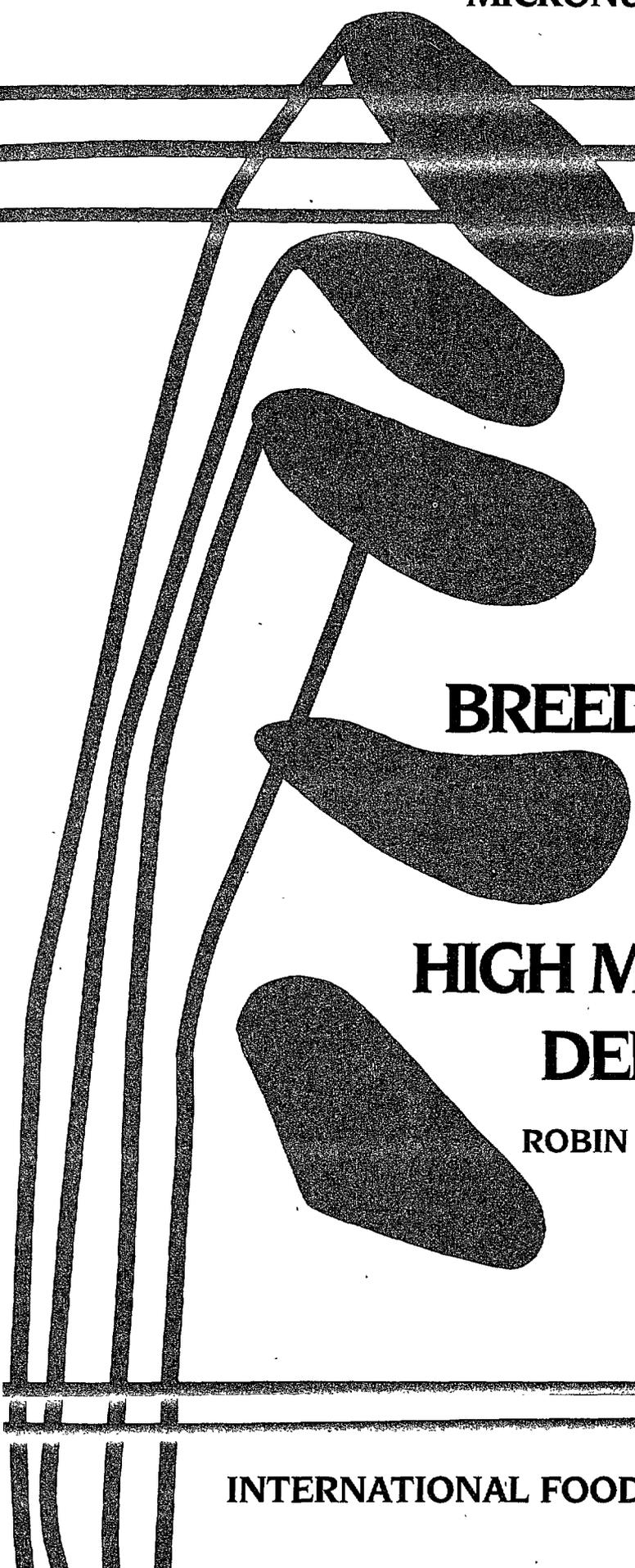


PN-ACH-933

AGRICULTURAL STRATEGIES FOR
MICRONUTRIENTS • WORKING PAPER **3**

A stylized, high-contrast illustration of a plant with several large, rounded leaves and a thick stem, positioned on the left side of the page. The plant is rendered in a dark, textured style against a white background.

**BREEDING FOR
STAPLE FOOD
CROPS WITH
HIGH MICRONUTRIENT
DENSITY**

ROBIN D. GRAHAM AND ROSS M. WELCH

INTERNATIONAL FOOD POLICY RESEARCH INSTITUTE

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Working papers of the International Food Policy Research Institute encompass a wide range of subjects drawn from its research programs. The papers—primarily data analyses, historical descriptions, or case studies—contain information that IFPRI believes may be of interest to others. Working papers undergo informal review but do not necessarily present final research results.

BREEDING FOR STAPLE FOOD CROPS WITH HIGH MICRONUTRIENT DENSITY

Robin D. Graham and Ross M. Welch

Working Papers on Agricultural Strategies for Micronutrients, No. 3

International Food Policy Research Institute
Washington, D.C.
April 1996

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FOREWORD

The International Food Policy Research Institute has been designated to take the lead in coordinating activities related to human nutrition across the 16 centers that constitute the Consultative Group on International Agricultural Research (CGIAR). A large part of that effort is organized around a five-year project initiated in May 1993 with funding from the Office of Health and Nutrition of the U.S. Agency for International Development. The objective of the project is to identify and implement cost-effective alternatives within the CGIAR for increasing micronutrient intakes. Taken together, micronutrient deficiencies affect a far greater number of people in the world than protein-energy malnutrition, with serious consequences for health, cognitive ability, and productivity.

This series of Working Papers on Agricultural Strategies for Micronutrients reports on activities undertaken on all aspects of the project, and so represents views and research findings from several disciplinary perspectives. The project will undertake activities in two broad areas. One component involves a coordinated effort between CGIAR centers and agricultural and nutrition research institutes in developing and developed countries to breed for nutritionally improved staple food crops. The second component, a collaborative effort between CGIAR centers and developing-country social science and nutrition research institutions, will undertake collection and analysis of household survey information to better understand the linkages between agricultural production, household resource allocation, and nutrition outcomes for improved policy formulation.

The first three papers in the series provide perspectives from the human nutrition, household economics, and plant nutrition/plant breeding disciplines. These papers were commissioned for presentation at an organizational workshop convened in Annapolis in 1994 and attended by an interdisciplinary group of persons, from within and outside the CGIAR, involved both in research on micronutrients and in implementing programs to reduce micronutrient malnutrition. Various research directions for the project were discussed. The authors of these three papers were asked to provide (1) a summary of what is known about micronutrients in their respective areas of expertise in a way that could be understood by those not trained in that discipline, (2) their judgments as to what are the significant gaps in knowledge, and (3) their opinions as to how project activities could best contribute to closing these gaps in knowledge and to reducing the micronutrient malnutrition problem.

This third paper is written jointly by Robin D. Graham, professor of plant science at the Waite Agricultural Research Institute, University of Adelaide, Australia, and Ross M. Welch, lead scientist and plant physiologist at the U.S. Department of Agriculture—Agricultural Research Service Plant, Soil, and Nutrition Laboratory in Ithaca, New York, U.S.A., and professor of plant nutrition in the Department of Soil, Crop, and Atmospheric Sciences at Cornell University. Professors Graham and Welch, who represent Australia and the United States, respectively, on the Council of the International Colloquium of Plant Nutrition and the Council of the International Symposium of Genetics and Molecular Biology of Plant Mineral Nutrition, argue in their paper that breeding for trace mineral-dense staple food crops as a strategy to improve human nutrition should improve plant nutrition and plant yields as well.

Also provided in this publication are formal comments on this monograph, which were presented at the organizational workshop by Maria José de Oliveira Zimmermann, a

researcher at the Brazilian Enterprise for Agricultural Research (EMBRAPA)—National Research Center for Rice and Beans (CNPAP), a professor of genetics at Goiás Federal University in Goiania, Brazil, and a member of the Technical Advisory Committee of the CGIAR.

Howarth E. Bouis
Series Editor

ACKNOWLEDGMENTS

The authors thank Drs. W. A. Norvell and B. McNeill for their valuable comments on the manuscript.

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1. INTRODUCTION

Micronutrient deficiencies affect over 2 billion people worldwide, largely in poorer developing countries. As a result, the world's human nutrition organizations have made fighting "hidden hunger" (that is, micronutrient deficiencies) a high priority (WHO 1992; FAO/WHO 1992). The micronutrients iron, iodine, and vitamin A have been targeted for intervention by the international human nutrition community, because of the immense magnitude of the problem of deficiencies of these micronutrients in the world's poor (WHO 1992; FAO/WHO 1992; Brown 1991). Estimates are that 2 billion people (about one-third of the world's population) are iron deficient with diminished work performance, impaired body temperature regulation, impaired psychomotor development and intellectual performance, detrimental behavioral changes (for example, significantly decreased responsiveness and activity and increased body tension and fearfulness), decreased resistance to infection, and increased susceptibility to lead poisoning (Dallman 1990). Women and children are particularly at risk of iron deficiency because of their elevated requirements for childbearing and for growth. Forty million preschool-aged children are vitamin A deficient (and, of these, about 14 million have clinically detectable eye damage), with increased risk of respiratory diseases and diarrhea leading to increased mortality rates (Sommer 1990). One billion people reside in iodine-deficient regions, with numerous inhabitants of these areas suffering from iodine deficiency disorders (IDD), including goiter, cretinism, lower intelligence quotients, and increased perinatal mortality (Hetzel 1990). Zinc deficiency, thought to be widespread (Gibson 1994), can lead to retarded growth, depressed immune function, anorexia, dermatitis, skeletal abnormalities, diarrhea, alopecia, and increased complications and mortality during childbirth if it is prolonged (ILSI 1990). Furthermore, zinc deficiency in humans has been linked to vitamin A underutilization (Kramer et al. 1993; Udomkesmalee et al. 1990). Even in developed countries, micronutrient deficiencies affect significant numbers of people (for example, 14 percent of all premenopausal women in the United States are iron deficient [USDHHS/USDA 1989]). "Taken together, micronutrient deficiencies (of iron, vitamin A, and iodine) affect a far greater number of people in the world than protein-energy malnutrition" (Chandra 1990).

World population, currently about 5.4 billion people, will double by the year 2050 if the earth's present population growth rate (that is, 1.7 percent, or 92 million people, per year) does not ebb significantly (Doughty 1993). Providing adequate sustenance to these additional masses will require that modern agricultural technologies almost triple food crop productivity by this time (Salunkhe and Deshpande 1991). But simply producing enough food energy to maintain the earth's population will not be enough, as implied by the statistics on micronutrient malnutrition cited above. Even if energy requirements are met, billions of malnourished, poor people will continue to live in poor health, with concomitant lower productivity and an inferior quality of life. Sustainable ways must be found to produce nutritious foods that meet minimum daily nutritional requirements, particularly for bioavailable forms of micronutrients, that is, essential trace minerals and vitamins.

Intervention programs, including supplementation, fortification, and education, have been successful in reducing micronutrient malnutrition in specific situations and will be needed in the future, but for most micronutrients such programs are expensive, incur ongoing annual

expenditures, and are unlikely to reach all of those at risk. Moreover, these intervention programs have often been suspended for economic, political, and logistical reasons (Gibson 1994). Lower-cost, long-term solutions to ending micronutrient malnutrition must be developed to complement existing interventions.

The poor already eat food staples in relatively large quantities. Can staple food production systems be changed to improve their micronutrient quality? More research is needed to answer this question definitively, but some information is available that gives hope that this can be accomplished. This paper presents a strategy that has the potential to reduce global micronutrient malnutrition substantially at low cost by breeding for micronutrient-dense staple food crops, a strategy that will at the same time boost agricultural productivity in an environmentally beneficial way.

This paper will argue that breeding for trace mineral-dense seeds improves plant nutrition, reduces input costs, and improves yields and profits on trace mineral-deficient soils. Results from Australia and elsewhere show that, where the soil is deficient in a particular micronutrient, seeds containing more of that nutrient have better germination, better seedling vigor, more resistance to infection during the vulnerable seedling stage, or all three. These benefits to crop establishment can, in turn, result in higher grain yields. Thus, priorities for human and plant nutrition may often coincide, and it can be expected that new cultivars with higher contents of micronutrient metals will have an agronomic advantage to ensure they are competitive in the marketplace.

Tolerance to micronutrient-deficient soils, as a genetic trait, is usually termed "micronutrient efficiency." It is defined for the purposes of this paper as applying to a genotype or phenotype that is better adapted to, or yields more in, a micronutrient-deficient soil than can an average cultivar of the species (Graham 1984). This is an agronomic definition that can be measured in terms of grain yield, and does not imply a mechanism. Nevertheless, nearly all micronutrient efficiency traits so far studied arise from a superior ability to extract the limiting micronutrient from soil, rather than a plant's capacity to survive on less of that micronutrient.

As an example, growing zinc-efficient plants on zinc-deficient soils represents the strategy of "tailoring the plant to fit the soil" in contrast to the older strategy of "tailoring the soil to fit the plant" (terminology according to Foy [1983]). The significance of such an approach should be assessed, bearing in mind that, of all agricultural innovations, farmers most readily accept new cultivars because these can improve yields without necessitating much change in agronomic practice (Little 1988).

However, the relatively slow progress in deciphering the genetics, physiology, and biochemistry of plant micronutrient efficiency has hampered development of genotypes of superior micronutrient metal efficiency. Currently, a combination of both strategies mentioned (for example, growing more micronutrient metal-efficient genotypes while fertilizing them less frequently and/or with smaller quantities of micronutrient metals) may be the most realistic approach to cropping on micronutrient-deficient soils.

Growing micronutrient metal-efficient genotypes of crops on micronutrient metal-deficient soils is an environmentally friendly approach that could reduce land degradation by diminishing the use of machinery for the application of chemicals like fertilizers and fungicides to agricultural land (see Thongbai et al. 1993). The danger of exhausting soil micronutrient resources ("land mining") would be minimal because the total supply of micronutrients in soils is sufficient

for hundreds of years of sustainable cropping by new, efficient genotypes, which are able to gain access to micronutrient pools generally considered unavailable for plant uptake.

This paper presents scientific evidence on the range of genetic variability known to be available for exploitation by future breeding programs, the agronomic advantages associated with micronutrient-dense seeds, the biochemistry and physiology of the plant transport mechanisms involved, and possible breeding strategies. Issues such as establishing the nutritional benefits of incremental changes in the micronutrient content of seeds, the significance of dietary zinc and vitamin A interactions, the important substances promoting micronutrient bioavailability in meals, maintaining balanced micronutrient composition, and the difficulties of devising efficient plant screening techniques for identifying micronutrient-dense genotypes are also discussed.

The micronutrients covered in this review include iron, zinc, and vitamin A. While iodine deficiency is also considered a world health issue, it was excluded from this discussion because dietary iodine supplementation programs, through the use of iodized salt, have proven effective in many countries. These programs are inexpensive and reach many of the populations most at risk (Hetzel 1990). Thus, breeding for iodine-dense staple grain crops is not as critical as for iron, zinc, and provitamin A. While zinc is not currently targeted by the World Health Organization/Food and Agriculture Organization of the United Nations (WHO/FAO) for nutritional intervention programs in poorer nations, it has been included in this review because of growing concern that it is also a major health problem in those regions (Gibson 1994). Other micronutrients (such as selenium, copper, vitamin E, and vitamin C) are also of increasing international concern for human nutrition but are not covered in this review because too little is currently known about the magnitude of deficiencies of these micronutrients in developing countries.

The inclusion of zinc in this review is important for reasons other than nutritional quality. In China and India, where the largest soil surveys in the developing world have been conducted, approximately 50 percent of the arable land used for cereal production is low in zinc or other of the plant micronutrient metals. Breeding cereals with enhanced zinc efficiency is perceived as a worthwhile objective that can decrease fertilizer requirements, improve seedling vigor, overcome yield losses from unrecognized and subclinical deficiencies, increase resistance to pathogens, and enhance the yield and nutritional quality of grain for human consumption. Zinc-efficient cultivars of wheat and maize also may be necessary to permit full exploitation of stored water from nutritionally inhospitable subsoils. Such a response may permit a breakthrough in the yield potential of such semi-arid, alkaline-soil areas and may also help to lessen the expansion rate of areas of dryland salinity.

The crops addressed specifically in this paper are wheat, rice, and maize, which together represent 54 percent of global food production and which, for the poor in developing countries, provide well over one-half of total calorie intake. Staple foods provide perhaps 50 percent or more of the total intake of iron and zinc in the diets of the poor. Thus, increasing the density of these trace minerals in staple foods and improving their bioavailability would be of significant benefit to the well-being of individuals largely dependent on these cereals for sustenance. For example, targeting irrigated lowland rice could increase the micronutrient density of 75 percent of all rice production, thereby improving the nutrition of essentially all of the rice-eating, urban poor in a significant number of target countries. For reasons to be outlined later, it should be relatively easy to genetically manipulate lowland rice cultivars for increased grain-iron content.

2. PLANT FOOD MICRONUTRIENTS AND HUMAN NUTRITION

Why do vast numbers of people suffer from “hidden hunger” in the poorer countries, even when adequate amounts of food are made available to them? Undoubtedly, the answer is linked primarily to insufficient diversity in their diets because of meager household incomes. Impoverished citizens often only have enough monetary resources to purchase staple foods to meet their energy requirements and stave off starvation. Globally, ways to significantly increase income in poor families, enabling them to purchase enough diverse foods to meet their nutritional requirements, have not been found. Therefore, the attempt to improve the micronutrient composition of their staple plant foods in sustainable ways must continue.

On a global basis, 88 percent of the caloric intake ultimately comes from plant food sources (Salunkhe and Deshpande 1991). Worldwide, cereal grains comprise 54 percent of the edible dry matter consumed. The primary staple cereals eaten by impoverished people are wheat (*Triticum aestivium* L.), rice (*Oryza sativa* L.), and maize (*Zea mays* L.)—foods that contain only meager available amounts of several micronutrients, including iron, zinc, and provitamin A carotenoids (Salunkhe and Deshpande 1991; Underwood 1971). Understanding why these foods are relatively poor sources of these micronutrients requires knowledge of daily dietary requirements (recommended dietary allowances [RDAs], or safe intakes) for these nutrients, along with some knowledge of the factors that influence their absorption from the digestive tract and utilization in the body.

Micronutrient bioavailability and associated physiological and dietary modifying factors, such as overall micronutrient status and the presence of dietary antinutrients (substances in foods that interfere with the absorption or use of nutrients) and dietary promoters (substances in foods that increase the absorption or use of nutrients) in meals as eaten, are of primary concern (National Research Council 1989). Often, it is difficult to make definitive conclusions concerning micronutrient bioavailability for diets composed of numerous food ingredients. Some information is available, however, and rough estimates have been reported.

DIETARY REQUIREMENTS

Estimating the human dietary requirements of iron, zinc, and vitamin A is beset with uncertainties. Homeostatic mechanisms are known to regulate the bodily absorption and excretion of micronutrients. Such mechanisms can maintain an individual in micronutrient balance even though daily dietary intake may be less than recommended as safe for a healthy balanced diet. However, extended periods of suboptimal micronutrient intake have dire consequences, including decreased resistance to infection and even increased mortality among infants and young children (ILSI 1990; Chandra 1990; Dallman 1990; Hetzel 1990; Olson 1990; Sommer 1990).

The amount of iron and zinc absorbed and used by a person can vary from less than 1 percent to more than 50 percent of the amount consumed and depends on a number of factors, including the types of food eaten, nutritional status, body demands, and general health. Homeostatic mechanisms in the intestinal mucosal cells control absorption, endogenous excretion, and body balance for these nutrients. Iron- or zinc-deficient people, or

individuals with low body stores of iron and zinc (typically women, infants, and children), absorb and retain iron and zinc with much greater efficiency than do those with adequate iron and zinc stores (for example, men and postmenopausal women) (ILSI 1990; Dallman 1990).

This paper focuses on plant food micronutrient nutritional quality and, therefore, does not attempt to adjudicate the complex issues and arguments involved in determining micronutrient bioavailability in humans. However, many experiments conducted to determine iron or zinc bioavailability in plant foods to human subjects are of little value to plant scientists interested in identifying potential antinutrients in plant foods, because the experiments did not select marginally iron- or zinc-deficient experimental subjects. If iron- or zinc-adequate individuals are used to determine the effect of a plant food substance on iron or zinc bioavailability, it is difficult to discern if the effect is a direct result of the substance or, instead, is due to the subject's homeostatic mechanisms controlling absorption or endogenous excretion processes in the digestive tract. Most likely, different results would be obtained, depending on the individual's nutritional status with respect to the micronutrients under study.

Table 1 lists the RDAs for iron, zinc, and vitamin A, as reported by the Food and Nutrition Board Subcommittee, National Research Council (NRC), U.S. National Academy of Sciences. Such safe intake values are only crude estimates, and significant changes in these values could either greatly enhance or negate concern over the adequacy of these nutrients in human diets in various developed countries. More research is necessary before more accurate estimates of micronutrient bioavailability are forthcoming for people consuming a wide variety of foods.

The Food and Agriculture Organization and the World Health Organization of the United Nations have recommended daily intakes of zinc that differ by a factor of four, depending on the proportion of dietary zinc obtained from animal sources (that is, 22 milligrams of zinc per day for people deriving less than 10 percent of their energy from animal sources, 11 milligrams of zinc per day if between 10 and 20 percent, and 5.5 milligrams of zinc per day if between 20 and 40 percent) (Mertz 1987).

Table 1—Recommended daily dietary allowances of iron, zinc, and vitamin A for the United States

Population Group	Age Range (years)	Iron ^a (milligrams per day)	Zinc ^b (milligrams per day)	Vitamin A (RE ^c)
Infants	0–1	6	5	375
Children	1–10	10–12	10	400–700
Adult males	11–51	10	15	1,000
Adult females	11–51	15	12	800
Pregnant females	All ages	30	15	800
Lactating females	First six months	15	19	1,300
	Second six months	15	16	1,200

Source: National Research Council 1989.

^aAssumes that 12.5 percent of dietary iron is bioavailable.

^bAssumes that 20 percent of dietary zinc is bioavailable.

^cRetinol equivalent; 1 retinol equivalent = 1 microgram of retinol, 6 micrograms of beta-carotene, or 12 micrograms of mixed provitamin A carotenoids from plant food sources. The bioavailability of retinol is assumed to be high (that is, more than 80 percent) for people consuming at least 10 grams of fat in their diet (Olson 1990).

Globally, nonheme iron accounts for 85 percent or more of dietary iron, but utilization of nonheme iron is most affected by plant food substances that reduce iron absorption in the gut. Heme forms of iron (the hemoglobin and myoglobin in meats, poultry, and fish) make up a much smaller portion but play an important role, because they are absorbed much more efficiently than nonheme iron and are much less affected by dietary substances that interfere with iron absorption. In the United States adult males absorb approximately 6 percent of their total dietary iron ingested, and menstruating females absorb about 13 percent. People who eat diets rich in cereals and legumes and low in animal proteins and ascorbic acid (vitamin C)—diets that are typical of many of the people in developing countries—absorb approximately 5 percent of their dietary iron. People who eat varied diets that are rich in meat and ascorbic acid may absorb as much as 15 percent of their dietary iron. In the United States, an overall figure of 12.5 percent has been reported as reasonable (Dallman 1990).

Because the amount of iron required also varies with the amount of dietary calories obtained from animal meats, the FAO and WHO recommend that people receiving iron from meals in which more than 25 percent of their calories come from animal meats need less iron in their diets (for example, healthy menstruating females, 14 milligrams per day; healthy adult males, 5 milligrams per day). However, people who obtain less than 10 percent of their calories from animal foods may require somewhat more total dietary iron (for example, healthy menstruating females, 28 milligrams per day; healthy adult males, 9 milligrams per day) (Special Report 1975).

The FAO and WHO's recommendations for iron make some allowances for individuals whose iron needs are greater than average (based on a coefficient of variation of 15 percent). A factor of 1.25 (times actual need) was used to calculate the recommended dietary requirement. Thus the recommended levels would meet the iron needs of most of the world's population. However, these RDAs may still not provide enough iron to meet the requirements of women with menstrual blood losses in excess of 80 milliliters per month. Such women are at risk of developing iron deficiency even though they consume sound diets; they may require supplemental iron to meet their higher demands (Dallman 1990).

FACTORS AFFECTING MICRONUTRIENT BIOAVAILABILITY

Many staple plant foods are considered poor sources of micronutrients because they contain either inadequate amounts of some micronutrients or significant concentrations of antinutrients—that is, substances that can interfere with the absorption or utilization (bioavailability) of micronutrients in the body (Ashmead and Christy 1985; Burk and Solomons 1985; Fairweather-Tait 1992; Harland 1989). Table 2 lists some of the major antinutrients identified in plant foods. In addition, foods derived from animal protein sources contain promoters of micronutrient-metal bioavailability. The negative effects of many plant food antinutrients on the bioavailability of some micronutrient metals (for example, iron and zinc) can be largely eliminated if meals contain adequate levels of these promoter substances (Ashmead and Christy 1985; Harland 1989).

Table 3 lists some of the known promoters of iron, zinc, and vitamin A bioavailability. Not all promoters have been identified, however, and further research is needed to identify them and their mode of action in the gastrointestinal tract. With such knowledge, it should be possible to determine if these substances play a major role in correcting the negative influence of antinutrients in plant food products on micronutrient bioavailability.

Table 2—Antinutrients in plant foods reported to reduce the bioavailability of iron, zinc, or both

Antinutrient	Major Dietary Sources
Phytic acid or phytin ^a	Whole legume seeds and cereal grains
Fiber (for example, cellulose, hemicellulose, lignin, cutin, suberin) ^b	Whole cereal grain products (for example, wheat, rice, maize, oats, barley)
Tannins and polyphenols	Tea, coffee, beans, sorghum
Oxalic acid ^c	Spinach leaves, rhubarb
Hemagglutinins (for example, lectins)	Most legumes and wheat
Heavy metals (for example, cadmium, mercury, lead, gold) ^d	Plant foods obtained from crops grown on metal-polluted soils (for example, cadmium in rice)

Sources: Ashmead and Christy 1985; Bodwell 1987; Burk and Solomons 1985; Fairweather-Tait 1992; Harland 1989; Morris 1983; and Welch and House 1983.

Note: These antinutrients are reported to apply to humans eating meals containing complex food sources under some circumstances.

^aWhile sodium salts of phytic acid, when added to purified diets, inhibit iron and zinc bioavailability to humans, the naturally occurring form, phytin, may actually increase iron bioavailability to humans under some circumstances (such as monoferric phytate in whole wheat products when fed to humans) (Morris 1986).

^bNot all plant-fiber sources inhibit iron and zinc bioavailability to humans. Some sources of fiber (for example, sugar beet fiber) can actually enhance zinc bioavailability (Fairweather-Tait and Wright 1990).

^cWhile oxalic acid has been shown to reduce calcium bioavailability to humans, it has not been shown to inhibit iron and zinc bioavailability. Thus, it should not be considered an antinutrient for iron or zinc bioavailability, even though it is treated as such for these micronutrients in most modern textbooks on nutrition (Welch and House 1983).

^dThese substances have been studied only in animal models, not yet in humans.

Table 3—Promoters reported to enhance the bioavailability of iron, zinc, and vitamin A

Promoter	Micronutrient Affected	Major Dietary Sources
Certain organic acids (for example, ascorbic acid or vitamin C, fumaric acid, malic acid, citric acid)	Iron and/or zinc	Fresh fruits and vegetables
Certain amino acids (for example, methionine, cysteine, histidine, lysine)	Iron and/or zinc	Animal meats (for example, beef, pork, fish)
Long-chain fatty acids (for example, palmitic acid)	Zinc	Human breast milk
Fats and lipids	Vitamin A	Animal products
Vitamin E	Vitamin A	Vegetable oils

Sources: Chandra 1990; Burk and Solomons 1985; Erdman and Ponerros-Schneier 1989; Harland 1989; Morris 1983; National Research Council 1989; Torre, Rodriguez, and Saura-Calixto 1991; and Welch and House 1983.

Note: These promoters are reported to apply to humans eating meals containing complex food sources under some circumstances.

Iron

Antinutrients in various plant foods thought to reduce iron bioavailability include phytic acid (myoinositol hexaphosphoric acid), tannins and other polyphenols, and some types of plant fiber. Many nutrition texts also list oxalic acid as an antinutrient inhibiting iron bioavailability, but some reports indicate that iron oxalate is a good dietary source of iron and that oxalic acid alone does not reduce iron bioavailability (Welch and House 1983). Other studies, however, suggest that interactions of oxalic acid with other dietary constituents may inhibit iron absorption. One such constituent is dietary fiber, which can form fiber-iron-oxalate complexes that are not readily destroyed in the digestive tract (Harland 1989).

Phytic acid is a major metabolite in all mature seeds and grains and is the primary storage form of phosphorus in these plant components (Lott 1984). The effects of phytic acid, or its naturally occurring form, phytin (a mixed magnesium and potassium salt of phytic acid), on iron bioavailability has received the most attention from nutritional scientists, because the compound forms insoluble precipitates with several divalent metal cations (including Ca^{2+} , Fe^{3+} , and Zn^{2+}) in vitro. However, many of the experimental data concerning the negative effects of phytin in seeds and grains on iron bioavailability to humans are confusing and contradictory (Bodwell 1987; Erdman and Poneros-Schneier 1989; Morris 1983; Morris 1986; Torre, Rodriguez, and Saura-Calixto 1991). Indeed, monoferric phytate (a soluble salt of phytic acid), the major fraction of iron in wheat bran, is readily available to humans (Morris 1983). Researchers also have recognized that, besides phytate, other interacting dietary factors (possibly antinutrients, such as fiber, and promoter substances, such as ascorbic acid) both negatively and positively affect iron bioavailability to humans eating whole cereal grain and legume seeds in mixed diets (Bodwell 1987; White 1992). More research is required to delineate the mechanisms of phytate inhibition of iron bioavailability to humans consuming varied diets, the interaction of phytate with various dietary constituents, and subsequent effects on iron bioavailability.

Some beverages derived from plant sources, such as tea and coffee, also contain substances that inhibit iron absorption in humans. Tannins and other polyphenolic substances, which also occur at high levels in some forms of plant food, such as sorghum (*Sorghum bicolor* [L.] Moench.) and bean (*Phaseolus vulgaris* L.) seed coats, are thought to be responsible (Harland 1989; Morris 1983; Radhakrishnan and Sivaprasad 1980).

Tannins and other polyphenolic compounds (such as flavonols, chalcones, and anthocyanins) that occur in significant amounts in many plant foods, including several cereal and bean crops, have also been implicated in reduced iron bioavailability. Some condensed tannins are known to depress growth and feed efficiency ratio in animals and increase the amount of feed required for unit weight gain. Some chemical forms of tannins can also damage the mucosal lining of the gut, alter excretion of certain cations, and increase the excretion of proteins and essential amino acids. The deleterious effects of tannins are thought to be primarily the result of their interaction with dietary proteins (Salunkhe and Deshpande 1991). Most of the research on iron-tannin interactions relates to heme iron absorption, but the tannins in tea and coffee beverages have been shown to bind nonheme iron by forming insoluble iron tannates within the lumen of the human intestine (Harland 1989; Morris 1983; Thompson 1988). However, some chemical forms of tannins and polyphenols can also play an important beneficial role for both plants and humans.

Plant fiber is another factor thought to inhibit iron bioavailability to humans. Generally, plant fiber is composed of numerous plant cell wall constituents that are not readily hydrolyzed by intestinal digestive enzymes. These substances include not only polysaccharides and lignin, but also cutin, suberin, β -glucans and galactomannans (gums), water soluble polysaccharides (mucilages), polyphenols and other phenolic compounds, and starch and proteins that are resistant to enzymic degradation (Torre, Rodriguez, and Saura-Calixto 1991). Some of these constituents of dietary fiber have the capacity to tightly bind polyvalent cations such as Fe^{3+} . Additionally, large amounts of fiber can dilute intestinal contents and increase the transit rate of digesta past mucosal cell absorption sites in the intestine. These properties of fiber can each reduce iron bioavailability to humans under some circumstances.

Fiber from diverse plant sources is known to have different chemical structures and different polyvalent cation binding capacities. Because of this complication, it is almost impossible to draw definite general conclusions concerning the relationship between plant food fiber and iron bioavailability. It can only be concluded that plant fiber can affect iron bioavailability under some, but not all, circumstances. Clearly, the benefits of dietary fiber outweigh its potential negative effects on iron bioavailability, based on the currently available literature.

Zinc

The factors affecting the bioavailability of zinc in plant foods include the same ones discussed for iron: phytic acid, fiber, tannins, oxalic acid, hemagglutinins, and heavy metals (Hambidge, Casey, and Krebs 1986; Welch 1993; and Welch and House 1983 review the literature concerning these factors).

As with iron, phytic acid forms insoluble precipitates with zinc when added to purified diets, and sodium phytate has been shown to decrease zinc absorption in a number of monogastric animal species, including humans (Hambidge, Casey, and Krebs 1986; Kirchgessner and Weigand 1982; Prozialeck and Niewenhuis 1991; Welch and House 1983). High dietary calcium accentuates the negative effects of phytate on zinc bioavailability. Some workers report that zinc \times phytate/zinc molar ratios in diets are better predictors of zinc bioavailability than dietary phytate content alone. For humans, Fordyce et al. (1987) reported that ratios above 0.5 moles per kilogram dry diet or 200 millimoles per 1,000 kilocalories may be cause for concern.

Despite the great interest in phytin as an antinutritive factor inhibiting zinc absorption by humans, many of the experimental data concerning the potential negative effects of naturally occurring phytin in seeds and grains on zinc bioavailability are equivocal (Hambidge, Casey, and Krebs 1986). Certainly, soluble salts of phytate reduce zinc bioavailability when added to purified diets. Apparently, other dietary factors such as calcium and protein may interact with phytin as well, reducing the bioavailability of zinc under some, but not all, circumstances. Again, as with iron, more research is required before a complete understanding of the complex role of phytin in the bioavailability of zinc to humans consuming mixed diets is obtained.

Plant fiber has also been implicated in reduced zinc bioavailability. Usually, products containing significant amounts of natural fiber from major food crops (for example, whole cereal grain) are associated with depressed zinc bioavailability to humans (Harland 1989). However, the ability of fiber to reduce zinc bioavailability depends on the chemical nature of the fiber and the chemical composition of other dietary constituents eaten. While certain

sources of fiber (for example, those that also contain phytate, such as cereal brans) are associated with reduced zinc bioavailability (Torre, Rodriguez, and Saura-Calixto 1991), other fiber sources may have little or no effect (that is, fiber-rich vegetables such as carrots, turnips, cabbage, or potatoes) or may even enhance zinc bioavailability (Harland 1989). For example, rats fed semi-synthetic diets supplemented with sugar beet (*Beta vulgaris* L.) fiber experienced a 39 percent increase in zinc absorption, while those fed diets supplemented with wheat bran experienced a 9 percent reduction in zinc absorption (Fairweather-Tait and Wright 1990).

Vitamin A

Deficiencies of retinol, a form of vitamin A, are found most often in children under the age of five years and are usually the result of insufficient dietary intake (National Research Council 1989). Most carotenoids are less efficiently absorbed than retinol (12 micrograms of mixed provitamin A carotenoids and 6 micrograms of dietary beta-carotene are nutritionally equivalent to 1 microgram of retinol) (Olson 1990). The absorption and utilization of carotenoids and vitamin A are also depressed by the presence of peroxidized fat and other oxidizing agents in food. Furthermore, deficiencies of other nutrients, including protein, vitamin E (alpha-tocopherol), iron, and zinc, also reduce the effectiveness of vitamin A transport, storage, and utilization in humans. Promoters of vitamin A bioavailability include dietary fat, protein, and vitamin E (National Research Council 1989).

Breeding Strategies for Antinutrients

Because antinutrients can inhibit micronutrient bioavailability, should plant scientists attempt to reduce the amounts of these substances in staple food crops in order to improve their nutritional quality? Rigorous consideration reveals that this approach could lead to undesirable consequences for both crop growth and human health. This is especially true for certain antinutrients that are major metabolites in reproductive organs, such as phytic acid.

Phytin, as the primary storage form of phosphorus in most mature seeds and grains, is an important compound required for early seed germination and seedling growth (Welch 1986). Phytin is accumulated as part of globoid crystals in membrane-bound protein bodies of certain cell types within the developing seed, such as within protein bodies occurring in cells of the aleurone layer of cereal grains (Lott 1984). Phytin deposits within globoid crystals of protein bodies also are associated with the accumulation of other minerals, including potassium, magnesium, iron, zinc, copper, manganese, and, in some seed cell types, calcium. As such, phytin plays an important role in determining mineral nutrient reserves of seeds and thus contributes to the viability and vigor of the seedling produced (Welch 1986, 1993). Selecting for seed and grain crops with substantially lower phytin content could have unacceptable effects on agricultural production, especially in regions of the world with soils of low phosphorus status, poor micronutrient fertility, or both.

Furthermore, recent reports have suggested that phytic acid may be an anticarcinogen for certain types of colon cancer (Jariwalla 1992; Messina 1991; Shamsuddin 1992). Additionally, several current reports suggest that tannins and other polyphenols in plant foods (for example, ellagic acid) show anticarcinogenic and antimutagenic properties (Gali et al. 1993;

Maas, Galletta, and Wang 1992; Miyamoto, Murayama et al. 1993; Miyamoto, Nomura et al. 1993; Murayama et al. 1992).

Attempts to significantly lower the antinutrient content of seeds and grains require a major shift in seed or grain composition. Indeed, because most of the antinutrients known to occur in seeds and grains are major organic constituents of these organs, they may play additional, but yet unrecognized, beneficial roles in plant growth and human health. Currently, the consequences of significantly lowering their content cannot be predicted but could include decreased resistance to crop pests or pathogens (Beart, Lilley, and Haslam 1985) or diminished worth to human health (Welch 1993). Plant breeders and molecular biologists must use caution before they attempt to modify the antinutrient content of food crops and must fully research the repercussions of such actions before releasing new crop varieties. Therefore, restraint is recommended when considering reductions in the antinutrient content of staple plant foods, until sufficient research is available to predict the overall ramifications for human health of such endeavors.

Breeding Strategies for Promoters

Numerous reports show that both iron and zinc are more bioavailable from meat products (that is, animal protein sources such as beef, pork, fish, and poultry) than from plant food products (Dallman 1990; Hambidge, Casey, and Krebs 1986; Morris 1983; Thompson 1988; Welch 1993). Additionally, vegan or cereal-based diets generally contain lower amounts of bioavailable iron and zinc to humans than do diets containing significant amounts of animal proteins, because they contain antinutrients and lack sufficient quantities of promoter substances. Indeed, including enough animal meat products in diets containing significant amounts of plant food antinutrients, such as phytate and tannins, can eliminate the negative effects of these antinutrients on iron and zinc bioavailability to humans (Harland 1989; Thompson 1988). Some reports show that certain amino acids (such as methionine, cysteine, and lysine) enhance iron or zinc bioavailability. These same amino acids are contained in lower concentrations in many staple plant food products derived from bean seeds and wheat, rice, and maize grains than in animal meat products.

It may not be necessary to substantially increase the concentration of these amino acids in plant foods to have a positive effect on iron and zinc bioavailability to humans. Iron and zinc only occur in micromolar amounts in plant foods, so only micromolar increases in the amounts of these compounds may be required to counteract the negative effects of antinutrients on iron and zinc bioavailability. Furthermore, these compounds are essential nutrients for plants as well as for humans, so relatively small increases of their concentrations in plant tissues should not have adverse consequences on plant growth. Additionally, many plant foods do not contain sufficient quantities of these essential amino acids to meet human dietary requirements for these nutrients. For example, normal commercial maize varieties are low in lysine, and soybean varieties are low in methionine and cysteine. By increasing the concentration of sulfur-containing amino acids and lysine in legume seeds and cereal grains, it may be possible to overcome the negative effects of antinutrients that occur in these plant foods without harming plant production or human health. Thus, it is highly recommended that plant scientists closely scrutinize the strategy of increasing promoter levels in food crops before embarking on changes in the nutritional quality of food crops with respect to iron and zinc.

3. THE MICRONUTRIENT POTENTIAL OF TARGET SOILS

A soil is said to be deficient in a given nutrient when addition of fertilizer produces better growth even though the amount of fertilizer added may be small compared with the total amount of the nutrient in the soil as determined by complete analysis. Only a small part of the nutrient in the soil is available to plants. Alternatively, the view can be taken that there is a genetic deficiency in the plant rather than a deficiency in the soil. Rye is an example of a highly nutrient-efficient crop for which few of the notoriously deficient soils of South Australia are low enough in nutrients to limit its production (Graham, Anderson, and Ascher 1981). Table 4 gives the analysis of both total and extractable micronutrients in one such trace element-deficient sandy soil in comparison with the nutrients removed in the grain of an average crop grown on that soil. The picture presented for nitrogen is quite different from that for the micronutrients: the ratio of amounts of nitrogen removed by the crop to amounts of nitrogen present in the soil is much higher than it is for the micronutrients, with phosphorus being intermediate. Depletion of soil nitrogen would thus take only a few years if there were no replacement, whereas, for the micronutrients, depletion may take hundreds or even thousands of years. In fact, depletion may never occur at all, owing to various inadvertent additions and other processes (Graham 1991). Although the soil of Table 4 is only one representative of infertile sandy soils, few soils of the world can sustain high yields for long periods without additional supplies of nitrogen, either from rotation of the crop with nitrogen-fixing legumes or from mineral fertilizer additions. Thus, it is pointless to breed for greater tolerance to nitrogen-deficient soils. The micronutrients are present in much greater concentrations (compared with plant needs) even in a nutrient-impoverished soil such as this, however, and it is logical to concentrate breeding efforts on these elements with low requirements or low availability but large reserves in the soil, or both.

Table 4—Nutrient balances in a wheat-growing soil of South Australia

Element	Amount Removed in Grain		Total Amount in Deficient Soil		Equivalent Crops ^a	Amount Extracted from Deficient Soil ^b		Equivalent Crops ^a
	(milligrams per kilogram)	(grams per hectare)	(milligrams per kilogram)	(grams per hectare)		(milligrams per kilogram)	(grams per hectare)	
Nitrogen	20,000	30,000	1,200	2 X 10 ⁶	67	12	20,000	0.67
Phosphorus	2,000	3,000	250	3.8 X 10 ⁶	1,250	5	75,000	25
Copper	2	3	3	45,000	15,000	0.3	4,500	1,500
Zinc	20	30	5	75,000	2,500	0.3	4,500	150
Manganese	33	50	10	150,000	3,000	1	15,000	300
Molybdenum	0.1	0.15	1	15,000	100,000	0.05	750	5,000

Source: Graham 1978.

Note: The data are based on a uniform profile 1 meter deep (except for nitrogen, which declines rapidly at depth and is based on the 0–5 centimeter value shown). Grain yield was taken as 1.5 metric tons per hectare, about the average for this soil and climate. Bulk density was set at 1.5 grams per cubic centimeter.

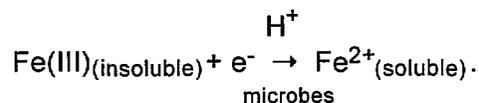
^aThe equivalent number of crops is simply the ratio of amounts of nutrient in the soil profile to the amounts in the grain.

^bExtractable with 1 molar KCl for nitrogen; 0.5 molar NaHCO₃ for phosphorus; 0.05 molar EDTA in 1 molar NH₄OAc, pH 6.0 for copper, zinc, and manganese; and ammonium oxalate for molybdenum (King and Alston 1975).

IRON

Iron, in particular, is abundant in soils. At 5 percent, it is the fifth most abundant element in the earth's crust, but its concentration in plants is only 0.005 percent, or 1,000 times less. (For this reason, soil contamination of food materials analyzed for iron can cause serious error if precautions are not taken.) Yet the fraction of the soil iron that may be in soluble forms suitable for absorption by plants at any instant in time may be only 10^{-15} of the total. Thus, depletion of soil iron is never an issue; it is always a matter of availability and of the amount of chemical attack on the soil that each crop genotype can mount to dissolve the iron. The small grain cereals have a highly efficient mechanism for solubilizing and extracting iron from soil. In contrast, there is considerable room for improvement in maize and sorghum, as well as the grain legumes, which normally have a less efficient system and are usually quite sensitive to iron deficiency.

A special case exists for paddy rice, for which the soil is flooded during much of the growing season. Under these conditions, the low oxygen status in the soil causes, by chemical reduction, more of the soil iron to become soluble. As a result, uptake of iron by the paddy rice crop is high. When such soils become acidic as a result of, among other things, considerable use of nitrogen fertilizer, the solubility of iron increases dramatically, and toxic levels can be found in the rice plant.



In spite of this, little of the foliar iron ends up in the grain of modern cultivars. Nevertheless, the potential to genetically manipulate the ability of a rice genotype to move more of its iron to the grain must be great. For this crop, then, the task may be simpler than for wheat, maize, cassava, or grain legumes: the iron is already in the plant in large amounts. In this case, it is necessary to breed not for agronomic iron efficiency (the ability to absorb more iron from the soil) but for improved phloem translocation efficiency, a task that should be considerably simpler.

With upland rice, maize, cassava, and grain legumes, there is also a strong case for improving the agronomic iron efficiency of the crop, for deficiency in these crops is widespread (De and Chatterjee 1976; Ponnampetuma 1974). Such an improvement should help the crop better adapt to nonacid soils, and, with this trait, the grains should also gain higher iron density (the pleiotropic effect).

For wheat, the best approach lies between the approaches for maize and paddy rice. Wheat is already extremely iron efficient, but iron deficiency is known to occur for wheat under two circumstances:

1. In soils that are consistently iron deficient toward wheat; such soils are rare but have been recognized in Texas and in Madhya Pradesh, India (Katyal and Vlek 1985); and
2. When the crop is under stress from waterlogging, herbicide damage, or low temperatures.

These conditions probably inhibit the expression of iron efficiency. Whether any attempt should be made to improve the iron efficiency of wheat for these situations is debatable at this stage.

ZINC

Zinc deficiency is probably the most widespread micronutrient deficiency in cereals. Many millions of hectares of soils used for cereal production are deficient in zinc since, unlike other micronutrients, zinc deficiency is a common feature of both cold and warm climates, drained and flooded soils, acidic and alkaline soils, and high clay and sandy soils. Unlike iron deficiency, zinc deficiency is quite common in acid soils (for example, in Cerrado soils of Brazil, as reported in Lopes and Cox 1977). Genotypic differences in tolerance of crop plants to zinc deficiency are thus of universal interest.

The extent of zinc deficiencies in comparison with those of other micronutrients is shown in Table 5. In a global sample of 190 soils from 25 countries, 49 percent were low in zinc. A field experiment conducted at each site showed that approximately one-half of the soils identified as low were, in fact, acutely deficient and that the remainder were potentially responsive when other problems were resolved. Taking the data for all elements together, it is obvious that the extent of micronutrient deficiencies is disturbingly large. The problem is exacerbated by the heightened sensitivity of modern cultivars of the major crops to this deficiency. The largest known contiguous area of zinc deficiency occurs in 8 million hectares of cropped land in Western Australia. About half of Australia's wheat belt, like the global sample, is low in zinc. In India, the figure also is coincidentally around 50 percent of all soils (Katyal and Vlek 1985; Welch et al. 1991), with zinc-deficient areas representing as much as 74 percent of the rice-growing soils of Andhra Pradesh (Katyal and Vlek 1985) and 69 percent of the wheat-growing soils (Rathore et al. 1980). Soepardi (1982) categorized 29 percent of the northern central plains of West Java as zinc deficient, and Zheng et al. (1982) published maps of micronutrient-deficient soil areas for China, in which soils low in zinc appeared to represent about one-third of that country's vast area. Areas low in boron, molybdenum, and manganese were of similar size but had markedly different distribution, and the area of soils low in copper represented only about 10 percent of the country.

Data for the continents of Africa and South America are much fewer (Kang and Osiname 1985; Leon, Lopez, and Vlek 1985), but maps for all elements show that instances of deficiency are widely distributed across both continents. There seems little reason to believe that micronutrient status of soils in these continents is any better than in Asia, though the balance of deficiencies among the various micronutrients may be different (Sillanpää 1982).

Table 5—Percentage of nutrient-deficient soils among 190 soils in a global survey across 25 countries

Estimate of Deficiency Level	Nitrogen	Phosphorus	Potassium	Boron	Copper	Iron	Manganese	Molybdenum	Zinc
Acute	71	55	36	10	4	0	1	3	25
Latent	14	18	19	21	10	3	9	12	24
Total	85	73	55	31	14	3	10	15	49

Source: Sillanpää 1990.

Note: The results are based on a field experiment conducted at each site, including analyses of the soil and of the crop grown in the experiment.

4. RATIONALE FOR A BREEDING PROGRAM

Plant breeding is a numbers game, and any new objective, such as improved iron or zinc efficiency, represents a considerable escalation of the breeder's workload or else a diversion of effort away from traditional targets such as yield, processing quality, and disease resistance. A strong case is therefore essential. Until now little effort has been made to adapt crop plants to micronutrient-deficient soils, primarily because of the lack of compelling arguments for doing so. Earlier papers (Graham 1984, 1988a, 1988b, 1991) demonstrated genetic diversity for micronutrient characters within wheat and further argued that nearly all soils (no matter how poor) had sufficient amounts of micronutrients stored. The problem thus was usually one of availability, a problem related partially to soil and partially to genotype. This brings us back to agronomic arguments.

Because both iron and manganese exist in soil redox systems, they are rapidly oxidized and become relatively unavailable to plant roots (unless localized reducing conditions exist because the soil is waterlogged). The same fate awaits these two elements when they are added to soil as soluble (available) fertilizers, which, in turn, means that deficiencies of these two nutrients are difficult to cure by agronomic means. Hence, the case for a breeding approach to iron- and manganese-deficient soils is particularly strong. Besides the ineffectiveness of iron and manganese fertilizers, other agronomic considerations support the case for breeding micronutrient-efficient cultivars. For example, nutrient-efficient genotypes generally also have higher seed content of the limiting nutrient.

Soluble zinc does not undergo redox reactions in soil but remains absorbable as the Zn^{2+} ion. It can, however, be effectively immobilized in soils in a number of ways that are quite widespread, as demonstrated by the worldwide extent of zinc deficiency. Although zinc fertilizers are considered relatively effective, there are a number of agronomic arguments in favor of a breeding approach (in conjunction with proper fertilizer practice) in order to achieve the most productive and sustainable system possible and, at the same time, deliver a grain richer in zinc.

Many of the agronomic arguments in support of a breeding approach for micronutrient efficiency apply equally well to all the microelements, but only for iron and manganese (which are such ineffective fertilizers) is the case considered easily established from an agronomic point of view.

THE AGRONOMIC ADVANTAGES OF NUTRIENT-DENSE SEEDS

Two particular advantages accrue to micronutrient-efficient varieties if by virtue of their efficiency they also accumulate more of the limiting nutrient in the grain. First, they offer better human nutrition if consumed; iron and zinc are especially low in cereal grains, which form the major part of the diet in many places (Welch and House 1983). Second, such varieties have markedly better seedling vigor when resown on deficient soils. Further, the degree of micronutrient efficiency currently known to exist in certain varieties is generally adequate to avoid subclinical deficiency. Such deficiency is not usually treated, because it is not usually recognized. Here, there appears to be a win-win situation: the agronomic and human nutritional requirements coincide and reinforce the breeding strategy. An efficient cultivar with

high seed micronutrient density will thus drive market forces simultaneously from both the consumer's and the producer's perspectives.

Nutrient Density and Seedling Vigor

An important function of the seed is to supply the young seedling with minerals until it has developed a root system large enough to take over this role, but in nutrient-poor soils seed reserves may be insufficient to last while the extra roots are being developed to compensate for low nutrient supply. The result is a transient and critical period of deficiency, when the seedling is particularly vulnerable; the plant may never recover its lost potential.

The nutrient density of seeds is important to agricultural production on nutrient-poor soils and consequently has a complicating effect on breeding varieties for such soils, an effect that is not widely appreciated by plant breeders. Basically, seeds that have a high density of a limiting nutrient produce more vigorous seedlings in the next generation. This effect can extend through the growing season and result in higher grain yields at harvest. The effect has been reported for phosphorus and for the micronutrients, all of which are diffusion-limited in their transport from soil to root surfaces. Table 6 summarizes the minimum concentrations found necessary by various workers for healthy seedling growth.

Manganese. The effect of the manganese content of seeds on the vigor of subsequent seedlings is the most documented of any of the micronutrient effects (Hannam et al. 1984; Longnecker, Marcar, and Graham 1991; Marcar and Graham 1987). Insufficient manganese in the seeds of the grain legume narrow-leaved lupin caused seed-coat splitting and nonviability when the seed manganese content fell below 10 milligrams per kilogram (Hannam et al. 1984). In cereals, a loss of seed germinability has been observed when the manganese content falls below 3 milligrams per kilogram.

Such effects on viability are independent of soil type or fertility, though seedling vigor in a manganese-deficient soil may require more than five times as much manganese in the seed, as can be deduced from Table 7 (an average-sized barley grain with a manganese concentration of 15 milligrams per kilogram would contain 0.5 micrograms of manganese). Three features emerge from the data in Table 7: (1) the range of seed manganese content is quite wide; (2) the effect on seedling vigor is seen throughout the growing season and manifest as increased grain yield; and (3) most surprising, the effect of seed manganese is observed even

Table 6—Critical levels of nutrients in seeds for normal seedling growth

Nutrient	Species	Critical Level (milligrams per kilogram)	Reference
Molybdenum	Maize	0.08	Weir and Hudson 1966
Nickel	Wheat	0.1	Brown, Welch, and Cary 1987
Boron	Green gram	15	Rerkasem, Bell, and Loneragan 1990
Boron	Black gram	14	Rerkasem, Bell, and Loneragan 1990
Manganese	Lupin	10	Hannam et al. 1984
Manganese	Soybean	16	Boswell et al. 1981
Manganese	Wheat	5	Marcar 1986
Zinc	Wheat	5–10	Al-Samerria 1984

when both soil and foliar fertilizers are used. An example of the effect of seed manganese content on the resistance of seedlings to pathogens when grown in manganese-deficient soil is given in Table 8 (Wilhelm, Graham, and Rovira 1988). In this case, seed manganese was varied by soaking the seed briefly in $MnSO_4$ solution. The same effect can be observed when the seed manganese content is varied naturally (Pedler 1994). The take-all fungus is the most important root disease of wheat worldwide and is ubiquitous in soils used for wheat production. It is particularly virulent in manganese-deficient soils.

Zinc. Although it has been observed for some time that seeds high in zinc seemed to be more vigorous, no one has documented this response until recently (Table 9). The importance of seed zinc for seedling vigor in zinc-deficient soil is quite strong and increases over time. This may be because pathogens have managed to infect the weaker, zinc-deficient seedlings at the vulnerable emergence stage and continued to retard seedling growth after six weeks. However, fertilizer supplements mixed throughout the soil can compensate for the lack of adequate seed zinc.

Molybdenum. Maize breeders have been aware of the effect of molybdenum in seeds for some time and make use of a hybrid seed production system to solve the problem of molybdenum-deficiency in maize. Because a plant's requirements for molybdenum are so low (about 100 micrograms per kilogram), it is possible to load sufficient molybdenum in the

Table 7—Effect of manganese content of seeds on vegetative and grain yields of Galleon barley grown in manganese-deficient soil on Eyre Peninsula, South Australia, with and without fertilizer manganese applied

Manganese Content of Seeds (micrograms per seed)	Vegetative Yield at 66 Days (grams per square meter)		Grain Yield (kilograms per hectare)	
	With No Manganese Applied	With Manganese Applied	With No Manganese Applied	With Manganese Applied
Low (0.08–0.26)	3.1	10.8	92	1,865
Medium (0.34–0.62)	8.2	11.3	212	2,352
High (0.74–1.20)	8.5	14.5	365	2,525

Source: Longnecker, Marcar, and Graham 1991.

Note: Manganese was applied both as soil and foliar dressings.

Table 8—Effect of manganese supplied via the seed or the soil on the severity of infection of wheat roots by the take-all fungus, *Gaeumannomyces graminis tritici*, inoculated into pots of manganese-deficient soil

Manganese Source	Lesion Length	Number of Lesions	Seminal Roots with Lesions
	(millimeters per plant)	(per plant)	(percent)
Nil	22	6.0	80
Seed	11	4.9	71
Soil	6	3.1	51

Source: Wilhelm, Graham, and Rovira 1988.

Table 9—Effect of zinc content of wheat seeds on the vigor of seedlings grown in zinc-deficient soil in pots

Zinc Content of Seeds (nanograms per plant)	Seedling Yield			
	Three Weeks		Six Weeks	
	With No Zinc Applied	With Zinc Applied	With No Zinc Applied	With Zinc Applied
	(milligrams per plant)			
Low (250 nanograms)	66	92	167	558
High (700 nanograms)	88	99	319	527
Ratio of low yield to high yield	0.75	0.93	0.52	1.06

Source: Rengel and Graham 1995.

large seed of the maize plant to supply the needs of the plant for its entire life cycle. The fact that the resulting commercial grain is still low in molybdenum does not appear to be a recognized problem in human nutrition. Thus, hybrid seed producers for molybdenum-deficient soils supply large amounts of molybdenum to the seed mother crop, resulting in high amounts in the commercial hybrid seed. This is sufficient to eliminate molybdenum-deficiency in farmers' fields (Weir and Hudson 1966).

Nickel. Higher plants' need for nickel remained unproven until 1983, because normal nickel contents of seeds were enough to supply the needs of the next generation entirely (Eskew, Welch, and Cary 1983). However, several generations of culture in a low-nickel environment progressively decreased the nickel content of barley and wheat seeds until not only seedling vigor was affected, but germination itself (Brown, Welch, and Cary 1987). Figure 1 shows that, as the nickel level fell to less than 10 micrograms per kilogram (that is, parts per billion), the seeds became inviable. The average nickel content of cereal grain needs to be at least 100 micrograms per kilogram to ensure 100 percent germination. More is probably required for maximum rate of seedling growth in low-nickel environments, but these are rare in nature.

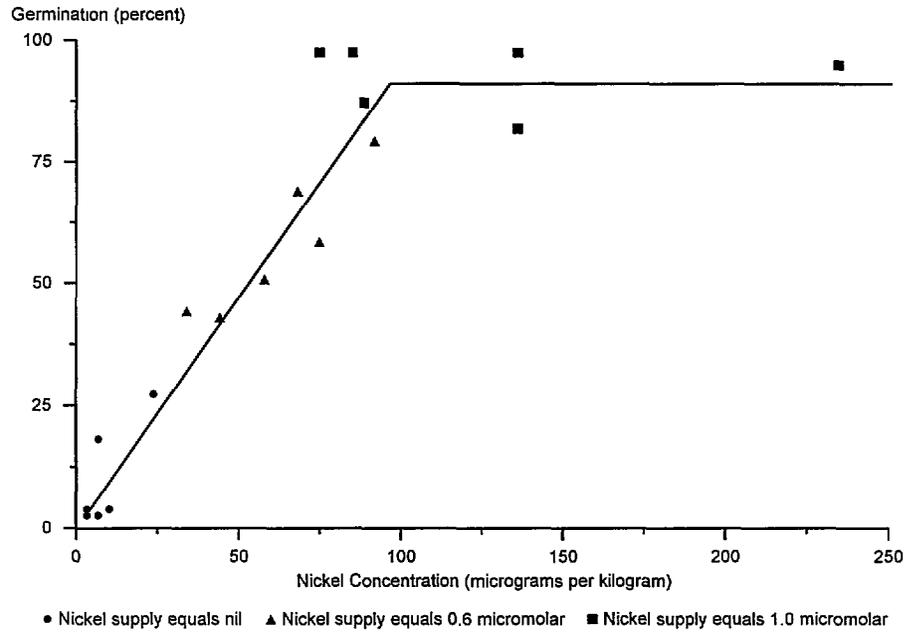
Phosphorus. Although phosphorus is not a micronutrient and thus not part of the human nutrition agenda of this paper, it is mentioned here briefly to illustrate that the effects of seed nutrient density occur also with macronutrients. (There is a sound agronomic and environmental case for breeding for phosphorus efficiency, but success has proved elusive because of the presumably quantitative genetics, and particularly because of the lack of an efficient screening procedure.) Figure 2 shows the effect of seed phosphorus content on the vigor of seedling growth in phosphorus-deficient soil (Bolland and Baker 1988); as with the micronutrients, these effects are known to extend to grain yield (Bolland, Paynter, and Baker 1989).

AGRONOMIC ISSUES

Disease Resistance

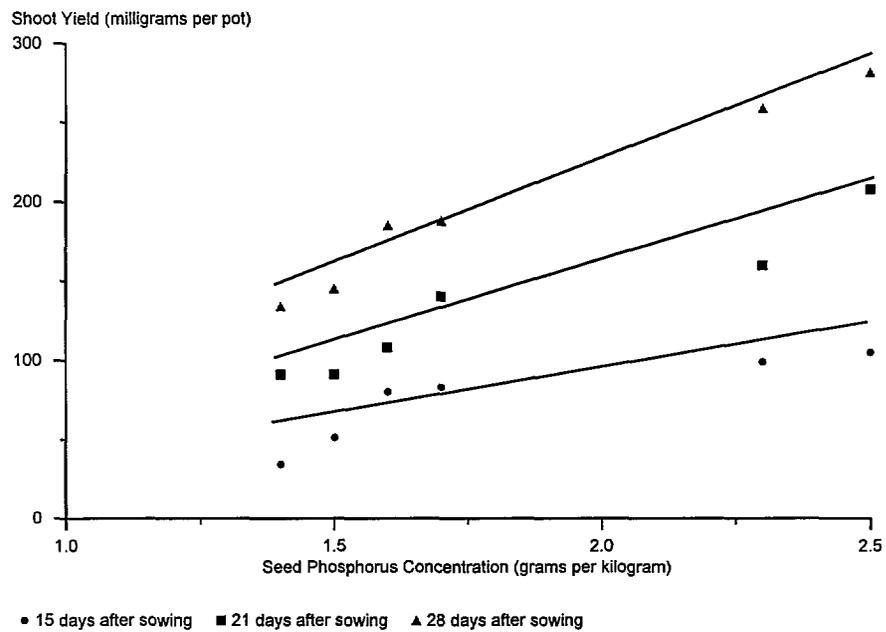
Good nutritional balance is as important to disease resistance in plants as it is in humans. In particular, micronutrient deficiency in plants greatly increases their susceptibility to diseases and especially to the fungal root diseases of the major food crops (Graham and Webb

Figure 1—Effect of seed nickel concentration on the viability of barley seeds



Source: Brown, Welch, and Cary 1987.

Figure 2—Effect of seed phosphorus concentration on dry matter yield of Jacup wheat sown in pots



Source: Adapted from Bolland and Baker 1988.

1991). As plant nutrient status is raised from severe deficiency, the disease resistance also increases, until the plants reach sufficiency. Further additions of the nutrient beyond sufficiency generally do not enhance disease resistance further. However, there is evidence that the nutrient requirement may be greater in the presence of pathogens (Webb and Graham 1990). Iron has not been convincingly linked to disease resistance in plants as have copper, manganese, zinc, nickel, and boron (given a soil deficiency in each case). Some examples of micronutrient effects on disease are shown in Tables 10 and 11 and Figure 3. These data have established that, in all cases studied, the deficiency predisposes the plant to infection rather than that the infection, through its effect on root pruning, causes the deficiency (Graham and Rovira 1984; Sparrow and Graham 1988; Thongbai et al. 1993). For example, zinc deficiency decreased the resistance of wheat to *Fusarium graminearum*, the crown rot fungus, and the presence of zinc strongly inhibited the upward spread of infection (Table 12). The implication is that nutrient-efficient genotypes in deficient soil, enjoying better nutrient status than inefficient types, should have greater resistance to such root pathogens. As evidence in support of this idea, a strong association of resistance to crown rot fungus (Burgess, Klein, and Liddell 1984) with zinc efficiency (Graham, Ascher, and Hynes 1992) is apparent from the following varietal rankings:

Table 10—Suppression of severe powdery mildew (*Erysiphe graminis*) infection of manganese-deficient wheat plants in pots in the greenhouse by small applications of manganese to the soil

Manganese Supply (milligrams per pot)	September 27		October 18		Grain Yield (grams per pot)
	Total Pustules (per pot)	Pustules (per gram of dry weight)	Total Pustules (per pot)	Pustules (per gram of dry weight)	
0	50	100	0.5
10	657	94	1,150	165	6.9
50	492	30	135	8	16.2
100	558	34	164	10	16.6
LSD ^a (manganese)	43	1.2			

Source: Graham, Ascher, and Mills 1984.

Note: Leaders (. . .) indicate no data.

^aLeast significant difference at a probability level of 5 percent.

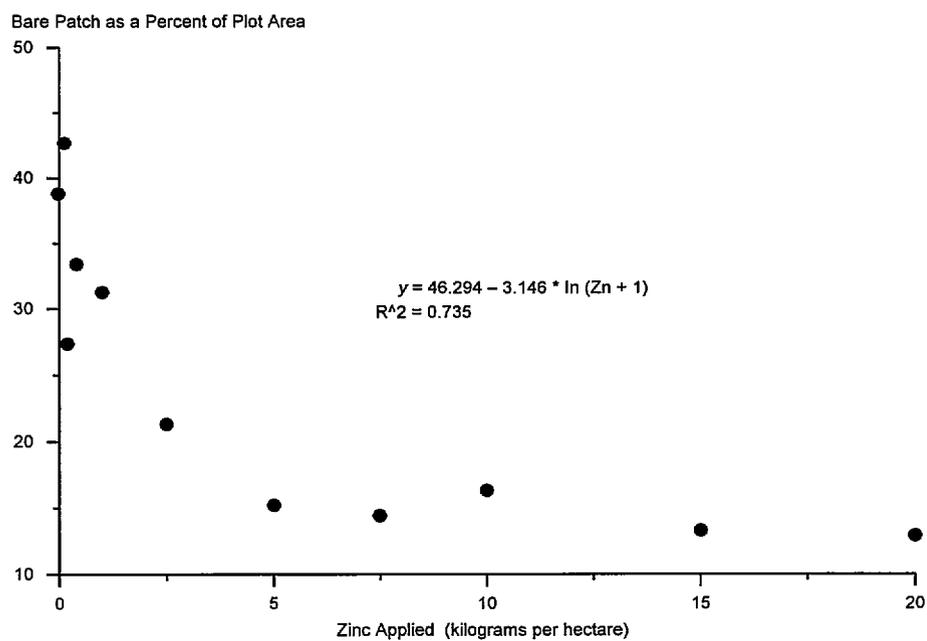
Table 11—Effect of nutrition on, and the importance of nutritional balance to, yield and incidence of ergot (*Claviceps purpuria*) for barley in Finland

Fertilizer	Grain Yield	Ergot
	(kilograms per hectare)	(percent of grains)
Nil	575	23.2
NPK	900	3
NPK + copper	625	24.1
NPK + boron	1,450	0.35
NPK + copper + boron	1,500	0.33

Source: Tainio 1961.

Note: NPK is nitrogen + phosphorus + potassium fertilizer.

Figure 3—Effect of zinc fertilizer on the severity of Rhizoctonia root rot in wheat growing in zinc-deficient soil



Source: Thongbai et al. 1993.

Note: Data were collected at Lameroo, South Australia.

Table 12—Effect of the rate of zinc supply on the severity of crown rot fungus (*Fusarium graminearum*) on six-week-old wheat plants growing in zinc-deficient sandy soil in pots

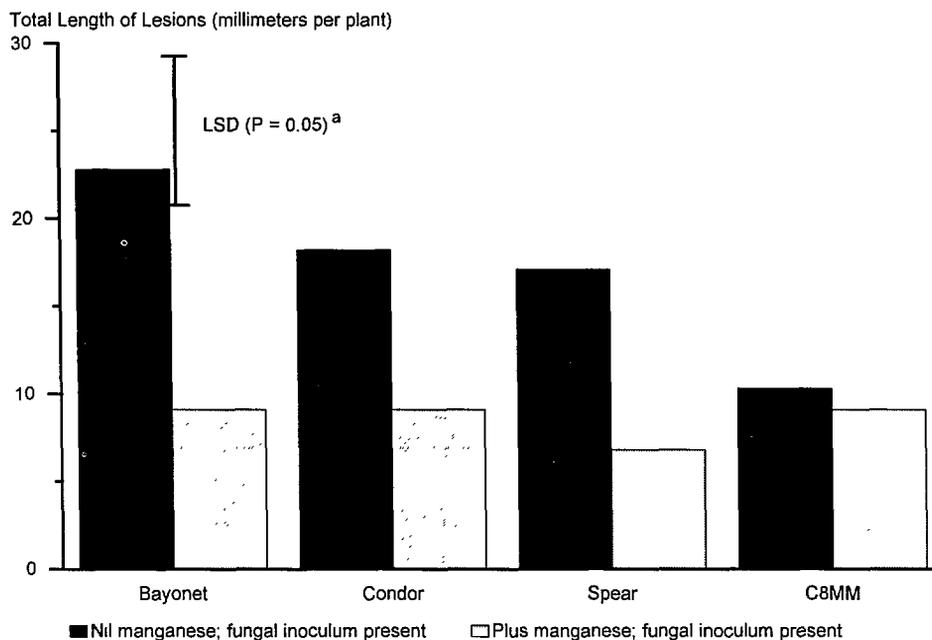
Evaluation	Zinc Added		
	0.0	0.2	6.0
	(milligrams per pot)		
Percent of all stem sections infected ^a	47	45	28
Percent of upper stem sections infected ^a	66	34	6
Concentration of zinc in shoots ^b	7.4	8.2	35

Source: Sparrow and Graham 1988.

^aF value for zinc effect = 14.4.

^bF value for zinc effect = 734.

Figure 4—Manganese efficiency and resistance of wheat cultivars to take-all fungus (*Gaeumannomyces graminis* var. *tritici*) in manganese-deficient soil



Source: Wilhelm, Graham, and Rovira 1990.

^aLeast significant difference at a probability level equal to or less than 5 percent.

Zinc efficiency: Cook = Takari > Kite > Gatcher > Songlen >> Durati
 Disease resistance: Cook > Kite > Takari > Gatcher > Songlen >> Durati

This hypothesis is supported by recent studies of take-all fungus for wheats having a range of manganese efficiencies and growing in manganese-deficient soil (Figure 4). The greater the manganese efficiency of the genotype, the more resistance it shows to the take-all fungus under the same conditions in infested, manganese-deficient soil. Collectively, these results suggest causality in the concurrence in southern Australia, for example, of some of the world's most severe root disease and micronutrient deficiency problems. They also demonstrate that breeding for micronutrient efficiency can confer resistance to root diseases that had previously been unattainable (Scott and Hollins 1985). This means, in turn, that a lower dependence on fungicides will be needed for efficient cultivars in nutrient-poor soils—in other words, a more sustainable system results.

Interactions with Water in the Soil Profile

Topsoil drying is another problem affecting wheat production in infertile soils of the seasonally humid zone. Many of the available micronutrients (and phosphorus) are in the topsoil by virtue of fertilizer additions and nutrient cycling. Leaching of the heavy metals is generally negligible (Jones and Belling 1967). When the topsoil dries as a result of a week

or two of dry weather, roots in the nutrient zone are largely deactivated, and the plant must rely on deeper roots or upon retranslocation for its further nutrition. With phloem-immobile micronutrients and inefficient genotypes, deficiency can result; this was demonstrated in a study using deep pots, in which adequate copper was added but located in the topsoil layer, which was allowed to dry out selectively (Table 13). The high straw yields for the drying treatments at 4–5 and 10–13 weeks showed that lack of water was not limiting growth; the associated sterility, however, is evidence of copper deficiency induced by the drying. Such effects have also occurred in the field, where copper deficiency from topsoil drying at the early boot stage caused severe sterility problems (Grundon 1980). These may, however, be overcome by copper-efficient genotypes such as triticale (Grundon and Best 1981).

The fertility of subsoils also limits production, though the subsoil is too deep to fertilize economically. In 1986, on 10 soil types scattered across South Australia, various nutrient treatments were applied to the subsoils as they were excavated and returned to grave-sized pits in their original layers. The topsoils, previously removed, were then also replaced and the sites sown as part of the farmers' fields. They received all the farmers' usual treatments and fertilizers, including, in some cases, micronutrients, placed in the topsoil. Responses to the subsoil nutrients were immediate and often spectacular (Figure 5), but there was generally little response to the physical disturbance only or to gypsum. This underlines the nutritional nature of the problem. These responses continued over seven years (Figure 5 shows 50–100 percent yield increases due to nutrients after six seasons). With the original nitrogen largely dissipated in the first year, the residual responses are principally due to phosphorus and trace elements. (Analyses not presented show that the effects of subsoil treatments on shoot nitrogen disappear within three years.) Indeed, at some sites, the micronutrient treatment seems to have relatively greater residual value as time passes.

In pot studies, it has been shown that wheat grows poorly in subsoil, even when fertilized with nitrogen and phosphorus. Although experiments are being conducted with deep injection of micronutrients through tubes welded down the back of deep-ripping tines, it is believed that the correct approach to this problem of the seasonally dry regions is to breed wheats with

Table 13—Effect of drying of topsoil containing adequate added copper on the sterility of wheat plants (a symptom of copper deficiency): A deep pot study using copper-deficient sandy soil in the greenhouse

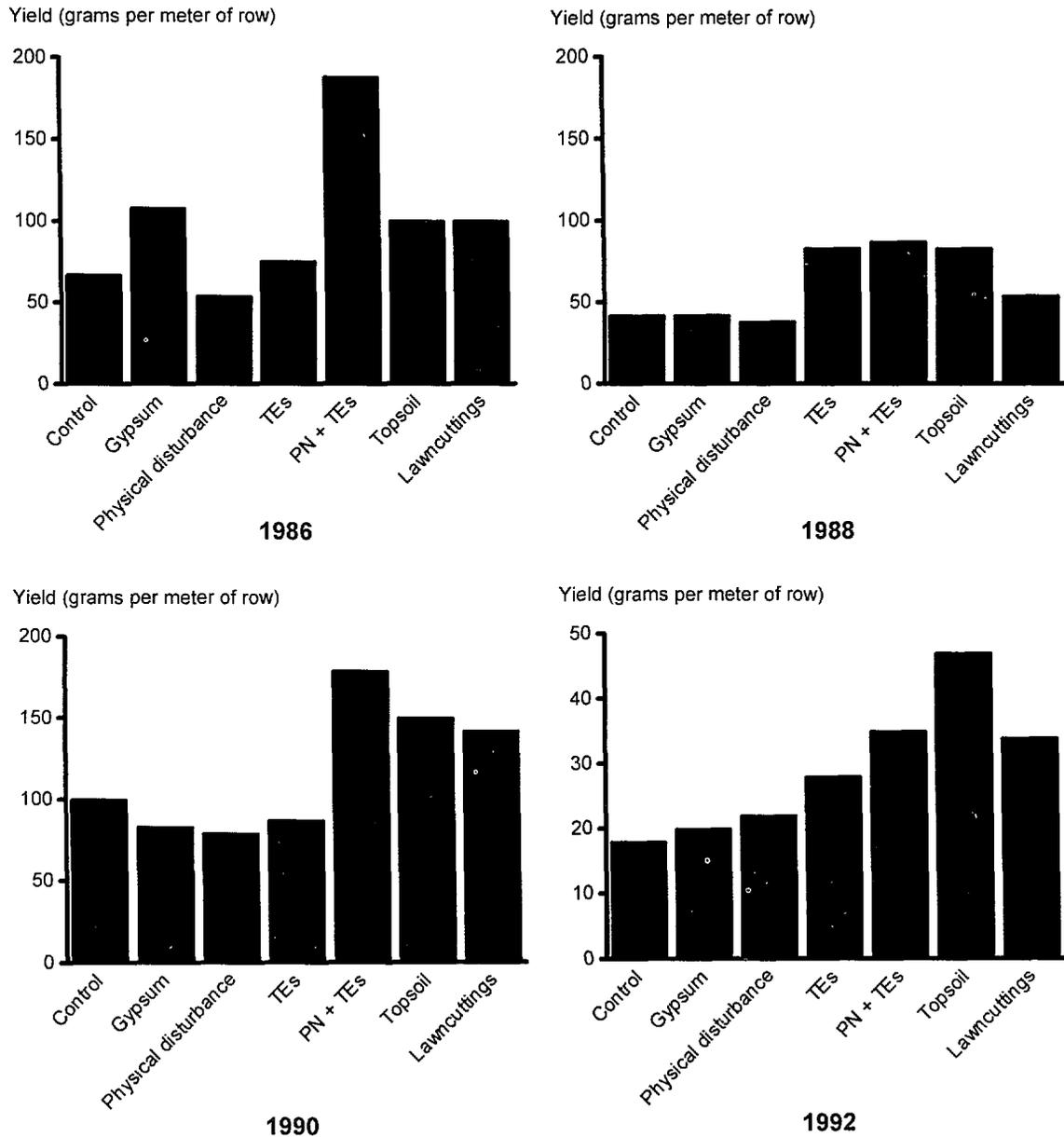
Soil Condition	Straw Yield		Number of Grains per Spikelet	
	With No Copper Added	With Copper Added	With No Copper Added	With Copper Added
	(grams per pot)			
Topsoil continuously wet	6.3	10.3	0.0	1.6
Topsoil dry after 2–3 weeks	5.5	7.7	0.0	0.6
Topsoil dry during development of foliar symptoms (weeks 4–5)	n.a.	9.6	n.a.	0.6
Topsoil dry during early stem elongation (weeks 10–13)	n.a.	9.8	n.a.	0.8
LSD ^a (drying treatments)	n.a.	1.1	n.a.	0.6

Source: Graham 1991.

Note: n.a. = not applicable; no treatments exist.

^aLeast significant difference at a probability level of 5 percent or less.

Figure 5—Barley yields over seven years on plots with various subsoil treatments, 1986–92



Source: Graham and Ascher 1993.

Notes: Grain yields are given for 1986, 1988, and 1990, and vegetative yield at 10 weeks is given for 1992. The barley was grown in calcareous sandy soil at Marion Bay, South Australia. The farmer cultivated the site, fertilized the topsoil layer (with micronutrient soil applications, seed coatings, and foliar sprays as part of his normal operations), and cropped the site four times after treatment in 1986. Gypsum = gypsum mixed through subsoil. TEs = trace elements mixed through subsoil. PN = phosphorus and nitrogen mixed through subsoil. Topsoil = topsoil material replacing subsoil. Lawncuttings = chopped grass mixed through subsoil.

root systems that will grow through subsoils of low phosphorus and micronutrient availability. In most respects, phosphorus and micronutrients behave similarly for the arguments of this paper, but genetic progress for phosphorus efficiency has been particularly slow (Graham 1984).

Relevant to this argument as well is the picture emerging from physiological studies of roots spanning four decades. From the papers of Bowling, Graham, and Dunlop (1978); Epstein (1972); Graham, Anderson, and Ascher (1981); Haynes and Robbins (1948); Loneragan, Kirk, and Webb (1987); Nable and Loneragan (1984); Pollard, Parr, and Loughman (1977); and Welch, Webb, and Loneragan (1982), it appears that the elements boron, calcium, manganese, phosphorus, and zinc are all required in the external environment of the root for membrane function and cell integrity. In particular, phosphorus and zinc deficiencies in the external environment promote leaking of cell contents such as sugars, amides, and amino acids, which serve as chemotactic stimuli to pathogenic organisms and may explain some of the disease results presented above. Although phosphorus is phloem-mobile, the other elements are not or are poorly so. Therefore the root tips may not be adequately supplied from elsewhere in the root system, such as, for example, from those roots contacting a fertilizer band in the topsoil. Moreover, in the case of zinc, a high internal zinc content does not prevent leakiness due to a deficiency of zinc external to the membrane (Welch, Webb, and Loneragan 1982). It follows that the roots of those cereal genotypes that have a greater capacity to mobilize nutrients strongly bound to soil particles in the rhizosphere will probably be better able to penetrate an infertile, alkaline subsoil. This view was confirmed by Nable and Webb (1993) in a pot experiment that compared the wheat cultivars Excalibur (zinc efficient) and Gatcher (zinc inefficient) in pots of zinc-deficient sand divided into upper and lower layers (Table 14). Gatcher plants withdrew more water from the bottom layer if they were treated with zinc. As expected, the advantage of subsoil zinc treatment was smaller for the zinc-efficient cultivar, Excalibur.

Table 14—Effect of subsoil zinc treatments and genotypic zinc efficiency on yield and removal of water from the subsoil by wheat cultivars grown in deep pots in the greenhouse

Cultivar and Fertilizer Regime	Grain Yield	Water Use
	(grams per pot)	(liters per pot)
Gatcher (zinc inefficient)		
Zinc supplied to top 10 centimeters only	23	9.1
Zinc supplied to topsoil and subsoil	29	10.3
Excalibur (zinc efficient)		
Zinc supplied to top 10 centimeters only	33	9.4
Zinc supplied to topsoil and subsoil	34	9.6

Source: Nable and Webb 1993.

5. MECHANISMS OF MICRONUTRIENT TRANSPORT

An understanding of the processes responsible for (1) micronutrient uptake by roots, (2) translocation of micronutrients to shoots, and (3) remobilization of micronutrients to and deposition in edible organs, along with the genetic potential for controlling these processes, is essential before efficient strategies can be developed to screen vast numbers of food crops for enriched micronutrient contents. This section reviews current concepts concerning these processes and delineates those areas where further research is needed.

This brief overview is limited to discussions of iron and zinc. However, much of what is covered also may apply to other transition metal cations, including copper, manganese, and nickel. It does not attempt to cover all of the information available on this topic, but only summarizes the most important, generally accepted concepts. Other reviews are available for those interested in more detailed discussions of this topic (Kochian 1991; Welch 1994).

Progress in understanding micronutrient absorption processes has made rapid advances in the last decade. Most of what is known is the result of active research directed at understanding iron absorption mechanisms for microorganisms and higher plants. At least three physiological strategies (based on iron transport studies) are known for divalent, transition-metal, root-uptake mechanisms in higher plants. Strategy I (for dicots and nongrass monocots) consists of root-cell influx of free divalent transition metal ions; efflux of organic acids, reductants, and H^+ ions; and inducible root-cell plasma membrane Fe(III)-chelate reductases. Strategy II (for *Poaceae* [*Graminaceae*] species) consists of root-cell influx of free divalent transition metal ions, root-cell efflux of free phytometallophores, and root-cell influx of transition metal-phytometallophore complexes. Strategy III (for all higher plants) relies on influx of microbial metallophore-metal complexes. Strategy III, which will not be discussed in this review, can have both positive and negative effects on iron and zinc uptake by higher plants, depending on the plant species, rhizosphere environment, and soil type (Crowley et al. 1991; Römheld 1991). Some researchers include root epidermal transfer-cell development as part of Strategy I iron-efficiency mechanisms, but this may be an experimental artifact resulting from rhizosphere acidification and root injury during experimentation. Hence, it will not be discussed here (Welch 1994). Figures 6 and 7 present current models for Strategy I and Strategy II plants.

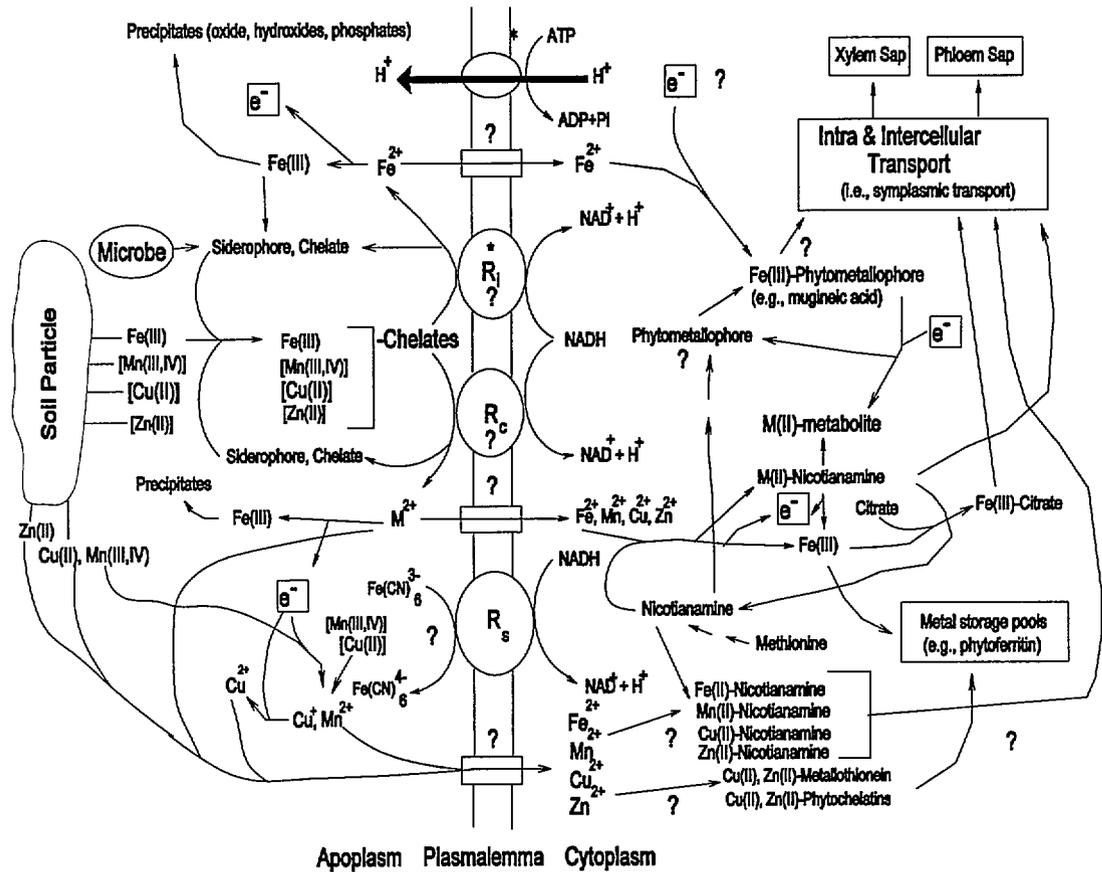
ROOT-RHIZOSPHERE INTERACTIONS AFFECTING IRON AND ZINC AVAILABILITY

Various soil factors (for example, soil chemical composition, microbial activity, and root activity) control the available supply of iron and zinc to plant roots. Limited availability is the first, and most important, barrier to surmount in striving to increase the iron and zinc content of food crops; that is, the size of available pools for iron and zinc in soils must increase if food crops grown on micronutrient-poor soils are to accumulate more of these essential nutrients.

Iron

Most primary minerals in soils contain iron in the Fe(II) oxidation state. During soil formation, under aerobic conditions, these minerals dissolve, and Fe^{2+} ions are released and

Figure 6—Micronutrient cation uptake model for dicotyledonous and nongrass monocotyledonous plants



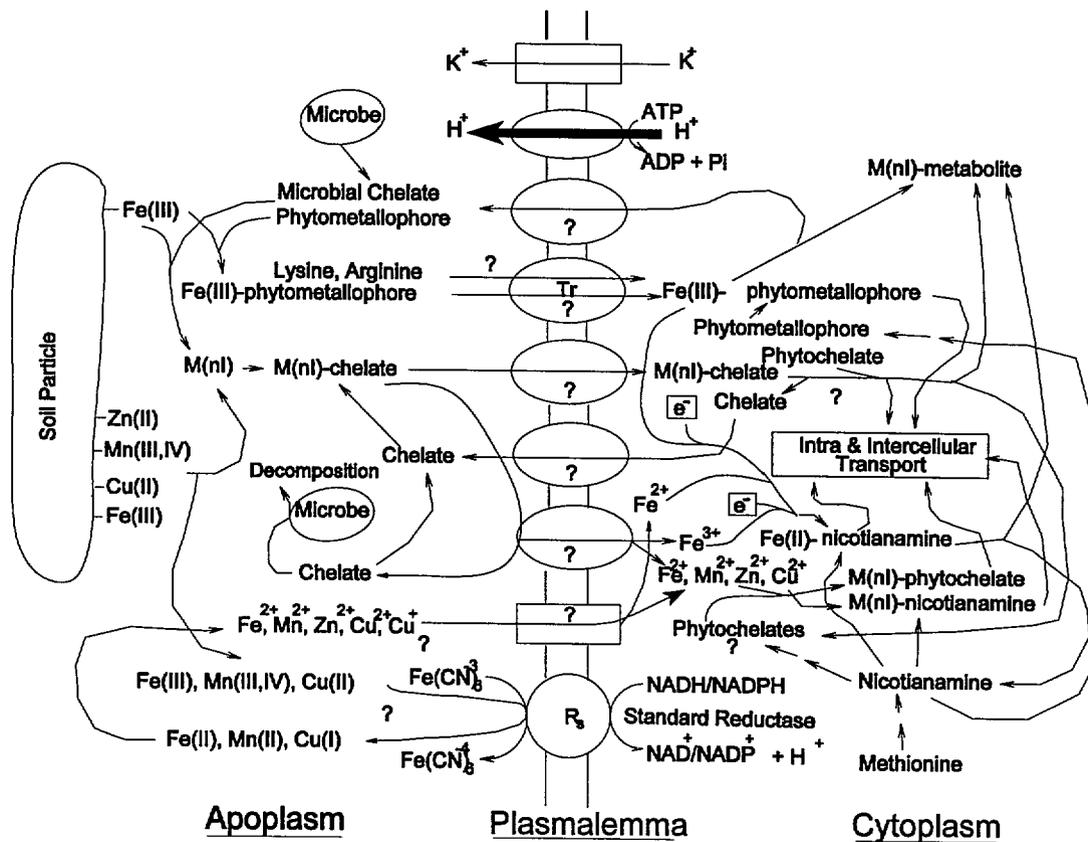
Source: Welch 1994.

Note: R_i = inducible reductase; R_c = constitutive reductase; R_s = standard reductase; rectangular box = transport protein (channel); circle = reductase; oval = primary H^+ -translocating ATPase; e^- = electron; ? = not known or speculative; * = increased activity in response to micronutrient metal deficiency stress.

rapidly undergo oxidation to Fe^{3+} ions, which precipitate as highly insoluble, mixed Fe(III) oxides. Thus, the solubility of iron in most aerobic soils is largely controlled by various Fe(III) oxides, including amorphous forms, maghemite, ferrihydrite, and lepidocrocite (Lindsay 1991). The ion activity product of $Fe(OH)_3$ in soils (that is, soil-iron) has been estimated to be near $10^{39.3}$ and likely represents the best estimate for iron solubility in aerobic soils.

Plants require in excess of 10^{-8} molar soluble iron in soil solution to meet their nutritional needs (Welch 1994). Unfortunately, soil-iron in fertile soils with pH values over 5.5 cannot maintain this concentration of soluble iron, so pH must be lowered, reduction reactions resulting from soil biotic activity must take place, or both to increase the solubility of soil-iron above this critical level in order to meet the growth requirements of plants (Welch 1994). Roots of many plant species have evolved mechanisms to modify the rhizosphere to increase

Figure 7—Micronutrient metal uptake model for grasses



Source: Welch 1994.

Note: R_s = standard reductase; Tr = transport protein; e⁻ = electron; circle = reductase; oval = transport protein; rectangular box = divalent ion channel; M(nl) = variable oxidation state dependent on metal species; ? = not known or speculative.

the solubility of soil-iron and meet this critical level. These root mechanisms will be discussed further below.

Zinc

The form of free zinc ions in soil solution absorbed by most plant roots is the Zn²⁺ ion, although some plant species apparently absorb Zn(II)-phytometallophores complexes in toto (Welch 1994). The critical concentration of Zn²⁺ in soil solution required to meet plant growth requirements has been determined to be about 10^{-10.2} to 10^{-10.6} molar for many plant species (Welch 1994). Some suggest that the mineral franklinite could be responsible for controlling Zn²⁺ activity in the soil solution of most fertile aerobic soils (Lindsay 1991).

The solubility of zinc in soils is highly dependent on soil solution pH. For example, the soil solution concentration of Zn^{2+} in a normal aerated soil at pH 5 is estimated to be about 10^{-4} molar. At pH 8, this value drops to 10^{-10} molar. Below pH 7.7, Zn^{2+} ions predominate in soil solution. At higher pH values, $ZnOH^+$ followed by $Zn(OH)_2^0$ species dominate. Zinc ion pairs formed with inorganic anions such as $ZnSO_4$ can also occur. Soluble zinc species, other than Zn^{2+} ions, do not appear to be absorbed by plant roots. Zinc-deficient conditions normally occur in alkaline soils, but zinc deficiency can also occur on a number of other soil types (Lindsay 1991).

Root Activity

Root mechanisms that alter water movement and ion gradients to the root, microbial activity, pH, and/or the redox state of the rhizosphere will influence the amounts of Fe^{2+} and Zn^{2+} ions available to root systems for absorption. All of these mechanisms have common effects on both zinc and iron availability.

Roots are not just static organs penetrating soils and functioning only as suppliers of minerals and water to plant shoots. Roots also play an active role in modifying the rhizosphere to make mineral nutrients more available for absorption. They interact dynamically with soil constituents, modifying the rhizosphere environment dramatically. They act as sinks for iron and zinc transport through mass flow and diffusion pathways; release H^+ ions (or HCO_3^-), thereby changing pH; consume and release O_2 , thereby altering redox potential; exude various organic and inorganic substances including organic acids, amino acids, reductants, phytosiderophores, and mineral nutrients (for example, potassium and phosphorus); and supply carbon and energy substrates to some microbes for their growth (Marschner 1986; Römheld and Marschner 1986). Indeed, root modifications to the rhizosphere are crucial to the uptake of iron and zinc by plants and, therefore, ultimately to the nutrient quality of food crops. It is imperative to understand each of these root processes and the extent to which each influences iron and zinc availability before striving to develop efficient strategies for breeding iron and zinc efficiencies into food crops to enhance the nutritional quality of their edible products.

Root-Affected pH Changes in the Rhizosphere

Root-provoked changes in rhizosphere pH occur primarily through excretion of H^+ and HCO_3^- ions, evolution of respiratory CO_2 , and efflux of organic acids. Usually, pH changes resulting from these processes are not large in magnitude. Of these processes, net excretion of HCO_3^- and H^+ ions is the predominant factor influencing rhizosphere pH. Their efflux is related, in turn, to the balance between net root-cell cation and anion influx rates. Among the macronutrients, nitrogen form has the greatest effect on rhizosphere pH, with NO_3^- inducing increased net HCO_3^- efflux and higher rhizosphere pH, while NH_4^+ and N_2 fixation each result in net H^+ efflux and lower rhizosphere pH (Marschner 1986).

Should researchers attempt to select for increased H^+ efflux (that is, lower the pH of the rhizosphere) from roots as a means of increasing the availability of iron and zinc to food crops? Because the rates of H^+ efflux by roots are so dynamic and are controlled by a myriad of environmental and plant physiological factors, attempts to breed for, or genetically alter, plants with regard to this trait could be risky. Such a strategy could lead to unpredictable

negative consequences for crop production. Thus, breeding for changes in rhizosphere pH through increased activity of H^+ ion efflux by roots is not recommended at present.

Root Exudates

Actively growing roots secrete or leak considerable amounts of inorganic ions and organic substances into the rhizosphere. These substances include exudates composed of low molecular weight organic compounds and higher molecular weight gelatinous mucilages. Debris and lysates, derived from sloughed root cells, root hairs, root cap cells, and dead or dying roots, also contribute to the effect of root activity on rhizosphere chemistry. Mucigels include the mixture of gelatinous material, microorganisms, and soil particles that form around roots as a result of the secretion of mucilages (Marschner 1986).

The primary root exudates include sugars, organic acids, amino acids, and phenolic substances. These exudates can undergo further chemical modification during microbial assimilative and degradative activities in the rhizosphere. Both iron and zinc deficiency are associated with increased root-cell efflux of amino acids (including several nonprotein amino acids termed phytometallophores), organic acids (particularly malic and citric acid), phenolic compounds, and mineral nutrients such as potassium (Welch 1994).

The solubilization and mobilization of iron and zinc in the rhizosphere can be greatly enhanced by root exudates and microbial by-products. Of particular interest are the organic acids, such as citrate, tartrate, malate, and oxalate, which form stable soluble complexes with iron and other polyvalent cations; and the phytometallophores, which form stable complexes with transition-metal, polyvalent cations, including Fe^{3+} and Zn^{2+} (Stevenson 1991). The occurrence of phytometallophore root exudates is restricted to cereal roots. These will be discussed separately in the section on phytometallophores, for Strategy II species.

Some have proposed that root-exuded organic acids, such as citrate, form soluble complexes with insoluble Fe(III)-hydroxyphosphate precipitates in the rhizosphere (Marschner 1986). These soluble complexes can then diffuse to the root surface, where Fe(III) is reduced to Fe^{2+} and the phosphate, and the Fe^{2+} liberated can be absorbed by root cells. The organic acid then is recycled back into the rhizosphere, according to this model, to perform the solubilization function again. Such processes also could affect zinc availability because zinc can be adsorbed to or co-precipitated with soil Fe(III)-hydroxyphosphate as well as with hydroxides and mixed, amorphous iron oxides (Shuman 1991).

Rhizosphere mucigels (composed mainly of polysaccharide and polygalacturonic acids in association with soil particles and certain microorganisms) perform important functions for plant roots, including protecting roots from dehydration, lubricating root movement through the soil, and increasing the absorptive surface area of roots in contact with soil particles (Marschner 1986; Kochian 1991). Increasing root-soil contact can lead, in turn, to increased solubilization of insoluble iron and zinc pools in soils, thereby increasing availability of iron and zinc to roots. For roots growing in dry regions of a soil profile, mucigels also can provide a microenvironment for ion absorption, provided that roots have access to water from other roots growing in wetter regions of the soil profile, including the subsoil.

The proliferation of beneficial microorganisms within mucigels can also have positive effects on plant growth and micronutrient uptake under some (but not all) circumstances. For example, some pseudomonad bacteria and certain ectomycorrhizal fungi produce very stable hydroxamate Fe(III)-siderophore complexes (for example, pseudobactin and fer-

richrome). These compounds are capable of solubilizing iron from minerals that contain little plant-available iron and may be important sources of iron for some plant species that have evolved in alkaline, calcareous environments (Neilands 1986). However, there appear to be plant species-specific root recognition mechanisms for siderophore uptake (Neilands 1986; Welch 1994). Thus, not all plant species in a given soil environment will benefit from the same microbial siderophores; some plant species may suffer from micronutrient metal deficiencies because they cannot absorb the siderophores produced by certain microorganisms in some rhizosphere environments. Important crop species that lack these siderophore recognition systems could possibly be genetically modified to absorb these siderophores by incorporating the genes responsible for the manufacture of these siderophore uptake systems into their genome (Neilands 1990a). Plant breeding and genetic engineering efforts could address this possibility if the strategy proves effective through further research.

Reductants in Root Exudates

While root cells can release reducing substances, such as phenolics, such reductants account for little of the total Fe(III)-reducing capacity of iron-deficient roots, unless excessive rhizosphere acidification occurs, causing root-cell injury and increased efflux of reductants (Welch 1994). Therefore, breeding for increased Fe(III)-reductant release by roots does not appear to be a viable option for plant breeders. The focus of research efforts should be, instead, on regulatory mechanisms controlling the membrane-bound Fe(III)-reductases, which operate at the surface of root-cell plasma membranes in Strategy I species. The activity of these reductases can have large effects on iron availability.

Because the major cause of iron deficiency in plants grown on most aerobic soils is the insolubility of Fe(III)-oxides, root and microorganism redox potential or pH modifications of the rhizosphere are critical to controlling iron availability to roots for most plant species (Bienfait 1989; Moraghan and Mascagni 1991). Biotic redox processes within the rhizosphere can reduce Fe(III) in Fe(III)-oxides and soluble Fe(III)-chelates to Fe(II), with ensuing disassociation of Fe(III) from these unavailable sources and release of soluble Fe^{2+} for subsequent uptake by plant roots (Welch 1994; Welch and Kochian 1992). Root redox processes are highly regulated and under genetic control in Strategy I plant species (Kochian 1991; Welch 1994). Apparently cereal species (that is, Strategy II species), which evolved in more alkaline soils, developed mechanisms other than redox and pH systems to increase iron availability (for example, phytometallophore uptake systems) (Crowley et al. 1991; Römheld 1991). Therefore, the following discussion is restricted to dicots and nongrass monocots (see the subsequent section on phytometallophores in Strategy II species for a discussion of cereal mechanisms).

Root Reductases in Strategy I Species

Briefly, root cells of Strategy I species contain at least two types of Fe(III) reductases. The standard reductase system, present in all higher plant root-cell plasma membranes, can reduce Fe(III) to Fe(II) from various soluble complexes, including ferricyanide, but not from Fe(III) in synthetic chelates such as Fe(III)-EDTA, or from Fe(III)-siderophores such as ferrichrome. A second class of root-cell plasma membrane reductases, termed constitutive, inducible, or "turbo" reductases, can reduce Fe(III) in soluble iron complexes such as

ferricyanide and also the iron in synthetic Fe(III)-chelates such as Fe(III)-EDDHA, Fe(III)-EDTA, and Fe(III)-DTPA. They can also reduce Fe(III) from naturally occurring Fe(III)-chelates such as the phytosiderophores produced by cereal roots (Kaji et al. 1993) but not from extremely stable Fe(III)-microbial siderophores such as aerobactin and enterobactin.

The Fe(III)-chelate reductase system is highly regulated and under environmental control. It responds to the nutritional status of the plant. Recent research has shown that this system is also effective in reducing Cu(II)- and Mn(III,IV)-chelates in solution. Deficiencies of these micronutrient metals result in increased activity of the reductase, and, additionally, the reductase activity is directly linked to root-cell H⁺ ion excretion rates. Single-gene, Strategy I plant mutants are known that either lack the ability to synthesize the reductase system (for example, the *fer* mutant in tomato, *Lycopersicon esculentum* Mill., cv T3820fer, genotype *fer*) or cannot turn off the reductase system (for example, the *brz* mutant of pea, *Pisum sativum* L., genotype E107 [*brz*, *brz*]) (Welch 1994).

The *fer* mutant of tomato develops iron deficiency early after germination unless it is supplied with an adequate level of Fe²⁺. The single-gene, E107 pea mutant will accumulate toxic concentrations of iron and manganese in its older leaves (that is, more than 1 percent iron by dry weight) unless grown on substrates containing very low levels of these micronutrients. Additionally, this mutant accumulates high shoot concentrations of many other cations, including zinc and magnesium. Thus, the activity of the constitutive reductase system in Strategy I species root cells appears to regulate the uptake of iron and other micronutrient metals by these species when they are grown under aerobic conditions (Welch and Kochian 1992; Welch 1994). Because the regulation of micronutrient metal uptake by Strategy I species appears to be controlled by only a few or even a single recessive gene, breeding for increased root reductase activity in micronutrient metal-inefficient Strategy I plant species may be a viable approach to increasing the accumulation of iron and zinc by these species.

Interestingly, the plant hormone ethylene has been linked to activity of the constitutive reductase system. Roots of plant-provided inhibitors of ethylene's action cannot increase Fe(III)-chelate reducing capacity even when grown under iron-deficiency conditions, and plants grown with adequate iron supplies have enhanced Fe(III)-chelate reductase activity if supplied with promoters of ethylene action (Welch 1994), even though they are not iron deficient. Thus, determining the amount of ethylene evolved from plant roots may provide a simple method of screening for iron- and zinc-efficient genotypes when cultured under iron- and zinc-deficient conditions. However, more research is required before an understanding of the effects of ethylene on reductase activity is fully understood.

ROOT IRON AND ZINC ABSORPTION MECHANISMS

The Driving Force behind Fe²⁺ and Zn²⁺ Uptake

The uptake of free Fe²⁺ and Zn²⁺ ions from soil solution by plant root cells is driven by electrical chemical potential gradients that exist across the root-cell plasma membrane. The driving force for absorption is generated by a root-cell, plasma membrane-bound, H⁺-translocating ATPase that actively pumps H⁺ ions, at the expense of ATP hydrolysis, from the cytosol across the membrane and into the root-cell apoplasmic spaces (that is, intercellular free space equilibrated with rhizosphere soil solution in contact with root-cell plasma membrane surfaces), forming an electrical potential gradient (Welch 1994). The activity of

the H⁺ transport system is highly regulated, and various factors, both nutritional and environmental, can influence its activity. The balance between net anion and net cation influx is of greatest consequence (Kochian 1991). According to the strict biophysical definition of active transport, the absorption of Fe²⁺ and Zn²⁺ is not an active transport process, but instead a passive process, because the electrical potential of the plasma membrane provides more than sufficient energy to drive such uptake. There is no need to link the hydrolysis of cellular energy substrates (for example, ATP or NADH) directly to their absorption.

Transmembrane Movement of Fe²⁺ and Zn²⁺

While the actual path of free Fe²⁺ and Zn²⁺ transport across the plasma membrane is not known with certainty, evidence is accumulating that their transport across this cellular barrier occurs through transport proteins that form divalent cation channels or conduits that traverse the plasma membrane (Welch 1994). Older hypotheses that invoked membrane carrier proteins specifically for Fe²⁺ or Zn²⁺ transport are being discarded in favor of the broader ion-channel hypotheses. Transport through these channels is driven by the membrane electrical potential and can be a passive process, as already discussed. Ion movement through these channels can, in turn, be controlled by channel gates (modified regions within the transport protein complexes) that open and close in response to a wide variety of cellular and environmental stimuli (Welch 1994). Ca²⁺ or Mg²⁺ channels or both may be the primary routes of entry for Fe²⁺ and Zn²⁺ into root cells. Interestingly, preliminary evidence shows higher specificity for Fe²⁺ and Zn²⁺ transport through Ca²⁺ or Mg²⁺ channels or both than for Ca²⁺ or Mg²⁺ transport through these same conduits (Huang and Kochian 1994). Concerns over ion specificity during divalent-ion channel transport are really not an issue, because the concentrations of Fe²⁺ and Zn²⁺ in soil solutions are exceedingly low in aerobic soil solutions compared with the corresponding concentrations of Ca²⁺ and Mg²⁺ ions. There is no need to be concerned about lack of specificity resulting in the accumulation of toxic levels of iron or zinc under normal soil conditions, if these species enter root cells via Ca²⁺ or Mg²⁺ channels or both.

Root Reductase Control and Divalent Ion Uptake

Recent studies suggest that the activity of reductase systems (that is, standard, constitutive, or inducible reductases or all three) may be involved in controlling gating phenomena in divalent cation channels, thereby regulating influx of metals, such as Fe²⁺ and Zn²⁺, into root cells (Welch 1994; Welch, Norvell et al. 1993). Increased activity of these reductases may reduce disulfide groups to sulfhydryls in channel-gating proteins, changing channel transport protein conformation from the closed to the opened state. This would allow the movement of cations down their electrochemical potential gradients into the cell. If true, manipulating the activity of root-cell plasma membrane reductase through plant breeding or genetic engineering techniques may be a key to improving the absorption efficiency of iron and zinc by normally iron- or zinc-inefficient food crop species.

Phytometallophores in Strategy II Species

The term “phytosiderophore” has been used to define a class of low-molecular-weight compounds synthesized by grass root cells in response to iron-deficiency conditions (see Table 15). These molecules are biosynthesized from cellular methionine via S-adenosyl-L-

Table 15—Some phytometallophores identified in root washings of iron-deficient cereals

Phytometallophore	Cereal Species
Mugineic acid	<i>Hordeum vulgare</i> L. (barley)
Avenic acid A and B	<i>Avena sativa</i> L. (oat)
2'-deoxymugineic acid	<i>Triticum aestivum</i> L. (wheat) and <i>Avena sativa</i> L. (oat)
3-hydroxymugineic acid	<i>Secale cereale</i> L. (rye)
Distichonic acid	<i>Hordeum vulgare</i> L., var. <i>distichum</i> (beer barley)
3-epihydroxy-mugineic acid	<i>Oryza sativa</i> L. (rice)

Source. Mori et al. 1991; Sugiura and Nomoto 1984.

methionine (SAM) and nicotianamine (Shojima et al. 1990) and form highly stable, hexadentate chelates with iron (Sugiura and Nomoto 1984). However, they also form stable chelates with other polyvalent transition metal ions, including zinc, copper, nickel, cobalt, and manganese. Cereal root cells are capable of completely absorbing these metal chelates (Welch 1994). Thus, in this review, the more general term "phytometallophore" is used to delineate these compounds.

The release of metal-free phytometallophores by cereal roots is stimulated by both iron and zinc deficiencies (Römheld and Marschner 1990; Zhang, Römheld, and Marschner 1989). Their release is accompanied by increased mobilization of iron and zinc from insoluble sources both in the rhizosphere and within root-cell apoplasmic spaces. Thus, their release is associated with increased availability of these micronutrients to cereal roots.

Some research suggests that phytometallophore metal-chelate absorption into root cells occurs through an H⁺-amino acid cotransport system (Mihashi and Mori 1989; Welch 1994). These phytometallophores may be absorbed across the plasma membrane by way of a transport system that normally functions for the transport of amino acids such as lysine and arginine. According to this mechanism, the driving force for phytometallophore uptake would be provided by both the H⁺ gradient and the K⁺ gradient that exist across the root-cell plasma membrane.

Aerobic microorganisms in soils (both fungi and bacteria) have genes that encode for inducible, iron-deficiency-stress, Fe(III)-siderophore transport systems (Crowley et al. 1991). These systems consist of specific Fe(III)-siderophore-ligand membrane-binding sites (siderophore receptors), membrane transport proteins (Fe[III]-siderophore transport protein and Fe²⁺ plasma membrane channels), plasma membrane-bound reductases, regulatory proteins controlling these inducible systems, and biosynthetic processes for siderophore synthesis and Fe(III)-siderophore cytosolic degradation (Crowley et al. 1991; Neilands 1990a). The genes responsible for regulating these systems are few in number (no more than four) and are incorporated into an iron operon region of a chromosome (Neilands 1990a, 1990b). If iron transport systems were conserved during higher plant evolution, then these types of genes could be manipulated to improve iron and zinc uptake by rice, wheat, and maize—important staple food crops for developing countries. Interestingly, nicotianamine biosynthesis, the direct biochemical precursor of phytometallophores, has been shown to be under the control of a single recessive gene in all higher plants investigated. This lends support to simple genetic control of the synthesis for these compounds (Welch and Kochian 1992).

IRON AND ZINC TRANSLOCATION MECHANISMS

The second barrier to the passage of iron and zinc to edible portions of food crops is the regulation of intracellular storage (organelle storage, such as in vacuoles) and intercellular transport (that is, symplasmic transport) processes in plant cells. Unregulated, free Fe^{2+} and Fe^{3+} ions can be toxic to cells because these ions can undergo redox reactions with many redox-sensitive cellular metabolites, producing toxic free radicals (for example, the highly reactive hydroxyl radical). Cellular damage or even death can result from such reactions. High Zn^{2+} cytoplasmic activities are also potentially toxic to cells, because they can interfere with the interaction of other required metals with essential plant metabolites. For example, excessive free Zn^{2+} ions can compete for free sulfhydryl groups at active sites in numerous metal-activated enzymes, interfering with the ability of proteins to bind metals, such as Mg^{2+} , Fe^{2+} , Mn^{2+} , Ni^{2+} , and Cu^{2+} , for activation. Thus, the free activity of iron and zinc ions in the cytoplasm of plant cells must be maintained at a very low level to prevent such harmful repercussions (Welch 1994).

Intra- and Intercellular Transport

Plants have evolved mechanisms to prevent the accumulation of excessive amounts of free iron and zinc ions in their cytosol. One such mechanism is chelation with low-molecular-weight, nonprotein amino acids, such as nicotianamine. Another is by binding to small peptides such as the class III metallothioneines or phytochelatins (poly[-glutamyl cysteinyl]-n-glycine; n = 2–7). Still another is via storage in subcellular pools, such as within the vacuole as precipitates with polyphosphate or as complexes with organic acids. Once iron or zinc is sequestered within root-cell organelles, it is difficult to remobilize it back into the symplasmic stream leading to vascular tissues, unless deficiencies of these metals are encountered during plant growth. Increasing a plant's ability to synthesize the low-molecular-weight metal-complexing agents, such as nicotianamine and phytochelatin, within its root-cell symplasmic spaces would possibly result in increased movement of iron and zinc to vascular tissues. Further research is needed to test this hypothesis (Welch 1994).

Xylem Transport

Once iron and zinc pass into vascular tissue via the symplasm, they encounter a third barrier to their movement to shoots. Again, this barrier consists of a plasma membrane (that is, the plasma membranes that enclose xylem parenchyma cells abutting against xylem vessels and tracheid elements within the root stele) (Welch 1994). For iron and zinc to enter the xylem stream, they must cross this membrane. This step in iron and zinc transport to shoots has been little studied and remains largely a "black box" at present (Welch 1986). What, if anything, can be done to increase the transport of iron and zinc across this barrier is not known with any certainty, but increasing their symplasmic concentrations would increase the gradient for their movement to this barrier and thus would appear to be a logical approach to increasing transport of iron and zinc to shoots.

Once they have entered the xylem stream, iron and zinc do not move as free ions but are bound to various organic acids and phytometallophores that exist in this aqueous fluid (Welch

1994; White et al. 1981; White, Chaney, and Decker 1981; White, Decker, and Chaney 1981). Their complexed forms are transported upward in the transpiration stream, driven by either the water vapor gradient developed within leaf stomates or root pressure or both (Welch 1986). Increasing the xylem sap concentration of phytometallophores through genetic manipulations might be another viable approach to increasing the movement of iron and zinc to plant leaves if sufficient genetic diversity can be found for this trait. Further research is needed to establish this possibility.

Phloem Transport

Xylem sap does not directly supply iron and zinc to developing seeds and grains, because there exists a discontinuity between mature xylem elements and such edible plant parts. Phloem sap is responsible for delivering iron and zinc to maturing seeds and grains (Welch 1986). Therefore, phloem transport mechanisms are of primary importance in the movement of iron and zinc into these important plant food organs.

Before exiting the xylem sap, iron and zinc must be transported across at least one other plasma membrane barrier before they can enter phloem tissues (Welch 1986). In their simplest form, the plasma membranes surrounding the phloem sieve tube companion cells and positioned next to the apoplasm associated with mature xylem elements in leaf vascular bundles are the membranes that iron and zinc must cross before entering phloem tissues. Once absorbed into these companion cells, iron and zinc could then move through the symplasm of these cells via plasmodesmata into sieve tube elements and the phloem sap. To the authors' knowledge, the processes involved in this step of iron and zinc movement have never been studied, and more research is needed to identify the mechanisms involved.

Movement within the phloem sap is driven under pressure by mass flow processes, with iron and zinc moving with phloem solutes from regions of phloem-solute loading (mature or source leaves) to regions of phloem-solute unloading that include reproductive organs, roots, meristematic tissues, and newly formed leaves (Welch 1986). Therefore, source-sink relationships should dominate the movement of iron and zinc to these organs. However, iron and zinc exchange between phloem sap and adjoining cells (that is, transfer cells) in various plant tissues en route to these organs could influence the delivery of iron and zinc.

The chemical composition, pH, and redox potential of phloem sap differ greatly from those found in xylem sap (Welch 1986, 1994). Because of such differences, the dominant forms of iron and zinc in phloem sap should be quite different from those found in xylem sap. Also, the free ionic activity of iron and zinc in phloem sap would be exceedingly low, because of the high pH (ranging from 7.5 to over 8) and high phosphate level (for example, 14 millimolar Pi) of phloem sap (that is, at pH values greater than 7.5). These metals in their free form would precipitate as mixed oxides, hydroxides, and phosphates.

Relatively high concentrations of phytometallophores (for example, 2.26 millimolar deoxymugineic acid and 0.67 millimolar epihydroxymugineic acid) have been found in phloem sap collected from rice shoots (Mori et al. 1991). Nicotianamine has also been recognized as a mobile form of Fe(II) in phloem sap. Indeed, nicotianamine is essential for iron transport in phloem sap of the single-gene tomato (*Lycopersicon esculentum* Mill.) mutant, chloronerva (Stephan and Scholz 1993). These findings suggest that genetic selection for improved phloem transport of iron and zinc may be possible with identification of the genes responsible

for phytometallophore synthesis and regulation. Research should be undertaken to determine the feasibility of such approaches.

Deposition and Storage

Once iron and zinc are unloaded from the phloem into developing seed or grain tissues, they are incorporated into plant metabolites or transported for storage to deposition sites in specific regions of these organs. Their highest levels are normally found in globoid crystals, which accumulate in membrane-bound protein bodies that occur in the aleurone layer and developing embryo of cereal grains, and within the cotyledons and embryonic tissues of seeds. Their storage is associated with the occurrence of phytin deposits (a mixed salt of phytic acid—myo-inositolhexaphosphoric acid) in the globoid crystals. Lower concentrations are also found in other regions of the endosperm in cereals and within other tissues of developing seeds. Here, they probably occur in association with essential metabolites that require their presence to function, such as the iron and zinc in metal-activated enzymes or possibly within certain sulfhydryl rich storage proteins within the developing embryo and associated tissues (Welch 1986, 1994). Unfortunately, storage pools associated with phytin may contain little bioavailable iron and zinc for people who do not eat significant amounts of animal protein in their diets.

More research should be carried out to delineate the forms of iron and zinc in seeds and grains. Identifying these forms and their associated storage sites should aid, in turn, in developing food-processing techniques to limit the effect of these techniques on the nutritional quality of edible products produced from seeds and grains. Additionally, research should be undertaken to determine if any of these forms have potential to provide storage pools of highly bioavailable iron and zinc for people. If such storage pools are identified, efforts might be taken to increase the level of these substances in staple seeds and grains to improve the bioavailability of iron and zinc in these crops, although adverse effects of such actions on plant production should also be studied concurrently.

6. GENOTYPIC VARIATIONS IN THE MICRONUTRIENT DENSITY OF SEEDS

The micronutrient content of seeds can be viewed as having a number of heritable components. Concentration and content are technical terms used by plant nutritionists to mean milligrams per kilogram and micrograms per seed, respectively (or equivalent units). The relation between these two measures of micronutrient density is as follows:

$$\text{content} = \text{concentration} \times \text{weight of seed.}$$

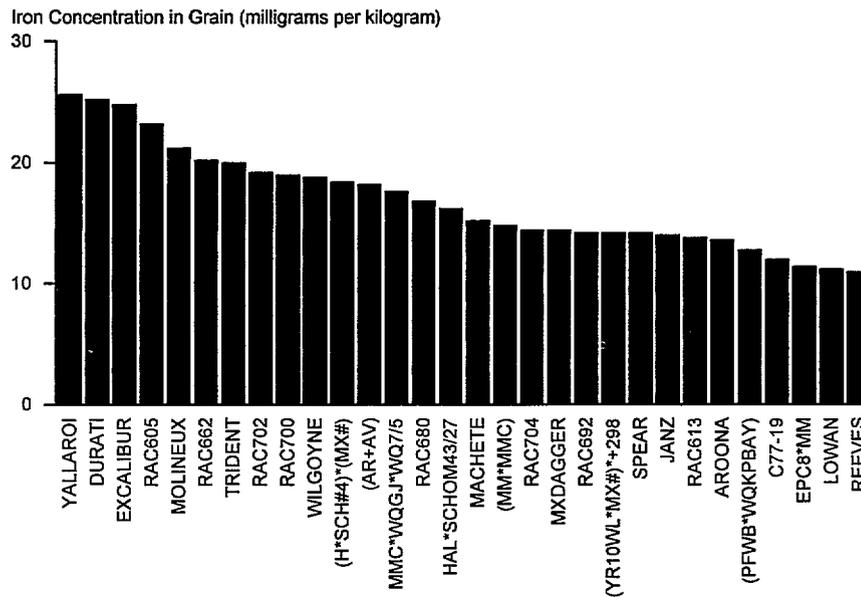
Plump seeds filled out with starch under the best growing conditions tend to have a lower concentration because of "dilution" by the extra starch, though their content may be the same or even greater. From the point of view of human nutrition, high concentration (density) appears to be the best criterion, since grains are sold for food almost exclusively by weight. For the nutrition of the seedling that may grow from the seed if it is planted rather than eaten, seed content is probably more important. Both are under genetic control but are also influenced strongly by many factors in the environment of the mother plant.

The concentration and content of micronutrients in seeds are the result of transport through living tissues (the phloem) from vegetative parts of the plant. Thus, seed density depends both on the micronutrient density of vegetative tissues and on the efficiency of the transport process itself. Both can be under genetic control, but there is considerable homeostasis built into the transport process. Thus, even when the soil and the vegetative plant are high in micronutrients, the levels in the seed are always relatively low. Over a range of genotypes, micronutrient density of seeds varies by a factor of 3 from lowest to highest, while vegetative parts may vary in concentration up to 100 times more than that. Still, simple calculations on food intakes for people whose diet is predominantly grains suggest that even a 50 percent increase in density could make a major contribution to nutrient balance and to health. As some of the following data will show, this goal should be entirely achievable.

Where vegetative parts are high in the target nutrient, as paddy rice grown on acid soils is high in iron, the strategy should be to select for improved internal transport to the grain. Where the soils themselves are low in available nutrients, the strategy should be to exploit genetic variations that improve absorption of nutrients from the soil. Most micronutrient efficiency traits for deficient soils so far explored have been expressed as a greater ability to extract the nutrient from the soil instead of a greater ability to survive on less of the nutrient in vegetative tissues or in grain.

EVIDENCE FOR GENETIC VARIATION IN SEED NUTRIENT LOADING

Although the transport mechanisms are unknown in most cases, it is assumed that, where the data are collected from sites adequate in the target nutrient, variation in seed density due to genotype is the result of variation in the transport system. In Figure 8, seed iron concentration varies over a wide range although the soil is not deficient in iron or, probably, in any other nutrient. These data suggest that an improvement of 50 percent in iron concentration is readily achievable if the variation is, as is believed, primarily genotypic. The first two ranked

Figure 8—Seed iron concentration in 30 wheat cultivars and breeders' lines

Source: J. van Beusichem and R. D. Graham, unpublished data.

Note: Wheat was grown in sandy soil at Yeelanna, Australia, in 1991.

varieties are acutely sensitive to zinc deficiency. This could explain their high iron contents. Therefore, attention ought to be focused on the third ranked line, which is highly zinc efficient as well.

Similarly, useful ranges in iron and zinc concentrations are shown for Phaseolus beans, a staple of about 300 million people in Africa and South America (Table 16). The relatively high concentrations of iron in dry beans (compared with cereals) contrast with the low values reported for cooked beans. It is likely that these large differences are primarily the result of their origin (soil type) rather than of genotype or cooking, since the Popayan soil is acidic and therefore relatively high in available iron and zinc. This site is certain to have adequate iron for beans.

Table 16—Iron and zinc concentrations in whole seeds of four cultivars each of Nuñas (popping) and dry beans grown at Popayan, Colombia, in the same season, and of four types of cooked beans of different origins

Nuñas Beans			Dry Beans			Cooked Beans		
Cultivar	Iron	Zinc	Cultivar	Iron	Zinc	Cultivar	Iron	Zinc
	(milligrams per kilogram)			(milligrams per kilogram)			(milligrams per kilogram)	
Pava	73	23	Magdalena 3	83	24	Pinto	22	10
Parcoyana	71	23	ICA Viboral	96	35	Navy	25	10
Mani Roja	61	25	ICA Llanogr	87	23	Red	22	10
Limona	60	34	Mortiño	107	28	Lima	16	9

Sources: van Beem, Kornegay, and Lareo 1992; Pennington and Young 1990.

GENOTYPIC DIFFERENCES IN MICRONUTRIENT EFFICIENCY

The genetic potential for improvement of nutrient efficiency is readily apparent from inspection of a diverse literature, including the proceedings of international symposia on the subject (Brown 1982; Christiansen and Lewis 1982; Graham 1984, 1988b; Jung 1978; Saric and Loughman 1983; Wright 1976), and various reviews (of which Clark 1982 lists 20).

Among the cereals, rye is especially nutrient efficient and may be taken as a standard of efficiency to be achieved in all small-grain cereals. Rye has proved to be copper efficient (Graham 1984; Harry and Graham 1981), zinc efficient (Harry 1982) and manganese efficient (Graham et al. 1983). The copper efficiency of triticale and rye is shown in Table 17. Wheat was affected vegetatively and was totally male sterile under conditions in which native soil copper was adequate for near-maximal yield of rye. Analysis of the above-ground parts showed that rye translocated roughly twice as much copper to shoots as did wheat, with triticale being intermediate. This efficiency of uptake translated into similar increases in grain density of copper (shown in parentheses).

Differences in efficiency within species are also spectacular. Examples of differences among wheat cultivars in copper efficiency were reported by Nambiar (1976) and Smilde and Henkens (1967), in zinc efficiency by Randhawa and Takkar (1976), and in manganese efficiency by Graham et al. (1983). Iron efficiency also varies widely between and within species of cultivated plants (Brown 1982; Clark 1982) but appears to be inherently high in cereals other than maize and sorghum. This is true even for the most alkaline or calcareous soils used for wheat and barley production.

By far the most extensive survey of efficiency factors was carried out at the International Rice Research Institute (Ponnamperuma 1982). Over a period of 10 years, some 80,000 lines from the world collection were screened for types tolerant of a number of soil stresses, including micronutrient deficiencies. Tolerant types gave a yield advantage of about 2 metric tons per hectare under any of seven different soil limitations (Table 18).¹ Ponnamperuma noted that zinc deficiency was widespread in paddy rice, and iron deficiency in dryland rice.

Table 17—Grain yield responses of wheat, rye, and triticale to copper added to copper-deficient sandy soil in the greenhouse

Genotype	Amount of Copper Added		Relative Yield ^b
	None	4 Milligrams per Pot ^a	
	(yield in grams per pot)		
Wheat cv. Halberd	0	11.3 (2.4)	0
Triticale, Armadillo	2.1	11.7 (3.0)	18
Rye cv. S.A. commercial	4	4.3 (4.9)	93

Source: Harry and Graham 1981.

Note: Data shown are means of results for two soil pH treatments, 7.0 and 8.4, recalculated from the data of Harry and Graham 1981. Self-incompatibility in the rye lowers its yield potential in the greenhouse.

^aFigures in parentheses are grain copper concentrations.

^bRelative yield = 100 (yield with no copper added/yield with copper added).

¹All tons in this paper are metric tons.

Table 18—Yield range of a selection of rice lines from the world collection, in response to soil stresses

Stress	Total Number			Mean Yield		
	Tests	Sites	Races	Minimum	Maximum	Difference
				(metric tons per hectare)		
Iron toxicity	14	4	58	2.1	4.7	2.6
Aluminum/manganese toxicity	4	1	36	1.8	3.6	1.8
Phosphorus deficiency	14	2	118	1.9	4.3	2.4
Zinc deficiency	31	10	107	0.8	2.9	2.1
Iron deficiency	9	3	69	0.8	2.7	1.9

Source: Ponnampereuma 1982.

Manganese deficiency has been reported for diverse crops (Table 19). It is difficult to generalize about tolerance among either plant families or species. Variations in tolerance levels within species (Table 20) can be almost as great as those between species (Table 19). In regions where farmers recognize manganese-deficient soil, they can use more manganese-efficient cultivars to achieve overall superior performance, although they may not recognize the physiological basis for such performance.

The most extensive literature on genotypic variation for zinc efficiency in crop plants appears to concern India, where Takkar (1991) pointed out that nearly half of more than 100,000 soil samples were considered low in zinc. A recent review of the Indian literature covers 16 crops (Takkar 1993). Within a crop, cultivars varied from tolerant (no or little response to zinc fertilizer on deficient soil) to sensitive, where the response to zinc in the sensitive types was severalfold. The largest single screening exercise was of 3,703 lines of rice (IRRI 1979; Ponnampereuma 1976), where 388 lines were judged to be tolerant and a strongly responsive soil was used. Like the cereals rice and wheat, soybean varieties show a yield range of two- to threefold in their response to zinc fertilizer (Rao, Gangwar, and Rathore 1977; Rose, Felton, and Banke 1981; Saxena and Chandel 1992). Again, such a result is suggested to be a consequence of differential efficiency of zinc absorption; the distribution of F₃ lines from the cross between zinc-efficient and zinc-inefficient genotypes (for 330 F₃ lines tested) suggests that only a few genes control the zinc efficiency trait (Hartwig, Jones, and Kilen 1991). In recent attempts to breed for zinc efficiency in soybeans, Hartwig, Jones, and Kilen (1991) found that concentrations of zinc in fully developed trifoliate leaves differed by a factor of two to three between tolerant and intolerant lines, with the latter showing a significant yield response to zinc in Minnesota.

In southern Australia, zinc deficiency is also widespread. According to a survey of farms in the Mallee district, 96 percent of crops were low in zinc (Hannam 1991). The range in sensitivity to zinc deficiency for wheat is similar to that reported by Takkar and others in India: zinc-efficient cultivars yield up to three times that of zinc-inefficient types (Table 21; Graham 1988a, 1991). Although the zinc concentration is low in the highest-yielding cultivar, its zinc content is high (about 11.5 grams per hectare).

Large differences in micronutrient efficiency can be demonstrated, stepwise, in pots and in the field among the following cereals:

ryes > triticales > bread wheats > durum wheats.

Table 19—Genotypic variation in response to manganese deficiency among various plant taxonomic groups

Source of Variation	Number of Entries	Plant Name(s)	Basis of Assessment	Extent of Variation	Experimental Conditions	Reference
Intraspecific						
Cultivar + breeder's lines	4	Oat	Grain yield	11–56 (+Mn)	Water culture	Brown and Jones (1974)
Cultivar	8	Oat	Tissue (Mn)	64–100	Sand culture	Deb and Scheffer (1971)
Cultivar	6	Spring wheat	Grain yield	11–85 (+Mn)	Pots	Gallagher and Walsh (1943)
Cultivar + breeder's lines	72	Barley	Grain yield	0–178	Field	Graham et al. (1983)
Breeder's lines	21	Sorghum	Tissue (Mn)	79–147	Field	Duncan (1983)
Cultivar + breeder's lines	4	Rice	Tissue	67–189	Field	Kunbhar and Sonar (1987)
Cultivar + breeder's lines	44	Spinach	Tissue (Mn)	0–69		Karvanek and Bantova (1966)
Cultivar + breeder's lines	73	Peas	Percent of plants affected	0–35 (+Mn)	Field	Glassoch (1941)
Cultivar + breeder's lines	10	Sweet lupine	Grain yield	26–100 (+Mn)	Field	Walton (1978)
Cultivar + breeder's lines	4	Soybean	Grain yield	65–100	Pots	Nair and Prabhat (1977)
Cultivar	8	Soybean	Grain yield	55–100	Field	Parker et al. (1981)
Genera, families, etc.						
Genera + species + cultivar	7	Cereals	Grain yield	56–100 (+Mn)	Field	Graham et al. (1983)
Genera + species + cultivar	8	Cereals	Grain yield	0–105 (+Mn)	Pots	Nyborg (1970)
Genera	4	Spinach, barley, peas, and flax	Green weight	71–95 (+Mn)	Pots	Takeuchi (1909)
Genera + species	6	Vegetables	Yield	89–94 (+Mn)	Field	Gilbert (1934)

Note: The extent of variation is expressed on a relative basis, where 100 is considered to be the score of an agriculturally standard genotype or group, or the score of plants subject to a +manganese treatment.

Table 20—Manganese efficiency of barley lines grown in manganese-deficient soil at Wangary, South Australia, 1985

Genotype	Grain Yield		Manganese Efficiency ^b	Manganese Concentration in Grain	
	With No Manganese Applied	With Manganese Applied		With No Manganese Applied	With Manganese Applied
	(metric tons per hectare)		(percent)	(milligrams per kilogram)	
WA73S276	3.5	4.8	73	10	13
Galleon	0.5	3.3	15	5	9

Source: Unpublished data of Graham.

^aSix kilograms of manganese were applied per hectare.

^bManganese efficiency = 100 (–Mn/+Mn).

Table 21—Zinc efficiency of five wheat genotypes grown in a farmer's field at Lameroo, South Australia, 1988

Genotype	Grain Yield		Zinc Efficiency ^b	Zinc Concentration in Grain	
	With No Zinc Applied	With Zinc Applied ^a		With No Zinc Applied	With Zinc Applied ^a
	(metric tons per hectare)		(percent)	(milligrams per kilogram)	
Excalibur	1.43	1.76	81	8	25
Warigal	1.00	1.22	82	13	26
Kite	0.95	1.37	69	10	24
TJB*MKR	0.63	1.30	48	12	30
Kamilaroi	0.45	1.29	34	9	23
LSD	0.3			3	

Source: Graham and Ascher 1993.

^aTwo-and-a-half kilograms of zinc were applied per hectare.

^bZinc efficiency = $100 (-Zn/+Zn)$.

Durum wheats, in the authors' experience, are exceptionally poorly adapted to micronutrient-deficient soils (Graham 1988a, 1991; Graham and Pearce 1979), and their zinc inefficiency (see Kamilaroi in Table 21) is typical of their reaction to other deficiencies. Zinc efficiency varies among durum wheats depending on the soil type, suggesting that the mechanisms involved in handling complex zinc deficiency in heavy clays are different from those involved in handling simple deficiency in deep sands.

Zinc efficiency is not well linked to other efficiency traits (for example, for manganese); this suggests that different efficiency traits have independent mechanisms and are subject to genetic control not tied to root-system geometry. Zinc-efficient genotypes absorb more zinc from deficient soils and produce more dry matter and more grain yield but do not necessarily have the highest zinc concentrations in tissue or grain. Although high zinc concentration in grain also appears to be under genetic control, it is not tightly linked to agronomic zinc efficiency traits and may have to be selected for independently.

PROVITAMIN A

The prospects for improving the provitamin A density of target crops are very good indeed. All the starchy, generally white food staples have varieties that are yellow. Although the genetics are complex and somewhat obscure, rapid progress is possible since genetic gain in carotene content can be visually estimated with accuracy (Simon 1992). The carotenoids tend to be stored in the endosperm and are thus less subject to the milling losses seen for mineral content. It is necessary, therefore, to characterize the parental material to establish that the sources of pigmentation do have high provitamin A activity in humans, as nonactive yellow pigments also occur in grains. Analysis for active pigments should be carried out during the initial survey of germplasm, so the intrinsic value of the sources is known from the beginning.

Yellow wheats are already in demand for pasta-making worldwide and for yellow noodles in parts of Asia, whereas white noodles are preferred elsewhere. Methods of handling pigments in durum wheat breeding are thus well known and could be introduced into bread

wheats in response to created demand. The common pigments in durum wheats do not have provitamin A activity and would require slight changes in molecular formula, perhaps by biotechnological transformation, before classical breeding work were to begin. Alternatively, it is necessary to explore wheat germplasm bank variability for active pigments, as it appears beta-carotene can be synthesized in wheat grains and may or may not be transferred to other pigments. Likewise, yellow rices exist and could be exploited. In Islamic countries yellow rice is highly regarded, and safranine (an expensive dye) is used to color rice yellow for special occasions. Wider preferences for yellow rice and other staples could be created through television advertising and education. "Prebreeding" projects should establish the potential genetic systems and selection criteria to enable breeders to respond quickly to future demand, whenever and however it is created.

Provitamin A content of plant food products varies genetically in many crops and by large amounts, up to 10 times the mean values (Haytowitz and Matthews 1984; Klaui and Bauernfeind 1981). In maize, total carotenoids varied by a factor of 2.5 between the means of six high- and six low-carotene inbred lines, and their provitamin A activity varied by a factor of 4.4. Even wider variation was found between means of single crosses made within these groups (Table 22). Yellow maizes have been recognized for decades as the main source of provitamin A for hogs and other farm animals relying on winter feed rations, and some effort has already been put into breeding for this trait. Since yellow maizes are acceptable in the marketplace, further enhancement of provitamin A content could immediately improve dietary intakes (Simon 1992).

Table 22—Provitamin A content of maize presented as the means of six high- and six low-carotene inbreds and as the means of nine single crosses made within each group

Maize Parents	Provitamin A Content of Kernel		
	Selfed	Single Crosses	High/Low Single Cross
	(milligrams per kilogram)		
High carotene	6.5	7.5	4.1
Low carotene	1.3	1.7	

Source: Brunson and Quackenbush 1962.

Note: Provitamin A content was calculated from the contents of beta-carotene + beta-zeacarotene + cryptoxanthin

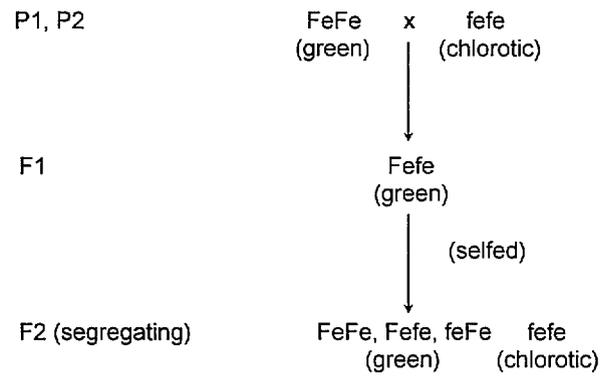
7. THE GENETICS OF MICRONUTRIENT EFFICIENCY

The recurring feature of micronutrient efficiency characters is single, major-gene inheritance (Epstein 1972). This was first demonstrated by the classic work of Weiss (1943) on iron efficiency in soybeans, resulting from the discovery of an iron-inefficient mutant. A genetic analysis of the progeny of the iron-efficient × iron-inefficient cross established that efficiency was due to a single, major, dominant gene that controlled the reducing power of the root surface (Figure 9), even though the phenotype of this double recessive trait is most easily seen in the leaves (Brown, Holmes, and Tiffin 1958). The efficient allele controls the ability of the roots to reduce Fe³⁺ to Fe²⁺ (Brown, Holmes, and Tiffin 1961).

Since Weiss's pioneering study, several minor additive genes have been discovered that contribute to iron efficiency for soybeans (Fehr 1982). This situation of both a major and several minor genes is likely to be the case, in general, for the micronutrients. Early reports were reviewed by Epstein (1972), who noted apparently simple genetic control of boron efficiency in tomato and celery, iron efficiency in maize and tomato, and magnesium efficiency in celery. Each of these results was derived from studies of recessive mutants. More recently, iron efficiency in tomato has been shown to be based on a major gene, coding for an iron-transporting amine, nicotianamine, and a string of minor genes (Brown and Wann 1982; Ripperger and Schreiber 1982). Since nicotianamine is also found in cereals, it is possible that differences among alleles at a corresponding locus will permit enhancement of nicotianamine synthesis and thereby also iron and zinc transport to grain. In a study of recurrent selection to improve iron efficiency of soybeans, Beeghly and Fehr (1989) found that, with the possible exception of an increase in lodging, there were no detrimental effects on the agronomic potential of the population after seven cycles of selection, while the seed iron concentration had increased significantly.

From a study of eight crosses by Hoan, Prasada Rao, and Siddiq (1992), iron efficiency in rice appears to be controlled by two pairs of non-allelic genes with complementary interaction. Basic gene *Ic*₁ requires *Ic*₃ to confer tolerance to iron deficiency; likewise, the pair *Ic*₂ and *Ic*₄, non-allelic to *Ic*₁ and *Ic*₃, together confer moderate tolerance. The recessive

Figure 9—Segregation in the F2 generation of a cross of iron-efficient (FeFe) and iron-inefficient (fefe) soybeans



Source: Weis 1943.

genes, ic_1 and ic_2 , regardless of the alleles at the other loci, are susceptible. ic_1 is closely linked to Ts, the tall seedling locus. The fact that these authors found a highly significant correlation between soluble Fe^{3+} -reduction capacity (their primary screening technique) and field resistance to chlorosis suggests that these efficiency genes may function independently from, and perhaps additively to, those studied by Mori et al. (1991). The latter work showed that iron efficiency was linked to synthesis and release of the phytosiderophore, deoxymugineic acid.

For dry beans (*Phaseolus vulgaris* L.), Coyne et al. (1982) also found two loci involved in iron efficiency, with dominant alleles being required at both loci for full expression. Although the data were not clear-cut on this point, it appeared to the authors that the interaction of these two genes was more additive than complementary, as had been true for the data of Hoan, Prasada Rao, and Siddiq (1992) above. As Beeghly and Fehr (1989) found with soybeans, wide variability in iron concentration had little effect on the level of other nutrients in vegetative parts. These studies did not analyze seeds.

Copper efficiency in rye appears to be a dominant trait controlled at a single locus on the long arm of chromosome 5R (Graham 1984). It has been possible to transfer copper efficiency from rye to wheat by means of a translocation of part of 5RL to a chromosome of wheat (Table 23). Efficiency in the 5RL/4A β genotype was not due to deletion of part of the 4Ab chromosome arm, because the 2R/4Ab genotype was inefficient and thus served as a control. Crosses of efficient \times inefficient rye produced evidence that efficiency is a dominant trait and probably controlled at a single locus, since the F2 segregants showed a ratio not significantly different from 3:1. Several such translocations exist but the 5RL/4A translocation appears to be the most satisfactory agronomic type and has been successfully incorporated into adapted cultivars for South Australia (Graham et al. 1987). The 5RL chromosome arm also confers copper efficiency on triticale (unless a rare copper-inefficient rye was used in the cross). Triticales generally show agronomically useful copper efficiency, being intermediate between wheat and rye, and thus have been used for this reason on many copper-deficient peats and sands. They have also been used on deficient clayey soils in Queensland to counter the effects of topsoil drying mentioned earlier (Grundon 1980).

Work with these 5R materials has shown that copper efficiency in rye is clearly not linked to zinc efficiency or manganese efficiency; thus, the 5R locus is specific for copper efficiency, independent and relatively specific genes are involved, and root system geometry or size does not appear to be critical. Although rye has a much longer and finer root system than

Table 23—Copper efficiency conferred on wheat by the trait located on the long arm of chromosome 5 of rye

Genotype	Grain Yield		Copper Efficiency ^b	Copper Concentration in Grain	
	With No Copper Applied	With Copper Applied ^a		With No Copper Applied	With Copper Applied ^a
	(metric tons per hectare)		(percent)	(milligrams per kilogram)	
Warigal	0.5	2.0	24	0.69	0.87
Warigal 5R/4A	1.6	2.7	61	0.77	1.01

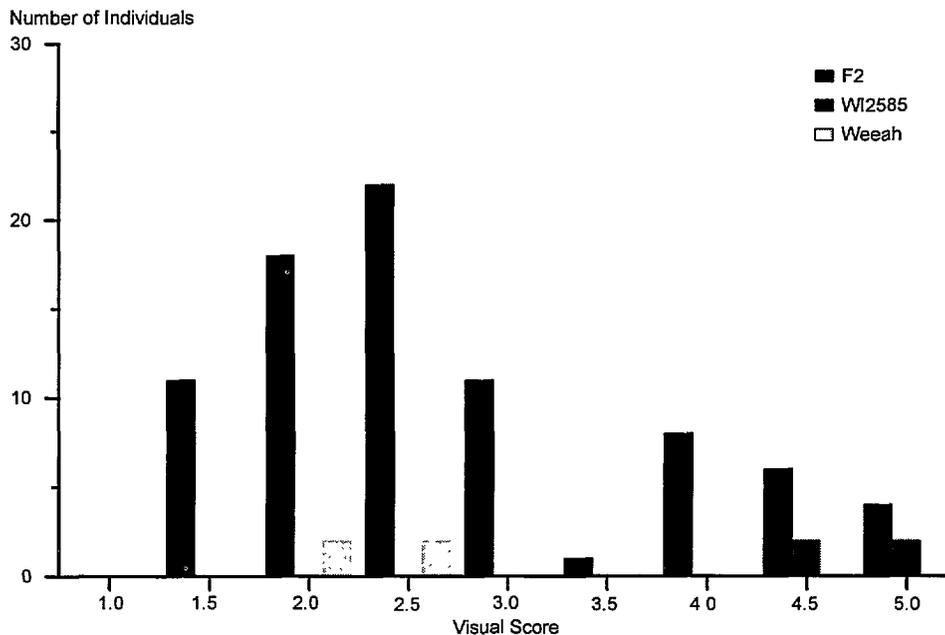
Source: Graham and Ascher 1993.

^aFour kilograms of copper were applied per hectare.

^bCopper efficiency = 100 (-Cu/+Cu).

wheat, triticale generally does not (Graham, Anderson, and Ascher 1981), yet it is usually more efficient for all three elements than its wheat parent (Cooper, Graham, and Longnecker 1988; Graham 1987; Graham et al. 1987; Harry 1982). Studies of rye addition lines suggest that 6R contributes somewhat to efficiency for all three elements, perhaps by way of a root geometry feature, but the major genes are elsewhere. Manganese efficiency is located on 2R, a conclusion supported by the poor performance on manganese-deficient soils of the cultivar Coorong, an Armadillo type triticale lacking the 2R chromosome. Manganese efficiency in barley (the best-studied species) appears to be simply inherited, based on the cross of Weeah (efficient) and Galleon (inefficient) (McCarthy et al. 1988). However, the line WA73S276, which has common parentage with Weeah, is significantly more efficient than Weeah. This suggests that other loci may be involved. Figure 10 shows the frequency distribution of F2 individual plants of a Weeah (efficient) × WI2585 (inefficient) cross. Assessment of efficiency was based on severity of symptoms (chlorosis) in the tops of five-week-old seedlings grown in deficient soil. Manganese efficiency appears to be controlled by a single dominant gene. Moreover, an important parent in the barley-breeding program at the Waite Institute, CI3576 from Alexandria, Egypt, is exceptionally susceptible to manganese deficiency, as are a high percentage of its progeny. At this stage, it is unclear whether this is some type of dominant manganese inefficiency trait or simply close linkage to a desirable trait in CI3576 that has been selected for in the breeding program. A low percentage of wheat

Figure 10—Frequency distribution of the severity of manganese-deficiency symptoms of F2 generation seedlings from a cross of Weeah × WI2585 barley grown in manganese-deficient soil



Source: McCarthy et al. 1988.

Note: Visual scores range from 1, signifying healthy, to 5, signifying extremely chlorotic.

cultivars also have exceptional sensitivity to manganese deficiency, the genetic basis of which is unclear as well.

By way of contrast, zinc efficiency in rye does not appear to be clear-cut (based on studies of rye addition lines of wheat by the authors) and may be spread across four or five chromosomes: 2R, 3R, 7/4R, and, to a lesser extent, 5RL and 6R appear to contribute (Graham 1988a). In comparison with iron, copper, and manganese efficiency, less is known of the genetics of zinc efficiency. Four additive genes appeared to be segregating among two zinc-efficient and two zinc-inefficient maize inbred lines for zinc concentration in the ear-leaf (El-Bendary et al. 1993). The ear-leaf zinc concentration could be increased more than 50 percent, but concentrations in the grain were not reported. A few genes are involved in zinc efficiency in rice, supporting the studies for rye. Following diallel analysis, a recent report suggested that mostly additive and, to a lesser extent, dominant gene effects are responsible for the zinc efficiency trait in rice (Majumder, Rakshit, and Borthakur 1990). The various mechanisms of zinc efficiency are likely to be additive (as suggested for rice), putting considerable emphasis on a breeding program based on stepwise compounding of genetic information (Yeo and Flowers 1986) for a number of zinc-efficiency mechanisms that are expressed at different levels of the plant organism (molecular, physiological, structural, or developmental) into one locally adapted crop cultivar (see Rengel 1992 for an analogous effect of this type). In such a breeding program, genotypes having genes controlling a particular mechanism for zinc efficiency may be highly important even though they themselves may not show phenotypically high overall zinc efficiency. One example is durum wheat, which shows varying performance in different soil types (Table 24), suggesting there may be different efficiency factors for heavy and light soils. Using genotypes from the local crop germplasm would be advantageous, because development of cultivars with improved zinc efficiency may be expedited without severely disrupting the broad adaptation previously selected.

Little definitive information on the genetics of inheritance of carotenoid content is available, except for carrots. Three major genes controlling primary color classes and other conditioning loci have been described for carrots (Gabelman and Peters 1979), and three basic biosynthetic enzymes are involved (Camara, Schantz, and Moneger 1992). Researchers have also characterized seven major biosynthetic steps and more than 20 genes in the synthesis of carotenoids by tomato (Porter, Spurgeon, and Sathyamoorthy 1984).

Table 24—Wheat grain yield of Kamilaroi relative to that of its parent, Durati, in contrasting soils of New South Wales and South Australia

Grain Yield	New South Wales (Black Clay)		South Australia (Light Sand)	
	With No Zinc Applied	With Zinc Applied	With No Zinc Applied	With Zinc Applied
Grain yield of Kamilaroi as a percent of the grain yield of Durati	130	105	95	110

Source: Data for New South Wales kindly supplied by Dr Ray Hare, Agricultural Research Centre, Tamworth, Australia. Data for South Australia are from the author.

Note: Kamilaroi = Durati × Leeds.

Hauge and Trost (1928) described a major gene for carotene content in maize and designated it the Y (yellow) locus. It is incompletely dominant.

As previously mentioned, rapid genetic progress is possible in this area despite a complex genetic system because of the ease of selection.

8. PLANT BREEDING STRATEGIES

To increase iron density in paddy rice and wheat, the starting point is the vegetative plant containing significant levels of the nutrient. The strategy here is simple: select types with a high capacity to move iron from the vegetative tissues to the grain (high harvest index). Screening is also simple: measure the concentration in the seed (or edible fractions of it) and select the highest ones. The only problem arises when the plant breeder wants to select in the seed or seedling stage, to minimize the number of plants in segregating populations that must be carried to maturity. Techniques may be developed to measure the nutrient content in half a seed and then to plant the other (embryo) half. With further work, if the mechanism of iron remobilization proves to be based on the plant's nicotianamine level, it may be easy to assay for this molecule in the leaf of the seedling. Promoter and inhibitor molecules can also be used as sole selection criteria for all crops.

After some preliminary work to establish that provitamin carotenoids are sources of color in the donor parent (not true, for example, for alkaline noodle wheats), selection for provitamin A may be based solely on product (seed) color, assuming that color is a desirable trait in the marketplace, as it is for some pasta wheats, or that education or government policy intervenes to influence the market.

In rice and corn, there are special opportunities to explore types with multiple aleurone layers that store more micronutrients in the seed, and microscopic examination may be useful in screening these after some initial analytical work has been done on the seeds.

Since zinc and iron deficiencies are common in corn, beans, upland rice, and cassava, the breeding strategy must concentrate on agronomic efficiency traits as well as seed-loading traits, as both should lead to higher seed micronutrient contents, and the agronomic advantages of the efficiency traits are important to the primary objective of raising micronutrient density. Selection for agronomic efficiency, however, is more difficult and time consuming.

SELECTION CRITERIA FOR AGRONOMIC EFFICIENCY TRAITS

The ultimate assessment of micronutrient efficiency is a field experiment comparing a new genotype with a standard cultivar where the soil is deficient enough in the limiting element to seriously limit the yield of some lines. Such tests are often conducted without any nutrient treatments when there are a large number of genotypes to be screened, but genotypes being tested may be located within an array of plots of a check genotype to provide a covariate on heterogeneity of the site. Differences in general adaptation and yield potential are assumed to be small compared with differences in efficiency; if not, a treatment with an adequate amount of the nutrient will define the yield potential and allow the calculation of an efficiency index. For example,

$$\text{zinc efficiency} = 100 (GY-/GY+),$$

where $GY-$ is the yield with no zinc applied and $GY+$ is the yield with zinc applied.

Such normalization removes much of the background genetic effect but does not exclude $Zn \times G \times E$ effects, which can occasionally be important (Graham 1991, Table 6); also, the data of Table 24 are an example of this interaction.

Field Testing

Selection in terms of yield is always imprecise and fraught with difficulties, for almost everything in the genome contributes to yield either directly or indirectly. If deficiency is severe and is the primary limiting factor, then selection based on yield will identify efficient genotypes, but the possibility that significant interactions will cloud selection is always strong.

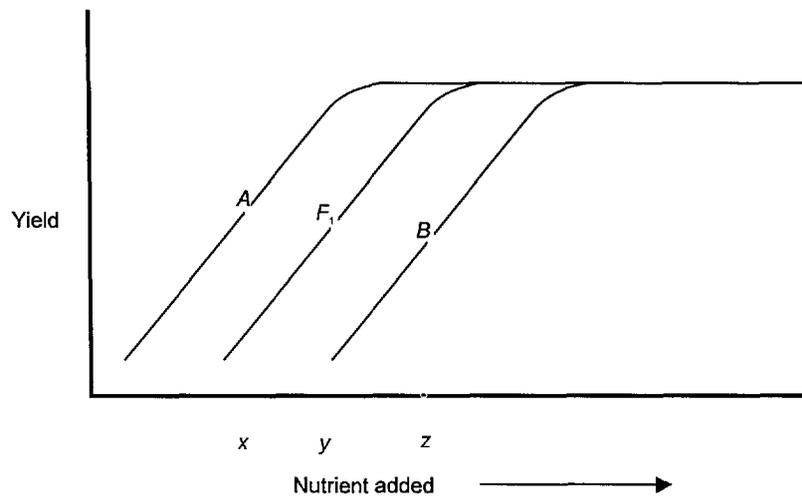
The authors' main approach to screening is to use plus-and-minus fertilizer plot pairs to calibrate the performance of a genotype in deficient soil against its own potential with the limiting element supplied. The primary efficiency index is thus micronutrient efficiency as defined in the previous section. However, this quotient is not the only concern. For some purposes, the lines with the highest grain yield in the nutrient-deficient plots (GY^-) may be of interest when they have outstanding yield potential yet still respond significantly to zinc (for example, Excalibur, shown in Table 21). Also of independent interest is GY^+ , potential grain yield. A "belt-and-braces" approach to zinc-deficient soils is the combination of zinc fertilization and a zinc-efficient genotype, which not infrequently outyields either alone (Graham 1988a). It may be that $100 (GY^-/GY^+)$ is the best basis for identifying parental material for a breeding program, whereas GY^- may be of most immediate interest to producers where the problem is subsoil deficiency or topsoil drying, or where they are not aware of the need for micronutrients.

Obviously, a genotype can be better characterized by a yield response curve to increasing rates of fertilizer than by simple plus-and-minus treatments. However, such studies can handle only a few lines before the task becomes too large. Particularly in field experiments, the increased area means increased spatial variability, which is a serious problem with micronutrients. The inherent variability in soil availability for micronutrients appears to be intrinsically greater than that for macronutrients (Graham 1991). Moreover, the critical comparison between any given two rates (whether it be the plus-and-minus pair or any other pair) will be spread by randomization requirements over greater distances, with other treatments between. Provided, therefore, that the site chosen has the degree of deficiency to be targeted in the breeding program, the paired-plot system has the advantage of putting the two treatments in close proximity, thereby allowing the most precise determination of $100 (GY^-/GY^+)$. Minimal size also permits comparison of the greatest number of lines. This is the system used most in South Australia, but its justification depends both on the selection of relevant sites and on a perception of the number and nature of the genes involved. Thus Paull (1990) found a number of genes involved in resistance to boron toxicity and proposed a model (to which Figure 11 is analogous for deficiencies) to account for the variable expression of dominance at different selection pressures. In his work, to select for and against all possible segregants in a tetragenic system, he selected at three different levels of stress.

In addition to analyses of grain yield data and mid-season vegetative yields, another valuable index involves calculating the total uptake of the test nutrient into vegetative growth and into grain based on chemical analysis. The most efficient genotypes absorb more of the test nutrient and often (but not always) maintain higher concentrations of the nutrient in tissues, grain, or both.

Test nutrient uptake = yield \times test nutrient concentration.

Figure 11—Model of the response to an added micronutrient of two parents and their F₁ progeny showing how, when screening them at a single level of stress, the genetic interpretation could be that at z, A is dominant; at y, A shows partial dominance; and at x, B is dominant (sensitive parent)



Source: Based on Paull 1990.

Uptake frequently shows greater variation among genotypes than yield. (By comparison with yield, variation in concentration is conservative.)

Some authors have stressed the importance of screening over a full range of stress, which necessitates a number of rates of fertilizer application and much larger and more expensive experiments (Blum 1988; Boken 1966; Paull 1990). Paull (1990), in studying the genetics of tolerance to boron toxicity, found that several loci were involved, each operating at a different level of stress. The presence or absence of tolerance alleles for a given locus can only be detected at an appropriate level of stress. Clearly, in this case multilevel testing is necessary, and as it has already been noted that several genes may be involved in efficiency, full-range response curves may be required to make adequate progress with this trait. Graham, Ascher, and Hynes (1992, Figure 1) showed therein several different responses to zinc rates among five cultivars of wheat grown in pots, but multirate field experiments are rare.

In deficiency work, it is common to discuss the relative merits of the genotypes with yield-fertilizer rate responses like those in Figure 11. Genotype A is seen to be desirable, for it reaches its potential at the lowest level of nutrient supply. But with micronutrients it is common to add 10–100 times as much fertilizer as is needed by the crop, and this fertilizer may last (iron and manganese excepted) for several years. Thus, the paired-plot system does not determine the lowest rate of nutrient to achieve the yield potential. It can be argued that such a determination is not critical and the plus nutrient treatment must reflect only a point safely on the yield plateau while the nil fertilizer treatment needs to represent a meaningful degree of deficiency for the region targeted in the breeding program (for example, point y in Figure 11). Until researchers have a better idea of the genes and mechanisms involved, this paired-plot system seems to be the most practical approach for field purposes.

One paired-plot screening of this type has been described by Graham (1991). This design, which is essentially a split-plot factorial, may be subjected to a nearest-neighbor or other spatial analysis of variance to detrend inherent soil variability (Wilkinson et al. 1983).

The exploitation of micronutrient efficiency factors in a breeding program is limited, however, by the high spatial variability of the limiting nutrient in field sites, which remains a major experimental problem. For this reason, better selection criteria are needed to allow laboratory screening based on early-generation segregating materials, but the development of such criteria is restricted because the mechanisms of efficiency factors are poorly understood. Real progress has been made in the last few years, and more work is needed in this area. Meanwhile, selection may best continue to be done in the field.

The ideal is to understand the mechanisms involved and to select for the desired characters by means of their gene products, whether this is phytosiderophore release as in the case of iron efficiency in wheat (Marschner, Römheld, and Kissel 1986), binding affinity in the membrane (Km), root geometry, or composition of simple root exudates that may control the rhizosphere. Where iron efficiency is required, it appears to be a simple matter to select for greater ability of the roots to release deoxymugeneic acid under standard conditions of iron stress (as Marschner Römheld, and Kissel [1986] have stated). The all-important advantage of this approach is that it is not dependent on yield, with all its potential for interactions, but instead directly measures the intensity of expression of the efficiency alleles present in that genotype.

Pot Studies

Simple, fast, and inexpensive techniques are needed in breeding programs to permit the assessment of hundreds or thousands of segregants from crosses of micronutrient-efficient × micronutrient-inefficient parents, without recourse to expensive field testing. The better the efficiency mechanism is understood, the easier it is likely to be to develop an ideal screening technique; hence, mechanistic studies are a top priority.

Screening in pots, less expensive and faster than field work, permits the soil to be made more uniform across all treatments. However, the environment is less realistic, and it has been found that low temperature, especially low soil temperature, is often essential to expressing micronutrient deficiencies. Considerable work has been done in pots, usually vegetative growth studies, and the rankings are sometimes quite different from field-based grain yield rankings. While these discrepancies may suggest that midseason or later effects can be important (for example, Marcar and Graham 1987), it has been found that root binding in small pots seriously limits the expression of micronutrient efficiency traits. The bigger the pot, the closer the rankings come to field results, but ultimately the value of pot work is limited. Screening for copper efficiency in pots seems to be satisfactory (Graham and Pearce 1979). The micronutrient content of seed grown on micronutrient-deficient soil is frequently too low for optimum seedling vigor, and this dictates that screening seedlings for micronutrient efficiency (in pots and in the field) must use seeds that are all from the same source.

One of the best leads on the mechanism of zinc efficiency is the induction under stress in efficient phenotypes of the synthesis in roots of phytosiderophores and their subsequent release to the rhizosphere (Cakmak et al. 1994, Table 3; see also the section on mechanisms, and Table 15). Since these are easily detected using high pressure liquid chromatography (HPLC), a simple screening technique presents itself for use in solution-culture-grown plants.

The level of zinc stress can be efficiently controlled to exacting and reproducible limits by the chelate-buffering technique (Norvell 1991; Parker, Aguilera, and Thomason 1992), and the level of phytosiderophores produced can be measured by the standard technique of Treeby, Marschner, and Römheld (1989). Another related approach is that of Zhang, Römheld, and Marschner (1989), in which the phytosiderophores release zinc bound to a heavy-metal-binding resin, Chelite 100. Yang, Römheld, and Marschner (1993) have used this approach to screen rice genotypes for zinc efficiency. Such techniques show genuine promise, but the correlation between zinc stress-released phytosiderophores and field- assessed zinc efficiency may be poor, at least in some cases.

Molecular Methods

In the not too distant future, molecular methods will probably dominate selection for these traits. The use of RFLP mapping techniques or of PCR leading to cDNA probes for regions of the genome segregating with micronutrient efficiency traits in doubled haploid populations should lead to rapid and efficient selection, prospects that appear to be only a few years away and that will radically improve the chances of reaching the goals foreshadowed in this paper.

9. CONCLUSIONS

Breeding varieties of food staple plants that load high amounts of iron and zinc in their seeds holds great promise for making a significant, low-cost, and sustainable contribution to reducing iron and zinc deficiencies in humans in developing countries. This strategy also may well increase yields on deficient soils in an environmentally beneficial way. For some crops, beta-carotene content may also be substantially improved, although this will impart no agronomic advantages and may affect the color of the marketed product. This paper has sought to summarize existing scientific knowledge to substantiate these claims. Based on the evidence presented, specific conclusions may be summarized as follows:

1. From the exploration conducted to date, adequate genetic variation appears to exist to enable breeding of cultivars of the major food crops for higher micronutrient density.
2. The genetics of these traits are generally simple, making the task for breeders comparatively easy, given adequate selection criteria to speed their work.
3. Where soils are deficient in one or more micronutrients, such high-density and high-efficiency genotypes will have a yield advantage, even while requiring fewer seeds, fertilizers, and fungicides. These agronomic advantages will help to secure their place in the market, as well as make a step toward the goal of sustainability.
4. Of the major food crops, corn, sorghum, and upland rice are significantly affected by iron deficiency, with beans, cassava, and some vegetables being susceptible only on soils of high pH. Zinc deficiency significantly affects all major staple food crops.
5. As much as one-half of all soils in all developing countries are deficient or low in available micronutrients, making benefits in productivity a significant outcome of the program.
6. Efficient genotypes generally extract more nutrient from the deficient soil, rather than survive on less of the limiting factor; thus, they can pass on more of the nutrient to the consumer.
7. The evidence indicates that “mining” of the soil by efficient genotypes is unlikely to cause significant depletion of the soil reserve for hundreds or thousands of years; indeed because of “contamination” from other sources, it is likely that most soils stay essentially in balance for the heavy-metal micronutrients.
8. The primary selection criterion is a simple and efficient one—the micronutrient content of the seed—and does not involve selecting for known physiological mechanisms, but it has the disadvantage of requiring plants grown to maturity.
9. A major constraint to rapid breeding progress for agronomic efficiency is the lack of knowledge about how micronutrients are transported into and through the plant; such information leads immediately to selection techniques that breeders can use. The recent discovery of several nonprotein amino acids that have critical roles is, however, encouraging.

10. In a few years, biotechnological methods are likely to produce gene probes for the efficiency and transport traits involved. These probes are already under study in several laboratories.
11. Important inhibitors to micronutrient bioavailability—phytates, fiber, and tannins—are also significant to human health as anticarcinogens or antimutagens and to plant nutrition as nutrient storage molecules and pest- and disease-suppressant substances. Decreasing their levels in seeds may not be an optimal breeding strategy for increasing the density of bioavailable micronutrient minerals.
12. On the other hand, important dietary nutrients—the essential amino acids methionine, lysine, and cysteine—are comparatively low in seeds but are promoters of iron and zinc bioavailability to humans. Increasing these amino acids in seeds may be a safer approach to human health and an important component of the overall breeding program.

Because of the comparatively long lead times involved in bringing the results of plant-breeding research to bear on micronutrient deficiency problems in humans, this strategy will not contribute to meeting the end-of-decade goals for reducing micronutrient malnutrition, reaffirmed in the World Declaration and Plan of Action for Nutrition and endorsed by 159 countries at the International Conference on Nutrition in 1992. However, if the necessary resources are found to implement this strategy soon at a high level of effort, nutrient-enriched seed technologies could become available just a few years after the major push to meet the end-of-decade goals through higher-cost strategies has run its course.

While plant breeding will not eliminate the need for supplementation, fortification, and nutrition education programs in the future, this strategy does hold promise for significantly reducing expenditures required for these higher-cost, short-run programs by significantly reducing the numbers of people requiring treatment. It would seem prudent to invest now in a plant-breeding strategy to sustain the gains made by the end of the decade and to maintain momentum for further reductions in micronutrient deficiencies.

REFERENCES

- Al-Samerria, I. 1984. The effect of nitrogen supply on the response of wheat to zinc. Ph.D. diss., University of Western Australia, Nedlands, Australia.
- Ashmead, D., and H. Christy. 1985. Factors interfering with intestinal absorption of minerals. *Animal Nutrition and Health* 40: 10–13.
- Beart, J. E., T. H. Lilley, and E. Haslam. 1985. Plant polyphenols—Secondary metabolism and chemical defense: Some observations. *Phytochemistry* 24: 33–38.
- Beeghly, H. H., and W. R. Fehr. 1989. Indirect effects of recurrent selection for iron efficiency in soybean. *Crop Science* 29: 640–643.
- Bienfait, H. F. 1989. Prevention of stress in iron metabolism of plants. *Acta Botanica Neerlandica* 38: 105–129.
- Blum, A. 1988. *Plant breeding for stress environments*. Boca Raton, Fla., U.S.A.: CRC Press.
- Bodwell, C. E. 1987. Nutritional implications of cereals, legumes, and their products. In *Cereals and legumes in the food supply*, ed. J. Dupont and E. M. Osman, 259–275. Ames, Iowa, U.S.A.: Iowa State University Press.
- Boken, E. 1966. *Studies on methods of determining varietal utilization of nutrients*. Copenhagen: DSR Vorlag-Boghandel, Royal Veterinary Agricultural College.
- Bolland, M. D. A., and M. J. Baker. 1988. High phosphorus concentrations in seed of wheat and annual medic are related to higher rates of dry matter production of seedlings and plants. *Australian Journal of Experimental Agriculture* 28: 765–770.
- Bolland, M. D. A., B. H. Paynter, and M. J. Baker. 1989. Increasing phosphorus concentration in lupin seed increases grain yields on phosphorus deficient soil. *Australian Journal of Experimental Agriculture* 29: 797–801.
- Boswell, F. C., K. Ohki, M. B. Paker, L. M. Shuman, and D. O. Wilson. 1981. Methods and rates of applied manganese for soybeans. *Agronomy Journal* 73: 909–912.
- Bowling, D. J. F., R. D. Graham, and J. Dunlop. 1978. The relationship between the cell electrical potential difference and salt uptake in the roots of *Helianthus annuus*. *Journal of Experimental Botany* 29: 135–140.
- Brown, J. C. 1982. Summary of symposium. *Journal of Plant Nutrition* 5: 987–1001.
- Brown, J. C., and W. E. Jones. 1974. Differential response of oats to manganese stress. *Agronomy Journal* 66: 624–626.
- Brown, J. C., and E. V. Wann. 1982. Breeding for iron efficiency: Use of indicator plants. *Journal of Plant Nutrition* 5: 623–635.

- Brown, J. C., R. S. Holmes, and L. O. Tiffin. 1958. Iron chlorosis in soybeans as related to the genotype of root stalk. *Soil Science* 86: 75–82.
- _____. 1961. Iron chlorosis in soybeans as related to the genotype of the root stalk. 3. Chlorosis susceptibility and reductive capacity at the root. *Soil Science* 91: 127–132.
- Brown, K. M. 1991. The importance of dietary quality versus growth for weanlings in less-developed countries: A framework for discussion. *Food and Nutrition Bulletin* 13: 86–94.
- Brown, P. H., R. M. Welch, and E. E. Cary. 1987. Nickel: A micronutrient essential for higher plants. *Plant Physiology* 85: 801–803.
- Brunson, A. M., and F. W. Quackenbush. 1962. Breeding corn with high provitamin A in the grain. *Crop Science* 2: 344–347.
- Burgess, L. W., T. Klein, and C. M. Liddell. 1984. Crown rot of wheat. In *Research on root and crown rots of wheat in Australia*, Australian Wheat Industry Research Council, 62–64. Adelaide.
- Burk, R. F., and N. W. Solomons. 1985. Trace elements and vitamins and bioavailability as related to wheat and wheat foods. *American Journal of Clinical Nutrition* 41: 1091–1102.
- Cakmak, I., K. Y. Gülüt, H. Marschner, and R. D. Graham. 1994. Effect of iron and zinc deficiency on phytosiderophore release in wheat genotypes differing in zinc efficiency. *Journal of Plant Nutrition* 17: 1–17.
- Camara, B., R. Schantz, and R. Moneger. 1992. Enzymology and genetic regulation of carotenoid biosynthesis in plants. In *Biotechnology and nutrition*, ed. D. D. Bills and S.-Y. Kung, 301–314. Boston, Mass., U.S.A.: Butterworth-Heinemann.
- Chandra, R. K. 1990. Micronutrients and immune functions. *Annals of the New York Academy of Sciences* 587: 9–16.
- Christiansen, M. N., and C. F. Lewis, eds. 1982. *Breeding plants for less favorable environments*. New York: Wiley.
- Clark, R. B. 1978. Differential response of maize inbreds to zinc. *Agronomy Journal* 70: 1057–1060.
- _____. 1982. Plant genotype differences to uptake, translocation, accumulation, and use of mineral elements. In *Genetic specificity of mineral nutrition of plants*, Scientific Assemblies Vol. 13, ed. M. R. Saric, 41–55. Belgrade: Serbian Academy of Sciences and Arts.
- Cooper, K. V., R. D. Graham, and N. E. Longnecker. 1988. Triticale: A cereal for manganese-deficient soils. In *International symposium on manganese in soils and plants 1988*, ed. M. J. Webb, R. O. Noble, R. D. Graham, and R. J. Hannam, 113–116. Adelaide, Australia: Manganese Symposium.
- Coyne, D. P., S. S. Korban, D. Knudsen, and R. B. Clark. 1982. Inheritance of iron deficiency in crosses of dry beans (*Phaseolus vulgaris* L.). *Journal of Plant Nutrition* 5: 575–585.

- Crowley, D. E., Y. C. Wang, C. P. P. Reid, and P. J. Szaniszlo. 1991. Mechanisms of iron acquisition from siderophores by microorganisms and plants. In *Iron nutrition and interactions in plants*, ed. Y. Chen and Y. Hadar, 213–232. Dordrecht, the Netherlands: Kluwer Academic Publishers.
- Dallman, P. R. 1990. Iron. In *Present knowledge in nutrition*, ed. M. L. Brown, 241–250. Washington, D.C.: International Life Sciences Institute, Nutrition Foundation.
- De, N. K., and B. N. Chatterjee. 1976. Effect of trace elements on the growth and yield of groundnut in leached sandy loam soil. *Indian Journal of Agronomy* 21: 209–216.
- Deb, D. L., and F. Scheffer. 1971. Effects of the amino acid fraction of root-exudate on the absorption of manganese by eight varieties of oat (*Avena sativa*) in sterile and non-sterile media. *Agronchmica* 15: 74–84.
- Doughty, J. 1993. World population: Time bomb has exploded. *Business Korea* 10: 48.
- Duncan, R. R. 1983. Concentration of critical nutrients in tolerant and susceptible sorghum lines for use in screening under acid soil field conditions. In *Genetic aspects of plant nutrition*, ed. M. R. Saric and B. C. Loughman, 101–104. The Hague: Martinus Nijhoff/Dr. W. Junk.
- El-Bendary, A. A., M. M. El-Fouly, F. A. Raksa, A. A. Omar, and A. Y. Abou-Youssef. 1993. Mode of inheritance of zinc accumulation in maize. *Journal of Plant Nutrition* 16: 2043–2053.
- Epstein, E. 1972. *Mineral nutrition of plants: Principles and perspectives*. New York: Wiley.
- Erdman, J. W., Jr., and A. Poneros-Schneier. 1989. Phytic acid interactions with divalent cations in foods and in the gastrointestinal tract. *Advances in Experimental Medicine and Biology* 249: 161–171.
- Eskew, D. L., R. M. Welch, and E. E. Cary. 1983. Nickel: An essential micronutrient for legumes and possibly for all higher plants. *Science* 222: 621–623.
- Fairweather-Tait, S. J. 1992. Bioavailability of trace elements. *Food Chemistry* 43: 213–217.
- Fairweather-Tait, S. J., and A. J. A. Wright. 1990. The effects of sugar-beet fibre and wheat bran on iron and zinc absorption in rats. *British Journal of Nutrition* 64: 547–552.
- FAO/WHO (Food and Agriculture Organization of the United Nations/World Health Organization). 1992. *Preventing specific micronutrient deficiencies*. Theme Paper 6, International Conference on Nutrition. Rome.
- Fehr, W. R. 1982. Control of iron deficiency chlorosis in soybeans by plant breeding. *Journal of Plant Nutrition* 5: 611–621.
- Fordyce, E. J., R. M. Forbes, K. R. Robbins, and J. W. J. Erdman. 1987. Phytate \times calcium/zinc molar ratios: Are they predictive of zinc bioavailability? *Journal of Food Science* 52: 440–444.

- Foy, C. D. 1983. Plant adaptation to mineral stress in problem soils. *Iowa State Journal of Research* 57: 355–391.
- Gabelman, W. H., and S. Peters. 1979. Genetical and plant breeding possibilities for improving quality of vegetables. *Acta Horticulturae* 93: 243–263.
- Gali, H. U., E. M. Perchellet, X. M. Gao, V. Bottari, and J.-P. Perchellet. 1993. Antitumor-promoting effects of gallotannins extracted from various sources in mouse skin *in vivo*. *Anticancer Research* 13: 915–922.
- Gallagher, P. H., and T. Walsh. 1943. The susceptibility of cereal varieties to manganese deficiency. *Journal of Agricultural Science* 33: 197–203.
- Gibson, R. S. 1994. Zinc nutrition and public health in developing countries. *Nutrition Research Reviews* 7: 151–173.
- Gilbert, B. E. 1934. Normal crops and supply of available soil manganese. *Rhode Island Agricultural Experiment Station Bulletin* 246.
- Glassoch, H. H. 1941. Varietal susceptibility of peas to marsh spot. *Annals of Applied Biology* 28: 310–324.
- Graham, R. D. 1978. Nutrient efficiency objectives in cereal breeding. In *Proceedings of the 8th international colloquium on plant analysis and fertiliser problems*, 165–170. New Zealand Division of Scientific and Industrial Research, Information Series no. 134. Wellington, New Zealand: Government Printer.
- _____. 1984. Breeding for nutritional characteristics in cereals. *Advances in Plant Nutrition* 1: 57–102.
- _____. 1987. Triticale, a cereal for micronutrient-deficient soils. *International Triticale Newsletter* (University of New England, Armidale, Australia) 1: 6–7.
- _____. 1988a. Development of wheats with enhanced nutrient efficiency: Progress and potential. In *Wheat production constraints in tropical environments*, ed. A. R. Klatt, 305–320. Mexico City: Centro Internacional de Mejoramiento de Maiz y Trigo.
- _____. 1988b. Genotypic differences in tolerance to manganese deficiency. In *Manganese in soils and plants*, ed. R. D. Graham, R. J. Hannam, and N. C. Uren, 216–276. Dordrecht, the Netherlands: Kluwer Academic Publishers.
- _____. 1991. Breeding wheats for tolerance to micronutrient deficient soil: Present status and priorities. In *Wheat for the nontraditional warm areas*, ed. D. A. Saunders, 315–332. Mexico City: Centro Internacional de Mejoramiento de Maiz y Trigo.
- Graham, R. D., and J. S. Ascher. 1993. Nutritional limitations of subsoils. In *Plant nutrition: From genetic engineering to field practice*, ed. N. J. Barrow, 739–742. Dordrecht, the Netherlands: Kluwer Academic Publishers.

- Graham, R. D., and D. T. Pearce. 1979. The sensitivity of hexaploid and octoploid triticales and their parent species to copper deficiency. *Australian Journal of Agricultural Research* 30: 791–799.
- Graham, R. D., and A. D. Rovira. 1984. A role for manganese in the resistance of wheat to take-all. *Plant and Soil* 78: 441–444.
- Graham, R. D., and M. J. Webb. 1991. Micronutrients and resistance and tolerance to disease. In *Micronutrients in agriculture*, 2d edition, ed. J. J. Mortwedt, F. R. Cox, L. M. Shuman, and R. M. Welch, 329–370. Madison, Wis., U.S.A.: Soil Science Society of America.
- Graham, R. D., G. D. Anderson, and J. S. Ascher. 1981. Absorption of copper by wheat, rye, and some hybrid genotypes. *Journal of Plant Nutrition* 3: 679–686.
- Graham, R. D., J. S. Ascher, and S. C. Hynes. 1992. Selecting zinc-efficient cereal genotypes for soils of low zinc status. *Plant and Soil* 146: 241–250.
- Graham, R. D., J. S. Ascher, and R. M. Mills. 1984. Manganese and resistance of wheat to powdery mildew. *Biennial report*. Waite Agricultural Research Institute, Glen Osmond, South Australia 1982–83, p. 83.
- Graham, R. D., J. S. Ascher, P. A. E. Ellis, and K. W. Shepherd. 1987. Transfer to wheat of the copper efficiency factor carried on rye chromosome arm 5RL. *Plant and Soil* 99: 107–114.
- Graham, R. D., W. J. Davies, D. H. B. Sparrow, and J. S. Ascher. 1983. Tolerance of barley and other cereals to manganese-deficient calcareous soils of South Australia. In *Genetic aspects of plant nutrition*, ed. M. R. Saric and B. C. Loughman, 339–345. Dordrecht, the Netherlands: Martinus Nijhoff Publishers.
- Grundon, N. J. 1980. Effectiveness of soil dressings and foliar sprays of copper sulphate in correcting copper deficiency of wheat (*Triticum aestivum* L.) in Queensland. *Australian Journal of Experimental Agriculture and Animal Husbandry* 20: 717–723.
- Grundon, N. J., and E. K. Best. 1981. Tolerance of some winter and summer crops to copper deficiency. In *Copper in soils and plants*, ed. J. F. Loneragan, A. D. Robson, and R. D. Graham, 360. Sydney: Academic Press.
- Hambidge, K. M., C. E. Casey, and N. F. Krebs. 1986. Zinc. In *Trace elements in human and animal nutrition*, 5th edition, ed. W. Mertz, 1–137. New York: Academic Press.
- Hannam, R. J. 1991. Nutrition and zinc update. In *Agronomy technical conference*, 90–94. Adelaide: South Australia Department of Agriculture.
- Hannam, R. J., W. J. Davies, R. D. Graham, and J. L. Riggs. 1984. The effect of soil- and foliar-applied manganese in preventing the onset of manganese deficiency in *Lupinus angustifolius*. *Australian Journal of Agricultural Research* 35: 529–535.
- Harland, B. F. 1989. Dietary fibre and mineral bioavailability. *Nutrition Research Reviews* 2: 133–147.

- Harry, S. P. 1982. Tolerance of wheat, rye, and triticale to copper and zinc deficiency in soils of low and high pH. Master's thesis, University of Adelaide, Adelaide, Australia.
- Harry, S. P., and R. D. Graham. 1981. Tolerance of triticale, wheat, and rye to copper deficiency and low and high pH. *Journal of Plant Nutrition* 3: 721–730.
- Hartwig, E. E., W. F. Jones, and T. C. Kilen. 1991. Identification and inheritance of inefficient zinc absorption in soybean. *Crop Science* 31: 61–63.
- Hauge, S. M., and J. F. Trost. 1928. An inheritance study of the distribution of vitamin A in maize. *Journal of Biological Chemistry* 80: 107–115.
- Haynes, J. L., and W. R. Robbins. 1948. Calcium and boron as essential factors in the root environment. *Journal of the American Society of Agronomy* 40: 795–803.
- Haytowitz, D. B., and R. H. Matthews. 1984. *Composition of foods: Vegetables and vegetable products. Handbook*. Washington, D.C.: United States Department of Agriculture.
- Hetzel, B. S. 1990. Iodine deficiency: An international public health problem. In *Present knowledge in nutrition*, ed. M. L. Brown, 308–313. Washington, D.C.: International Life Sciences Institute, Nutrition Foundation.
- Hoan, N. T., U. Prasada Rao, and E. A. Siddiq. 1992. Genetics of tolerance to iron chlorosis in rice. *Plant and Soil* 146: 233–239.
- Huang, J. W., and L. V. Kochian. 1994. Evidence for the transport of Zn^{2+} via a divalent cation channel in the root cell plasma membrane. U.S. Plant, Soil and Nutrition Laboratory, Cornell University, Ithaca, N.Y. Personal communication.
- ILSI (International Life Sciences Institute), Nutrition Foundation. 1990. Zinc. In *Present knowledge in nutrition*, ed. M. L. Brown, 251–260. Washington, D.C.
- IRRI (International Rice Research Institute). 1979. *Annual report*. Los Baños, Philippines.
- Jariwalla, R. J. 1992. Anticancer effects of phytate. *American Journal of Clinical Nutrition* 56: 609.
- Jones, G. B. and G. B. Belling. 1967. The movement of copper, molybdenum, and selenium in soils as indicated by radioactive tracers. *Australian Journal of Agricultural Research* 18: 733–740.
- Jung, G. A., ed. 1978. *Crop tolerance to suboptimal land conditions*. Madison, Wis., U.S.A.: American Society of Agronomy.
- Kaji, T., A. Mishima, C. Yamamoto, M. Sakamoto, and H. Kozuka. 1993. Zinc protection against cadmium-induced destruction of the monolayer of cultured vascular endothelial cells. *Toxicology Letters* 66: 247–255.
- Kang, B. T., and O. A. Osiname. 1985. Micronutrient problems in tropical Africa. *Fertilizer Research* 7: 131–150.

- Karvanek, N., and J. Bantova. 1966. Scientific papers of the Institute of Chemical Technology Prague E11:73–82. In *Genetic specificity in relation to plant mineral nutrition*, ed. M. R. Saric. *Journal of Plant Nutrition* 3: 743–766.
- Katyal, J. C., and P. L. Vlek. 1985. Micronutrient problems in tropical Asia. *Fertilizer Research* 7: 69–94.
- Kennedy, E., and H. Bouis. 1992. Agriculture/nutrition linkages: Implications for policy and research. Paper prepared for the Food and Agricultural Organization of the United Nations/World Health Organization International Conference on Nutrition, December, Rome.
- King, P. M., and A. M. Alston. 1975. A survey of soils near Wharminda and Stokes, Eyre Peninsula, South Australia. Special Land Description SB5. Adelaide: Soil Conservation Branch, South Australia Department of Agriculture.
- Kirchgessner, M., and E. Weigand. 1982. Zinc absorption and excretion in relation to nutrition. In *Metal ions in biological systems*. Vol. 15, *Zinc and its role in biology and nutrition*, ed. H. Sigel, 319–361. New York: Marcel Dekker.
- Klaui, H., and J. C. Bauernfeind. 1981. Carotenoids as food colors. In *Carotenoids as colorants and vitamin A precursors*. New York: Academic Press.
- Kochian, L. V. 1991. Mechanisms of micronutrient uptake, translocation, and interactions in plants. In *Micronutrients in agriculture*, 2d edition, ed. J. J. Mortvedt, F. R. Cox, L. M. Shuman, and R. M. Welch, 229–296. Madison, Wis., U.S.A.: Soil Science Society of America.
- Kramer, T. R., E. Udomkesmalee, S. Dhanamitta, S. Sirisinha, S. Charoenkiatkul, S. Tuntipopipat, O. Banjong, N. Rojroongwasinkul, and J. C. Smith, Jr. 1993. Lymphocyte responsiveness of children supplemented with vitamin A and zinc. *American Journal of Clinical Nutrition* 58: 566–570.
- Kunbhar, D. D., and K. R. Sonar. 1987. Genetic variation in concentration and uptake of Fe and Mn in rice cultivars grown under upland conditions. Paper presented at the National Symposium on Micronutrient Stresses in Crop Plants: Physiological and Genetical Approaches to Control Them, December 16–18, Mahatma Phule Agricultural College, Rahuri, India.
- Leon, L. A., A. S. Lopez, and P. L. G. Vlek. 1985. Micronutrient problems in tropical Latin America. *Fertilizer Research* 7: 95–129.
- Lindsay, W. L. 1991. Inorganic equilibria affecting micronutrients in soils. In *Micronutrients in agriculture*, 2d edition, ed. J. J. Mortvedt, F. R. Cox, L. M. Shuman, and R. M. Welch, 89–112. Madison, Wis., U.S.A.: Soil Science Society of America.
- Little, R. 1988. Plant-soil interactions at low pH: Problem-solving genetic approach. *Communications in Soil Science and Plant Analysis* 19: 1239–1257.

- Loneragan, J. F., G. J. Kirk, and M. J. Webb. 1987. Translocation and function of zinc in roots. *Journal of Plant Nutrition* 10: 1247–1254.
- Longnecker, N. E., N. E. Marcar, and R. D. Graham. 1991. Increased manganese content of barley seeds can increase grain yield in manganese-deficient conditions. *Australian Journal of Agricultural Research* 42: 1065–1074.
- Lopes, A. S., and F. R. Cox. 1977. A survey of the fertility status of surface soils under “Cerrado” vegetation in Brazil. *Soil Science Society of America Journal* 41: 742–747.
- Lott, J. N. A. 1984. Accumulation of seed reserves of phosphorus and other minerals. In *Seed physiology*. Vol. 1, *Development*, ed. D. R. Murray, 139–166. Sydney: Academic Press.
- Maas, J. L., G. J. Galletta, and S. Y. Wang. 1992. Ellagic acid enhancement in strawberries. In *Biotechnology and nutrition*, ed. D. D. Bills and S.-Y. Kung, 345–362. Boston, Mass., U.S.A.: Butterworth-Heinemann.
- Majumder, N. D., S. C. Rakshit, and D. N. Borthakur. 1990. Genetic effects on uptake of selected nutrients in some rice (*Oryza sativa* L.) varieties in phosphorus deficient soil. *Plant and Soil* 123: 117–120.
- Marcar, N. E. 1986. Genetic variation for manganese efficiency in cereals. Ph.D. thesis, University of Adelaide, Adelaide, Australia.
- Marcar, N. E., and R. D. Graham. 1987. Tolerance of wheat, barley, triticale, and rye to manganese deficiency during seedling growth. *Australian Journal of Agricultural Research* 38: 501–511.
- Marschner, H. 1986. *Mineral nutrition of higher plants*. London: Academic Press.
- Marschner, H., V. Römheld, and M. Kissel. 1986. Different strategies in higher plants in mobilization and uptake of iron. *Journal of Plant Nutrition* 9: 695–713.
- McCarthy, K. W., N. E. Longnecker, D. H. B. Sparrow, and R. D. Graham. 1988. Inheritance of manganese efficiency in barley (*Hordeum vulgare* L.). In *International symposium on manganese in soils and plants*, ed. M. J. Webb, R. O. Nable, R. D. Graham, and R. J. Hannam, 121–122. Adelaide, Australia: Manganese Symposium.
- Mertz, W. 1987. The practical importance of interactions of trace elements. In *Trace substances in environmental health* 21, 526–532. Proceedings of the University of Missouri’s 21st Annual Conference on Trace Substances in Environmental Health, May 24–28, St. Louis, Mo., U.S.A. Columbia, Mo., U.S.A.: University of Missouri, Columbia.
- Messina, M. 1991. Phytate’s potential role in reducing colon-cancer risk. *American Journal of Clinical Nutrition* 54: 762–763.
- Mihashi, S., and S. Mori. 1989. Characterization of mugineic-acid-Fe transporter in Fe-deficient barley roots using the multi-compartment transport box method. *Biology of Metals* 2: 146–154.

- Miyamoto, K., T. Murayama, M. Nomura, T. Hatano, T. Yoshida, T. Furukawa, R. Koshiura, and T. Okuda. 1993. Antitumor activity and interleukin-1 induction by tannins. *Anticancer Research* 13: 37–42.
- Miyamoto, K., M. Nomura, M. Sasakura, E. Matsui, R. Koshiura, T. Murayama, T. Furukawa, T. Hatano, T. Yoshida, and T. Okuda. 1993. Antitumor activity of oenothien B, a unique macrocyclic ellagitannin. *Japanese Journal of Cancer Research* 84: 99–103.
- Moraghan, J. T., and H. J. Mascagni. 1991. Environmental and soil factors affecting micronutrient deficiencies and toxicities. In *Micronutrients in agriculture*, 2d edition, ed. J. J. Mortvedt, F. R. Cox, L. M. Shuman, and R. M. Welch, 371–425. Madison, Wis., U.S.A.: Soil Science Society of America.
- Mori, S., N. Nishizawa, H. Hayashi, M. Chino, E. Yoshimura, and J. Ishihara. 1991. Why are young rice plants highly susceptible to iron deficiency? In *Iron nutrition and interactions in plants*, ed. Y. Chen and Y. Hadar, 173–188. Dordrecht, the Netherlands: Kluwer Academic Publishers.
- Morris, E. R. 1983. An overview of current information on bioavailability of dietary iron to humans. *Federation Proceedings* 42: 1716–1720.
- _____. 1986. Phytate and dietary mineral bioavailability. In *Phytic acid: Chemistry and applications*, ed. E. Graf, 57–76. Minneapolis, Minn., U.S.A.: Pilatus Press.
- Murayama, T., N. Kishi, R. Koshiura, K. Takagi, T. Furukawa, and K.-I. Miyamoto. 1992. Agrimoniin, an antitumor tannin of *Agrimonia pilosa* Ledeb., induces interleukin-1. *Anticancer Research* 12: 1471–1474.
- Nable, R. O., and J. F. Loneragan. 1984. Translocation of manganese in subterranean clover (*Trifolium subterraneum* L. cv. Seaton Park). II. Effects of leaf senescence and of restricting supply of manganese to part of a split root system. *Australian Journal of Plant Physiology* 11: 113–118.
- Nable, R. O., and M. J. Webb. 1993. Further evidence that zinc is required throughout the root zone for optimum plant growth. *Plant and Soil* 150: 247–253.
- Nair, K. P. P., and G. Prabhat. 1977. Differential response of tropical maize genotypes to zinc and manganese nutrition. *Plant and Soil* 47: 149–159.
- Nambiar, E. K. S. 1976. Genetic differences in the copper nutrition of cereals. I. Differential responses of genotypes to copper. *Australian Journal of Agricultural Research* 27: 453–463.
- National Research Council. 1989. *Recommended dietary allowances*. Washington, D.C.: National Academy Press.
- Neilands, J. B. 1986. Siderophores in relation to plant growth and disease. *Annual Review of Plant Physiology* 37: 187–208.

- _____. 1990a. Molecular aspects of regulation of high affinity iron absorption in microorganisms. *Advances in Inorganic Biochemistry* 8: 63–90.
- _____. 1990b. Molecular biology and regulation of iron acquisition by *Escherichia coli* K12. In *The bacteria*, Vol. 11, ed. B. H. Iglewiski and V. L. Clark, 205–223. San Diego, Calif., U.S.A.: Academic Press.
- Norvell, W. A. 1991. Reactions of metal chelates in soils and nutrient solutions. In *Micronutrients in agriculture*, 2d edition, ed. J. J. Mortvedt, F. R. Cox, L. M. Shuman, and R. M. Welch, 187–227. Madison, Wis., U.S.A.: Soil Science Society of America.
- Nyborg, M. 1970. Sensitivity to manganese deficiency of different cultivars of wheat, oats, and barley. *Canadian Journal of Plant Science* 50: 198–200.
- Olson, J. A. 1990. Vitamin A. In *Present knowledge in nutrition*, ed. M. L. Brown, 96–107. Washington, D.C.: International Life Sciences Institute, Nutrition Foundation.
- Parker, D. R., J. J. Aguilera, and D. N. Thomason. 1992. Zinc-phosphorus interactions in two cultivars of tomato (*Lycopersicon esculentum* L.) grown in chelator-buffered nutrient solutions. *Plant and Soil* 143: 163–177.
- Parker, M. B., F. C. Boswell, K. Ohki, L. M. Shuman, and D. O. Wilson. 1981. Manganese effects on yield and nutrient concentration in leaves and seed of soybean cultivars. *Agronomy Journal* 73: 643–646.
- Paull, J. G. 1990. Genetic studies on the tolerance of wheat to high concentrations of boron. Ph.D. thesis, University of Adelaide, Adelaide, Australia.
- Pedler, J. F. 1994. Resistance to take-all disease by Mn-efficient wheat cultivars. Ph.D. thesis, University of Adelaide, Adelaide, Australia.
- Pennington, J. A. T., and B. Young. 1990. Iron, zinc copper, manganese, selenium, and iodine in foods from the United States total diet study. *Journal of Food Composition and Analysis* 3: 166–184.
- Pollard, A. S., A. J. Parr, and B. C. Loughman. 1977. Boron in relation to membrane function in higher plants. *Journal of Experimental Botany* 28: 831–839.
- Ponnamperuma, F. N. 1974. Micronutrient limitations in acid tropical rice soils. In *Soil management in tropical America*, ed. E. Bornemisza and A. Alverado, 330–347. Raleigh, N.C., U.S.A.: North Carolina State University.
- _____. 1976. Screening rice for tolerance to mineral stresses. In *Plant adaptation to mineral stress in problem soils*, ed. M. J. Wright, 341–353. Ithaca, N.Y., U.S.A.: Cornell University Agricultural Experiment Station.
- _____. 1982. Genotypic adaptability as a substitute for amendments on toxic and nutrient-deficient soils. In *Plant Nutrition 1982: Proceedings of the Ninth International Plant Nutrition Colloquium*, ed. A. Scaife, 467–473. Slough, England: Commonwealth Agricultural Bureaux.

- Porter, J. W., S. L. Spurgeon, and N. Sathyamoorthy. 1984. Biosynthesis in carotenoids. In *Isopentenoids in Plants: Biochemistry and Function*, 161–183. New York: Marcel Dekker.
- Prozialeck, W. C., and R. J. Niewenhuis. 1991. Cadmium (Cd^{2+}) disrupts intercellular junctions and actin filaments in LLC-PK₁ cells. *Toxicology and Applied Pharmacology* 107: 81–97.
- Radhakrishnan, M. R., and J. Sivaprasad. 1980. Tannin content of sorghum-vulgare varieties and their role in iron bioavailability. *Journal of Agricultural and Food Chemistry* 28: 55–57.
- Randhawa, N. S., and P. N. Takkar. 1976. Screening of crop varieties with respect to micronutrient stresses in India. In *Plant adaptation to mineral stress in problem soils*, ed. M. J. Wright, 393–400. Ithaca, N.Y., U.S.A.: Cornell University Agricultural Experiment Station.
- Rao, V. S., M. S. Gangwar, and V. S. Rathore. 1977. Genotypic variation in distribution of total and labelled zinc and availability of zinc (A and L values) to soybeans grown in Mollisol. *Journal of Agricultural Science* 8: 417–420.
- Rathore, G. S., R. S. Khamparia, G. P. Gupta, and S. B. Sinha. 1980. Availability of micronutrients in some alluvial soils and their effect on wheat. *Journal of the Indian Society of Soil Science* 28: 248–250.
- Rengel, Z. 1992. Role of calcium in aluminum toxicity. *New Phytologist* 121: 499–513.
- Rengel, Z., and R. D. Graham. 1995. Importance of seed zinc content for wheat growth on Zn-deficient soil. I. Vegetative growth. *Plant and Soil* 173: 259–266.
- Rerkasem, B., R. W. Bell, and J. F. Loneragan. 1990. Effects of seed and soil boron on early seedling growth of black and green gram (*Vigna mungo* and *V. radiata*). In *Plant nutrition, physiology, and applications*, ed. M. L. van Beusichem, 281–285. Dordrecht, the Netherlands: Kluwer Academic Publishers.
- Ripperger, H., and K. Schreiber. 1982. Nicotianamine and analogous amino acids, endogenous iron carriers in higher plants. *Heterocycles* 17: 447–461.
- Römheld, V. 1991. The role of phytosiderophores in acquisition of iron and other micronutrients in graminaceous species: An ecological approach. In *Iron nutrition and interactions in plants*, ed. Y. Chen and Y. Hadar, 159–166. Dordrecht, the Netherlands: Kluwer Academic Publishers.
- Römheld, V., and H. Marschner. 1986. Mobilization of iron in the rhizosphere of different plant species. *Advances in Plant Nutrition* 2: 155–204.
- _____. 1990. Genotypical differences among graminaceous species in release of phytosiderophores and uptake of iron phytosiderophores. *Plant and Soil* 123: 147–153.

- Rose, I. A., W. L. Felton, and L. W. Banke. 1981. Response of four soybean varieties to foliar zinc fertilizer. *Australian Journal of Experimental Agriculture and Animal Husbandry* 21: 236–240.
- Salunkhe, D. K., and S. S. Deshpande. 1991. *Foods of plant origin: Production, technology, and human nutrition*. New York: Van Nostrand Reinhold, AVI Book.
- Saric, M. R., and B. C. Loughman, eds. 1983. *Genetic specificity in mineral nutrition of plants*. Dordrecht, the Netherlands: Kluwer Academic Publishers.
- Saxena, S. C., and A. S. Chandel. 1992. Effect of zinc fertilization on different varieties of soybean (*Glycine max*). *Indian Journal of Agricultural Sciences* 62: 695–697.
- Scott, P. R., and T. W. Hollins. 1985. Role of plant breeding in controlling soil-borne diseases of cereals. In *Ecology and management of soil-borne plant pathogens*, ed. C. A. Parker, A. D. Rovira, K. J. Moore, and P. T. W. Wong, 157–159. St. Paul, Minn., U.S.A.: American Phytopathological Society.
- Shamsuddin, A. M. 1992. Phytate and colon-cancer risk. *American Journal of Clinical Nutrition* 55: 478.
- Shojima, S., N.-K. Nishizawa, S. Fushiya, S. Nozoe, T. Irifune, and S. Mori. 1990. Biosynthesis of phytosiderophores: *In vitro* biosynthesis of 2'-deoxymugineic acid from L-methionine and nicotianamine. *Plant Physiology* 93: 1497–1503.
- Shuman, L. M. 1991. Chemical forms of micronutrients in soils. In *Micronutrients in agriculture*, 2d edition, ed. J. J. Mortvedt, F. R. Cox, L. M. Shuman, and R. M. Welch, 113–144. Madison, Wis., U.S.A.: Soil Science Society of America.
- Sillanpää, M. 1982. *Micronutrients and the nutrient status of soils: A global study*. FAO Soils Bulletin 48. Rome: Food and Agriculture Organization of the United Nations.
- _____. 1990. *Micronutrient assessment at the country level: An international study*. FAO Soils Bulletin 63. Rome: Food and Agriculture Organization of the United Nations.
- Simon, P. W. 1992. Genetic improvement of vegetable carotene content. In *Biotechnology and nutrition*, ed. D. D. Bills and S.-Y. Kung, 291–300. Boston, Mass., U.S.A.: Butterworth-Heinemann.
- Smilde, K. W., and C. H. Henkens. 1967. Sensitivity to copper deficiency of different cereals and strains of cereals. *Netherlands Journal of Agricultural Science* 15: 249–258.
- Soepardi, G. 1982. The zinc status of Indonesian agriculture. Contrib. Cent. Research Institute. Food Crops, Bogor. The Institute. 68: 10–31, maps.
- Sommer, A. 1990. Vitamin A status, resistance to infection, and childhood mortality. *Annals of the New York Academy of Sciences* 587: 17–23.
- Sparrow, D. H., and R. D. Graham. 1988. Susceptibility of zinc-deficient wheat plants to colonization by *Fusarium graminearum* Schw. Group 1. *Plant and Soil* 112: 261–266.

- Special Report: FAO/WHO Handbook on Human Nutritional Requirements 1974. 1975. *Nutrition Review* 33: 147–156.
- Stephan, U. W., and G. Scholz. 1993. Nicotianamine: Mediator of transport of iron and heavy metals in the phloem? *Physiological Plant Pathology* 88: 522–529.
- Stevenson, F. J. 1991. Organic matter-micronutrient reactions in soil. In *Micronutrients in agriculture*, 2d edition, ed. J. J. Mortvedt, F. R. Cox, L. M. Shuman, and R. M. Welch, 145–186. Madison, Wis., U.S.A.: Soil Science Society of America.
- Sugiura, Y., and K. Nomoto. 1984. Phytosiderophores: Structures and properties of mugineic acids and their metal complexes. *Structure Bonding* 58: 107–135.
- Tainio, A. 1961. *Can ergot be controlled by trace element fertilization?* Mass Tulev Supp Finland No. 12, Boron and Ergot. London: Borax Consolidated Ltd.
- Takeuchi, T. 1909. On differences of susceptibility of plant to stimulation. *Journal of the Tokyo Imperial University College of Agriculture* 1: 207–210.
- Takkar, P. N. 1991. Zinc deficiency in Indian soils and crops. In *Zinc in crop nutrition*, 55–64. New Delhi: Lead Zinc Research Organization Incorporated and Indian Lead Zinc Information Center.
- _____. 1993. Requirements and response of crop cultivars to micronutrients in India: A review. In *Genetic aspects of plant mineral nutrition*, ed. P. J. Randall, E. Delhaize, R. A. Richards, and R. Munns, 341–348. Dordrecht, the Netherlands: Kluwer Academic Publishers.
- Thompson, D. B. 1988. Iron. In *Trace minerals in foods*, ed. K. T. Smith, 157–208. New York: Marcel Dekker.
- Thongbai, P., R. J. Hannam, R. D. Graham, and M. J. Webb. 1993. Interaction between zinc nutritional status of cereals and Rhizoctonia root rot severity. *Plant and Soil* 153: 207–214.
- Torre, M., A. R. Rodriguez, and F. Saura-Calixto. 1991. Effects of dietary fiber and phytic acid on mineral availability. *Critical Reviews in Food Science and Nutrition* 1: 1–22.
- Treeby, M., H. Marschner, and V. Römheld. 1989. Mobilization of iron and other micronutrient cations from a calcareous soil by plant-borne, microbial, and synthetic metal chelators. *Plant and Soil* 114: 217–226.
- Udomkesmalee, E., S. Dhanamitta, J. Yhounng-Aree, N. Rojroongwasinkul, and J. C. Smith, Jr. 1990. Biochemical evidence suggestive of suboptimal zinc and vitamin A status in school children in Northeast Thailand. *American Journal of Clinical Nutrition* 52: 564–567.
- Underwood, E. J. 1971. *Trace elements in human and animal nutrition*, 3rd edition. New York: Academic Press.

- USDHHS/USDA (United States Department of Health and Human Services and United States Department of Agriculture). 1989. *Nutrition monitoring in the United States: An update report on nutrition monitoring*. DHHS Publication No. (PHS) 89-1255. Hyattsville, Md., U.S.A.
- van Beem, J., J. Kornegay, and L. Lareo. 1992. Nutritive value of the Nuña popping bean. *Economic Botany* 46: 164-170.
- Walton, G. H. 1978. The effect of manganese on seed yield and the split seed disorder of sweet and bitter phenotypes of *Lupinus angustifolius* and *L. consentinii*. *Australian Journal of Agricultural Research* 2: 1177-1189.
- Webb, M. J., and R. D. Graham. 1990. Supra-optimal Mn suppresses the effect of *Gaeumannomyces graminis* var. *tritici* on grain yield of wheat. Paper presented at the Australian Agronomy Conference, February 19-21, Perth, Australia.
- Weir, R. G., and A. Hudson. 1966. Molybdenum deficiency in maize in relation to seed reserves. *Australian Journal of Experimental Agriculture and Animal Husbandry* 6: 35-41.
- Weiss, M. G. 1943. Inheritance and physiology of efficiency in iron utilization in soybeans. *Genetics* 28: 253-268.
- Welch, R. M. 1986. Effects of nutrient deficiencies on seed production and quality. *Advances in Plant Nutrition* 2: 205-247.
- _____. 1993. Zinc concentrations and forms in plants for humans and animals. In *Zinc in soils and plants*, ed. A. D. Robson, 183-195. Dordrecht, the Netherlands: Kluwer Academic Publishers.
- _____. 1994. Micronutrient nutrition of plants. *Critical Reviews in Plant Sciences* 12.
- Welch, R. M., and W. A. House. 1983. Factors affecting the bioavailability of mineral nutrients in plant foods. In *Crops as sources of nutrients for humans*, ed. R. M. Welch and W. H. Gabelman, 37-54. Madison, Wis., U.S.A.: American Society of Agronomy.
- Welch, R. M., and L. V. Kochian. 1992. Regulation of iron accumulation in food crops: Studies using single gene pea mutants. In *Biotechnology and nutrition*, ed. D. D. Bills and S.-Y. Kung, 325-344. Boston, Mass., U.S.A.: Butterworths-Heinemann.
- Welch, R. M., M. J. Webb, and J. F. Loneragan. 1982. Zinc in membrane function and its role in phosphorus toxicity. In *Plant Nutrition 1982: Proceedings of the Ninth International Plant Nutrition Colloquium*, ed. A. Scaife, 710-715. Slough, England: Commonwealth Agricultural Bureaux.
- Welch, R. M., W. H. Allaway, W. A. House, and J. Kubota. 1991. Geographic distribution of trace element problems. In *Micronutrients in agriculture*, 2d edition, ed. J. J. Mortvedt, F. R. Cox, L. M. Shuman, and R. M. Welch, 31-57. Madison, Wis., U.S.A.: Soil Science Society of America.

- Welch, R. M., M. E. Smith, D. R. Van Campen, and S. C. Schaefer. 1993. Improving the mineral reserves and protein quality of maize (*Zea mays* L.) kernels using unique genes. *Plant and Soil* 155/156: 215–218.
- Welch, R. M., W. A. Norvell, S. C. Schaefer, J. E. Shaff, and L. V. Kochian. 1993. Induction of iron(III) and copper(II) reduction in pea (*Pisum sativum* L.) roots by Fe and Cu status: Does the root-cell plasmalemma Fe(III)-chelate reductase perform a general role in regulating cation uptake? *Planta* 190: 555–561.
- White, C. L. 1992. Zinc deficiency in man and animals: Endemic or imaginary? *Proceedings of the Nutrition Society of Australia* 17: 115–124.
- White, M. C., R. L. Chaney, and A. M. Decker. 1981. Metal complexation in xylem fluid. III. Electrophoretic evidence. *Plant Physiology* 67: 311–315.
- White, M. C., A. M. Decker, and R. L. Chaney. 1981. Metal complexation in xylem fluid. I. Chemical composition of tomato and soybean stem exudate. *Plant Physiology* 67: 292–300.
- White, M. C., F. D. Baker, R. L. Chaney, and A. M. Decker. 1981. Metal complexation in xylem fluid. II. Theoretical equilibrium model and computational computer program. *Plant Physiology* 67: 301–310.
- WHO (World Health Organization). 1992. *National strategies for overcoming micronutrient malnutrition*. Geneva.
- Wilhelm, N. S. 1991. Investigations into *Gaeumannomyces graminis* var. *tritici* infection of manganese-deficient wheat. Ph.D. diss., University of Adelaide, Adelaide, Australia.
- Wilhelm, N. S., R. D. Graham, and A. D. Rovira. 1988. Application of different sources of manganese sulphate decreases take-all (*Gaeumannomyces graminis* var. *tritici*) of wheat grown in a manganese deficient soil. *Australian Journal of Agricultural Research* 39: 1–10.
- _____. 1990. Control of Mn status and infection rate by genotype of both host and pathogen in the wheat take-all interaction. *Plant and Soil* 123: 267–275.
- Wilkinson, G. N., S. R. Eckert, T. W. Hancock, and O. Mayo. 1983. Nearest neighbor (NN) analysis of field experiments. *Journal of the Royal Statistical Society* B45: 151–211.
- Wright, M. J., ed. 1976. *Plant adaptation to mineral stress in problem soils*. Ithaca, N.Y., U.S.A.: Cornell University Agricultural Experiment Station.
- Yang, X., V. Römheld, and H. Marschner. 1993. Effect of bicarbonate and root zone temperature on uptake of Zn, Fe, Mn, and Cu by different rice cultivars (*Oryza sativa* L.) grown in calcareous soil. In *Plant nutrition: From genetic engineering to field practice*, ed. N. J. Barrow, 657–660. Dordrecht, the Netherlands: Kluwer Academic Publishers.

- Yeo, A. R., and T. J. Flowers. 1986. Salinity resistance in rice (*Oryza sativa* L.) and a pyramiding approach to breeding varieties for saline soils. *Australian Journal of Plant Physiology* 13: 161–173.
- Zhang, F., V. Römheld, and H. Marschner. 1989. Effect of zinc deficiency in wheat on the release of zinc and iron mobilizing root exudates. *Zeitschrift für Pflanzenernährung und Bodenkunde* 152: 205–210.
- Zheng, L., Z. Qi-qing, T. Li-hua, X. Jung-xiang, and Y. Chu-liang. 1982. Geographical distribution of trace elements-deficient soils in China. *Acta Pedologica Sinica* 19: 210–223.

COMMENTARY

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Graham and Welch covered many aspects of the improvement of micronutrient density (iron and zinc as well as vitamin A) in wheat, maize, and rice. These comments will focus on additional aspects that deserve consideration from the breeder's point of view as well as from nutritional and economic points of view.

ECONOMIC CONSIDERATIONS

Robellen (1977, 46–47) stated

Plant breeding is the cheapest way for the nutritional improvement of cereal (food) grains (if that can be accomplished without loss of yield or acceptability). The new variety produces the better quality automatically without further expenditures. But it is generally accepted that quality breeding can only be successful when conducted as part of an overall breeding program in which yield factors have top priority. The nutritionist who requests changes in nutritional values of cereals from the plant breeder must keep in mind that the development of a variety takes 10 or even 15 years. By then, the plant breeder has invested around one million of U.S. dollars; he has planted tens of thousands of progenies and has run similar numbers of various determinations and analyses before the first kilogram of sold seed gives him the first financial return. Deciding upon the breeding aims 15 years in advance is a high risk and needs utmost knowledge and responsibility. It is indispensable that the breeding priorities are still accepted and valid after completion of the breeders program, thus securing a wide cultivation and market for the new variety. With the permanently expanding knowledge in the field of nutrition, and the rapid economic fluctuations, this basic requirement is not always fulfilled in quality breeding. This may explain why quantity still has priority in agricultural production and why internal quality of the product normally receives much less attention. In the European Economic Community (as well as in the U.S.), the marketing system of cereals (but not necessarily of forages or vegetables) is almost exclusively based on yield units. Financial stimuli for better qualities are insufficient or lacking. Yet the potential of plant breeding to improve nutritional values is at least as high as the chances for yield increase.

This statement is still valid. There is no financial stimulus for increasing the quality of cereals, and there is no reassurance that, in the time it takes to develop a new variety, new ways to economically and efficiently supply the population with the needed nutrients will not be developed and implemented, making the new variety obsolete even before it is released.

On the other hand, education programs to encourage more balanced diets, coupled with programs to increase the volume and lower the costs of vegetable production, may be a more efficient approach to solve the problem of vitamin and mineral deficiency. The increased food

and income security that results from the diversification of agricultural production will also improve micronutrient status.

The economic and social trade-offs involved in the decision to start a breeding program for nutritional quality are enormous. It is especially important to consider that while the breeding program is in effect, other measures must be taken to overcome the nutritional problems, because no population can afford to wait more than 10 years for the solution of such an important health problem.

PROCESSING FOR HUMAN CONSUMPTION

The three cereals under consideration need some processing before use by humans. In many cases the processing procedures do not change the nutritional content of the cereal much, but in others that may not be the case.

Of the three cereals, the worst case is that of rice. We know that for rice grains, vitamins and minerals are concentrated in the embryo, the integument, and the aleurone (external layers of the grain). The integument and the aleurone are eliminated in the processing that results in "polished" rice (the way it is most commonly eaten). Losses of proteins, vitamins, fat, and minerals may be as high as 87 percent. Rice loses about 50 percent of its original iron just in the polishing, and more mineral salts are lost later, in the washing before cooking (Brandão 1974). When trying to create a breeding program to select for nutrient-dense cereal, the analysis of content in the selection procedure must be done for the part that will be effectively consumed. In the case of rice, if consumption habits could be changed (for example, avoiding washing before cooking), some of the mineral deficiencies under consideration will have already been overcome.

Moreover, in Latin America and other areas, rice and maize are not the only components of the diet. They are usually eaten together with beans, which have nutritional properties that complement those of the cereals. Beans, for example, are a source of iron and of some amino acids that cereals lack. The decision on whether to pursue a breeding program, besides taking into account economic and educational factors, must be based on the eating habits of the population, so that researchers understand which crop would be best suited to improvement for which factor.

PLANT BREEDING ASPECTS OF INCREASING MICRONUTRIENT AVAILABILITY

Increasing micronutrient availability through breeding means increasing the absorption of such micronutrients from the soil, or improving "agronomical efficiency." To increase absorption of a particular micronutrient, plant breeding offers two approaches: the first involves improving the root system of the plants to allow the exploitation of larger volumes of soil, as well as deeper areas of soil profile; the second involves changing the absorption pattern of a plant, since all nutrients tend to be absorbed together.

Improvement of Root Systems

Roots are by far the least well known plant part, even though they were long ago recognized as the main organ for a plant's absorption of water and nutrients. They stay hidden

in the soil, and at some stage in the life cycle of some crops, they start to degenerate. Even when roots are dug from the soil, it is hard to precisely evaluate the root system. Thin roots and root hairs, which tend to break when being dug, may be the most important parts for the absorption process, because they are the areas of active root growth.

Mineral absorption and water absorption go hand in hand, because nutrients must be in solution to be taken up by the roots. A larger root system is able to take up larger volumes of water and minerals for the plant (Yoshida and Hasegawa 1982; O'Toole and Soemartono 1981).

Experiments in artificial conditions (tall tubes full of soil, large pots, rhizotrons, and nutrient solution) all have a common problem: they do not thoroughly simulate a real soil profile with gradual changes in chemical and physical composition with depth. Roots of plants that are grown in such conditions may not react the same way in the field. Besides, limitations in the diameter and length of containers may change the root morphology.

Change in Absorption Pattern

In the same species and soil conditions, different cultivars may absorb some nutrients and water differently. This is the basis for the argument that such traits reflect genetic diversity (Malavolta and Fornasieri 1983; Clark 1978). It is known that plants absorb all nutrients jointly: there is no selective absorption, and as long as a minimum concentration of all nutrients (macro- and micronutrients) is present in the soil solution, the plant survives. What varies is the minimum level for each plant. In cases where plants are able to live with very small concentrations of a nutrient, it is because they are able to extract more of that nutrient than other plants in the same conditions.

Graham and Welch report, "In our experience, all micronutrient efficiency traits for deficient soils so far explored have been expressed as a greater ability to extract the nutrient from the soil rather than a greater ability to survive on less of that nutrient in vegetative tissues or in grain." It is difficult to establish, under natural or artificial conditions, the absolute minimum for a particular micronutrient in order to proceed with efficient selection for that trait.

The physiological mechanism for cellular absorption of each micronutrient may be different: some will be preferentially absorbed through a potential gradient while others may require some sort of enzyme-mediated active transport process. Different ions also compete for absorption. The mechanisms for each nutrient and their interactions are still largely unknown. To modify these mechanisms, the first step is to gain a better understanding of them. No efficient procedure has been established to improve the ability of plants to selectively absorb more or less of one particular ion.

PLANT BREEDING ASPECTS OF CHANGING THE CONCENTRATION OF NUTRIENTS AMONG PLANT ORGANS

The accumulation of minerals and vitamins in certain parts of the plant may be advantageous for the plant, since it results from thousands of years of natural selection. When a nutrient is selectively increased in seeds, for example, the nutrient in some other part or component of the seed will decrease. The decrease may or may not be reflected in germination, survival of seedlings, seed filling, and, ultimately, yield. If the micronutrient increase in seeds is very small, it will probably do little damage to the seed's agronomical qualities, but it may not be enough to overcome subclinical deficiencies in humans.

The same caveat applies to accumulating promoters or decreasing antinutritional factors in seeds: if the change is a large one, seed viability and yield may change. Until a better understanding of seed physiology for such cases is developed, this is shaky ground for plant breeders. One example is the case of the gene Opaque 2 in maize. That gene enhances the concentration of lysine in maize grains, by replacing some of the zein, the main storage protein in maize, with other proteins that are richer in lysine residues (Axtell 1981). Lysine enhances iron and zinc in addition to improving nutritional balance through the higher biological value of such protein compared with regular zinc. The increase in lysine, however, generally results in lighter-weight grains that translate into decreased yield for such varieties compared with regular maize varieties.

To compensate for its increased nutritional value, maize with more lysine should ideally have a higher market value. That may happen in areas of the world where people are well educated. In developing countries, however, such improvement represents a decreased profit for the farmers.

PLANT BREEDING PROCEDURES

Genetic Control of Characteristics

The genetics of root traits (morphology and traits related to absorption efficiency) is, to some extent, known. Researchers have reported that root length, thickness, and root dry weight in rice are polygenic traits, with moderate to high heritabilities (Bhaduri and Ghosh 1965; Armenta-Soto et al. 1983).

For traits other than root morphology, the pathways of absorption of nutrients may be defined by a few major genes or by many genes. The pathway for vitamin A production (provitamin A, cofactors, and so forth) in tomatoes is complex, and mutants at different points of it have been identified (Axtell 1981).

When it is said that the inheritance of a particular phenotype is determined by one major gene, it means that, for the particular constraining phenotypes studied, the difference among them was caused by a single gene. The gene may not be the same for all crosses and cases.

Cases in which many genes influence the determination of nutrient absorption and accumulation should be more common than reported in the literature, considering the complexities of such traits and the number of factors that may influence absorption. The existence of many different genes, even when they have small effects, offers a possibility of obtaining transgressive segregants: homozygous plants from segregating populations that may achieve higher efficiency than either of the parents used in the crosses.

For achieving progress by plant improvement, more important than the number of genes involved is the heritability of the characteristic. Heritability varies from 0 to 1 (or from 0 to 100 percent) and represents the proportion of the total phenotypic variation that is determined by the genotype of the plant. In mathematical terms, phenotypic effects are composed by the genotypic effects (G) added to the environmental effects (E) and to the genotype by environmental interaction effects (GE). Heritability (H^2) then, is

$$H^2 = \frac{G}{(G + E + GE)}$$

Heritability indicates how easy or difficult it would be to produce changes in a given trait by applying selection. The closer it is to 1, or 100 percent, the less environmental influence the characteristic is subject to and the easier it is to make progress through selection. So the environmental effects and the genotype by environmental interaction over a given characteristic are the factors that most reduce the possibilities of success that may be achieved by selection in each generation. The better the environmental control that can be achieved (the more uniform the environment), the greater the progress that can be made. In an ideal situation in which there is no environmental effect over a trait, the only limitation to progress is caused by the plant genotypes. Genotypic effects (G) are formed by the additive effects (A), which can be fixed through selection, added to the dominant effects (D), which cannot be fixed:

$$G = A + D.$$

A characteristic is said to be quantitative when it shows continuous variation, and if any classes may be formed, they are arbitrary (nondiscrete). Generally, such a characteristic is subject to large environmental effects. Quantitative traits may be determined by a large number of genes subject to small effects or by a small number of genes subject to large effects. Most characteristics of agronomical importance are quantitative.

The hypothesis that iron efficiency involves one or a few major genes and several minor additive genes suggests that this trait has an inheritance pattern that is a mixture of quantitative and qualitative. For zinc efficiency in rice, a few additive genes may be involved (Majumder, Rakshit, and Borthakur 1990), so the trait is quantitative.

Selection Procedures

Improvement of Concentration of Iron, Zinc, and Vitamin A in Seeds. Direct selection for any characteristic is always better than indirect selection. The strategy of measuring concentration in seeds and selecting the higher one, recommended by Graham and Welch, is a direct selection method that is valid for micronutrient density as well as for high provitamin A content for wheat and maize. In the case of rice, contents should be determined for the polished grain, allowing selection for concentration of the nutrient in the edible part of the plant. Differences in concentration among different plant parts (leaves versus roots, for example) will arise as long as the nutrient is available in the plant. Graham and Welch discussed strategies for indirect or direct selection in relation to plant age and ways to overcome the difficulties caused by differences that arise due to small variations (a few days) in plant age. Any plant improvement procedure that is adequate for the mating system of the crop and that increases micronutrient content can be used, provided it is based on the determination of the content of such products in the edible parts.

If, to precisely determine the amounts of such nutrients and vitamins in seeds, a large volume of seeds is needed, selection must be done after plant maturity. This is the case for polished rice. In the case of maize, an allogamous crop, in any generation, selection will be based on the female parent only, and the pollen will be a random sample of the population, slowing, to some extent, the progress that can be achieved.

Improvement of Agronomic Efficiency Traits. Graham and Welch's discussion of the genetic control of traits indicates that to achieve progress in selection programs to improve iron or zinc efficiency, researchers must overcome the difficulty of obtaining a uniform micronutrient-deficient environment (or a nutrient solution with micronutrient deficiency). Such an environment is particularly difficult to obtain and to maintain because the plants need only very small amounts of these micronutrients. Besides, as Graham and Welch argue, nutrients interact with each other and their availability is heavily influenced by low pH (indicating soil acidity that usually accompanies aluminum, which is toxic to plants). In soils, it is almost impossible to obtain the ideal selective conditions for each micronutrient separately, without influences of high or low pH and deficiencies in macronutrients or other micronutrients. Thus breeding for agronomic efficiency will not be easy, and environmental control will be the main problem.

Graham and Welch discussed aspects of selection based on an efficiency index, selection for yield using plus and minus plots, and response curves (minus plots and different micronutrient levels, as already mentioned, are very difficult to obtain in the field, and soil specialists have doubts about whether they are ever feasible). As Graham and Welch properly point out, a lack of understanding of the basic mechanisms involved in micronutrient efficiency hampers the development of better screening techniques.

Researchers have proposed improvement of root systems as a way of increasing drought resistance in all species and soils; they should also pursue it as a way of increasing the nutrient status of plants. It will be difficult because of the virtual nonexistence of adequate procedures for evaluating individual plants in segregating populations, since all current methods for root studies are destructive, awkward, and labor-intensive.

The molecular marker technology that is now available may help to develop efficient selection procedures for modifying and improving root morphology to increase efficiency of water and nutrient absorption. DNA probes for roots (RFLPs technique) are already available for rice (Champoux et al. forthcoming). The ability to map DNA probes that are closely associated with traits of interest makes marker-assisted selection a promising alternative for plant improvement, especially for characteristics that are inaccessible or hard to evaluate or visualize, such as roots. Marker-assisted selection combines the traditional plant improvement procedures of crossing and selecting in segregating generations with the molecular technique to make selection efficient (McCouch and Tanksley 1991; Tanksley et al. 1989). Improvement of root systems may now be the most promising approach for improvement of micronutrient efficiency.

REFERENCES

- Armenta-Soto, J. L., T. T. Chang, C. G. Loresto, and J. C. O'Toole. 1983. Genetic analysis of root characters in rice (*Oryza sativa* L.). *Sabrao J* 15, no. 1: 103–116.
- Axtell, J. D. 1981. Breeding for improved nutritional quality. In *Plant Breeding II*, ed. K. J. Frey, 365–432. Ames, Iowa, U.S.A.: Iowa State University Press.
- Bhaduri, P. N., and M. Ghosh. 1965. Excised embryo culturing: The study of inheritance of root types in rice. *Botanical Magazine* 78, no. 2: 348–352.

- Brandão, S. S. 1974. *Cultura do Arroz*. Viçosa, Brazil: Imprensa Universitária, Universidade Federal de Viçosa.
- Champoux, M. C., G. Wang, S. Sarkarung, D. J. Mackill, J. C. O'Toole, N. Huang, and S. R. McCouch. Forthcoming. *Locating genes associated with root morphology and drought avoidance in rice via linkage to molecular markers*.
- Clark, R. B. 1978. Differential response of maize inbreds to zinc. *Agronomy Journal* 70, no. 4: 1057–1060.
- Majumder, N. D., S. C. Rakshit, and D. N. Borthakur. 1990. Genetic effects on uptake of selected nutrients in some rice (*Oryza sativa* L.) varieties in phosphorus deficient soil. *Plant Soil* 123, no. 1: 117–120.
- Malavolta, E., and F. D. Fornasieri. 1983. Nutrição mineral. In *Cultura do Arroz de Sequeiro: Fatores afectando a produtividade*, ed. M. E. Ferreira, T. Yamada, and E. Malavolta, 95–144. Piracicaba, Brazil: Instituto da Potassa e Fosfato, Instituto Internacional da Potassa.
- McCouch, S. R., and S. Tanksley. 1991. Development and use of restriction fragment length polymorphism in rice breeding and genetics. In *Rice biotechnology*, ed. G. S. Khush and G. H. Toenniessen, 109–134. Biotechnology in Agriculture 6. Los Baños, Philippines: CAB International and International Rice Research Institute.
- O'Toole, J. C., and J. Soemartono. 1981. Evaluation of a simple technique for characterizing rice root systems in relation to drought resistance. *Euphytica* 30, no. 2: 283–290.
- Robellen, G. 1977. Possibilities and limitations of breeding for nutritional improvement of cereals. In *Nutritional evaluation of cereal mutants*, 46–57. Vienna: International Atomic Energy Agency.
- Tanksley, S. D., N. D. Young, A. H. Paterson, and M. W. Bonierbale. 1989. RFLP mapping in plant breeding: New tools for an old science. *Biotechnology* 7, no. 2: 257–264.
- Yoshida, S., and S. Hasegawa. 1982. The rice root system: Its development and function. In *Drought resistance in crops with emphasis on rice*, 97–114. Los Baños, Philippines: International Rice Research Institute.

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defined food sector. The Institute's research program reflects worldwide collaboration with governments and private and public institutions interested in increasing food production and improving the equity of its distribution. Research results are disseminated to policymakers, opinion formers, administrators, policy analysts, researchers, and others concerned with national and international food and agricultural policy.

IFPRI is a member of the Consultative Group on International Agricultural Research and receives support from the Andina Foundation, the Asian Development Bank, Australia, Belgium, Canada, China, Denmark, Food and Agriculture Organization of the United Nations, Ford Foundation, France, German Agency for Technical Cooperation (GTZ), German Federal Ministry for Economic Cooperation (BMZ), India, Inter-American Development Bank, International Development Research Centre (Canada), International Fund for Agricultural Development, Italy, Japan, Netherlands, Norway, Overseas Development Administration, the Philippines, Rockefeller Foundation, Rural Industries Research and Development Corporation (Australia), Spain, Sweden, Switzerland, United Kingdom, United Nations Development Programme, United Nations Children's Fund, United States, and the World Bank.