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"Plant Breeding Solutions for Environmental Stress Problems in Crop Production"

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FROM THE EDITORS

The Plant Science Lectures are presented annually at Iowa State University on subject matter areas that seem destined to contribute to success in plant breeding and crop production research and which relate ultimately to human welfare. The Departments of Agronomy, Botany, Forestry, Genetics, Horticulture, and Plant Pathology, Seed and Weed Science all cooperate in sponsoring this endeavor.

The theme for the Plant Science Lecture Series for 1982 was "Plant breeding solutions for environmental stress in crop production." This issue of the *Iowa State Journal of Research* is a compilation of the papers presented in this lecture series.

Breeding for consistency of crop yields and to extend a crop into areas with serious production stresses are important objectives for future food and feed production. In fact, they may be more important objectives than breeding for higher yield potential. If breeders are successful, much crop production in the decades ahead can come from land areas that are subject to production stresses, some so severe that crop production on them is currently impossible. For example, it is estimated that 15% of the earth's land surface has highly saline soils, and 60 million hectares of current and potential rice land in south and southeast Asia contain toxic amounts of salt. These estimates provide some appreciation of the enormity of today's salinity problem in crop production, and the problem continues to grow. Further, several million hectares of land in the temperate wheat-producing areas of the world have aluminum toxicity problems that are directly related to low soil pH. And, in Iowa each year, about a half million hectares of soybeans grown on alkaline soil with pH greater than 7.6 may suffer severe chlorosis and stunting due to iron deficiency. Many additional examples could be given to illustrate that almost every current or potential crop production area has some environmental stress that reduces crop productivity.

Fortunately, genetic tolerance to salinity is readily available in the cultivated species of rice, and progress has been made in placing tolerance genes into cultivars. Also, the aluminum tolerance that has been bred into wheat cultivars has made the farmers free from the need for liming soil in order to produce four to five tons of wheat per hectare. And recently, soybean cultivars have been released that are resistant to the iron chlorosis that occurs on the aklaline soils in the Corn Belt. Such genetic solutions to environmental stress conditions in crop production are especially beneficial because they are nonpolluting, have no input cost to the farmer, and are easy to manipulate by breeding.

Certainly, plant breeding is not a cure for all crop production stresses, but research and successes have shown that much progress can be made in developing stress-tolerant cutlivars of plants.

The papers included in this volume, written by leaders in this research effort, provide a record for future students and scientists who were unable to attend the Plant Science Lecture Series for 1982. It will be an important document historically because it presents a partial record of the status of breeding for stress tolerances in 1982 and gives insight into future areas of this research arena that should be lucrative for plant breeding success.

Kenneth J. Frey, Coordinator, Plant Science Lecture Series

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PLANT ADAPTATION TO MINERAL STRESS IN PROBLEM SOILS

C. D. Foy[1]

ABSTRACT. This paper reviews some of the soil fertility problems that may be amenable to plant breeding efforts and discusses the possible benefits and constraints of a plant genetic approach to such problems. Specific topics include acid soil infertility, with emphasis on Al and Mn toxicities, Fe deficiency in calcareous soils, differential plant tolerances to mineral stress, current knowledge concerning genetic control of mineral stress tolerance, and current activity in the development and release of stress-tolerant germplasm. It is suggested that the plant breeding approaches discussed may have great potential in alleviating present and anticipated food shortages throughout the world.

Index Descriptors: acid soil infertility, alkalinity, salinity, drought, subsoils, rooting depth, Al tolerance, Mn tolerance, Fe efficiency, genetic control of stress tolerance, release of stress-tolerant germplasm, world food shortages, and minimum input agriculture.

INTRODUCTION

Several years ago a colleague of mine, upon returning from an International Congress of Soil Science, said to me, "Soil science is dead; there is nothing new coming out." I agreed with him and added, "That is because you have left the plant out of the soil science picture." Crop production depends upon interactions between the genetic potentials of plants and all factors of the production environment. Until recently, plant genotype-mineral stress relationships have been largely ignored by researchers.

In the past, the approach to soil fertility problems has emphasized "changing the soil to fit the plant." Soil scientists have said to plant breeders, "Develop a variety with climatic adaptation, insect and disease resistance, high yield potential, and high quality. Soil fertility factors can be adjusted to optimum levels for the plant." As a result, many crop cultivars have been developed for nearly ideal conditions of soil fertility and pH. Such cultivars are like incubator babies, i.e., they thrive inside the incubator but cannot tolerate stresses of the outside world. They may develop mineral deficiency or toxicity problems when grown on soils that are only slightly different from those on which they were developed.

Examples of plants having adaptation limitations are: the famous Green Revolution wheat (*Triticum aestivum*) cultivars 'Sonora 63' and 'Gaines'; the latter is a USA variety that holds the world yield record of 14.3 t/ha. Neither of these will tolerate the strongly acid, Al-toxic soils of Brazil, and this explains

[1] Research Soil Scientist, USDA, ARS, Plant Physiology Institute, Plant Stress Laboratory, Beltsville, Maryland 20705.

why Gaines yields poorly when moved from the Palouse Region of Washington State to more acidic soils. Wheat cultivars developed at the Ohio Research and Development Center at Wooster, Ohio, are generally more tolerant to Al-toxic soils than those developed at the Indiana Agricultural Experiment Station at Lafayette, IN. The Green Revolution rice (*Oryza sativa*) cultivar 'IR-8' is much more sensitive to Fe toxicity than cultivars native to Southeast Asia. 'Wayne' soybean (*Glycine max*), selected for high yield (5.3 t/ha), shows Fe-deficiency chlorosis on calcareous soils of the midwestern USA. Some maize (*Zea mays*) and tomato (*Lycopersicon esculentum*) inbreds cannot absorb and/or use Fe efficiently, even in soils at pH 5.0 or less. One maize inbred line cannot obtain adequate Mo from a strongly acid soil (Foy, 1981; Foy et al., 1978; Foy and Fleming, 1978).

The practice of liming and fertilizing soil to optimum levels was profitable on the moderately acid soils of the USA when lime, fertilizer, and fuel were cheap. However, in many parts of the world this approach has never been practical, and even in the developed countries of the world, current energy costs and fear of environmental pollution are causing a re-examination of liming and fertilization practices. Furthermore, in both developing and developed countries, some soil conditions are not economically correctable with current technology. There is simply a need to seek great accomodations with nature rather than attempting to change it. For example, in some parts of the tropics, scientists are developing technology based on crop production with minimum input rather than maximum output (Sanchez and Salinas, 1981) for marginal soils. In such cases, tailoring the plant to fit the soil may be more economical than changing the soil to fit the most exacting plant. Native farmers on marginal soils live with a minimum input system, and a high input technology in such situations is often both unprofitable and harmful.

SOIL STRESS PROBLEMS AMENABLE TO PLANT BREEDING SOLUTIONS

Soil situations and problems for which plant breeding solutions are appropriate are: (a) acid, Al-toxic subsoils that are difficult to lime, (b) strongly acid mine-spoil areas where rapid plant cover is needed at minimal cost; (c) steep pasture lands that are strongly acid, infertile, and difficult to lime, even in the surface layer; (d) strongly acid, P-fixing surface soils and subsoils of the tropics (e.g., Campo Cerrado of Brazil and the Llanos of Eastern Colombia); (e) saline soils; (f) soils polluted with heavy metals; (g) calcareous soils with Fe unavailability or other micronutrient problems; (h) wet soils; (i) dry soils; and (j) even hard soils.

Plant breeding may also be used to improve the efficiency of fertilizer nutrients, particularly for P and N, on good soils. For example, N-efficient genotypes would make more efficient use of energy and fertilizer and reduce ground-water pollution.

Plant breeding can regulate mineral composition of crops to improve the quality of food and feed. For example, forages that will accumulate sufficient Mg could prevent grass tetany in grazing animals (Allen and Robinson, 1980), and plants that exclude Cd would reduce the accumulation of this element in humans (Foy et al., 1978).

For plant breeding to be successful in alleviating soil stress problems in crop production, there must be genetic variability between and within crop species for reaction to factors causing the stress problems. And as will be documented later, there is much genetic variation that can be used in this area. First, however, the benefits and objections to this approach will be discussed.

BENEFITS OF A PLANT BREEDING APPROACH TO SOIL STRESS PROBLEMS

Perhaps the most important benefits to a plant breeding approach to solving soil fertility problems are that it is ecologically clean, energy conserving, and cheaper than amending the soil. Thus, it is compatible with national and international goals of economical food production, conservation of fertilizers and energy, and control of pollution.

More specificially, the benefits are:

1. The introduction of a cultivar specifically adapted to a prevailing stress can increase crop yields on the stressed production areas. Examples are the selection of Fe-efficient strains of weeping lovegrass (*Eragrostis curvula*) and soybean for calcareous soils (Voigt et al., 1982; Bahrenfus and Fehr, 1980).
2. Crop acreage of a species can be expanded to marginal soils not presently suited to the crop. For example, wheat growing has been extended into the Campo Cerrado of Brazil only because Al-tolerant cultivars were bred and made available to growers in that region (Silva, 1976).
3. Plant breeding can develop cultivars of new and more profitable crop species for areas with very specific problems. An illustration of this was the introduction of an Al- and cold-tolerant strain of limpograss (*Hemarthria altissima*) for possible use on strongly acid, high-altitude mine spoils or on acid soils in northern climates (Oakes and Foy, 1980).

OBJECTIONS TO THE PLANT BREEDING APPROACH TO SOIL STRESS PROBLEMS

1. It has been said that mineral-tolerant genotypes may be low yielding in the absence of stress. However, research has not shown this to be true. For example, Al-tolerant cultivars of

snapbean (*Phaseolus vulgaris*), cotton (*Gossypium hirsutum*), tomato, wheat, and barley (*Hordeum vulgare*) produce high yields in the absence of Al.

2. Some of the Al-tolerant wheat cultivars are tall, and hence, they may lodge under high N fertilization. However, Camargo et al. (1980) reported a source of Al tolerance in the short wheat cultivars 'Tordo' and 'Siete Cerros', so Al-tolerant cutivars need not be tall.
3. The mineral content, and thus crop quality, of stress-tolerant cultivars may be low. Again, this is not an absolute relationship as shown by the Al-tolerant wheat 'BH 1146', which is more Mg efficient than Al-sensitive 'Sonora 63.' There is also evidence that Al-tolerant plants are more efficient in absorbing Ca and P when concentrations of these elements are low in the growth medium.
4. Breeding stress-tolerant and element-efficient cultivars of crops may discourage the use of lime and fertilizer. In reality, the opposite may occur because such cultivars will promote the use of marginal land which received no previous treatment. With stress-tolerant genotypes such land could become economically productive with low to moderate inputs of lime and fertilizer. Plant breeding solutions to soil stress problems will not mean the abolition of lime and fertilizer use but rather will establish another route to solving difficult problems of soil fertility. Lime and fertilizer inputs would still be required but at lower levels than those used in current agriculture.

   The related charge that the use of stress-tolerant and element-efficient cultivars will bleed soil fertility to zero levels is also invalid. The use of such plants would only promote more effective use of fertilizers already fixed in the soil or those used as amendments. The goal is to produce profitable (not necessarily maximum) yields of acceptable quality with lower inputs.
5. Breeding for high levels of tolerance to one stress may increase vulnerability to other stresses. This is a valid concern, and the breeder must relate available genetic resources to prevailing economic and production constraints.
6. An endless number of specific genotypes could be developed to fit specific soil stress conditions. Obviously, there is a practical limit to the number of plant cultivars that should be produced for soil stress situations. For example, a farmer growing wheat on the acid soils of the Campo Cerrado region in Brazil should use only an Al-tolerant cultivar, such as 'BH 1146', and in doing so, he will use less lime and P fertilizer than would be

needed with an Al-sensitive cultivar to produce a profitable yield. But seemingly, no more than two or three cultivars would be needed for all farmers in the Campo Cerrado region. At any time that the application of lime, gypsum, and superphosphate could be afforded to reduce the Al saturation and increase pH of subsoils (Ritchey et al., 1980; Messick et al., 1981), the grower might profit from switching to 'Sonora 63' which is a higher-yielding cultivar.

OBJECTIVES OF RESEARCH

At the Plant Stress Laboratory at Beltsville, we have the following objectives for mineral-stress research:

1. To identify both present and potential mineral stress factors in problem soils;
2. To screen plant collections for lines with stress tolerance;
3. To collaborate with plant breeders in developing superior genotypes for specific problem soils;
4. To determine the physiological mechanisms of differential plant adaptations to mineral stress;
5. To use plant physiological traits to refine screening procedures and improve soil-plant management practices;
6. To use plant genotypes as indicators of potential mineral stress problems;
7. To determine interactions between mineral stress and the other environmental factors, i.e., water, light, temperature, air pollution, pathogens, rhizobia, and mycorrhizae.

The writer has specialized in studies on Al and Mn toxicities and nutrient unavailability in acid soils and Fe-unavailability in calcareous soils. Hence, these areas of mineral stress will be emphasized in the following discussion.

ACID SOIL INFERTILITY

Soil acidity is a major growth-limiting factor for plants in many parts of the world. Acid soil injury (particularly Al toxicity) is an insidious problem, which may reduce fertilizer and water efficiency of plants and be mistaken for nutrient deficiency, drought, herbicide injury, low-temperature damage, or plant disease. Aluminum toxicity in subsoils is particularly harmful because it causes shallow rooting, drought susceptibility, and poor use of subsoil nutrients.

Acid soil toxicity is a complex of factors that may affect the growth of various plants through different physiological pathways, probably being controlled by different genes. Furthermore, several acid soils with the same pH may cause different mineral stress problems for a given genotype.

The causes of reduced plant growth on acid soils may vary with soil pH, clay mineral types and amounts, contents and kinds of organic matter, concentrations of salts, and particularly with plant species or genotype. Growth-limiting factors associated with soil acidity include toxicities of Al, Mn, and other metal ions, low pH (H^+ toxicity), and deficiencies or unavailabilities of essential elements such as Ca, P, Mg, and Mo. Even Fe deficiency has been reported in upland rice (*Oryza sativa*) on acid soils of pH 5.1 to 5.8. In acid coal mine spoils and soils polluted with industrial wastes, Zn, Cu, Cd, Ni, or Pb may be toxic to some plants. Acid soil factors may act independently, or together, to affect the growth of plants. Also, they may promote or inhibit the survival and function of rhizobia, mycorrhizae, and other soil microflora.

At a soil pH $>$ 4.2, low pH (H^+ toxicity) probably does not limit plant growth directly, so the detrimental effects of soil acidity in these cases are largely indirect. However, low pH *per se* can increase the Ca requirement of plants and may restrict the growth of rhizobia or other soil microflora. In general, only the most sandy and highly leached (low CEC) acid soils are deficient in Ca and Mg for higher plants, but certain legume rhizobia require higher Ca levels than do their host plants. Also, excesses of Al and certain other elements may interfere with plant uptake and the use of Ca, Mg, P, or other essential elements. Molybdenum is more available in limed soils than in strongly acid soils; hence, liming usually prevents Mo deficiency, but some soils (Australia) may be so low in this element that they require Mo fertilization for growing legumes.

Aluminum and Mn toxicities are the major growth-limiting factors in many acid soils. Al toxicity is particularly severe with a soil pH $<$ 5.0, but it may occur with pH = 5.5 in kaolinitic soils. Within this pH range, clay minerals, which are aluminosilicates, become unstable and some Al, normally bound within the clay crystal structure, moves to exchange positions on clay surfaces. Thus, in strongly acid soils the surfaces of clay crystals are saturated with exchangeable Al and not H ions. Some Al released from structural positions reacts with water in the soil solution to produce H ions, which keeps the pH low. Poor root development (and drought susceptibility) in soils with acid (pH 5.0) subsoil layers may be due to Al toxicity, which limits both rooting depth and branching.

Manganese toxicity generally occurs in soils having pH values of 5.5 or below, if the soil parent materials contain sufficient total Mn (Foy, 1973, 1981). However, it can occur at pH of 6.0 or above in poorly drained or compacted soils where reducing conditions produce divalent Mn, which is most available to plants. Thus, Mn toxicity can occur at pH values too high to

cause Al toxicity. Soils of the Atlantic coastal Plains of the USA are lower in total Mn than those of the Gulf Coastal Plains. Hence, at a given low pH, Mn toxicity is less likely in the former than the latter.

DIFFERENTIAL PLANT TOLERANCES TO MINERAL STRESSES

Aluminum Tolerance

Plant species and cultivars within species differ greatly in tolerance to mineral stresses caused by toxicity or deficiency. Publications that document this statement are numerous, and for those who wish to pursue the subject, many review papers serve as sources of literature. Herein, I will emphasize differences in plant tolerances to excess Al and Mn in acid soils and to Fe-related chlorosis in calcareous soils (Clark, 1981, Foy, 1982; Rorison, 1980; Vose, 1981, 1982a, 1982b; Devine, 1981).

Aluminum-tolerant plant species include azalea (*Azalea* spp.), datura (*Datura* spp.), rye (*Secale cereale*), cranberry (*Oxycoccus* spp.), tea (*Thea sinensis*), weeping lovegrass, bermudagrass (*Cynodon dactylon*), stargrass (*Aletris farinosa*), buckwheat (*Fagopyrum esculentum*), and peanuts (*Arachis hypogea*) (Foy, 1982). Other species that tolerate strongly acid soils with a high degree of Al saturation are pangolagrass (*Digitaria decumbens*) (Blue and Rodriguez-Gomez, 1975); rubber (*Hevea brasiliensis*) (Santana and Braga, 1977); blueberries (*Vaccinium* spp.) (Cummings, 1978); and Norway spruce (*Picea abies*) (Ogner and Tiegen, 1980). Tanaka and Hayakawa (1975) concluded that in general, the *Gramineae* and *Leguminoseae* were more tolerant to Al than the *Crucifereae* and *Chenopodiaceae*. McCormick and Steiner (1978) reported differential Al tolerance among 11 species of trees. Differential tolerances to acid soils among species of tropical legumes (Andrew and Hutton, 1974, Munns and Fox, 1977) and ornamentals (Foy and Wheeler, 1979) are probably also due in large part to differences in Al tolerance.

Differential Al tolerances among cultivars within species are often greater than species differences based on random cultivar comparisons. For example, differences exist among strains of barley, wheat, triticale, rice, alfalfa (*Medicago sativa*), tomato, soybean, ryegrass (*Lolium perenne*), snapbean, cotton, maize, rye, sunflower (*Helianthus anuus*), pea (*Pisum sativa*), sweet potato (*Ipomoea batatas*), green algae, and even soil-borne pathogens (Foy, 1974, 1982).

Lopez et al. (1976) reported that the diploid and tetraploid wheats were highly sensitive to Al; hexaploid wheats differed widely in tolerance, but all were injured by 25 ppm Al in solution, and rye cultivars varied from sensitive to 1 ppm Al to tolerant to 30 ppm in solution. Additional recent references to differential Al tolerances of plant genotypes within species are as follows: wheat (Magalhaes, 1979; Muzilli et al., 1978, Aniol and Kaczkowski, 1979;

Sousa et al., 1977; and Mugwira et al., 1981); barley and wheat (Graves et al., 1980); triticale (Aniol and Kaczkowski, 1979; Mugwira et al., 1981); rye (Aniol and Kaczkowski, 1979); ryegrass (Nelson and Kiesling, 1980); rice (Fageria and Zimmerman, 1979); cotton (Foy et al., 1980); sorghum (*Sorghum bicolor*) (Brown and Jones, 1977b); turfgrasses (Murray and Foy, 1978); bermuda grass (Lundberg et al., 1978); sweet potato (Munn and McCollum, 1976); apple rootstocks (*Malus pumila*) (Kotze, 1976); soybean (Muzilli et al., 1978; Sartain and Kamprath, 1975; Devine et al., 1979); cassava (*Manihot esculenta*) (Edwards and Kang, 1978); and cocoa (*Theobroma cacao*) (Garcia and Leon, 1978).

Manganese Tolerance

Plant species and cultivars differ widely in their tolerances to excess soluble or exchangeable Mn. Maize and rice are more tolerant than lespedeza (*Lespedeza striata*), soybean, or barley. Alsike clover (*Trifolium hybridum*) and oats (*Avena sativa*) are more tolerant than cowpea (*Vigna sinensis*), lespedeza, and sweet clover (*Melilotus alba*). The ornamental plants, calendula (*Calendula officinalis*), snapdragon (*Anterrhinum majus*), and chrysanthemum (*Chrysanthemum indicum*) are sensitive to excess Mn, whereas carnation (*Dicanthus caryophyllus*), poinsettia (*Poinsettia pulcherrina*), and rose (*Rosa carolina*) are Mn-tolerant. Other species rankings according to Mn tolerance are tomato > lettuce (*Lactuca sativa*) > barley, and bean > clover (*Trifolium* spp.) > potato (*Solanum tuberosum*) (Foy, 1973, 1982; Foy et al., 1978). Berg and Vogel (1976) observed that several plant species differed in tolerance to Mn toxicity in acid coal mine spoils. Lespedeza, birdsfoot trefoil (*Lotus corniculatus*), and black locust (*Robinia pseudoacacia*) showed Mn toxicity symptoms only on soils with pH < 4.0. Two other lespedezas, bicolor lespedeza (*Lespedeza bicolor*) and Korean lespedeza (*Lespedeza stipulacea*), developed Mn toxicity symptoms at pH < 5.0 and sometimes at pH 5.0 to 5.4. As with Al tolerance, ranking of plant species according to Mn tolerance depends upon the cultivars selected to represent the species.

In other studies on differential Mn tolerance between plant species, Benac (1976) found maize was more tolerant than peanut; Dionne and Pesant (1976) reported birdsfoot trefoil more tolerant to flooding and Mn toxicity than alfalfa; Rayment and Verrall (1980) found kikuyu grass (*Pennisetum clandestinum*) more tolerant to a high Mn soil than white clover (*Trifolium repens*); Ward (1977) noted that tomatoes tolerated a wide range of Mn concentrations; Tanner (1977) found maize tolerant to high Mn concentrations and yellow lupine (*Lupinus luteus*) more tolerant than horse bean (*Vicia faba*) and sorghum; and Mahmoud and Grime (1977) reported the order of Mn tolerance as common hair grass (*Deschampsia flexuosa*) > > sheep's fescue (*Festuca ovina*) > bentgrass (*Agrostis tenuis*) > tall oat grass (*Arrhenatherun elatius*). Mahmoud

and Grime (1977) also observed that Mn tolerance correlated with ability to colonize acid soils. Hutton et al. (1978) reported differences in Mn tolerance among species of tropical legumes.

Recent reports of differential Mn tolerance within plant species include wheat (Andrade et al., 1976), apple (Miller and Schubert, 1977), triticale (Mugwira et al., 1981), soybeans (Brown and Jones, 1977a; Jones and Nelson, 1979; Heenan and Campbell, 1980; Ohki et al., 1980; Heenan and Campbell, 1981), cotton (Foy et al., 1981), and flax (*Linum usitatissimum*) (Moraghan and Ralowicz, 1979).

Iron Efficiency

The unavailability of iron in alkaline soils is a good example of a soil stress that can be ameliorated via plant breeding. In fact, for field crops like sorghum, breeding Fe-efficient (chlorosis resistant) genotypes is probably the only economical, long-term solution to the stress. Plant species and genotypes within species differ widely in their abilities to resist Fe-related chlorosis (Mortvedt, 1975; Clark, 1981, 1982; Vose, 1982a, 1982b).

GENETIC CONTROL OF MINERAL STRESS TOLERANCE

Aluminum Tolerance

In barley, Al tolerance is controlled by one major dominant gene (Reid, 1971), whereas in wheat, two and possibly three major, dominant genes and several modifiers appear to be involved (Campbell and Lafever, 1981; Lafever and Campbell, 1978). In maize, Al tolerance is inherited via one locus with multiple alleles (Rhue, 1979). Lafever and Campbell (1978) and Campbell and Lafever (1981) could find no genetic difference for reaction to Al among the sensitive cultivars 'Gaines' from Washington State and 'Redcoat' and 'Arthur' from Indiana, nor among tolerant cultivars 'Atlas 66' from North Carolina and 'Seneca' and 'Thorne' from Ohio. Prestes et al. (1975) concluded that the Al tolerance locus in wheat was located on chromosome 5D.

Lopez-Benitez (1977) suggested that the D genome from hexaploid wheat and the R genome from rye contain the loci that condition Al tolerance for triticale. Sapra et al. (1978) found that 'Beagle' triticale, which has a full complement of rye chromosomes, showed high Al tolerance, but 'Armadillo' and 'Rosner', which lack chromosome 2R, and 'Cimmaron', which lacks 2R and 4-7R, showed considerable tolerance. Apparently, the genes on rye chromosomes that control or modify Al tolerance interact with the D genome of wheat to express the trait in substituted hexaploid triticales.

Hanson and Kamprath (1979) found no clear-cut genetic control of Al tolerance in soybeans. Differential Al tolerance has been reported in callus cell cultures of tomato (Meredith, 1978a, 1978b). Callus from VNT cherry tomato

was less inhibited by Al than that of Marglobe. The Al-resistant variants probably resulted from mutation, but they may have an epigenetic basis.

Manganese Tolerance

Mn tolerance in alfalfa has been attributed to additive genes having little or no dominance (Dessureaux, 1959), whereas in lettuce, it is controlled by one to four genes, depending upon the species (Eenink and Garretsen, 1977). In soybeans, Mn tolerance is also under polygenic control with evidence for cytoplasmic inheritance (Brown and Devine, 1980).

Iron Efficiency

Resistance to Fe-deficiency chlorosis is controlled by one major, dominant gene, with modifiers in oats (McDaniel and Brown, 1982), and by major genes with complete dominance at two loci in dry beans (Coyne et al., 1982). In tomato Fe-chlorosis resistance is controlled by one dominant gene (Brown and Wann, 1982). In soybean, Weiss (1943) found one major gene involved in Fe efficiency, but Fehr (1982) found that this trait was quantitatively inherited.

DEVELOPMENT AND RELEASE OF STRESS TOLERANT GERMPLASM

Aluminum Tolerance

Selection for a high degree of tolerance to Al in Brazilian wheat cultivars has been conducted on strongly acid, Al-toxic soils for the past 50 years (Silva, 1976; Foy, 1976). 'Titan' wheat, released for use in eastern Ohio (Lafever, 1978, 1979), is not as tolerant to Al as the Brazilian wheat 'BH 1146', but it is much more tolerant than Indiana cultivars like 'Abe', 'Arthur', and 'Redcoat', or the Green Revolution cultivar 'Sonora 63.' Camargo et al. (1980) reported Al tolerance in the semidwarf wheats 'Tordo' and 'Siete Cerros', so Al tolerance is not necessarily associated with tallness. Two Al- and drought-tolerant sorghum cultivars have recently been released in Brazil (Robert Schaffert, pers. comm.), and Duncan (1981) has developed an acid-soil tolerant (presumably Al-tolerant) sorghum germplasm population 'GPIR' in Georgia, USA. Reid et al. (1980) have recently released the Al-tolerant barley population composite cross XXXIV for experimental purposes.

In alfalfa, recurrent phenotypic selection was effective for increasing tolerance to acid, Al-toxic Bladen and Tatum soils (Devine et al., 1976). J. H. Elgin (pers. comm.) subjected 'Arc' alfalfa to four cycles of recurrent selection (two each in acid soil and nutrient solutions containing Al), and the resulting population was significantly more tolerant to Al than Arc. This

difference did not persist in field studies on acid soils, however. Individual clones that survived in field plots at pH 4.2 are being tested further. Bouton and Sumner (1981) found that an alfalfa population developed by recurrent selection on an acid (pH 4.4) Al-toxic soil produced significantly higher yields on a soil with pH 4.8 than did a population selected on limed soil with pH 6.5 or the cultivar 'Apollo' used as a check.

J. J. Murray (pers. comm.) has used recurrent selection to increase the acid soil (Al) tolerance of tall fescue by 40% in the first cycle and by 2-3% in the second cycle. A third cycle is in progress.

A cold-tolerant strain ('PI 364344') of limpograss has exceptional tolerance to acid soils and Al and, hence, has potential for use on acid, high altitude mine spoils (Oakes and Foy, 1980).

Iron Efficiency

Fehr and Cianzio (1980) released a soybean population (Ap 9 (SI) C_2) with superior resistance to Fe chlorosis. In addition, soybean cultivars (example 'Weber') with high yields and improved resistance to Fe-deficiency chlorosis have been developed for Northern Iowa (Bahrenfus and Fehr, 1980). Fehr (1982) stated that "no genetic limitations to future progress have been identified in developing new cultivars with high yield and high resistance to Fe-deficiency chlorosis." Iowa State University will shortly release a germplasm line of soybean, 'A7', which has the highest known resistance to Fe-deficiency chlorosis (W. R. Fehr, personal communication).

CONCLUSIONS

Mineral stresses of plants in problem soils cannot always be economically ameliorated with current technology. An alternative is to breed plant cultivars having greater tolerance to mineral toxicity or deficiency. This approach does not propose the elimination of liming and fertilization or the bleeding of soil fertility to zero levels. Instead, it makes more effective use of genctic diversity to solvc some of the more difficult problems of soil fertility. The result will be more specific combinations of plant genotypes, soils, and soil fertility practices to optimize net returns.

The most productive soils of the world do not necessarily occur in areas where food needs are greatest. Furthermore, attempts to move food (worldwide) from regions of plenty to those of need have not been successful. The only workable crop production strategy is to produce more food in localities where it is needed. This often entails using marginal land and low input technology, at least initially. Hence, breeding crop cultivars that are tolerant or resistant to soil stress problems is especially valuable for developing countries.

The proposed plant breeding approach involves identification of both present and potential growth-limiting factors in problem soils, screening of germplasm for tolerance, elucidating the genetic, physiological and biochemical

mechanisms of stress tolerance, and the selection or breeding of stress tolerant cultivars. Such multidisciplinary research, which is already in progress at several research centers, has a tremendous potential for alleviating present and anticipated food shortages throughout the world.

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THE PHYSIOLOGY OF PLANT ADAPTATION TO MINERAL STRESS

C. D. Foy[1]

ABSTRACT. Mineral stresses of toxicity or deficiency in plants are not always clearly identifiable entities. Instead, they are often the results of complex interactions among the major toxic ions involved, other essential or non-essential ions, other environmental factors, and particularly the species or genotype of the plant. The ultimate expression of a given "problem soil syndrome" is determined by the plant genotype and its interaction with the environment.

Problems of mineral stress can be reduced or prevented by modifying the soil to fit a given plant or by selecting or breeding plant genotypes with greater tolerance to stress. The probability of success in either approach would be greatly increased by a better understanding of how plants tolerate or adapt to mineral stress.

This paper reviews our knowledge concerning the physiology of differential plant tolerance to mineral stress, with emphasis on Al and Mn toxicities in acid soils and Fe unavailability in neutral and alkaline soils.

Index Descriptors: soil acidity, shallow rooting, drought, Al toxicity, Mn toxicity, Fe deficiency, P efficiency, Ca efficiency, Mg efficiency, Al-Si and Mn-Si interactions, plant induced pH changes, rhizobia, NH_4^+-NO_3^- nutrition.

INTRODUCTION

Plant species and genotypes within species differ widely in tolerance to various mineral stresses (Foy et al., 1978; Alam and Adams, 1979; Foy, 1982a, 1982b; Clark, 1982a). In several species these differences are genetically controlled (Foy, 1982b). Considerable progress is being made in breeding plants that have greater tolerance to specific soil mineral stress problems that are not economically correctable. However, our knowledge about physiological mechanisms responsible for stress tolerance lags considerably behind plant breeding progress. Until recently, most research on stress physiology was done with a single plant species or variety. What we need now is research on comparative physiology, that is, studies with closely related genotypes (preferably isogenic lines) of the same species that are differentially tolerant to a given mineral stress. If the physiological mechanisms of differential stress tolerance are understood, breeding for tolerance traits can be done more precisely. Also, such knowledge may lead to improved chemical (fertilizer, lime, organic matter, etc.) and physical (tillage, drainage, and irrigation) management practices for problem soils.

This paper will be confined to Al, Mn, and other metal toxicities in acid soils and Fe deficiency (unavailability) on calcareous soils. Recent reviews on

[1] Research Soil Scientist, USDA, ARS, Plant Physiology Institute, Plant Stress Laboratory, Beltsville, Maryland 20705.

the subject have been published by Foy et al. (1978) and Clark (1982a) and prepared by Foy (1982a, 1982b). Plants are identified only by common name in the text of this paper except in instances where no common name is available These common names are equated with the appropriate scientific name in the Appendix.

ALUMINUM TOXICITY

Plant Symptoms

Symptoms of Al toxicity are not always easily identified (Foy et al., 1978; Alam and Adams, 1979; Clark, 1982a; Foy, 1982a, 1982b; Lee, 1982). In some plants the foliar symptoms resemble those of P deficiency (overall stunting; small, dark green leaves, and late maturity; purpling of stems, leaves and leaf veins; and yellowing and death of leaf tips), whereas for others they appear as an induced Ca deficiency or reduced Ca transport problem within the plant (curling or rolling of young leaves and a collapse of growing points or petioles). Roots injured by Al are stubby and brittle. Root tips and laterals become thickened and turn brown. The root system as a whole is coraloid in appearance and lacks fine branching. Such roots are inefficient in absorbing nutrients and water. Hence, plants growing in soils with strongly acid subsurface layers often suffer from Al-induced drought because roots cannot tap subsoil water. Aluminum can also reduce water use even when the root zone is moist. Cotton plants affected by Al toxicity sometimes wilt in nutrient solutions, especially at high temperature (C. D. Foy—personal observation). In general, young seedlings are more susceptible to Al toxicity than are older plants (Thaworuwong and Van Diest, 1974).

Physiological and Biochemical Effects of Aluminum

For plants in general, excess Al interferes with cell division in root tips and lateral roots, increases cell wall rigidity by cross-linking pectins, reduces DNA replication by increasing the rigidity of the DNA double helix, fixes P in less available forms in soils and on plant root surfaces, decreases root respiration, interferes with enzymes governing sugar phosphorylation and the deposition of cell wall polysaccharides, and interferes with the uptake, transport, and use of essential elements such as Ca, Mg, K, P, and Fe (Foy, 1974, 1982a, 1982b; Foy and Fleming, 1978; Foy et al., 1978; Lee, 1982).

Metal ions, such as Al, form strong complexes with nucleic acid; in fact, they are used to complex and isolate polynucleotides from leaves (Trim, 1959). Matsumoto et al. (1979) found that Al was bound to the P in DNA. Ulmer (1979) reported that in susceptible wheat cultivars, Al caused inhibition of root elongation and DNA synthesis. Naidoo (1976) found high Al concentrations in the nuclei of Al-injured snapbeans and suggested that Al is bound to

esteric P in nucleic acids and membrane lipids. Such binding would inhibit cell division by interfering with nucleic acid replication. McLean (1979) found that Al caused an abnormal distribution of ribosomes on the endoplasmic reticulum of barley root cells, which was postulated to interfere with protein synthesis. And, Schmandke et al. (1979) observed that $AlCl_3$ increased the firmness and decreased the solubility of protein casein fibers in broad bean. These authors postulated that trivalent Al formed coordination complexes with carboxyl and sulfhydyl groups of the protein, resulting in cross-linking.

Many effects of Al on plants are probably associated with the alteration of root membrane structure and function. Plant membranes are visualized as semi-fluid arrangements of proteins and lipids, either or both of which may be affected by Al. Vierstra and Haug (1978) found that Al decreased lipid fluidity in membranes of *Thermoplasma acidophilium*. Aluminum at 270 ppm in solution produced dramatic changes, but detectable effects occurred with only 0.27 ppm Al at pH 4.0. Gomez-Lepe et al. (1979) reported that Al binds to cell membrane proteins on the inner epidermal cells of onion.

Aluminum also affects water use by plants. For example, Horton and Edwards (1976) found that increasing concentrations of Al in nutrient solutions increased the diffusive resistance of peach seedlings. Kaufman and Gardner (1978) concluded that the water potential of wheat plants increased when root growth was reduced by soil acidity (probably Al toxicity).

Excess Al may reduce or increase the uptake of certain essential elements (Lau, 1979; Ben et al., 1976; Gurrier, 1979; Alam and Adams, 1979, 1980, Duncan et al., 1980). However, such variations in plant composition are difficult to interpret in terms of Al toxicity mechanisms, because no single pattern of element accumulation applies to all cases of Al injury. Duncan et al. (1980) found that decreased yields of sorghum in a soil at pH 4.4 (compared with pH 5.5) were associated with lower concentrations of Cu, Zn, Mg, and Ca and higher concentrations of Al, Fe, Mn, K, and P in plant tops. Aluminum toxicity was probably the dominant yield-limiting factor in this soil. Ali (1973) found that Al toxicity in wheat was prevented by increasing the concentrations of Ca, Mg, K, or Na, either individually or collectively, in nutrient solutions. Beneficial effects of increased concentrations of these elements were probably due to a competitive reduction in Al-root contact, rather than supplying deficient nutrients.

Aluminum accumulates on, or in, roots of Al-injured plants, often in association with P (McCormick and Borden, 1972), but it generally does not accumulate in the tops (Foy, 1974). Even when Al injury of a given plant is correlated with increased Al concentration in the plant top (James et al., 1978; Alam and Adams, 1979; Duncan et al., 1980), the Al in the tops may not be directly harmful to vegetative tissue itself. Rather, it may result from passive accumulation caused by prior injury to the roots. In fact, the entire array of

elements in the tops of Al-injured plants probably represents the accumulated, systemic effects of the initial root injury by Al. Such effects are generally so remote from the initial injury that they cannot reveal Al-toxicity mechanisms. For example, Thaworuwong and Van Diest (1974) concluded that the Al contents of plants were not useful indices of Al tolerance in rice.

Aluminum toxicity is associated with P nutrition of plants. James et al. (1978) concluded that Al-induced P deficiency reduced the growth of Sitka spruce in Scotland, because negative correlations occurred between growth, beaded roots (Al-injured), and foliar Al concentrations. Santana and Braga (1977) found that P, Ca, and Mg concentrations in rice tops decreased with increasing Al saturation of the soil, and Helyar (1978) concluded that Al toxicity effects were largely associated with Al interference in P metabolism and with Al binding to root cell wall pectins, which stopped root elongation. However, Ulmer (1979) found that neither increasing the P concentration in the pre-treatment nutrient solutions nor adding IAA affected the degree of injury or recovery of wheat subsequently exposed to Al.

Aluminum and Fe interactions are mentioned frequently in the literature. For example, Alam and Adams (1980) found that Al induced Fe deficiency in oats and postulated that Al interfered with the reduction of Fe^{3+} to Fe^{2+} within the plant, a process essential for normal Fe metabolism.

Aluminum and Ca interactions have been reported in peach seedlings where Al toxicity reduces Ca uptake, but not its transport (Edwards and Horton, 1977). Simpson et al. (1977) and Awad et al. (1976) attributed poor root growth of alfalfa and Kikuyu grass to Al-Ca interactions.

Aluminum has recently been implicated in animal and human health. Allen and Robinson (1980) found very high concentrations of Al (2,000 to 8,000 ppm) in annual ryegrass pastures on which grass tetany was prevalent. Samples from the rumen of animals that died of tetany contained an average of 2,373 ppm Al compared with 405 ppm from normal animals. Addition of Al to rumen fluid-ryegrass buffer solutions decreased Mg and Ca solubility by 56% and 74%, respectively, within 48 hr. It was concluded that high Al levels in forages and rumen contents are actively involved in the etiology of grass tetany. However, subsequent work at the same station (Cherney et al., in press) indicated that the very high Al levels reported previously in forage and rumen fluid samples collected at grass tetany sites were due to soil contamination of the forage (and ingestion by grazing animals) rather than high Al uptake by the plants. More recent work (D. L. Robinson, personal communication) has also shown that the administration of either Al sulfate or Na sulfate to cows depressed serum levels of Mg. Hence, the decrease in Mg solubility, induced by Al sulfate and previously attributed to Al, appears to be associated instead with the sulfate anion. Grass tetany is still consistently associated with high Al contents of rumen fluid, but the role of soil ingestion in the disease has not

been determined. It does appear, however, that the Al ion, *per se*, is not directly involved in the etiology of grass tetany as previously suggested.

Senile dementia (Alzheimer's disease) in humans is associated with Al accumulation in brain cells. For a detailed discussion of mechanisms by which Al may react with DNA see Karlik et al. (1980).

Aluminum and Rhizobia

Rhizobia of some legume species are more sensitive to Al than are their host plants. Pieri (1974) reported that the nodulation of groundnut was reduced when the Al saturation of the soil CEC reached 30% in sandy soils of Senegal, whereas higher levels of Al saturation were required for toxicity of the host. Carvalho et al. (1981) found that Al toxicity decreased the growth of *Stylosanthes* species more severely when plants were dependent on symbiotically fixed N than when N was applied in fertilizer. The Al tolerances of six *Stylosanthes* species appeared to be dependent both on the ability to nodulate and develop an efficient symbiosis in the presence of Al and on the inherent sensitivity of the host plant to Al.

The symbiotic N_2 fixation process, itself, is apparently less sensitive to Al than is the process of nodule formation. For example, Carvalho et al. (1982) found that a 10 to 20 day exposure to Al concentrations up to 2.7 ppm in nutrient solution did not affect the N_2 fixation of well nodulated plants of *Stylosanthes hamata, S. humilis* and *S. scabra*.

Mengel and Kamprath (1978) discovered that the shoot growth of soybeans on eight organic soils in North Carolina was significantly related to soil pH between 4 and 5. As pH increased, nodule numbers, weights, and N content and N uptake by plants increased markedly. The critical pH for shoot, root, and nodule growth was between 4.6 and 4.8 (salt pH). Growth response to lime was attributed to decreased exchangeable and water soluble Al, increased water soluble Ca, and providing favorable pH for rhizobia.

Keyser and Munns (1979a) found that Al toxicity and soil acidity *per se* were more important than Mn toxicity and Ca deficiency in limiting growth of rhizobia of cowpea and soybean. Aluminum concentrations of 0.68 and 1.35 ppm produced more severe stress than did high Mn (10.8 ppm) or low Ca (2 ppm). Furthermore, Ca added at 0.2 to 40 ppm did not protect rhizobia (2 strains) against Al toxicity. In general, cowpea rhizobia were more tolerant to Al than was *Rhizobium japonicum*. In another study, Keyser and Munns (1979b) discovered that 1.35 ppm Al was more harmful to rhizobia than was low pH (4.5) or low P (0.3 ppm). Keyser et al. (1979) concluded that cowpea rhizobia contained a large, and perhaps continuous, variation in symbiotic tolerance to soil acidity at pH 4.6. Sixty-five percent of the acid-soil sensitive strains were identified in nutrient solutions containing 1.35 ppm Al. No strain highly tolerant to the acid soil was identified as Al sensitive in solution.

The importance of N deficiency in limiting legume growth on acid soils is debatable. Munns et al. (1981) have suggested that soybeans, unlike other legumes, may be limited by factors other than nodulation failure. They used two soybean cultivars and 13 strains of inoculum and found that raising soil pH from 4.4 to 6.0 (aqueous paste) doubled growth, regardless of N source, cultivar, or rhizobial strain. Inoculated plants were nodulated, green, and high in N, even when growth was severely reduced by the acid soil. Plant symptoms indicated that soybeans grown on acid soils were limited by Al toxicity to the host plant. Studies with nutrient solutions in which the pH, Al, and Ca were controlled at levels similar to those in soil solution extracts, showed that soybean growth was unaffected by low Ca (8 ppm) or low pH (4.5) but was depressed by 0.8 to 1.6 ppm Al. Munns et al. (1981) concluded that increasing the acid soil tolerance of soybeans should center on the plant and not the rhizobia. Likewise, C. D. Foy and T. E. Devine (unpublished data) found that the relative acid soil (Al) tolerances of 'Perry' and 'Chief' soybean cultivars were the same with N of either symbiotic or fertilizer origin. Additional information on rhizobial growth in relation to acid-soil factors has been published by Andrew (1978).

Beneficial Effects of Aluminum on Plants

Aluminum is not regarded as an essential nutrient but low concentrations can sometimes increase plant growth or produce other desirable effects (Foy et al., 1978; Foy and Fleming, 1978; Lee, 1982). Plants that have shown positive growth responses to Al include rice (3 ppm; Howeler and Cadavid, 1976), tropical legumes (0.5 ppm; Andrew et al., 1973), eucalyptus (1 ppm; Mullette, 1975), tea (27 ppm, Matsumoto et al., 1976), peach (17.5 ppm; Edwards et al., 1976), sugarbeet (1 ppm; Kesar et al., 1975), maize (3-5 ppm; Clark, 1977), and wheat (3.0 ppm; Foy and Fleming, 1978). The growth stimulus from added Al was greater in Al-tolerant than in Al-sensitive rice cultivars (Howeler and Cadavid, 1976. An Al concentration (3 ppm at pH 4.5), highly detrimental to 'Sonora 63' wheat from Mexico, was beneficial to 'BH 1146' wheat from Brazil (Foy and Fleming, 1978). Kumar (1979) reported that 4 ppm Al in nutrient solution was beneficial to betel palm.

Mechanisms by which small quantities of Al benefit plants may vary among genotypes and growth media. Possible explanations involved are: (a) increasing the solubility and availability of Fe in calcareous soils (through hydrolysis of Al and lowering of pH); (b) correcting or preventing Fe deficiency (by displacing Fe from bound, metabolically inactive sites within plants as observed by Grime and Hodgson (1969) in *Scabiosa columbaria*); (c) blocking negative charges on cell wall sites and thereby promoting P uptake as observed in eucalyptus (Mullette, 1975); (d) correcting or preventing P toxicity as in Al-tolerant maize (Clark, 1977); (e) delaying root deterioration in low

Ca solutions by slowing growth and preventing absolute depletion of Ca from the medium as observed in sunflower (C. D. Foy, personal observation); (f) altering the distribution of growth regulator in roots of peach seedlings (Edwards et al., 1976); (g) preventing toxicities of Cu in citrus (Liebig et al., 1942) and of Mn in atriplex (Rees and Sidrak, 1961); (h) serving as a fungicide (Ko and Hora, 1972; Lewis, 1973; Muchovej et al., 1980); and (i) reducing undesirable top growth in N-rich nursery stock (Borkenhagen and Iyer, 1972).

Physiology of Differential Aluminum Tolerance

As shown in many reports, plant species and varieties within species differ widely in tolerance to excess Al in the growth medium. The exact physiological mechanisms of Al tolerance are still debated. They may be controlled by different genes through different biochemical pathways in different plants. Epstein (1969) stated that an element present in excess can interfere with plant metabolism via competition for uptake, inactivation of enzymes, displacement of elements from functional sites, or alteration of the structure of water. Many of these effects probably involve modification of membrane structure and function. Obviously, Al-tolerant plants can either prevent the absorption of excess Al or detoxify the Al after it has been absorbed, and thus consideration of plant physiological and biochemical factors related to differential Al tolerance is necessary.

Aluminum tolerance has been associated with pH changes in root zones, Al-trapping in non-metabolic sites within plants, P use efficiency, Ca and Mg uptake and transport, root cation exchange capacity, root phosphatase activity, internal concentrations of Si, NH_4^+ or NO_3^- tolerance or preference, organic acid content, Fe use efficiency, and resistance to drought (Foy, 1974; Foy et al., 1978; Foy and Fleming, 1978; Foy, 1982a, 1982b; Lee, 1982). These several facets of Al tolerance are reviewed sequentially as follows.

pH Changes in Root Zones

Certain Al-tolerant cultivars of wheat, barley, rice, peas, and maize increase the pH of their nutrient solutions and thus decrease the solubility and toxicity of Al (Foy and Fleming, 1978; Foy et al., 1978). In contrast, Al-sensitive cultivars of the same species decrease or have no effect on the pH of their nutrient cultures. In certain wheat genotypes, pH differences have also been found in thin layers of soil removed directly from the roots and even in the bulk soil from pot cultures. For this species differential pH changing abilities may be associated with differential anion-cation uptake (Foy and Fleming, 1978).

NH_4^+ vs NO_3^- Nutrition

In strongly acid soils nitrification is inhibited and NH_4^+ becomes an important source of N for plants (Raven and Smith, 1976). Many plants adapted to such soils and hence tolerant to Al, also tolerate NH_4^+ levels that are toxic to Al-sensitive plants, and in some cases, prefer NH_4^+ to NO_3^- as a source of N. Examples are cranberry (Greidamus et al., 1972; Medappa and Dana, 1970), sugarcane (Presad, 1976), birch (Rorison, 1972, 1975), certain grasses (Wiltshire, 1973), and blueberries (Townsend and Blatt, 1966; Havil et al., 1974). Both high bush and low bush blueberries use NH_4^+ effectively but are often injured by NO_3^-. Nitrate toxicity in low bush blueberry coincides with a lack of nitrate reductase activity in the leaves and roots (Townsend and Blatt, 1966). The ratio of NO_3^- to NH_4^+ in the nutrient solution determines the rate and direction of plant-induced pH changes in the presence or absence of Al. Superior Al tolerance in certain wheat cultivars is characterized by their ability to use NO_3^- efficiently in the presence of NH_4^+ and to increase the pH of the growth medium (Foy and Fleming, 1978; Foy et al., 1978). Mesdag et al. (1970) found that acid soil (Al) tolerance in winter wheat varieties was correlated with high grain protein. However, Aniol and Kaczkowski (1979) found no such relationship in 55 varieties of spring wheats.

Aluminum Uptake and Distribution

Aluminum-tolerant plants may be divided into three groups with respect to Al accumulation. In the first group, the Al concentrations in the tops are not consistently different from those in Al-sensitive plants, but the roots of tolerant plants contain less Al. Species in this category include certain cultivars of wheat, barley, soybean, snapbean, triticale, and pea (Foy, 1974; Mugwira et al., 1981; Ulmer, 1979; Klimashevski et al., 1976). In such cases Al tolerance apparently involves an exclusion mechanism. Shoji et al. (1980) reported that the degree of Al accumulation (as indicated by aluminon staining) in roots of three species was in the same order as their root yield decreases in acid soils; namely, burdock > barley > orchardgrass.

The second group includes plants for which Al tolerance is associated with lower levels of Al in plant tops and/or entrapment of excess Al in roots. Examples are azalea, cranberry, rice, triticale, rye, alfalfa, ryegrass, wheat, barley, and potato (Foy et al., 1978; Foy, 1982a), tomato (Baumgartner et al., 1976), paper birch (Steiner et al., 1980), and kikuyu grass (Huett and Menary, 1980).

In a third group of plants, Al tolerance is directly associated with Al accumulation by the tops, which means that such plants have high internal tolerance to Al. Examples of Al accumulators are tea, certain Hawaiian grasses, pines, mangrove (Foy et al., 1978), flowering dogwood (Crum and Franzmeir, 1980), river birch (Bartuska and Unger, 1980), and *Arabidopsis thaliana*

(Tingey et al., 1982). Seven clones of Norway spruce were extremely tolerant to artificial acid rain in pots of soil; their needles accumulated 1350 ppm Al without injury (Ogner and Teigen, 1980). Memon et al. (1981) concluded that Al-tolerant tea bound the Al to cell walls of epidermal and mesophyll cells which prevented it from reaching critical metabolic sites within the cell.

Differential internal tolerance to Al has been reported among rye genotypes (Aniol et al., 1980) and callus cell cultures of tomato (Meredith, 1978a, 1978b). Mugwira (1980) reported that rye had a higher internal tolerance to Al than either wheat or triticale, and Naidoo (1976) concluded that 'Dade' snapbean was superior to 'Romano' in Al tolerance because of its greater ability to tolerate Al within the root cell nuclei where this element presumably was associated with esteric P in nucleic acid and membrane lipids.

Huett and Menary (1979) concluded that Al uptake by Al-sensitive cabbage and Al-tolerant kikuyu grass was non-metabolic. In freeze-dried materials, Al was uniformly distributed along the roots of Al-sensitive cabbage and lettuce and Al-tolerant kikuyu grass. Highest concentrations of Al occurred in the epidermis and cortex. Aluminum occurred in the stele and protoplasm of cortical cells of all species (Huett and Menary, 1980). These investigators concluded that Al can enter the plant by moving into meristematic cells and the symplasm via the cortex; hence, it bypasses the endodermal barrier. They found poor association between the distributions of Al and P in plant roots. Calbo and Cambrai (1980) observed that the Al uptake curve in sorghum roots showed two distinct phases, one at 0 to 4 ppm Al and another at 4 to 8 ppm Al. They suggested that the inflection was associated with the synthesis of pectic substances which could increase the number of binding sites in the cell wall.

Efforts have been made to establish critical levels of Al in plant tops. Wallace and Romney (1977) reported that the threshold concentrations of Al for toxicity were 30 ppm in soybean leaves and 20 ppm in rice shoots. Aluminum concentrations of 70 ppm in soybean leaves were reported to produce severe toxicity, but it is unlikely that such concentrations were directly harmful to the tops. Normal alfalfa may contain 300 ppm Al in its tops, and yet this species is much more sensitive to Al than either soybeans or rice (Foy, 1964). Malavolta et al. (1979) stated that Al toxicity in sweet sorghum was associated with 640 ppm Al in lower leaves and 1220 ppm in upper leaves. Duncan (1981) found that sorghum genotypes tolerant to acid soil (and probably Al) contained lower concentrations of Al, Fe, and Mn than did lines that were sensitive. An acid soil-sensitive cultivar had 5021 ppm Al and 1820 ppm Fe in its tops whereas a tolerant cultivar contained 462 ppm Al and 403 ppm Fe. From other studies, Duncan (1982) concluded that no single element can be used to evaluate genotypes for tolerance or susceptibility to acid soils. Assignment of a critical Al level in plant tops is of questionable value. Accumulation of Al (and other elements, like Fe) under conditions of Al toxicity probably reflects prior

root damage, which results in differential element transport; Al concentrations directly harmful to the plant tops have not been determined.

Calcium Nutrition

Aluminum tolerance in certain cultivars of wheat. barley, soybean, and snapbean are associated with the ability to resist Al-induced Ca deficiency (Foy et al., 1978). Baumgartner et al. (1976) found that the Al-tolerant 'Santa Cruz Kada' tomato had a lower Ca requirement than the less tolerant 'Ronita.' Aluminum tolerant cultivars of soybeans accumulated more Ca, P, and Mg in their tops per unit root length than did the more sensitive cultivars (Sartain, 1974). Brauner (1979) noted that Al acted as a competitive inhibitor of Ca uptake in wheat, but his wheat cultivars did not differ in Ca uptake in the presence of Al. Huett and Menary (1980) found that 500 ppm Ca in nutrient solution completely overcame the detrimental effect of Al on yields of cabbage, lettuce, and kikuyu grass. Sartain (1974) concluded that Ca uptake by soybean from a limed soil zone was reduced by the presence of Al translocated from the unlimed zone. Aluminum affected the uptake of Ca more than that of P.

Phosphorus Nutrition

In many plant species, Al tolerance is closely related to P use efficiency. For example, in certain genotypes of wheat, tomato, and maize, Al tolerance coincides with tolerance to low P levels in nutrient solutions, either in the presence or absence of Al (Foy et al., 1978; Foy, 1982a). Salinas and Sanchez (1976) stated that Al toxicity and P deficiency are frequently associated on most Cerrado oxisols in Brazil. Also, tolerance to Al and tolerance to low P coincided in certain cultivars of wheat and beans. Aluminum tolerance in cultivars of pea is closely related to the ability to absorb and use P in the presence of Al (Klimashevski et al., 1979), and Baumgartner et al. (1976) found that Al tolerant Santa Cruz Kada tomato had a lower P requirement than the less tolerant Ronita. Aluminum-tolerant soybean cultivars absorbed more P per unit of root length than did Al-sensitive ones (Sartain, 1974). According to Garcia and Leon (1978), variable Al tolerances of cacao hybrids are probably due to differential abilities to extract P in the presence of Al. Fox (1979) mentioned that maize cultivars developed for tropical America (probably Al tolerant) are more tolerant to low P than those developed for temperate climates.

Cambrai and Calbo (1980) reported that pretreatment with Al decreased P uptake by 42% in the roots of the Al-sensitive sorghum 'CMS x S-903' but did not affect that of the Al-tolerant 'CMS x S-106.' In both cultivars, Al reduced the ATPase activity of the plasma membrane. Because Al reduced the accumulation of cations in the tops and roots of both cultivars and because ATPase

was regarded as being involved in the active transport of cations, it was suggested that Al affects plants primarily by inhibiting the ion carriers. Aluminum has been reported to greatly decrease inorganic P, lipid bound P, nucleic acid bound P, protein bound P, and total P concentration in the tops of sorghum plants (Edgar Cunha, Sete Lagoas, Brazil, 1981, pers. commun.). The decreases were significantly lower in the Al-tolerant 'CMS 297' than in the Al-sensitive 'CMS 244.' Soybean selections differing in Al tolerance had similar levels of metabolic intermediates when grown with no Al, but under Al stress, pyruvate and ATP levels were significantly increased in tolerant lines but not in sensitive lines (Hanson and Kamprath, 1979).

In contrast to the evidence cited above, Brauner (1979) found that Al tolerance in wheat cultivars was not related to concentrations of P, Ca, or Mg in plant tops.

Aluminum Related to Fe, Mg, Si, and K

Acid soil (Al) sensitivity in certain wheat and barley cultivars is associated with Al-induced Fe deficiency chlorosis in nutrient cultures at pH 4.1 (Otsuka, 1969). Aluminum sensitivity in a mutant strain (SKII) of *Bacillus megaterium* was attributed to Al-induced Fe deficiency caused by a loss in the ability to produce a secondary hydroxamate (skizokinen) which chelates Fe and makes it available to the normal strain (ATCC 192/13) (Davis et al., 1971). Brown and Jones (1977) found that sorghum lines which grew poorly and developed chlorosis and purple pigmentation in Al-toxic Bladen soil at pH 4.3 contained significantly less Fe and P than those that remained green. Aluminum concentrations were higher in chlorotic than in green plants, but the highest concentration was only 127 ppm.

Aluminum tolerance has been associated with greater uptake of K and Mg in potato cultivars and with greater Mg uptake in maize lines. Certain Al-tolerant rice cultivars accumulated higher levels of Si in the epidermal cells of their leaves than did Al-sensitive cultivars. Silicon is known to reduce the toxicity of Mn in barley leaves and could play a similar role in detoxifying Al (Foy et al., 1978).

Organic Aluminum Complexes in Plants

Jones (1961) suggested that naturally occurring organic acids in Al-tolerant species chelate Al and thereby reduce the Al-P precipitation expected at normal pH levels in plant sap. Small (1946) noted that acid-tolerant plants generally have strong organic acid buffer systems in their cells, but alkaline tolerant plants have phosphate buffers dominating the system. Phosphate systems would be more susceptible to disruption by Al-P interaction than would organic acid systems. Aluminum tolerance in certain calcifuge species

has been attributed to a chelating mechanism which has an affinity for Fe (Grime and Hodgson, 1969). Lee (1977) noted that hydroxy Al was toxic to maize seedlings, but Al citrate was not. The addition of humic acid also ameliorated Al toxicity at pH 4.7. Bartlett and Riego (1972) found that the toxicity of ionic Al for maize was prevented by complexing the Al with citrate, EDTA, or soil organic matter extract. High Al tolerance in the Al accumulating tea plant may be due to chelation (and detoxification) of Al by organic acids and phenols (Sivasubramaniam and Talibudeen, 1972), but the organic acid-Al chelation hypothesis of Al tolerance has not been adequately tested for plant genotypes within species.

Aluminum and Plant Membranes

Hofler (1958) concluded that Al tolerance in *Spirogyra* depended on the structure of the plasmalemma and was genotype specific. Certain *Spirogyra* cells showed plasmolysis when treated for a few seconds with a 0.1% $Al(NO_3)_3$ solution, but cells of another strain were essentially unaffected by the same treatment.

Aluminum-Water Relationships

Excess Al can reduce water use by restricting plant root penetration and proliferation in acid subsoils when surface soils are dry. In addition, Al toxicities can damage roots to the extent that they cannot absorb adequate water even in moist soils. Krizek and Foy (1981) found that the Al-sensitive 'Kearney' barley was more tolerant to soil water stress, *per se*, than was an Al-tolerant cultivar 'Dayton'; however, Kearney was much more sensitive to Al-induced water stress than was 'Dayton.' When plants are grown over acid, Al-toxic subsoils, both types of water stress tolerance are needed to prevent yield reductions from drought.

MANGANESE TOXICITY

Plant Symptoms

Excess Mn affects plant tops more directly than roots (Foy, 1973; Foy, 1982a). In addition, Mn produces more definitive symptoms in plant tops than does Al, and for a given plant, Mn accumulates somewhat in proportion to plant injury (Osawa and Ikeda, 1980). Symptoms of Mn toxicity include marginal chlorosis and necrosis of leaves in alfalfa, rape, kale, and lettuce, leaf puckering in snapbean, soybean, and cotton, and chlorosis of young leaves (resembling Fe deficiency) and necrotic spots on leaves in barley, lettuce, and soybeans. Necrotic spots in barley and chlorotic leaf margins in lettuce contain

much higher Mn concentrations than adjacent leaf tissues (Vlamis and Williams, 1973). In severe cases of Mn toxicity, plant roots turn brown, but only after the tops have been severely injured.

Specific physiological disorders associated with excess Mn are "crinkle leaf" of cotton (Foy, 1973), "stem streak necrosis" in potato (Lee and McDonald, 1978), "internal bark necrosis" of apples (Miller and Schubert, 1977; Scibisz and Sadowski, 1979), growth retardation and "leaf tip burn" in carnation (Ishida and Masui, 1976), and "fruit cracking" at the blossom end of muskmelon (Masui et al., 1976).

In several tropical pasture grasses, such as buffelgrass, Mn toxicity causes small, black or dark brown flecks on the lower leaves, sometimes with induced Fe deficiency (Smith, 1979). Buffelgrass had these symptoms, plus white bands 1-2 cm side, extending transversely across the leaves at regular intervals along the blades. No other species had this latter symptom. Manganese toxicity is characterized by interveinal chlorosis on upper leaves in bean, eggplant, pepper, tomato, and spinach, and by marginal leaf chlorosis on lower leaves of cabbage, lettuce, and celery. In Welsh onion, excess Mn induced chlorosis of the older leaves (Osawa and Ikeda, 1977, 1980).

Andrew and Pieters (1976) described Mn toxicity symptoms in *Glycine wightii* as interveinal chlorosis of young expanding leaves, similar to Fe deficiency, mottling and irregularly shaped brown spots near midrib and main veins of leaves, puckering of leaf surfaces, and, in severe cases, a browning of midribs and main veins (particularly on the upper leaf surfaces). Jones and Clay (1976) found that Mn injury in a tropical legume (*Stylosanthes humilis*) was characterized by an overall yellowing, particularly in the youngest leaves and stems, with some interveinal chlorosis and leaf puckering. Under severe Mn toxicity, tips of emerging leaves became necrotic before the leaf was fully expanded, and the necrosis proceeded from the leaf tip downward. Easter lilies grown in nutrient cultures with high Mn levels produced short plants, leaf chlorosis, chlorotic mottling, and leaf curling (Holmes and Coorts, 1980).

Manganese toxicity is common in burley tobacco, because this crop is frequently grown on high Mn soils maintained at a pH below 5.3 to control black root rot disease. Manganese toxicity causes slow growth of tobacco seedlings, and toxicity symptoms appear 4 to 5 weeks after transplanting (Link, 1975, 1979; Smiley, 1976). The symptoms are light green or yellowish mottling of leaves between the large veins, dark green leaf veins, and necrotic spots on older leaves. Leaves also may have a hard, semi-glossy appearance. Plants may recover but overall growth is reduced. Arnold (1977) described a disorder "grey leaf" in tobacco grown on shallow, poorly drained soils at pH 4.7-5.1; the condition is characterized by high concentrations of both Mn and Fe in plant tops.

Flax developed Mn toxicity symptoms (leaf scorch) even at a soil pH of 8.1 (Moraghan and Freeman, 1978). Symptoms were brownish spots on distal

portions of older leaves and subsequent necrosis. The leaf spotting progressed from older to younger leaves.

Plant symptoms of Mn toxicity are often detectable at stress levels which produce little or no reduction in vegetative growth. In contrast, Al toxicity can reduce yields greatly (by root damage) without producing clearly identifiable symptoms in plant tops. Hence in acid soils with high levels of both Al and Mn, plant growth reductions may be largely or wholly attributed to Mn toxicity when Al toxicity is the more important factor (Foy, 1976).

Physiological and Biochemical Effects of Excess Manganese

Manganese toxicity has been characterized by: (a) destruction of auxin (IAA) by increasing the activity of IAA oxidase in cotton and Japanese morning glory, (b) a postulated amino acid imbalance in potato, (c) increased replication of RNA in potato spindle tuber virus, (d) increased activities of peroxidase and polyphenol oxidase, decreased activities of catalase, ascorbic acid oxidase, gluthathione oxidase and cytochrome C oxidase, and lower ATP contents and respiration rates in cotton, (e) reversal of growth inhibition of sorghum roots caused by gibberellic acid which enhances auxin production, and (f) induction of erythromycin-resistant mitochondrial mutations in yeast. Manganese interacts with many other mineral elements in plant nutrition, and under certain conditions, the addition of Si, Fe, Ca, and P may alleviate Mn toxicity (Foy, 1973; Foy et al., 1978; Foy, 1982a).

Excess Mn also alters the activities of enzymes and hormones in plants. For example, Sirkar and Amin (1979) found that Mn toxicity reduced respiration in cotton seedlings and that this effect was reversed by adding IAA. Respiration rates of Mn-injured tissue were also increased by indole butyric acid, indole acetamide, and indole propionic acid.

Horst and Marschner (1978a) proposed a Mn toxicity hypothesis which involves auxin and Ca. They observed that excess Mn inhibited Ca transport into the leaves of beans and induced typical Ca deficiency symptoms (crinkling of leaves in shoot apices and younger leaves). Manganese toxicity also reduced the cation exchange capacity of leaf tissues and reduced the Ca movement into the free space of isolated leaf segments. Consequently, these investigators concluded that Mn toxicity increased the activity of IAA oxidase, which decreased the auxin level (proposed earlier in cotton by Morgan et al., 1966, 1976). It was reasoned that the lowered auxin level then reduced cell wall expansion and that the subsequent reduction in formation of new, negative sites decreased Ca translocation into the tissues.

Helyar (1978) suggested that Mn-toxicity problems are caused mainly when plants lose control of their Mn-activated enzyme systems. Heenan and Campbell (1981) found that Mn toxicity in soybeans was associated with reduced nitrate reductase activity. Aoba and Sekiya (1977) reported that in

Mn-injured Satsuma trees (the sweet orange), the activities of leaf catalase, peroxidase and polyphenol oxidase, and O_2 uptake were higher and that the activity of ascorbic acid oxidase was lower than in healthy trees. Also, Aoba et al. (1977) detected divalent Mn in epidermal and xylem tissues of roots from Mn-injured orange trees. Treating the fine roots with heat or sodium azide to inhibit their oxidizing powers greatly increased Mn absorption. By contrast, untreated, healthy roots oxidized divalent Mn to MnO_2, which remained on root surfaces. The site of greatest Mn oxidation was about 5 mm from the root apex and occurred in the epidermis, endodermis, and xylem. Masui et al. (1980) observed that Mn accumulation by muskmelon was much higher in fine roots than in coarse ones and was especially high in root hairs. Manganese accumulation was greater in older than in younger leaves and greater in the outer (rind) than in the inner portion of fruit. Memon et al. (1980) reported that in *Acanthopanax sciadophylloides*, an Mn accumulator, Mn accumulated markedly in peripheral cells of the leaves, petiolules, and petioles. There were high concentrations of Mn in the epidermis, palisade, and spongy parenchyma cells, but low Mn in vascular bundle cells. In the petiolule and petiole, most of the Mn was concentrated in the cell walls of the epidermis, collenchyma, and bundle sheath cells. Such compartmentalization of Mn may act as a protective mechanism against toxicity by keeping excess Mn from key metabolic centers.

Satsuma orange tissues injured by excess Mn were stained dark brown and gave positive tests for pyrogall, catechol, and ferrous Fe (Aoba et al., 1977) and excess Mn in soil or nutrient solution increased plant levels of phenolic derivatives. Polyphenolic compounds identified in new leaves included five flavonoids and three phenolic acid derivatives. The latter were 2,5 dihydroxybenzoyl glucose, feruloylglucose and feruloylrhamnoglucose. Rao and Gupta (1979) found that sugar cane affected by Mn deficiency or Mn toxicity accumulated more free amino acids than normal plants.

Manganese toxicity is often associated with a decrease in the Ca concentrations in plants (Smith, 1979; Horst and Marschner, 1978a; Takayanagi, 1976) and addition of Ca to the growth medium sometimes reduces Mn accumulation, decreases the severity of Mn-induced chlorosis, and alleviates growth reductions (Osawa and Ikeda, 1977). Masui et al. (1976) reported that Ca decreased Mn concentrations in muskmelon plants but did not prevent yield decreases by excess Mn.

Addition of Si to nutrient solutions reduced Mn toxicity in barley (Williams and Vlamis, 1957) and bean (Horst and Marschner, 1978b, 1978d). Apparently this detoxification is not due to reduced Mn uptake but to the prevention of localized accumulations of Mn associated with necrotic spots on the leaves. Horst and Marschner (1978c) found that necrotic spots on red kidney bean plants were precipitated Mn compounds which occurred primarily in the walls of cells adjacent to the vessels. Excess Mn induced secondary Fe and Ca

deficiencies. Kluthcouski and Nelson (1979) found that Si decreased Mn concentrations in the youngest fully developed soybean leaves. Effects of both Mn toxicity and deficiency were slightly moderated by the addition of Si. For additional information on Mn-Si interactions, see Foy et al. (1978).

Manganese and Fe are closely related in plant nutrition, and the Fe/Mn ratio in plant tops has been used as one indicator of toxicity. For example, Hati et al. (1979) found that Mn toxicity occurred when Fe/Mn ratios were 1.36, 1.40, and 0.8 for cotton, wheat, and soybean, respectively, grown on an acid soil at pH 4.1. Iron and Mn contents were negatively correlated. Excess Mn often produces plant symptoms resembling Fe deficiency (Horst and Marschner, 1978c) and increasing the Fe supply may decrease Mn uptake (Masui et al., 1976). However, not all Mn toxicity is Fe-related. Gupta and Rao (1977) concluded that Mn toxicity in sugarcane is not Mn-induced Fe deficiency, and Van der Vorm and Van Diest (1979) stated that under acid, aerobic conditions, absorption of Fe is little affected by large quantities of Mn. However, excess Mn uptake can interfere with normal Fe metabolism without reducing Fe uptake. For example, chlorosis of weeping lovegrass on calcareous soil was associated with high Mn/Fe ratios (Foy et al., 1977).

Flax shows some interesting interactions between Mn and Fe (Moraghan and Freeman, 1978; Moraghan, 1979). This species was a Mn accumulator and developed Mn toxicity on a calcareous soil at pH 8.1. Although the plant symptoms (brown spots starting on lower leaves) did not resemble Fe deficiency, the addition of 2 ppm FeEDDHA to the soil prevented the lesions, decreased Mn concentrations from 500 to 50 ppm in plant tops, and increased yields. Manganese-induced brown spots on flax leaves showed distortion of chloroplast membranes, absence of starch, decrease in grana membranes, absence of plastid ribosomes, and increased plastoglobulin. Manganese contents of plants were not closely related to Mn extracted from soil with several extractants but were significantly and inversely related to DTPA extractable Fe. It was suggested that plant Fe status influenced the ability of flax roots to use Mn in calcareous soil, possibly by changes in pH or redox potential at or near the roots.

Moraghan et al. (1980) found that 'Bragg' soybean (Mn sensitive, Fe efficient) developed Mn toxicity on calcareous soil. Leaves curled and dropped off prematurely but were not chlorotic, and low temperature intensified the problem. On the same soil (without added Fe) 'Lee' soybeans (Mn tolerant, Fe inefficient) developed typical Fe deficiency chlorosis. Such observations point out the complexities of plant-mineral-stress relationships and the difficulties involved in diagnosing problems and adapting plants to problem soils.

Temperature-Mn interactions affect the severity of Mn toxicity in plants. Moraghan et al. (1980) reported that Mn toxicity in 'Bragg' soybean was more severe at a lower temperature. Ruffy et al. (1979) found that in tobacco the lower the temperature, the lower the level of leaf Mn required for toxicity.

Leaf Mn concentrations associated with toxicity at different day/night temperatures were as follows: 700-1200 ppm at 22/28 C, 2000-3500 ppm at 26/22 C, and 5000-8000 ppm at 30/26 C. Manganese toxicity was more severe in young leaves, and the severity decreased with age. It was concluded that increased tolerance of tobacco to high leaf Mn contents in warm temperatures was associated with a more rapid rate of leaf expansion, accompanied by increased vacuolar capacity for accumulated Mn. Thus, although Mn uptake may be increased with higher temperatures, as reported for oats (Cheng and Pesant, 1977), the increased internal tolerance to Mn at higher temperature may prevent toxicity. Such interactions make it difficult to determine critical Mn concentrations in soils and plants.

Another factor affecting Mn toxicity is the form of N in the growth medium. Osawa and Ikeda (1980) concluded that this had a marked effect on Mn uptake and toxicity in vegetable crops. Manganese toxicity symptoms (interveinal chlorosis on upper leaves of tomato and spinach and marginal chlorosis on lower leaves of cabbage) were both less severe with $NO_3^- + NH_4^+$ than with NO_3^- alone, and in some cases, symptoms were less severe at pH 4.0 than at 6.0. The incidence of necrotic brown spots was reduced by increasing the proportion of NH_4^+ but all NH_4^+ reduced growth, even with normal Mn treatments. Osawa and Ikeda (1980) also found that NH_4^+ and H^+ at non-toxic levels greatly reduced Mn uptake and toxicity in vegetable crops. Another beneficial effect of NH_4^+ may have been a decreased pH (through root exchange for H^+) which protected plants against Fe deficiency, whether Mn-induced or not.

Manganese and Rhizobia

Dobereiner (1966) suggested that the sensitivity of many legumes to acid soils was due to specific Mn toxicity on the N-fixing process. She found that when bean plants were dependent on symbiotic N, 25 ppm Mn in sand culture-solutions decreased the total N contents of plants by 30 to 60%. However, when plants were adequately supplied with mineral N, the corresponding reduction was only 13%. For beans totally dependent on symbiotic N-fixation, the N contents of plants decreased linearly with the logarithm of the Mn concentrations in plant tops; however, this relationship was not observed in plants supplied with mineral N. Effects of Mn toxicity on N fixation varied with strains of *Rhizobium phaseoli* used as inoculum. Souto and Dobereiner (1971) found that excess Mn markedly decreased nodulation in tropical legumes. Kliewer (1961) reported that 20 ppm Mn in nutrient solutions reduced the number of nodulated alfalfa plants by 50% but had no effect on nodulation of birdsfoot trefoil. Dobereiner (1966) postulated that contradictory results regarding the response of legumes to lime may be due to variable concentrations of soluble Mn in acid soils and differences in tolerance to Mn toxicity among both host plants and rhizobial strains.

Freire (1975) concluded that soil pH values of 4.0 to 4.5 were not harmful to soybean nodulation in the absence of excess Al and Mn, but toxic Al and Mn, together with P and Ca deficiencies, were highly detrimental to nodule formation and N_2 fixation. Later Freire (1976) found that soybean cultivars with low tolerance to Mn showed high response to lime and had higher Ca uptake than Mn-sensitive cultivars.

Recent studies by Keyser and Munns (1979a, 1979b) with cowpea and soybean rhizobia indicate that on acid soils, Al toxicity and soil acidity *per se* are probably more important than Mn toxicity and Ca deficiency in restricting N fixation. Aluminum at 0.68 to 1.35 ppm in solution produced greater stress than high Mn (10.8 ppm) or low Ca (2 ppm). All rhizobial strains tolerant to Al were also tolerant to high Mn and low Ca. High Mn (10.8 ppm) and low Ca (2 ppm) reduced growth rates of some strains but did not stop the growth of any strain.

Physiology of Differential Manganese Tolerance

Manganese tolerance in plants has been associated with (a) oxidizing powers of plant roots, (b) Mn absorption and translocation rate, (c) Mn entrapment in non-metabolic centers, (d) high internal tolerance to excess Mn, and (e) the uptake and distribution of Si and Fe (Foy, 1973, 1982a; Foy et al., 1978).

Manganese Uptake and Transport

Concentrations of Mn in plant tissues required to produce Mn toxicity symptoms vary with plant species and genotype within species and with environmental conditions (Foy et al., 1978). Hence, "critical" Mn concentrations in plants are useful only when applied within a limited set of circumstances. Internal Mn concentrations that have been associated with toxicity symptoms are 120-600 ppm in apple leaves (Fisher et al., 1977; Scibisz and Sadowski, 1979), 171-181 ppm for Bragg soybean (Mask and Wilson, 1978), 450-500 and 900-1000 ppm, respectively for young and old leaves of tomato (Ward, 1977), 445-1400 ppm, respectively, for upper and lower leaves of sweet sorghum (Malavolta et al., 1979), 500 ppm for flax (Moraghan, 1979), 500-1960 ppm for cotton (Foy et al., 1981a; Hati et al., 1979), 2000 ppm for Easter lily (Holmes and Coorts, 1980), 2600 ppm for carnation (Ishida et al., 1977), and 2500-6500 ppm for maize (Tanner, 1977).

The difficulty of assigning "critical" internal Mn concentrations for toxicity is shown by the fact that Mask and Wilson (1978) reported that Bragg soybean had toxicity symptoms when leaves contained 171-181 ppm, but Jones and Nelson (1978) found no toxicity symptoms for the same variety when its leaves contained 320 ppm.

Hutton et al. (1978) concluded that Mn tolerance in tropical legumes is associated with reduced Mn uptake in some species and greater internal tolerance in others. Bromfield (1978) noted that rape was more efficient than oats in absorbing Mn from MnO_2, but it was also more sensitive to excess Mn, which may be related to greater Mn uptake. Flax is so efficient in absorbing Mn that it can accumulate sufficient Mn (500 ppm) in its tops to cause toxicity, even on a soil at pH 8.1 (Moraghan and Freeman, 1978). Superior Mn tolerance in maize as compared with peanuts has been attributed to reduced transport of Mn from roots and stems to leaves. Manganese-sensitive peanuts accumulate high levels of Mn in their leaves (Benac, 1976).

In some cases, differential Mn tolerances of plant genotypes within species are associated with differential Mn uptake. Holmes and Coorts (1980) found that 'Ace' Easter lily was much more tolerant to excess Mn and absorbed less Mn than 'Nellie White.' Gammon and McFadden (1979) reported that Mn accumulation by rose plants of *Rosa fortuniana* root stocks was five times that of plants on *R. odorata*. However, Mn accumulation was not related to flower production.

Internal Tolerance to Excess Mn

Some cultivars of wheat, cotton, soybean, and lettuce tolerate higher levels of Mn within their tops than do others (Foy et al., 1978). Jones and Nelson (1979) concluded that the differential Mn tolerance of Bragg (sensitive) and 'Lee 68' (tolerant) soybeans was not reflected in Mn concentrations of plant tops. Heenan and Campbell (1981) found that Lee and Bragg did not differ in Mn distribution to plant parts or in Mn concentrations in actively growing tissues. Brown and Devine (1980) reported that Mn-tolerant and Mn-sensitive soybean cultivars and F_2 progeny from their reciprocal crosses all contained about the same Mn concentrations in their tops (500 ppm). Similar results have been found for cultivars of wheat (Foy et al., 1973; Brauner, 1979), cotton (Foy et al., 1981a), and flax (Moraghan and Ralowicz, 1979).

Smith (1979) concluded that different mechanisms were involved in Mn tolerance in certain grasses. *Setaria* and *Paspalum* were much more tolerant to Mn toxicity than Rhodesgrass and buffelgrass. However, *Paspalum* was the highest Mn accumulator and *Setaria* was the lowest. In sand culture the Mn concentrations in plant tops were 4880 and 2830 ppm for *Paspalum* and *Setaria*, respectively, and in soil they were 2740 and 1530 ppm, respectively.

Some plants accumulate high concentrations of Mn in their tops without injury. Examples of Mn accumulators are sugar maple (Crum and Franzmeir, 1980); tropical plants of the genus *Rauwolfia* (Shchigelskii et al., 1979); *Acanthopanax sciadophylloides, Anacardiaceae* and *Acereceae* (Memon et al., 1979); *Vaccinium myrtillis* (Bouwens and Longin, 1979); and *Lupinus albus* (Oram et al., 1979). Jaffre (1979) found that the leaves of numerous New Caledonian

species of *Proteaceae* contained abnormally high Mn concentrations (up to 5.5% of dry weight and 55.2% of ash weight in *Macadamia neurophylla*), and these unusual levels of Mn uptake occurred in soils that were not high in Mn. Several *Proteaceae* also accumulated Al, but in general, the leaves of the Mn-accumulating species had relatively low Al contents. Species reported to be low in Mn accumulative ability are *Cryptomeria japonica* and certain coniferous trees (Memon et al., 1979).

Interactions of Manganese with Other Mineral Elements

Manganese interacts with Fe, Mo, P, Ca, and Si in affecting toxicity symptoms and growth of plants. In some plants, excess Mn produces symptoms that resemble Fe deficiency, and these can be prevented by adding soluble Fe to the plant or soil. In others, Mn-induced symptoms are quite different (Foy et al., 1978) and are not corrected by added Fe. Increasing Ca in the growth medium may decrease Mn uptake and toxicity. Phosphorus additions can reduce the toxicity of Mn by rendering it inactive within the plant. Silicon reduces Mn toxicity by preventing localized accumulations of Mn in leaves. Thus, toxicity of a given level of soluble Mn in the growth medium, or even within the plant, depends upon interactions between this element and other minerals, particularly Fe and Si. Such interactions may account for the wide variety of plant symptoms and different growth reductions produced by excess Mn in different plant species and cultivars (Foy, 1973; Foy et al., 1978).

The most interesting interactions reported in the current literature are among Mn, Fe, and plant genotypes. Moraghan and Ralowicz (1979) found that flax accumulated high concentrations of Mn in its tops (500 ppm) and developed Mn toxicity symptoms on calcareous soils (pH 8.1) that are low in available Fe. Iron chelate (FeEDDHA at 2 ppm) added to the soil prevented the lower leaf scorch associated with Mn toxicity, increased dry weight and Fe uptake, and decreased Mn uptake by all four genotypes studied. Dry matter yield responses to Fe treatment were 81, 33, 27, and 18%, respectively, for the four genotypes, and the yield responses were inversely related to Mn tolerance. Genotypes did not differ appreciably in Mn or Fe contents with or without Fe treatment of the soil. Hence, factors other than Mn uptake were responsible for differential Mn tolerance.

Weeping lovegrass strains differ widely in resistance to an Fe-related chlorosis in calcareous soils (Foy et al., 1977). Chlorosis-susceptible strains grown on calcareous soil were not lower in Fe content but were much higher in Mn and hence had lower Fe/Mn ratios than did chlorosis-resistant strains. There was also evidence of unfavorable Fe/Cu and Fe/Zn ratios in chlorosis-susceptible strains. Hence, chlorosis susceptibility seemed to be related to inhibited Fe metabolism, rather than reduced Fe uptake. In nutrient solutions

the superior chlorosis resistance of 'FQ 22' weeping lovegrass was associated with the ability to maintain a low pH in the root zone (which prevents Fe precipitation), a greater affinity for NH_4^+ nitrogen, more effective transport of Fe from roots to tops, and restricted transport of Mn and Ca (Foy et al., 1981b). Results suggested that excesses of Mn and Ca probably interfered with the metabolism of transported Fe in the tops of the chlorosis-susceptible in 'FQ 71' genotype. Thus, on calcareous soil, susceptibility to chlorosis appeared to be related to inhibited Fe metabolism (possibly caused by Mn, Cu, and Zn), whereas in nutrient culture, both reduced Fe transport and inhibited Fe metabolism (possibly by Mn and Ca) were involved. In contrast, Brown and Jones (1977) concluded that Mn tolerance was not related to Fe efficiency or inefficiency in soybean cultivars. The cultivar 'T203' is Mn tolerant and 'Forrest' is Mn sensitive, but both are susceptible to chlorosis (Fe inefficient).

Organic Manganese Complexes in Plants

The relationship between organic Mn complexes and Mn tolerance in plants has received little attention, but this appears to be a promising area for research. Sutcliffe (1962) suggested that organic complexes can reduce the transport of Mn to plant tops. Tiffin (1967) found that Mn in tomato xylem exudate showed no binding to organic compounds (moved as a cation under electrophoresis) even when the exudate Mn content was enriched by 18-fold. However, Hofner (1970) found a close relationship between Mn and an amino acid and carbohydrate fraction of sunflower stem exudate. Gel filtration fractionated the exudate into three associated components with molecular weights below 1500. Bremner and Knight (1970) and Bremner (1974) concluded that in rye grass, Mn occurred as a single, cationic, and perhaps noncomplexed form. In phloem sap of castorbean only limited organic binding of Mn was found (Van Goor and Wiersma, 1974). From 60 to 70% of the Mn behaved as a simple cation, but some organically bound Mn was associated with compounds having molecular weights between 1000 and 5000.

Ebeid and Kutacek (1979) indicated that Mn did not occur as a free cation in xylem exudates of 20-day-old maize seedlings. Manganese was associated with 3 UV (254 nM) absorbing fractions. Two fractions contained amino acids, and the third (MW about 600) was a carrier practically free of amino acids but containing 10.2% N. The addition of EDTA to xylem exudates favored Mn transport by fractions of relatively low molecular weight. High stability organic binding of Mn is infrequent in plants, but it does occur. Dieckert and Rozaky (1969) isolated from peanut seed a protein-Mn complex with a molecular weight of 56,300 and with one Mn atom; its function is unknown, however.

Overall data from widely different plants indicate that Mn occurs as a free cation or is bound in soluble form with low molecular weight complexes in xylem fluid. However, the significance of this in relation to Mn tolerance has not been determined. The form of Mn occurring in stem exudate may be quite different from that in plant leaves where toxicity occurs.

OTHER METAL TOXICITIES

General

Toxic levels of metals in soils may be caused by natural soil properties or by agricultural, manufacturing, mining, and waste disposal practices (Foy et al., 1978). Any metal can be toxic if soluble in sufficient quantities. In near-neutral soils the heavy metals occur as inorganic compounds or in bound forms with organic matter, clays, or hydrous oxides of Fe, Mn, and Al. This precipitation and absorption of metals by soils protects plants against toxicity. However, decreasing the soil pH can create metal toxicity problems. Iron toxicity can occur under flooded conditions where Fe occurs as the reduced soluble Fe^{2+} form (Foy et al., 1978). Zinc, Cu, and Ni toxicities occur frequently. Toxicities of Pb, Co, Be, As, and Cd occur only under unusual conditions. Lead and Cd are of particular interest, because they can move into the food chain and affect human and animal health. For further details, see Foy et al. (1978).

Plant Symptoms

Metal toxicities in plants are not always clearly identifiable entities; instead they are the results of complex interactions of the major toxic ions in question with other essential or non-essential ions and with environmental factors. The most common symptoms are stunting and chlorosis of plant tops. Chlorosis associated with excess Zn, Cu, Ni, and Cd appears to be caused by a direct or indirect interaction with foliar Fe, so painting leaves with $FeSO_4$ frequently corrects the chlorosis. Zinc increases the translocation of Mn to soybean tops, and both Zn and Mn can interfere with Fe utilization in the leaves for chlorophyll synthesis. However, not all Zn toxicity is expressed as an induced Fe deficiency. Excess Zn has also induced Mn toxicity in soybean (crinkle leaf) by promoting Mn transport within the plant.

For most metals the toxicity is first expressed at root tips, and much of what follows is a secondary, systemic effect expressed in many symptoms (Foy et al., 1978).

Physiology of Differential Tolerance

Mechanisms by which plants may avoid metal toxicity are: (a) exclusion of the metal by precipitation in the growth medium or on plant root surfaces;

(b) entrapment of excesses in cell walls or vacuoles; (c) chelation and detoxification by organic acids or other complexes in plant sap; and (d) alteration of enzyme structure which reduces metabolic susceptibility. For a review, see Foy et al. (1978).

Some fungi precipitate Cu at their cell walls by generating sulfide (Ashida, 1965; Kikuchi, 1965a, 1965b); they also apparently produce a thioenein protein which binds Cu (Naiki and Yamagata, 1976). Some *Chlorella* also exclude Cu (Foster, 1977). Bacteria apparently develop resistance to Ni and Co by reducing the activity of their Mg carrier, which suggests that these metals occupy this carrier when producing toxicity (Nelson and Kennedy, 1971; Webb, 1970). Differential metal tolerance of enzymes has been reported for ecotypes differing in resistance to toxicity (Cox et al., 1976).

Some plants resist injury by storing excess metals in their vacuoles (Ernst, 1969). Possible sites of metal binding within the cytoplasm include cysteinyl and histidyl side chains of proteins, purines, pteridines, and prophyrines (Vallee and Ulmer, 1972). According to Mathys (1975, 1977), Cu and Cd react with SH groups, but Zn showed a closer affinity for carboxyl groups. Malate has been suggested as a major factor in the development of heavy metal tolerance; it is believed that it complexes metals within the cytoplasm and that it may also detoxify excess metals by transporting them to cell vacuoles. Also, oxalate may serve as a terminal acceptor of excess metals (Mathys, 1977).

Differential tolerance of soybean cultivars to excess Zn has been associated with differences in susceptibility to Zn-induced Fe deficiency, Zn uptake and translocation, and susceptibility to Zn toxicity not related to Fe deficiency (White, 1976). Tolerance to excess Zn resides in the shoots of soybeans (White, 1976) and navy bean (Polson and Adams, 1970).

Although excesses of various metals may produce some common effects on a given plant (dead root tips, stunting, induced deficiencies of Fe and P, etc.), there is considerable evidence that tolerances to some metals are specific. Wu and Bradshaw (1975) observed that Cu decreased MDH (1-malate dehydrogenase) activity and protein synthesis in the roots of non-tolerant genotypes but had no such effect on a tolerant genotype of *Agrostis stolonifera*. They suggested that Cu tolerance is due to a mechanism which protects metabolism and which is maintained by natural selection. It was concluded that different mechanisms have evolved for different heavy metals. Karataglis (1980) also concluded that the toxicities of Cu and Zn were specific in clones of *Agrostis tenuis*. The coexistence of the two metals increased toxicity, even though uptake of Cu and Zn occurred rather independently, and the toxicity of one was not affected competitively by the presence of the other. Sensitive clones showed less ability to store metals in their roots than did tolerant clones.

Multiple tolerance to metals has also been reported. Cox and Hutchinson (1980) found that *Deschampsia cespitosa* grown around a Ni/Cu smelting complex were more tolerant to Ni, Cu, Al, Zn, and Pb than were populations

grown on uncontaminated sites. Individual plants from the smelter site actually demonstrated a growth requirement for Ni and Cu in solution culture. Germination and seedling survival from these populations were also better on contaminated than on uncontaminated soils.

Iron Deficiency

Iron-related chlorosis is a complex physiological disorder that generally occurs on naturally calcareous soils or those limed to a pH above 7.0. Plant stress (chlorosis) results from low Fe solubility in the soil and insufficient uptake by the plant or by interference in the transport and metabolism of Fe by numerous factors, including excesses of P, Zn, Cu, Ni, Mn, or other ions. For current literature on the Fe deficiency problem, see Clark (1982a, 1982b) and Vose (1982).

The weeping lovegrass strain FQ22 is much more resistant to Fe-related chlorosis in calcareous soil and nutrient solution than is the strain 'FQ71' (Foy et al., 1982a, 1982b). Superior Fe efficiency in the FQ22 strain has been associated with its ability to maintain a low pH in its root zone (which prevents Fe precipitation), and greater affinity for NH_4^+ than for NO_3^- (which lowers pH by release of H ions), more effective transport of Fe from roots to tops, and restricted transport of Mn and Ca. Excesses of Mn and Ca may interfere with the metabolism of transported Fe in the chlorosis-susceptible FQ71. In soybeans and tomatoes, Fe efficiency has been associated with a lowering of pH in the root zone and the release of a "reductant" that converts Fe^{3+} to Fe^{2+}, the form more available to plants. In tomato this "reductant" is caffeic acid or one of its derivatives (Olson et al., 1981).

DISCUSSION AND CONCLUSIONS

Mineral stresses of toxicity or deficiency in plants are not always clearly identifiable entities. Instead, they may be the results of complex interactions among the major toxic ions involved, other essential or non-essential ions, and environmental factors. For example, water stress can be caused by simple water deficit (high soil moisture tension) or by Al toxicity. Some Mn toxicities can be alleviated by Fe treatment of soils and/or plants and others cannot. Iron deficiency chlorosis can result from low Fe solubility at high soil pH and/or from excesses of P, Zn, Cu, Ni, Mn, Ca, or other ions. The ultimate expression of a given "problem soil syndrome" is determined by the plant genotype and environmental factors.

Problems of mineral stress in plants can be reduced or prevented by modifying the soil to fit a given plant or by breeding plant genotypes with greater tolerance to stress. The probability of success in either approach would be greatly increased by a better understanding of how plants tolerate or adapt

to stress. In the past, much of the research on mineral stress physiology has been confined to a single genotype of a particular plant species. A more promising approach for discovering stress tolerance mechanisms is to use stress tolerant and sensitive genotypes of closely related plants (preferably near-isogenic lines) of the same species. Knowledge concerning the physiology and biochemistry of such differential stress tolerances would be useful in modifying both plants and soils to achieve the desired results. Pairs of stress-tolerant and sensitive plant genotypes are valuable indicators of present and potential mineral stress problems in soils, particularly when used in conjunction with conventional soil testing procedures.

Differential tolerances of plant genotypes to mineral stress almost certainly involve differences in membrane structure or function. For example, Al is known to inhibit DNA replication, but in order to gain access to the DNA, the Al must cross the outer cell membrane (plasmalemma) and also the nuclear membrane (tonoplast) or membranes surrounding other DNA containing subcellular bodies that may be affected. In certain wheat cultivars the exclusion of Al appears to be important in tolerance, but in plants like tea, which accumulate high internal levels of Al without injury, other mechanisms are obviously involved. Oxidation-reduction reactions in the root zone can explain differential Fe efficiencies of tomato and soybean genotypes. Similar reactions may be implicated in differential tolerance to excess Mn, but these cannot explain wide differences in tolerance to high internal levels of Mn observed in certain soybean and cotton genotypes.

To increase our understanding of differential mineral stress tolerance in plants, the following lines of study are suggested: (a) characterization of plant cell membranes with respect to microstructure, electric potentials, enzyme activities, and permeabilities to various ions and molecules—with and without stress; (b) chemical and physical compartmentalization of mineral elements in various plant fractions; (c) levels and kinds of organic acids, amino acids or other ligands that may chelate metals and thereby regulate either mineral toxicity or deficiency; (d) nitrogen metabolism (nitrate reductase activity and protein accumulation), NH_4^+ or NO_3^- tolerance or preference in relation to pH change and metal ion activities in root zones and plant fractions; (e) the possible role of Si in explaining differential Al and Mn tolerances; (f) oxidation-reduction reactions in root zones as related to differential Mn tolerance; (g) the nature of Mn-Fe interactions in producing Mn toxicity or Fe deficiency; (h) the role of P and Ca metabolism in differential Al tolerance; (i) interactions of mineral stress with water stress, temperature, air pollutants, and pathogens; and (j) the role of rhizobia and mycorrhizae in differential plant adaptation to mineral stress in problem soils.

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APPENDIX

Plant Names

Alfalfa	*Medicago sativa* L.
Apple	*Malus pumila* Mill.
Azalea	*Rhododendron calendulaceum* (Michx.) Torr.
Barley	*Hordeum vulgare* L.
Bean, broad	*Vicia faba* L.
Bean, snap	*Phaseolus vulgaris* L.
Birch, European white	*Betula verucosa* Ehrh.
Birch, paper	*Betula papyrifera* Marsh.
Birch, river	*Betula nigra* L.
Blueberry, high bush	*Vaccinium corymbosum* L.

Plant Names

Blueberry, low bush	*Vaccinium pennsylvanicum* Ait.
Buffelgrass	*Pennisetum ciliare* (L.) Link
Burdock	*Arctium lappa* L.
Cabbage	*Brassica oleraceae* L.
Cacao	*Theobroma cacao* L.
Carnation	*Dianthus caryophyllus* L.
Castorbean	*Ricinus communis* L.
Celery	*Apium graveolens* (Mill.) Pers.
Corn (maize)	*Zea mays* L.
Cotton	*Gossypium hirsutum* L.
Cowpea	*Vigna unguiculata* (L.) Walp.
Cranberry, American	*Vaccinium macrocarpus* Ait.
Cranberry, small	*Vaccinium oxycoccus* L.
Dogwood, flowering	*Cornus florida* L.
Eggplant	*Solanum melongena* L.
Eucalyptus	*Eucalyptus* sp.
Flax	*Linum usitatissimum* L.
Kale	*Brassica oleracea* L.
Kikuyu grass	*Pennisetum clandestinium* Hochst.
Lettuce	*Latuca sativa* L.
Lily, Easter	*Lilium candidum*, L.
Mangrove	*Rhizophora mangle* L.
Maple, sugar	*Acer saccharum* Marsh.
Morning glory	*Ipomea* sp.
Muskmelon	*Cucumis melo* L.
Oats	*Avena sativa* L.
Onion	*Allium cepa* L.
Orange, Satsuma	*Citrus reticulata* Blanco.
Orchardgrass	*Dactylis glomerata* L.
Palm, betel	*Areca triandria* L.
Pea	*Pisum sativum* L.
Peach	*Prunus persica* (L.) Batsch.
Peanut (ground nut)	*Arachis hypogaea* L.
Pepper	*Capsicum annuum* L.
Pine	*Pinus* sp.
Potato	*Solanum tuberosum* L.
Rape	*Brassica napus* L.
Rhodesgrass	*Chloris gayana* Kunth.
Rice	*Oryza sativa* L.
Rye	*Secale cereale* L.
Ryegrass, Annual	*Lolium multiflorum* Lam.

Plant Names

Ryegrass, perennial	*Lolium perenne* L.
Sorghum	*Sorghum bicolor* (L.) Moench.
Soybean	*Glycine max* L.
Spinach	*Spinacia oleracea* L.
Spruce, Norway	*Picea abies* (L.) Karst.
Spruce, Sitka	*Picea sitchensis* (Bong.) Carr.
Sugarcane	*Saccharum officinarium* L.
Sunflower	*Helianthus annuus* L.
Tea	*Camellia sinensis* (L.) Ktze.
Tobacco	*Nicotiana tabacum* L.
Tomato	*Lycopersicon esculentum* Mill.
Trefoil, birdsfoot	*Lotus corniculatus* L.
Triticale	*X Tritosecale*
Weeping lovegrass	*Eragrostis curvula* (Schrad.) Nees.
Wheat	*Triticum aestivum* L.

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MODIFICATION OF MINERAL NUTRITION IN SOYBEANS BY PLANT BREEDING[1]

Walter R. Fehr[2]

ABSTRACT. The principles of plant breeding have been used to minimize yield loss of soybeans from iron-deficiency chlorosis when grown on calcareous soil. Yield loss, which is efficiently estimated by level of chlorosis, is sufficiently great to justify development of varieties with high yield and chlorosis resistance for use in areas with calcareous soils. Resistance to iron chlorosis can be considered a quantitative character for breeding purposes. Improved sources of genetic resistance have been developed through recurrent selection. Varieties with high yield and moderate chlorosis resistance have been developed and released for commercial use. Seed mixtures of susceptible and resistant varieties may minimize yield loss when the highest-yielding variety is susceptible to iron chlorosis.

Index Descriptors: Iron-deficiency chlorosis, calcareous soils, variety development, seed yield, breeding methods, and recurrent selection.

INTRODUCTION

A principal soil association found in north-central Iowa is Clarion-Nicollet-Webster (Fig. 1). Some soils in this glaciated area have a pH above 7.6 and are called high pH, alkaline, high lime, or calcareous soils. These calcareous soils, a total of about 563,000 ha (1.39 million acres), include primarily four soil types: 88,000 ha of Harps, 344,000 ha of Canisteo, 63,000 ha of Storden, and 68,000 ha of Talcot (Tom Fenton, Dept. of Agronomy, Iowa State University, Ames, pers. comm.). The calcareous soils usually are irregular patches within a field; they do not occupy an entire field. About half of the calcareous soil in the Clarion-Nicollet-Webster soil association is planted to soybeans.

It has long been known that soybean varieties differ in their ability to utilize soil iron when grown on calcareous soil (Weiss, 1943). Those that cannot effectively utilize the soil iron have leaves with dark green veins and yellow interveinal tissue. The yellowing may be only slight, or the leaves may become necrotic and drop from the plant. Yellowing can be observed on the first and later trifoliolate leaves. The unifoliolate leaves of these varieties, however, remain green and do not exhibit chlorosis symptoms.

There are two approaches to the control of iron-deficiency chlorosis, chemical and genetic. First, chlorosis on a susceptible variety can be minimized by the application of foliar iron before or as soon as symptoms are expressed (Randall, 1977). Foliar iron will not always completely prevent yellowing of a susceptible variety but can reduce the intensity of yellowing and the number

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[2]Professor, Department of Agronomy, Iowa State University, Ames, Iowa 50011.

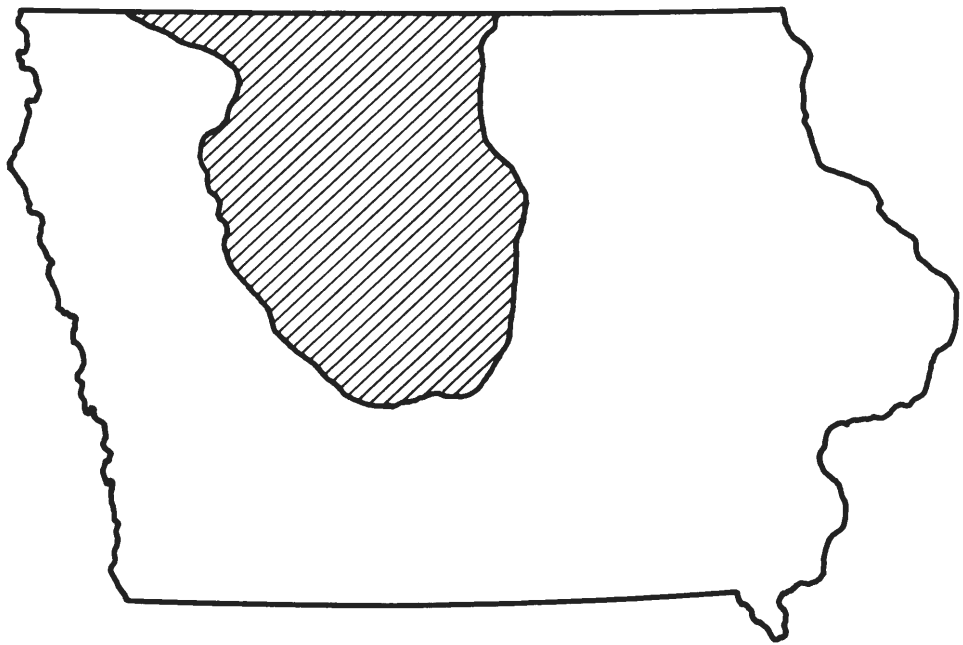

Figure 1. The shaded area is the Clarion-Nicollet-Webster soil association in Iowa where calcareous soils are present of the Harps, Canisteo, Storden, and Talcot type.

of leaves that express symptoms. Problems associated with application of foliar iron are the cost of the iron and its application and the fact that rainy weather may prevent satisfactory timing of applications.

The second approach is the development and use of varieties that are able to utilize the iron in calcareous soils. The use of such varieties eliminates the costs associated with application of foliar iron and is independent of the weather.

The purpose of this paper is to review research that has been conducted to find a genetic solution to iron-deficiency chlorosis. Questions to be considered whenever a plant breeder contemplates selection for a character include:

(a) Is the character important enough economically to justify any breeding effort?
(b) What is its inheritance?
(c) Is there a source of genes that controls the desired level of the character?
(d) What breeding method is most efficient for developing new varieties that are improved for the character?
(e) What are alternative strategies for utilizing improved varieties in commercial production?

Each of these questions will be addressed with respect to genetic improvement for resistance to iron-deficiency chlorosis on calcareous soils.

ECONOMIC IMPORTANCE OF IRON-DEFICIENCY CHLOROSIS

There are about 281,500 ha of soybeans grown on calcareous soils in Iowa each year. Substantial calcareous areas also are used for soybean production in southern Minnesota, and some calcareous soils are present in other states, e.g., North Dakota, Illinois, Nebraska, and Texas. The number of hectares of calcareous soils is adequate to justify breeding for iron-deficiency chlorosis in soybeans if yield reduction on those areas is significant.

A few soybean varieties are so susceptible to iron chlorosis that they will die on some calcareous soils. Rarely, however, is a large area of a field so severely affected. More commonly, soybeans have moderate yellowing early in their vegetative growth and are completely green later in their development. An assessment of economic loss must consider the relationship between yield reduction and level of chlorosis. Knowledge of the relationship between yield reduction and chlorosis also is important for the breeder in establishing realistic standards for the level of chlorosis resistance required in a variety. Selection for absence of yellowing in a breeding population would be unnecessary if a moderate level of chlorosis can be tolerated.

The relationship between level of chlorosis and yield was evaluated in Iowa by Froehlich and Fehr (1981). They selected 15 varieties representing a continuum from resistant to highly susceptible. The varieties were grown in replicated yield tests in five Iowa environments during two years. The planting sites were ones having calcareous soils where susceptible varieties had shown chlorosis in the past. The varieties also were grown on noncalcareous soil adjacent to the calcareous area to provide data on performance in the absence of chlorosis. Chlorosis was rated visually on a scale of 1 (no yellowing) to 5 (severe yellowing) based on symptoms of the first and second trifoliolate leaves (Cianzio, Fehr, and Anderson, 1979). Percentage yield reduction was calculated as 1-(yield on calcareous soil / yield on noncalcarous soil) X 100. There was a significant linear relationship between percentage yield reduction and level of chlorosis. The regression equation for data combined across environments was:

$$\text{Precentage yield reduction} = -25.56 + 20.26X$$

where X was the chlorosis score of the variety. The results indicated that, on the average, there was 20% yield reduction for each unit increase in chlorosis score. No reduction was associated with chlorosis symptoms rated as 1, 20% for a score of 2, 40% for a score of 3, 60% for a score of 4, and 80% for a score of 5.

Slight yellowing was observed on the most resistant varieties used by Froehlich and Fehr (1981). A2 and Agripro 1120 both had an average chlorosis score of 1.5, and the yield reduction was 6.4% for A2 and 7.9% for Agripro 1120. The yield reduction may have been due to stress caused by chlorosis. It also may have been caused by higher fertility on the noncalcareous soil with the result that the ratio of yield on calcareous vs. noncalcareous soil would be less than 1, even in the absence of chlorosis.

A study by Niebur and Fehr (1981) demonstrated that there is significant yield reduction whenever any yellowing occurs. They grew 19 soybean lines with a high level of chlorosis resistance on calcareous and noncalcareous soils in three Iowa environments. A split-plot design was used on each soil type, with iron treatments as whole plots and lines as subplots. The iron treatments were *no iron applied* and *sufficient iron chelate applied* so that no chlorosis symptoms were expressed. The iron treatment had no effect on yield on the noncalcareous soil. On the calcareous soils, the mean chlorosis score of the lines was 1.5, and their mean yield was 23.6 q/ha. Prevention of chlorosis with iron treatment was associated with a significantly higher yield of 24.9 q/ha. The yield decrease of 5.6% with a chlorosis score of 1.5 was similar to the yield reduction of 6.4% for A2 and 7.9% for Agripro 1120 observed by Froehlich and Fehr (1981).

These two studies have demonstrated that iron-deficiency chlorosis causes yield reduction, even when the yellowing is slight. To better appreciate the economic impact of the problem, consider the following example. Assume that a highly susceptible variety was grown on the 281,500 ha of calcareous soil planted to soybeans each year in Iowa. The variety yields 30 q/ha (44.6 bushels/acre) on noncalcareous soil and 20% of that amount (equivalent to a 5 rating) on calcareous soil (6 q/ha or 8.9 bushels/acre). The value of soybeans is $6 per bushel. The yield loss of 24 q/ha (35.7 bushels/acre) on 281,500 ha (695,586 acres) would be 6,756,000 q (24,832,420 bushels). The total economic loss for farmers in Iowa would be $148,994,500 each year.

Thus, these calculations indicate that iron-deficiency chlorosis can cause significant yield loss in Iowa. They also indicate that complete elimination of yield loss from iron chlorosis is contingent upon development of varieties that exhibit no yellowing when grown on a calcareous soil.

INHERITANCE OF RESISTANCE TO IRON-DEFICIENCY CHLOROSIS

The first genetic study on the inheritance of iron utilization of soybeans on calcareous soil was conducted by Weiss (1943). He classified varieties and plant introductions as either iron-efficient or iron-inefficient. Crosses were made between efficient and inefficient types, and the progeny were evaluated for iron utilization in nutrient solution. Weiss' interpretation (1943) was that efficiency is controlled by an allele *Fe* that was completely dominant to the allele *fe*. He also noted, "Although some variation of efficiency was noted among the inefficient varieties, the magnitude of expression of any modifying genes was negligible in comparison with that of the major gene involved."

In 1967, the Iowa Agriculture and Home Economics Experiment Station and USDA released the variety Corsoy (Weber and Fehr, 1970). It yielded about 4.7 q/ha (7 bushels/acre) more than the popular variety Hawkeye and was rapidly adopted by farmers in Iowa and states of comparable latitude. As the area planted to Corsoy increased, the prevalence and intensity of iron chlorosis on calcareous soil also increased. This, consequently, created concern about potential yield loss from iron chlorosis. As a result of this concern, all public and private varieties and brands in the 1971 Iowa Soybean Yield Test were evaluated for their susceptibility to iron chlorosis on calcareous soil located at the Agronomy and Agricultural Engineering Research Center near Ames (Clark et al., 1971). The chlorosis scores were based on a rating of 1 (little or no yellowing) to 5 (very severe yellowing) and were evaluated to the nearest 0.1 score. The entries displayed a continuum of chlorosis ratings from 2 to 5. The variety Corsoy had a rating of 4, and Hawkeye had a rating of 2.

The lack of discrete classes for reaction of varieties to iron chlorosis cast doubt upon the single-gene model proposed by Weiss (1943) for control of iron utilization. A genetic study was initiated by Cianzio and Fehr (1980)

to re-examine inheritance of the character. The most resistant genotype available to them was an experimental line, IVR Ex-5003 developed by Improved Variety Research, Inc., Adel, Iowa (Clark et al., 1971). They crossed it to a highly susceptible public variety Anoka (Lambert, 1971). One backcross was made to Anoka and four to IVR Ex-5003. The parents and lines derived from each single-cross and backcross population were evaluated on calcareous soils in Iowa. They reported, "The observed segregation can be explained by a single major gene and modifying genes. . . .The influence of environment and modifying genes on chlorosis expression suggested that resistance to chlorosis may be considered as a quantitative character in breeding programs. . . .Backcrossing should be an effective means of transferring the major gene from a resistant to a susceptible cultivar. A replicated progeny test on appropriate calcareous soils before each backcross should improve the probability of recovering favorable modifying genes from the resistant parent."

Cianzio and Fehr (1982) decided to evaluate the feasibility of developing varieties resistant to chlorosis by backcrossing. They chose Pride B216 as the recurrent parent and A2 as the donor. Pride B216 is a private variety of Pride Co., Inc., Glen Haven, Wisconsin, that has high yield and is extremely susceptible to iron chlorosis (Bahrenfus et al., 1975). A2 is a germplasm line that has a high level of resistance to iron chlorosis but is lower yielding than Pride B216 (Fehr and Bahrenfus, 1980). Enough F_1 seed of the single-cross Pride B216 x A2 was made to evaluate the F_1 generation and parents in replicated tests on calcareous soils. The F_1 had a mean chlorosis score of 3.3, compared with a midparent value of 3.2 for Pride B216 (4.4) and A2 (1.9). The results indicated no dominance for iron chlorosis resistance in the cross. Cianzio and Fehr (1982) then evaluated the chlorosis reaction of 200 random F_2-derived lines in F_3 from the cross, and none was as resistant as A2. These results indicated that chlorosis resistance in the cross was a quantitative character controlled by multiple genes with additive effects.

Additional evidence for the quantitative nature of inheritance of resistance to iron-deficiency chlorosis was provided in the recurrent selection study discussed in the next section. The genetic improvement obtained from recurrent selection is dependent on the presence of additive genetic variability for the character under selection (Prohaska and Fehr, 1981).

DEVELOPMENT OF IMPROVED GENETIC RESISTANCE TO IRON CHLOROSIS

From 1971 to 1975, plant introductions and public and private varieties and experimental lines were evaluated on calcareous soils by the soybean breeding project at Iowa State University. Some genotypes had a high level of chlorosis resistance, but none was completely free of yellowing in all the environments.

In 1975, on the basis of the assumption that resistance to iron chlorosis was quantitative and that additive genetic variability could be developed in a population, the decision was made to conduct recurrent selection for improved resistance to iron chlorosis. Ten varieties or experimental lines and ten plant introductions with the best chlorosis resistance then available were chosen as parents (Fehr and Cianzio, 1980). Varieties and experimental lines were chosen as a source of genes for both chlorosis resistance and high yield potential. Yield potential of the parents was considered because of future interest in recurrent selection for yield in the population, if selection for chlorosis resistance was successful. The ten plant introductions were used as a source of genetic variability for genes controlling chlorosis resistance. Three generations of intercrossing were used to develop the population, designated AP9.

Recurrent selection is a cyclic procedure for population improvement that includes development of a population, evaluation of individuals from the population, and selection of superior individuals that are used as parents to form an improved population for the next cycle of selection. Genetic improvement per year for the character of interest is strongly influenced by the number of years required to complete a cycle of selection (Eberhart, 1972; Fehr and Ortiz, 1975). The most rapid procedure available that includes evaluation of selfed progeny is S_1 line evaluation. The procedure was well suited to our objective because we could complete one cycle of selection each year. The two-step procedure adopted was:

Puerto Rico, Winter	Grow S_0 plants of the population and harvest 100 individually for evaluation of their S_1 progeny in Iowa.
Iowa, Summer	Grow a replicated test of 100 S_1 lines on calcareous soils in Iowa. Evaluate for chlorosis resistance before flowering and select the ten most resistant lines as parents. Cross the ten parents to form a new population for the next cycle of selection.

In 1979, after only two cycles of selection, S_1 lines from the cycle 2 population exhibited a high level of resistance when evaluated for chlorosis symptoms on calcareous soils at Ames, Knierim, and Meservey, Iowa. A seed mixture was then released to other breeders as AP9(S1)C2 that consisted of S_2 seed from 71 of the 100 S_1 lines that had a chlorosis score not greater than 1.5 in any of the replications at the three locations (Fehr and Cianzio, 1980). The following year, the 71 lines were re-evaluated individually on a calcareous soil near Humboldt, Iowa, where chlorosis usually is more severe than at the locations used in 1979. The ten most resistant lines in the 1980 test were crossed to form the cycle 3 population. One additional cycle of selection was conducted in 1981 to form the cycle 4 population by again using Humboldt as the test site for evaluation of chlorosis resistance.

Progress from selection in cycles 1 and 2 was evaluated by Prohaska and Fehr (1981). The parents of the cycle 0, 1, and 2 populations were compared in replicated tests on calcareous soils in four environments. The mean of the parents was 2.2 for cycle 0, 2.0 for cycle 1, and 1.8 for cycle 2. In 1981, the parents of the cycle 0, 1, 2, and 3 populations were compared in a separate study. The population means were 2.8 for cycle 0, 2.3 for cycle 1, 2.2 for cycle 2, and 2.0 for cycle 3. The chlorosis score of the best parent was 2.2 for cycle 0, 1.8 for cycle 1, 1.8 for cycle 2, and 1.6 for cycle 3. The results indicated that recurrent selection by S_1 line evaluation was successful for improving genetic resistance to iron chlorosis. The best parent of cycle 4 has resistance superior to any other known soybean genotype. It will be purified and released to breeders as a parent stock. It will be a useful source of genes for development of varieites with a high level of resistance.

DEVELOPMENT OF HIGH-YIELDING VARIETIES WITH RESISTANCE TO IRON-DEFICIENCY CHLOROSIS

The primary objective of soybean breeders is to increase the yield potential of varieties and incorporate resistance to serious production problems. The pertinent question about iron-deficiency chlorosis is whether it can be considered sufficiently serious and widespread to require that resistance be present in every variety developed and released. Each year about 11.7% of the soybeans in northern and central Iowa are grown on calcareous soils. The percentage for the Midwest as a whole is considerably less. Thus the breeder must conclude that resistance to chlorosis cannot reasonably be a requirement of all varieties. The major breeding emphasis should be placed on yield potential *per se* while a separate and smaller program for developing high-yielding varieties with chlorosis resistance would be appropriate.

We have used two procedures at Iowa State University for breeding varieties with high yield and chlorosis resistance. The first of these involves selection and evaluation of lines from two-parent populations. Our current procedure for selection from two-parent populations is as follows:

Step 1 (Iowa) Cross varieties or experimental lines that have the best combination of yield and chlorosis resistance available. Parents used generally have a chlorosis score of 3 or less.

Step 2 (two seasons in Puerto Rico) Advance the populations to the F_3 generation by single-seed descent.

Step 3 (Iowa) Grow the F_3 populations on calcareous soil and discard plants susceptible to chlorosis. Harvest individually the remaining F_3 plants of proper maturity.

Step 4 (Iowa) Evaluate F_3-derived lines in F_4 for chlorosis resistance in three replications on calcareous soil. Harvest an F_4 plant with acceptable maturity from those rows with adequate chlorosis resistance.

Step 5 (Iowa) Evaluate the F_4-derived lines in F_5 for yield on non-calcareous soil. Lines with superior yield are retained for further evaluation as potential new varieties.

The variety Weber was developed from a two-parent population. It was selected for chlorosis resistance as an F_5-derived line on calcareous soil, then for yield on noncalcareous soil.

Our second approach for variety development has involved backcrossing. The backcross we have evaluated most extensively involved Pride B216 as the recurrent parent and A2 as the donor parent. The strategy for backcrossing was as follows: (a) Evaluate the progeny of F_2 plants (F_2-derived lines in F_3) each backcross generation and use the most resistant progeny for backcrossing. (b) Evaluate the yield of the most resistant F_2-derived lines in comparison with the parents on noncalcareous soil. (c) Discontinue backcrossing when yield of the recurrent parent has been recovered in a line with acceptable chlorosis resistance.

We evaluated 200 F_2-derived lines in F_3 from the single-cross of Pride B216 x A2 on calcareous soil in 1979 (Cianzio and Fehr, 1982). Only seven of the lines had a chlorosis score equal to or better than A2. Each of the lines was backcrossed to Pride B216, and 40 BC_1F_2-derived lines in F_3 from each of the seven populations were evaluated on calcareous soil in 1980. None of the BC_1F_2-derived lines had chlorosis resistance equal to A2, and backcrossing was discontinued until an evaluation of yield could be made. Twenty-eight of the 280 BC_1F_2-derived lines with chlorosis scores of 3 or better were harvested individually in bulk. The BC_1F_2-derived lines in F_4 were evaluated for yield in two replications at four locations in Iowa during 1981. Fifteen of the lines had yields that were not significantly different from Pride B216, and 20 yielded significantly more than A2 (Table 1). None of the lines had chlorosis scores as good as A2, but all were better than Pride B216. The lines were compared on calcareous soils with Lakota, a variety released from our project in 1981 because of its high yield and good chlorosis resistance. Most of the lines were only slightly more susceptible than Lakota.

It is important to note the ancestory of the BC_1F_2-derived lines with the highest yield (Table 1). Six of the seven highest-yielding BC_1F_2-derived lines in 1981 traced to the same F_2-derived line, designated 8. If line 14 had been the only parent used to develop a BC_1F_2 population, none of the BC_1F_2-derived lines would have been equal in yield to Pride B216. The data emphasize the importance of using multiple F_2-derived lines for backcrossing when transferring resistance to chlorosis.

Table 1. Mean yields and maturities of BC_1F_2-derived lines in F_4 and parents of the mating Pride B216[2] X A2 when grown on noncalcareous soil at four Iowa locations and their chlorosis score on a calcareous soil and that of the check variety Lakota.

Genotype	Ancestory[a]	Yield[b]	Chlorosis[c] score	Maturity[d]
		q/ha		
A81-157005	8	44.1	3.5	18
A81-157003	8	43.5	3.4	18
A81-157007	8	43.3	2.7	16
A81-157009	12	43.3	3.0	24
A81-157001	8	43.0	3.1	18
A81-157004	8	43.0	3.3	17
Pride B216		42.5	4.3	18
A81-157002	8	42.4	3.3	16
A81-157024	19	42.1	3.5	10
A81-157008	8	41.7	3.0	14
A81-157027	19	41.6	3.2	14
A81-157014	15	41.6	3.1	16
A81-157025	19	40.9	3.3	14
A81-157015	15	40.6	3.1	14
A81-157006	8	40.3	3.3	12
A81-157026	19	40.2[e]	3.2	12
A81-157013	15	39.8	3.2	14
A81-157019	18	39.8	3.2	14
A81-157018	16	39.5	3.4	14
A81-157028	19	39.2	3.3	12
A81-157022	18	39.2	3.2	16
A81-157011	12	38.8	3.3	10
A81-157012	14	38.6	3.3	10
A81-157023	19	38.2	3.5	12
A81-157017	15	37.6	3.0	14
A81-157020	18	37.6	2.7	10
A81-157021	18	37.4	3.0	12
A81-157010	12	37.4	3.6	10
A81-157016	15	37.1	3.2	16
A2		36.5	2.5	12
Lakota		--	2.8	--
LSD (0.05)[f]		2.6		

[a] F_2-derived line used as a parent for the backcross to Pride B216. BC_1F_2-derived lines with the same number have the same F_2-derived line as a parent.

[b] Yield expressed in quintals per hectare (q/ha).

[c] Scores range from 1 (no yellowing) to 5 (severe yellowing).

[d] Days after August 31.

[e] Lines with a yield of 40.2 q/ha or above are not significantly different from Pride B216 at the 0.05 probability level.

[f] Least significant difference at the 0.05 probability level.

A third approach we plan to study for development of varieties with high yield and chlorosis resistance is recurrent selection. The difficulty with simultaneous selection for two characters is that the probability of a segregate with superiority for both characters is less than selection for superiority in only one of the characters. To minimize this problem, recurrent selection will be initiated with a population developed from parents with high levels of chlorosis resistance. Selection for yield can be the primary consideration because the majority of the individuals in the population will have acceptable chlorosis resistance.

Regardless of the breeding procedure chosen, it is not necessary to yield test resistant lines on calcareous soils to identify those with superior yield potential. Niebur and Fehr (1981) compared yield of 19 resistant lines on calcareous and noncalcareous soils in four Iowa fields. They found no significant genotype x soil type interaction for yield, maturity, height, lodging, seed weight, and protein and oil content of the seed. Their results indicated that yield and other agronomic characters of lines resistant to iron chlorosis can be evaluated as effectively on noncalcareous as on calcareous soil. The breeder can evaluate for chlorosis resistance with small plots on calcareous soil and evaluate for yield and other agronomic characters on noncalcareous soils that are more uniform and readily available.

MINIMIZING YIELD LOSS FROM IRON CHLOROSIS IN COMMERCIAL SOYBEAN FIELDS

Because the major emphasis in soybean breeding has been on yield without regard to chlorosis resistance, new varieties with superior yield are likely to be more susceptible to chlorosis than is desirable. How can such varieties best be utilized in areas that have calcareous and noncalcareous soils in the same field? Four alternatives are compared in Table 2. The following assumptions will illustrate the impact of each: (a) The farmer's field contains 60 ha, of which 6 ha are calcareous. (b) The susceptible variety yields 40 q/ha, and an available resistant variety 36 q/ha on noncalcareous soil. (c) The yield of the susceptible variety is reduced by 80% on the calcareous area. (d) Yield of the resistant variety is 36 q/ha on calcareous soil.

Alternative 1. The high-yielding susceptible variety is planted throughout the field, and the yield loss on the calcareous soils is disregarded. The yield in our example is 2,208 q/ha. The acceptability of this alternative is highest when the percentage of calcareous soil is relatively small.

Alternative 2. The lower-yielding resistant variety is planted in the entire field. A better yield is obtained on the calcareous

Table 2. Yield of a 60-ha soybean field with 6 ha of calcareous soil when four alternative management strategies are used.

Alternative	Yield		
	Calcareous area	Noncalcareous area	Total
	quintals		
1. Susceptible variety alone	48	2,160	2,208
2. Resistant variety alone	216	1,944	2,160
3. Susceptible variety on noncalcareous soil (54 ha) Resistant variety on calcareous soil (6 ha)	216	2,160	2,376
4. Seed mixture 70% susceptible variety 30% resistant variety	194	2,095	2,289

soil than from the susceptible variety, but a lower yield is obtained on the noncalcareous soil. As a result, total yield of the field in our example is 48 quintals less for this procedure than for the first one. The acceptability of planting a lower-yielding resistant variety in the entire field is greatest when the percentage of calcareous soil in a field is relatively large.

Alternative 3. The high-yielding susceptible variety is planted on noncalcareous soil and the lower-yielding resistant variety on calcareous soil. This, in theory, is the best option, because it maximizes yield on both types. In practice, however, it is inconvenient or perhaps impractical. A farmer must know the location of calcareous areas in the field. Seed in the planter must be changed for the two soil types; thus extra time must be spent planting the two areas.

Table 3. Yield of seed mixtures of A2, a line with good resistance to iron-deficiency chlorosis, and Northrup King S1492, a highly susceptible variety, when grown on calcareous soils at four locations in Iowa during 1980.

Frequency of components		Location				
A2	S1492	Ames	Humboldt	Knierim	Meservey	Combined
%	%	q/ha				
100	0	33	34	27	26	30
90	10	33	34	27	24	29
80	20	31	33	29	25	30
70	30	33	31	26	25	29
60	40	33	32	26	24	29
50	50	33	34	25	25	29
40	60	31	30	24	20	27
30	70	31	32[a]	24[a]	24[a]	28[a]
20	80	33	28	17	22	25
10	90	30[a]	25	13	18	21
0	100	25	14	0	13	13

[a] Blend with the lowest frequency of A2 that yielded within 10% of a pure stand of A2.

Alternative 4. A seed mixture of a high-yielding susceptible and lower-yielding resistant variety is planted over the entire field, thereby minimizing yield loss on the calcareous soil without sacrificing entirely the high yield of the susceptible variety on noncalcareous soil.

The yield of alternative 4 was less than that of alternative 3 but greater than the other two alternatives in our example. It was assumed that yield of the mixture on calcareous soil would be 90% of the resistant variety (32.4 q/ha). Yield on the noncalcareous soil was assumed to be the weighted mean of the two varieties (0.7 x 40) + (0.3 x 36) = 38.8 q/ha. These assumptions were based on a study in progress as part of the M.S. thesis of Michael Trimble to evaluate the optimum proportion of susceptible and resistant components of a blend for chlorosis control. Results for one combination of varieties studied in 1980 are presented in Table 3. A2 was the lower-yielding resistant variety, and Northrup King S1492 was the high-yielding susceptible variety. They were mixed in proportions varying by 10% increments. The mixtures and a pure stand of both varieties were grown in replicated plots on calcareous soils in four Iowa environments. Yield of a 30% A2: 70% S1492 mixture was within 10% of the resistant variety in all environments, including Knierim where S1492 had no yield in pure stand. The results indicated that the frequency of the resistant component in the blend can be kept relatively low without markedly reducing yield on calcareous soil compared with a pure stand of the resistant variety. The resistant plants are able to branch and compensate in yield for loss of the susceptible individuals.

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REDUCTION OF IRON BY SOYBEAN ROOTS: CORRELATION WITH IRON EFFICIENCY ON CALCAREOUS SOILS[1]

Carl L. Tipton and Joan Thowsen[2]

ABSTRACT. Roots of 10-day-old soybean (*Glycine max*) plants reduce Fe(III)EDTA to Fe(II) in a process that is stimulated by glucose and inhibited by caffeic and chlorogenic acids and by biosynthetic precursors of these acids. Iron reduction by roots of plants grown in the absence of iron was more rapid than by those supplied iron in the nutrient solution. In a series of ten soybean cultivars, the rate of iron reduction was correlated positively with seed yield on calcareous soils. Seemingly, the reduction is catalyzed by a cell wall enzyme in a reaction clearly different from previously reported reactions in tomatoes and other plants in which a non-enzymatic reductant is secreted by the roots.

Index Descriptors: soybean; *Glycine max*; iron nutrition; clorosis.

INTRODUCTION

Despite the abundance of iron in most soils, iron-deficiency chlorosis often is an important problem in crop nutrition, largely because of the limited solubility of iron compounds in soils. Iron deficiency chlorosis frequently appears in soybeans (*Glycine max*) grown on calcareous soils, but soybean cultivars vary greatly in the severity of chlorosis on a given soil (Cianzio and Fehr, 1980). Efforts of breeders to improve the resistance of soybean cultivars to iron deficiency chlorosis might be facilitated, if the biochemical events in iron utilization and the biochemical basis for genetic variation in the efficiency of iron utilization were known.

Previous research (reviewed by Brown, 1978) has shown that iron utilization by plants involves the reduction of soil Fe(III) to Fe(II), absorption of Fe(II) by the roots, re-oxidation in the roots to Fe(III), transport as the citrate chelate, and finally reduction again to Fe(II) in the leaves. The physiological responses of plants to iron deficiency commonly include secretion of acids and of organic reducing substances by the roots (Brown, 1978). In tomatoes (*Lycopersicon esculentum*), the major component of the reducing substances probably is caffeic acid (Olsen et al., 1981). Reduction of Fe(III) by caffeic acid is apparently non-enzymatic. Lowering of the pH and reduction of iron to the ferrous state both increase greatly the solubility, and presumably the availability, of iron.

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[2] Professor and Research Associate, Department of Biochemistry and Biophysics, Iowa State University, Ames, Iowa 50011, respectively.

We have found that these responses are inadequate to account for the observed variation in resistance of soybean cultivars to iron deficiency chlorosis and have undertaken research to improve understanding of these reactions.

MATERIALS AND METHODS

Seeds of twelve soybean cultivars, 'A2', 'Agripro 1120', 'Amsoy 71', 'Asgrow 2440', 'Corsoy', 'Hawkeye', 'Hodgson', 'Peterson 2447', 'PI 54-619', 'Pride B216', 'Weber', and 'Wells' were used in this study.[3]

Nutrient solutions used for growing soybean plants were prepared from reagent grade mineral salts (used without further purification) and deionized distilled water. Modified Steinberg's solution (Brown and Jones, 1976) had the composition: $Ca(NO_3)_2$, 18.9 mM; $MgSO_4$, 0.45 mM; NH_4NO_3, 1.2 mM; KCl, 1.8 mM; K_2HPO_4, 0.129 mM; $MnCl_2$, 1.18 μM; $ZnSO_4$, 0.31 μM; $CuSO_4$, 0.08 μM; H_3BO_4, 3.29 μM; and Na_2MoO_4, 0.5 μM. When added, iron was used in the form of Fe(III)EDTA, 35.8 μM. The final pH of the solution was 7.0. Hoagland's No. 1 nutrient solution without iron was modified as described by Brown and Jones (1976).

Ferrozine [3-(2-pyridyl)-5, 6-bis(4-phenylsulfonic acid)-1,2,4-triazine sodium salt] and biochemical reagents and buffers were obtained from Hach Chemical Co., Ames, IA, and Sigma Chemical Co., St. Louis, MO, respectively. Soybean seeds were germinated on moist germination paper in the dark at 29 C for three days, after which the seedlings were transferred to modified Steinberg's solution held in plastic containers. The nutrient solution was stirred and aerated with aquarium air pumps. Plants were grown in a growth chamber with a daily cycle of a 16-hr light period (1900-2000 lux) at 26 C and an 8-hr dark period at 20 C. Relative humidity was not controlled.

Secretion of reducing substance

Fe(II) formed by reduction of $FeCl_3$ by substances secreted into nutrient media was estimated spectrophotometrically as the Fe(II) complex of Ferrozine (Chaney et al., 1972). Ten-day-old plants were transferred to Hoagland's No. 1 solution without iron and grown with the same temperature and light regimes. Aliquots (2.5 ml) of the nutrient solution, sampled at 24-hr intervals, were mixed with 0.125 ml of 4 M sodium acetate, 0.125 ml of 4 mM $FeCl_3$ in 2 N HCl and 0.25 ml of 2.5 mM Ferrozine. Reaction mixtures were stored in the dark at room temperature, and the absorbance at 562 nm (A_{562}) was measured after 20 hrs. Concentration of Fe(II) produced was calculated from the molar extinction coefficient (27,900) (Chaney et al., 1972).

[3] Appreciation is expressed to Dr. W. R. Fehr, Department of Agronomy, Iowa State University, Ames, Iowa 50011, who supplied the soybean seeds.

Iron reduction by roots of intact plants

To assess the reduction of iron by roots, an intact soybean plant was placed in a foil-covered 125 ml Erlenmeyer flask so the root was submerged in 50 ml of Steinberg solution plus micronutrients to which 10^{-4} M Fe(III)-EDTA and 3 x 10^{-4} M Ferrozine had been added. The pH of the solution was adjusted to 6.6. Flasks were shaken at 26 C in a water bath under fluorescent lights (approximately 700 lux), and at intervals aliquots were removed and the absorbance at 562 nm was measured.

Iron reduction by excised roots

With some modifications the same general procedure was used on excised roots as described for intact plants. In some instances (indicated in the Results), the Steinberg solution was replaced by Hoagland's solution No. 1, and there were some instances when there was addition of glucose or adjustment of pH of the medium as indicated in the Results. Roots were pre-incubated in 50 ml of medium lacking Fe(III)EDTA and Ferrozine for 1 hr, after which they were transferred to 50 ml of the same solution to which 1.86 x 10^{-4} M Fe(III)EDTA and 6 x 10^{-4} M Ferrozine had been added.

Preparation of root plasma membranes

Root microsome fractions enriched in plasma membrane fragments were prepared by sucrose density gradient contrifugation and assayed for K^+, Mg^{++}-ATPase activity as described by Travis and Booz (1979).

Cell wall isolation

Excised roots were homogenized in 4 volumes of cold 0.1 M sodium acetate buffer (pH 5.0) for 2 min using a Brinkman Polytron (PCU-2), and the homogenate was centrifuged at 3900 x g for 20 min at 4 C. The cell wall material was washed with cold 0.1 M sodium acetate buffer (pH 5.0), filtered on a coarse fritted glass funnel, and the remaining liquid squeezed out through pre-moistened Miracloth (Calbiochem-Behring Corp., La Jolla, CA, USA).

Buffer-washed cell wall material was suspended in ice cold 80% acetone/water, stirred, and filtered on a coarse fritted glass funnel. The cell wall material was then washed with ice cold acetone, filtered, and air-dried.

Cell wall extractions

Acetone-treated cell walls (50 mg) were suspended in 20 ml of cold 0.1 M sodium acetate (pH 5.0), which contained either 1 M $NaClO_4$, 3 M LiCl, or 1% Zwittergent 3,14.

The three suspensions were kept in an ice bath for 2.5 hr and filtered through 4 layers of pre-moistened cheesecloth. Extracts were assayed for reduction of Fe(III)EDTA as follows: to 2.7 ml of 0.1 M sodium acetate buffer (pH 5.0) was added 0.1 ml of 8 mM Fe(III)EDTA, 0.1 ml of 24 mM Ferrozine, and 1 ml of extract. The reactions were initiated by adding 0.1 ml of 30 mM NADH. The reaction mixtures were incubated in the dark at room temperature for 20 hrs, after which the Fe(II) Ferrozine complex was estimated by measurement of the absorbance at 562 nm.

RESULTS

The first trifoliate leaves of the soybean seedlings grown hydroponically without iron were slightly chlorotic (days 10-11), and the second trifoliate leaves were severely chlorotic on full expansion (days 15-16). During the interval between the expansion of these leaves, the pH of the nutrient solution dropped and after one or two days, substances capable of nonenzymatic reduction of Fe(III) appeared in the nutrient solution (Figure 1). The four cultivars included in this experiment included two (A2 and Hawkeye) considered iron-efficient and two (Pride B216 and PI 54-619) considered iron-inefficient. The rate and extent of lowering of the pH did not correlate with the iron efficiency of the four cultivars, and the production of reducing substances was only roughly related to their iron-use efficiency. Also, of the plasmalemma-enriched membrane preparations from iron-efficient (A2) and iron-inefficient (Pride B216) cultivars, the K^+, Mg^{++}-ATPase activities were virtually identical both before and after chlorosis had appeared (Table 1).

Even though roots of iron-deficient plants did not begin to secrete a soluble reducing material until 12 days old, the roots of 10-day-old plants could reduce iron at the root surface (Table 2). The rate of reduction was greatest in the iron-efficient cultivar (A2), but the reaction was repressed by previous exposure to iron in the nutrient medium.

Excised roots also catalyzed the reduction of Fe(III) (Figure 2). The maximum rate occurred in roots from 9-day-old plants. The rate of reduction was greater with roots from the iron-efficient cultivar (A2), and the reaction was repressed by previous exposure to iron (Table 3). On the other hand, the reaction was stimulated by the addition of 1 mM glucose to the incubation medium (Table 4) and by gentle shaking (Table 5). During reduction, the pH dropped (Table 6), and addition of buffer to maintain pH 5.6 severely inhibited the reduction reaction.

Iron reduction rates by excised roots of ten cultivars, chosen to cover a wide range of seed yields and susceptibility to iron deficiency chlorosis on calcareous soils (Froehlich and Fehr, 1981), are shown in Figure 3 and Table 7. There was a degree of positive linear relationship between rate of iron reduction and seed yield for these cultivars when grown under conditions of iron

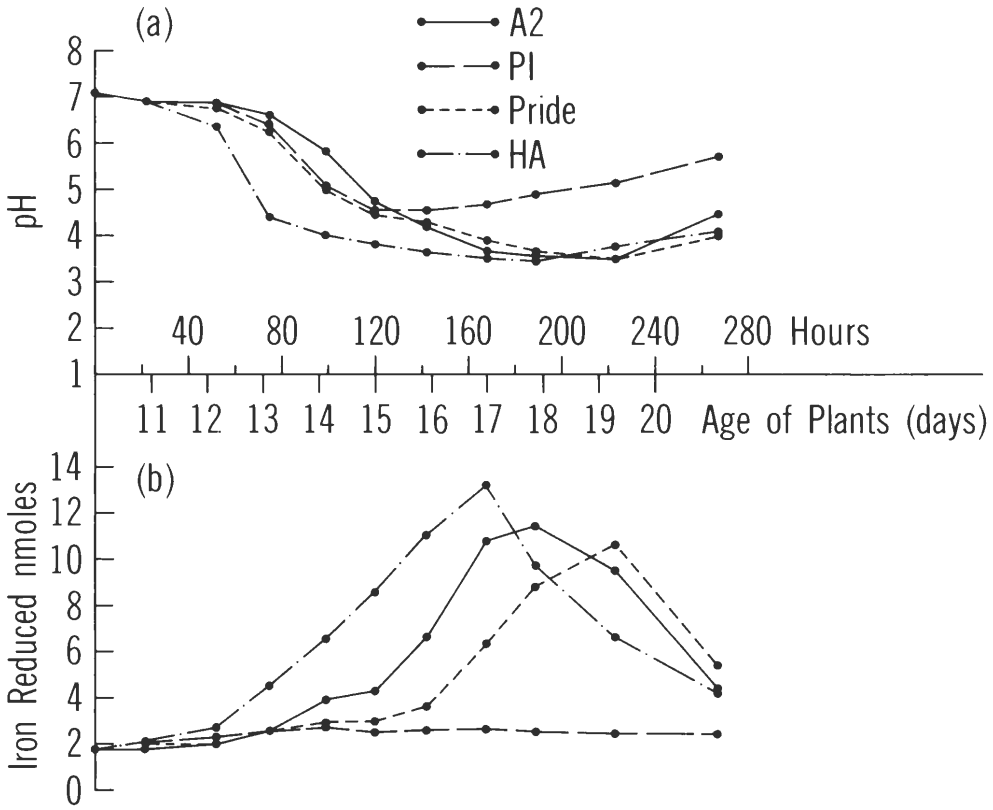

Figure 1. Responses of four cultivars of soybean to prolonged culture in iron-deficient nutrient media: a) pH of nutrient solution, b) secretion into the nutrient solution of substances capable of non-enzymatic reduction of $FeCl_3$.

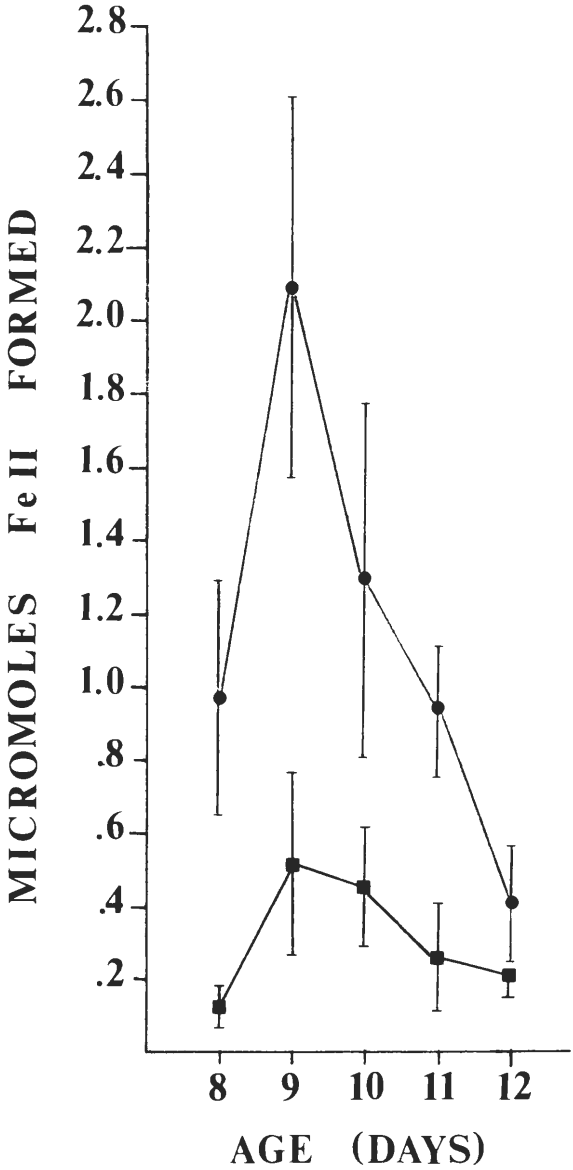

Figure 2. Iron reduced by excised roots from plants 8 to 12 days old. Reduction was measured over a 4 hr period in Hoagland's solution No. 1 with 1 mM glucose added, pH 5.6. Upper curve, variety A2, lower curve, Northrup-King Pride B-216.

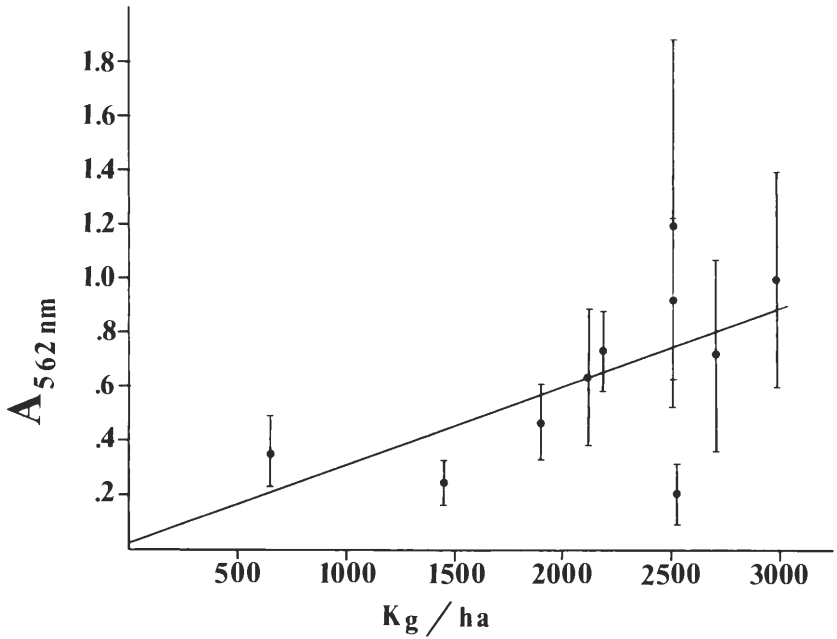

Figure 3. Iron reduction by excised roots of ten soybean cultivars, plotted against seed yields on calcareous soils. (Seed yields data from Froehlich and Fehr, 1981.) Experimental conditions as described for Figure 2.

deficiency stress. However, the relationship was not sufficiently good to permit prediction of iron-use efficiency of other varieties from iron reduction readings.

Addition of either caffeic or chlorogenic acid to excised soybean roots completely inhibited their ability to reduce iron (Table 8). Caffeic acid is biosynthesized from phenylalanine via *trans*-cinnamic acid and *p*-courmaric acid (as the coenzyme A ester). Of these precursors, *trans*-cinnamic acid, *p*-coumaric acid, and phenylalanine are complete, partial, and non-inhibitors, respectively (Table 8).

The ability of cell walls to catalyze reduction of Fe(III)EDTA by NADH was assayed visually, and these readings are reported as qualitative or semi-quantitative differences between various samples and treatments in Table 9. The results show that the cell wall preparations can catalyze the reduction of Fe(III)EDTA by NADH and that the reduction is not strongly inhibited by caffeic acid. Dehydration of the cell walls by extraction with acetone did not destroy the activity, but replacement of NADH by NAD plus malate resulted in no reduction.

When acetone-dried cell walls were extracted with salt solution or a detergent, the highest iron reduction activity in solution was obtained with 1 M LiCl, but in no case was all activity dissociated from the cell walls (Table 10). The reduction activity of cell walls isolated from 10-day-old seedlings of Agripro 1120 was 87% as great for plants grown without iron as those grown with it.

DISCUSSION

Soybean plants after extended iron deprivation show the same responses as tomatoes and other dicotyledonous plants (Brown, 1978; Olsen et al, 1981) by depressing the pH of the nutrient medium and secreting substances capable of nonenzymatic reduction of iron(III). The four soybean cultivars studied showed differences in the timing of these responses, but differences among them probably were related to their rates of development rather than to their iron-use efficiencies. Only PI 54-619, a highly iron-inefficient cultivar, showed complete absence of secretion of iron-reducing material. Furthermore, there was no difference in activity of K^+, Mg^{++}-ATPase, an enzyme involved in H^+ secretion, between efficient and inefficient cultivars. These responses appear when the plants become severely chlorotic and seemingly are related to the ability of plants to recover from a severe iron stress by increasing the solubility of soil iron in the vicinity of the roots. Froehlich and Fehr (1981) found that cultivars differing in severity of chlorosis, when grown on a calcareous soil, gave yield reductions proportional to their severities of chlorosis, even though all cultivars recovered and grew to maturity. For this reason we suggest that the biochemical pathway responsible for differences in iron use efficiency among soybean cultivars involves the ability of plants to reduce and absorb

Table 1. K^+, Mg^{++}-ATPase activity of plasmalemma-enriched fractions from roots of Pride B216 (Fe-inefficient) and A2 (Fe-efficient) soybean cultivars.

Variety	Age (days)	ATPase Activity	
		nmoles P_i/hr/μg Protein	nmoles P_i/hr/g fr. wt.
Pride B216	5	6.7	147
A2	5	5.3	133
Pride B216	21	1.2	71
A2	21	1.1	108

Table 2. Reduction of Fe(III)EDTA by roots of intact 10-day-old soybean plants.

Variety	Fe III in Nutrient Medium	A_{562} nm at 4¼ Hours
Pride B216	+	.04 ± .02*
	-	.20 ± .12
A2	+	.06 ± .06
	-	.48 ± .23

* Mean ± standard deviation; nine plants in each experiment.

Table 3. The effect of previous iron nutrition on the ability of excised roots of 12-day-old soybean plants to reduce Fe(III)EDTA.

Variety	Fe(III) in Nutrient Medium	A_{562} nm at 10 hours
Pride B216	+	.05 ± .03*
Pride B216	-	.09 ± .03
A2	+	.08 ± .07
A2	-	.40 ± .14

*Mean ± standard deviation; nine roots in each experiment.

Table 4. Effect of glucose on Fe(III)EDTA reduction by excised roots of 10-day-old Agripro 1120 soybean plants.

	A_{526} nm	
Glucose	2 Hours	4 Hours
0	.10 ± .06	.16 ± .09*
1 mM	.14 ± .08	.34 ± .19

*Mean ± standard deviation; nine roots in each experiment.

Table 5. Effect of shaking on Fe(III)EDTA reduction by excised roots of 10-day-old soybean plants.

Variety	Condition	A_{562} nm at 20 Hours
Pride B216	Shaken	.76 ± .23*
Pride B216	Stationary	.42 ± .09
A2	Shaken	.93 ± .26
A2	Stationary	.77 ± .21

*Mean ± standard deviation; nine roots in each experiment.

Table 6. Effect of MES buffer on reduction of Fe(III)EDTA by excised roots of soybean plants.

Variety	Buffer	A_{562} at 6 hr. per Root	pH at 6 hr.
A2	none	1.28 ± .23*	4.1
A2	10 mM MES	.39 ± .15	5.6
Corsoy	none	.77 ± .23	4.2
Corsoy	10 mM MES	.32 ± .11	5.5
Pride B216	none	.44 ± .12	4.6
Pride B216	10 mM MES	.11 ± .06	5.5
Weber	none	.35 ± .16	4.5
Weber	10 mM MES	.14 ± .05	5.6

*Mean ± standard deviation; nine roots in each treatment.

Table 7. Reduction of Fe(III)EDTA by 10 soybean cultivars compared with yield on calcareous soils.

Variety	Yield Kg/ha*	Absorbance at 562 mn at 4 hr Mean	S.D.	n
A2	2986	1.00	0.40	25
Amsoy 71	2512	1.21	0.68	25
Hodgson	2519	0.93	0.29	16
Agripro 1120	2795	0.73	0.35	34
Peterson 2477	2128	0.64	0.25	16
Wells	2186	0.74	0.15	16
Corsoy	1903	0.48	0.14	24
Weber	2528	0.21	0.12	24
Asgrow 2440	1447	0.25	0.08	34
Pride B216	650	0.36	0.13	35

*Data from Froehlich and Fehr (1981).

Table 8. Effects of phenolic acids and their metabolic precursors on Fe(III)-EDTA reduction by excised roots of 10-day-old soybean plants.

Additions	Pride B216 A_{562} at 4 hr.	% of Control	A2 A_{562} at 4 hr.	% of Control
None	.06 ± .04	100	0.60 ± .20*	100
Phenylalanine	.05 ± .02	85	0.64 ± .37	106
Trans-Cinnamic Acid	.01 ± .002	13	0.03 ± .01	4
p-Coumaric Acid	.04 ± .01	64	0.47 ± .28	77
Caffeic Acid	0.000	0	0.000	0
Chlorogenic Acid	0.000	0	0.01 ± .01	1

*Five roots in each treatment. Each addition 2×10^{-5} M. Mean ± standard deviation.

Each root was pre-incubated for 1 hour on Hoagland's solution No. 1 containing 1 m M glucose plus 2×10^{-5} M of each addition; the pH was 5.6.

Table 9. Reduction of Fe(III)EDTA by soybean root cell walls.

Cell Wall Preparation	Additions	Fe(III) Reduction
F	None	–
F	NADH	+++
F	NADH, Caffeic Acid	–
F (boiled)	NADH	–
F	Glucose	–
F	NAD^+	–
F	Glucose + NAD^+	–
A	NADH	+++
A	NADH, Caffeic Acid	++
A	NAD^+	–
A	Malate	–
A	Malate + NAD^+	–
–	NADH	(+)
–	Caffeic Acid	(±)
–	NADH + Caffeic Acid	(+)

F = Fresh cell walls; A = Acetone-dried cell walls.

Table 10. Extraction of Fe(III) reductase by salts and detergent.

Extractant	Fe(II) Formed, nmoles/g Cell Wall
1 M $NaClO_4$	1770
3 M LiCl	2340
1% Zwittergent 3,14	1340

soil iron under adverse conditions before chlorosis appears rather than the ability to recover from severe chlorosis.

Excised roots from 10-day-old seedlings, which were more convenient to use than were whole plants, showed that reduction of Fe(III)EDTA was stimulated by glucose and by shaking and was inhibited by growing seedlings in nutrient solution containing iron. These results suggest that carbohydrate metabolism is necessary to supply energy for the process and that the reaction is at least partially induced by iron stress.

The non-enzymatic reducing substance secreted by iron-deficient tomato plants is probably caffeic acid (Olsen et al., 1981). The process of iron reduction, we observed, is clearly distinguished from the production of non-enzymatic reducing material in several ways. First, soybean roots can reduce Fe(III)EDTA, whereas the non-enzymatic reductant requires $FeCl_3$ for rapid reaction. Second, secretion of reductant by iron-deficient soybean roots begins at about 12-13 days after germination, whereas the reduction by excised roots begins before 8 days and peaks at 9-10 days. Finally, the reduction by excised roots is severely inhibited by caffeic acid, cinnamic acid, and *p*-coumaric acid but not by L-phenylalanine, if the roots are pre-incubated in solutions containing these materials before measurement of iron reduction.

These observations suggest there are several possibilities explanatory of differences in iron-use efficiency among soybean cultivars: (a) the amount of the cell-wall enzyme may vary, (b) the supply of reducing agent from the cells to the cell-wall enzyme may vary, and/or (c) excessive production of caffeic acid or related catechols by iron-inefficient cultivars may inhibit iron reduction by the cell-wall enzyme. These possibilities are under current study.

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GENETIC VARIABILITY IN PHYSIOLOGICAL MECHANISMS OF DROUGHT RESISTANCE

Charles Y. Sullivan[1]

ABSTRACT. Although variability among genotypes to drought stress is well known, breeding by empirical methods has made limited progress. This report, using sorghum (*Sorghum bicolor*) as a model, examines some facets of drought resistance and suggests research methods.

Plant response is divided into tolerance and avoidance mechanisms, among which no single one will account for field drought resistance. Hydroponic studies demonstrate that sorghum genotypes differ in rooting characters. That deep rooting is a major avoidance mechanism is demonstrated by the correlative relationship of deep rooting with drought resistance and yield in the field. The line source sprinkler and gradient irrigation systems were found useful for field testing and the subsequent selection of drought resistant genotypes. These field studies show a linear relationship between yield and evapotranspiration, but the regression slopes differ dependent on the period of water stress.

Index Descriptors: Water stress, sorghum, root growth, hydroponics, breeding for drought resistance, irrigation gradient, and evapotranspiration-yield.

INTRODUCTION

Food supplies and grain reserves are generally in surplus in the United States, but food is not plentiful in much of the world that is arid or semiarid. Alleviating drought by timely irrigations is an increasing practice, but lack of economic resources, increasing real prices for energy, and insufficient available water in the arid crop-producing areas necessitate dryland crop production with its hazards.

One possible route for alleviating this complex of problems is the breeding of crop plants that are more resistant to drought, and indeed, much progress has been made in the past three decades. However, this progress has been slow, and sometimes the drought-tolerant cultivar is no more productive in dry seasons than is a reportedly less drought-resistant one. Although many questions about plant responses to drought are yet unanswered, our understanding of physiological mechanisms involved in drought resistance is increasing. This knowledge can be related to variability of responses among plant genotypes that may result in better selection procedures for characterizing drought resistance of plants.

My recent research has dealt specifically with grain sorghum (*Sorghum bicolor*) improvement in Nebraska with some comparisons to responses of maize (*Zea mays*) and pearl millet (*Pennisetum americanum*).

[1]Plant Physiologist, USDA, Agricultural Research Service, and Professor of Agronomy, University of Nebraska, Lincoln, Nebraska 68583.

According to Levitt (1972), drought resistance can be divided into drought avoidance and drought tolerance mechanisms, and I will use his terminology. Drought avoidance refers to a mechanism that permits a plant to live and produce under droughty conditions while maintaining high tissue water potentials, whereas drought tolerance occurs while the plant maintains low tissue water potentials (May and Milthorpe, 1962; Turner, 1979).

DROUGHT TOLERANCE

Drought and heat tolerance are jointly considered here because high temperatures often accompany droughts. The interactions of these stresses will be treated later.

We have evaluated cellular tolerance to desiccation and heat extensively by measuring electrolyte leakage from leaf discs that have been desiccated by bathing them in solutions of polyethylene glycol (Carbowax) (Sullivan and Ross, 1979) or by heating them in water baths (Sullivan, 1972; Sullivan and Ross, 1979). These treatments affect the stability of cellular membranes which control electrolyte leakage.

Significant differences have been shown in both desiccation and heat tolerance of sorghums via the leaf-disc method (Table 1). M.35-1 sorghum was previously found to have good heat and desiccation tolerance (Sullivan, 1972). Entry Tx406B was used to convert M.35-1 to shorter height. All other entries in Table 1 except RS 626 are selections from the first backcross to M.35-1. Line 4213 had significantly higher heat tolerance than M.35-1, but no derived line had greater desiccation tolerance. Desiccation and heat tolerance ratings also were made on 40 S_1 progenies from each of the sorghum populations NP3R, NP7BR, and NP9BR, via the leaf-disc method. NP9BR was selected under conditions of drought and high temperature stresses, and it had significantly greater heat tolerance than did the other two populations (Sullivan and Ross, 1979).

Good agreement between ratings from the leaf-disc procedure and other methods of evaluating stress tolerance has been shown. Visual estimates of drought injury made by Sullivan and Eastin (1974) on RS 626 and India hybrid CSH-1 agreed well with ratings from the leaf-disc method. Ninety percent of the plants of hybrid CHS-1 recovered from stress where leaf water potentials reached -33 bars, whereas no RS 626 plant recovered. Obviously, CSH-1 was much more desiccation tolerant than was RS 626.

Under conditions of reduced leaf water potential, photosynthesis by leaf sections of M.35-1 was maintained at higher levels than was that of RS 610 (Sullivan and Blum, 1970). Also, desiccation and heat tolerance ratings from leaf discs showed that M.35-1 had greater tolerances for both stresses. Among a broader set of sorghum lines, Sullivan and Eastin (1969) found good agreement between electrolyte leakage from leaf discs and stability of photosynthetic activity of chloroplasts from the same plants.

Also using the leaf-disc technique, Jordan and Monk (1980) measured the desiccation tolerances of 30 sorghum hybrids and their parents near anthesis. Leaf tissue, desiccated 8 hours in a solution of Carbowax 3000, showed an osmotic potential of -33 bars. The mean desiccation injury for all 30 hybrids was 43.8% (Table 2), with a range of 26.2 to 58.9% among all tests. Among females, A35-6 promoted the greatest desiccation tolerance (36.9% cellular damage) in its hybrids, whereas ATAM618 (51.1% damage) promoted the least. Among males, GPR148 gave hybrids with the greatest desiccation tolerance (34.1% damage), whereas 1790E (50.4% damage) and TX7000 (48.9% damage) contributed to hybrids that were most susceptible. These results show that selecting for increased heat and desiccation tolerance among sorghum lines is feasible.

The extent to which cellular stress tolerance of sorghum contributes to grain production is, however, yet to be demonstrated except for the report of Sullivan and Ross (1979) of one instance of a positive correlation between heat tolerance and yield. Intuitively, one would believe that plants with the inherent ability to endure and metabolically function under heat and drought stresses should produce greater yields than those that do not have this capability.

DROUGHT (CELLULAR DESICCATION) AVOIDANCE

Avoidance of cellular desiccation is an important factor in drought resistance of the major U. S. grain crops. When drought is severe, such mechanisms may become critical. Factors that contribute to drought avoidance are (a) an extensive and deep root system (certainly very important), (b) stomatal closure characteristics that decrease water loss, (c) leaf rolling which decreases exposed leaf area, and (d) epicuticular wax deposits that retard water losses. Osmotic adjustment is a form of desiccation avoidance (or tolerance at low leaf water potentials), since it acts to retain water in the cell and thus maintain cell turger.

IRRIGATION GRADIENT

Use of a line-source irrigation gradient system has aided drought resistance research in field experiments. Watts et al. (1979, 1980) modified the gradient irrigation system reported by Hanks, et al. (1974, 1976) by using two parallel sprinkler lines with the plots between the lines. When both lines are used simultaneously, the plots are uniformly irrigated, whereas when only one line is used, an irrigation gradient occurs.

Garrity et al. (1982a, 1982b) utilized this system to apply different irrigation treatments to sorghum at vegetative, reproductive, and grain filling stages. They applied gradient irrigation, designated a G treatment, and uniform

Table 1. Heat and desiccation tolerance values for grain sorghum lines averaged over June, July, and August (from Sullivan and Ross, 1979).

Sorghum line	% injury	
	Heat[a]	Desiccation[b]
4213	36.8 a[c]	67.3 bc[c]
4210	42.0 ab	63.6 abc
4196	44.3 ab	67.2 bc
4150	44.6 ab	64.1 bc
4146	45.2 ab	71.7 c
4104	46.1 b	62.5 ab
Tx406B	47.5 b	66.2 bc
4214	48.0 b	62.3 ab
M.35-1	48.5 b	55.5 a
4128	57.4 c	71.6 c
4184	59.6 c	72.1 c
RS 626	68.2 d	69.5 bc

[a]Heat treat for 15 minutes at 52C.
[b]Desiccated for about 16 hours in Carbowax 600.
[c]Values not followed by the same letter are significantly different at the 5% level of probability according to Duncan's multiple range test.

Table 2. Desiccation tolerances of 30 sorghum hybrids grown at Temple, Texas and measured near anthesis. Hybrids of the different male and female combinations are ranked from low (1) to high (6) tolerance within columns. Mean % cellular desiccation damage for each male and female are given at the side and bottom of the table (Jordan and Monk, 1980).

Male Parent	Female Parent					
	A35-6	A4R	ATAM618	Atx623	Atx5404	Mean (%)
1790E	1	1	1	2	3	50.4
Tx430	4	2	5	5	1	45.0
SCO170-6-17	5	5	4	4	4	40.7
Tx7078	3	3	3	3	5	43.9
Tx7000	2	4	2	1	2	48.9
GPR148	6	6	6	6	6	34.1
Mean (%)	39.6	42.7	51.1	44.0	41.8	43.8

irrigation, designated an I treatment, in various combinations during these three growth stages. For example, a GGG treatment received gradient irrigation during all three growth stages. A GGI treatment received gradient irrigation during the vegetative and reproductive stages and uniform irrigation during the grain filling stage, and an IIG treatment received uniform irrigation during the vegetative and reproductive stages and gradient irrigation during the grain filling stage, etc. These authors (Garrity et al, 1982b) corroborated the results of Stewart et al. (1975) and Inuyama et al. (1976) who found an approximately linear relationship between measured evapotranspiration (ET) and yield of grain and biomass. There were, however, differences in response among sorghum hybrids (Fig. 1), and different combinations produced different ET/yield relationships (Fig. 2). The lowest regression occurred when a gradient treatment was applied over the entire season (GGG treatment); i.e., stress increased as the season progressed. The slopes of response were steepest when stress occurred only during one period of growth; e.g., during the grainfilling stage in the IIG treatment. There was a stress conditioning response of the crops to increasing drought stress throughout the season. Water use efficiency decreased when the plants were drought stressed, but the decrease was least when the stress was gradually increased over the season (e.g., GGG treatment, Fig. 3). In a dissertation report about this work, Garrity (1980) concluded that the ability of the root systems to extract soil water at depths of 60 to 150 cm accounted for much of the difference in yield response between the GGG and IIG treatments. The roots from plants subjected to a drought stress that increased gradually during the season grew into the deeper layers of soil more readily than did those of plants that were not stressed prior to grain filling. Thus, although the IIG treatment started the grainfilling stage with soil water profile at field capacity, plants on the dry side of the GGG treatment extracted nearly as much water during grain filling (6.44 cm) as did those on the dry side of the IIG treatment (7.82 cm).

DEEP ROOTING

Genetic variability for root characteristics of sorghum has been reported by Bhan et al. (1973), Blum et al. (1977), Jordan et al. (1979), Jordan and Monk (1980), and Jordan and Miller (1980). Hurd (1964, 1974) improved drought resistance by selecting for increased depth of rooting in wheat (*Triticum aestivum*). Townley-Smith and Hurd (1979) discussed differences in rooting patterns associated with biomass and grain productivity. Jordan and Miller (1980) found that deep-rooted sorghums extracted soil water from deeper depths than did shallow-rooted ones, and Taylor (1980) concluded that increasing root depths held promise for eluding drought stress by soybeans (*Glycine max*) and cotton (*Gossypium hirsutum*).

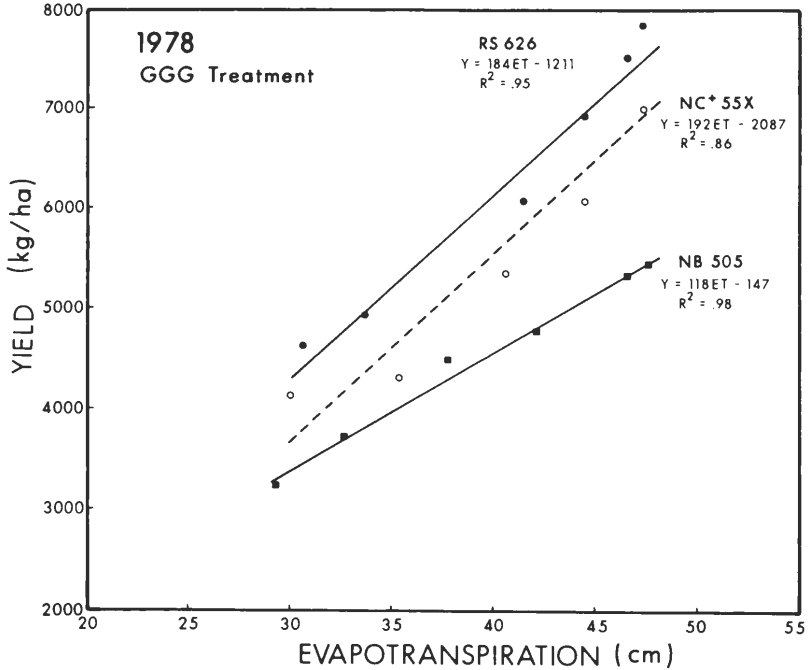

Figure 1. Relationships between evapotranspiration and grain yield for three sorghum hybrids grown on an irrigation gradient (from Garrity, et al., 1982b).

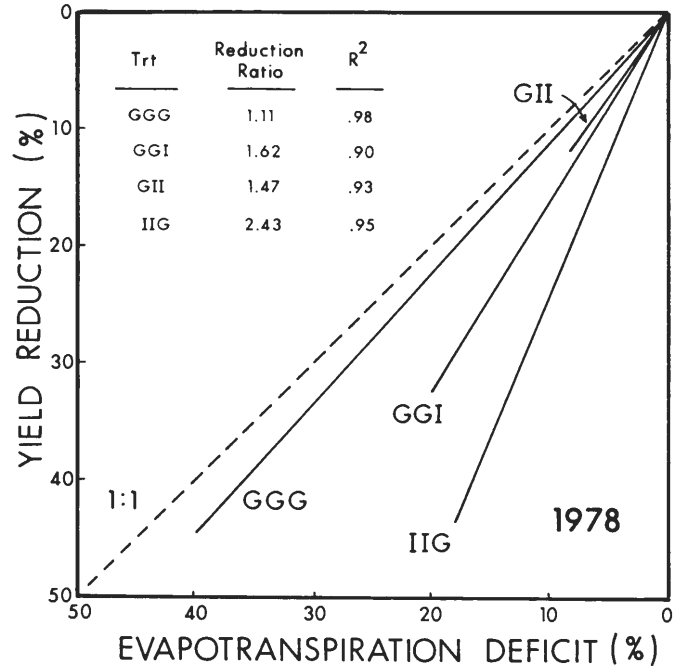

Figure 2. Relationships between evapotranspiration deficits and yield reductions for different irrigation treatments of sorghum hybrid RS 626. The Reduction Ratio is the ratio of % Yield Reduction / % Evapotranspiration Deficit (from Garrity, et al., 1982b).

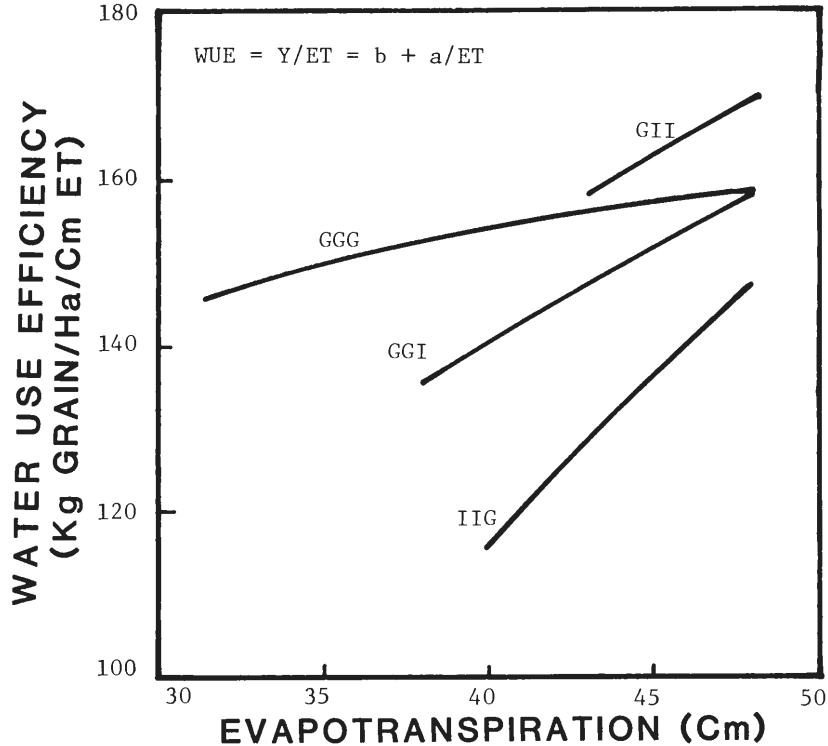

Figure 3. Relationships between water use efficiency (WUE) and evapotranspiration (ET) for different irrigation treatments of sorghum hybrid RS 626 (from Garrity, et al., 1982b).

Research on root characteristics has been inhibited because of the laborious nature of field investigations. Alternative procedures, however, have recently been developed. Sullivan and Ross (1979) grew sorghum utilizing hydroponic cultures in slender plastic tubes 10 cm in diameter and 1 m tall. Jordan et al. (1979), using a similar method to grow 53 sorghum cultivars, found that cultivars differed significantly for ratios of shoot to root, leaf area to root length, and root length to volume, but they did not relate these measurements to drought resistance. Nour and Weibel (1978) showed, however, that low shoot/root ratios of 10 sorghum genotypes they studied were associated with drought resistance.

The relation of these findings to drought stress in the field, however, is uncertain, and Jordan and Monk (1980) cautioned that deep rooting in the field may not occur unless extended drought occurs.

We have developed a technique to simulate drought stress of plants grown in hydroponic cultures (Sullivan and Ross, 1979, Bennett and Sullivan, 1979). Sorghum plants were grown in the slender tubes until near the bloom stage, when addition of nutrient solution to the tubes was discontinued for half of the plants. Transpiration lowers the level of water (nutrient solution) in the tubes, and progressively less of the total root system is in contact with it. In one experiment, root length in the nutrient declined for 22 days until only 12% of the maximum root length was in contact with nutrient solution. During this period, however, the roots became longer and heavier at the bottom compared to controls (Fig. 4).

Results given in Table 3 show that root weights for the control plants and from plants with only root tips in nutrient solution differed only by 12%, and the number of main roots did not noticeably differ. The lowered availability of moisture reduced plant height by 25% and the shoot/root ratio by 12%. But, even though only about 12% of the maximum root length remained in contact with water and nutrient, and these roots represented only 3% of the total weight of the root system, they supplied enough water and nutrients to support nearly normal plant growth.

Subsequently, water use was studied for plants with 10 to 20 cm of root tips in contact with nutrient solution and for those with entire root systems submerged. Nutrient solution was withheld completely for 17 days until ca 20-cm length of the root tips remained in contact with the nutrient. Water use was measured until the nutrient level declined to the point that only 10 cm of root length were in the nutrient solution. The nutrient level was then adjusted so that again 20 cm of root length were in the nutrient solution, and, finally, all tubes were filled so that the entire root systems were in the nutrient solution. Then water use was measured for a period of 7 days, after which the plants were harvested, shoot weight and leaf area were measured, and root segments were weighed. Plants were in the early boot stage when the experiment was begun, so all leaves were fully expanded during the time when water use

Figure 4. Root systems of grain sorghum grown in normal hydroponic culture on the left and with only the tip portion of the root system in contact with nutrient solution on right.

Table 3. Effects on grain sorghum of lowered nutrient level resulting in tips only of root systems in contact with nutrient solutions, compared to controls with entire root systems in contact with nutrient solutions.

Parameter	Control	Root tips only in nutrient
Shoot dry weight (g)	83.7	62.6
Plant height (cm)	118.0	88.0
Root dry weight (g)	13.6	12.0
Max. length of roots (cm)	51.4	65.6
Number of main roots	23.0	24.0
Shoot/root ratio (g/g)	6.2	5.2

was measured. Means of total leaf area of controls and plants grown with a lowered nutrient level were 29.7 and 23.3 dm^2, respectively, and mean maximum root lengths of controls and plants grown with a lowered nutrient level were 56.2 and 63.5 cm, respectively.

Water use (volume used per unit of leaf area) was affected by amount of root system in contact with nutrient. When ca 20 cm of root length were in contact with nutrient, water use was 62 to 64% of the control value. When ca 10 cm of root tips were in the nutrient solution, water use was 47% of control. The tip 10 cm of roots, which represented 19% of the total root dry weight, supplied ca 50% of the potential water use of these plants. The tip 20 cm of roots represented 39% of the total root dry weight and supplied over 60% of the potential water use. When the entire root system was again submerged, water use was 80% of the control, indicating that, in regard to water uptake, only a small loss of root function occurred during the desiccation process. Biomass production for plants grown with roots in the lowered nutrient level was 90% of that of the controls. Water use was reduced by 37 and 53% when only 19 and 39%, respectively, of the root dry matter were in the nutrient solution.

The root response just described presumably parallels what happens under drought conditions in the field. It thus is a method for evaluating the genetic potentials for roots to follow a receding level of water availability, for roots to recover from drought, and for water use efficiency. Currently, we are comparing root growth under natural dryland and irrigated field conditions with that of plants grown hydroponically in tubes.

Table 4. Grain yields and percentages of yield reduction when drought stressed (yield at 5% ET replacement level: yield at 95% ET replacement level) for 20 S_2 sorghum progenies of population NP9BR and cultivar Martin, grown in sandy soil with an irrigation gradient (Watts et al., 1980).

Genotype	ET Replacement (%)		Yield Reduction (%)
	95	5	
	Yield (Kg/Ha)		
121	6129 a*	2879 a	53
22	5985 a	1772 abcde	70
272	5625 ab	2690 ab	52
142	5349 abc	2787 a	48
279	4705 abcd	2005 abcde	57
145	4579 abcd	1908 abcde	58
30	4364 abcd	1487 abcde	66
251	4178 abcd	1135 cde	73
146	4113 abcd	1601 abcde	61
98	3877 abcd	1307 bcde	66
174	3792 abcd	909 de	76
9	3771 abcd	1753 abcde	54
126	3498 abcd	2513 abc	28
Martin	3492 abcd	1200 cde	66
117	3440 abcd	1119 cde	67
51	2918 bcd	1198 cde	59
226	2888 bcd	2294 abcd	21
103	2554 cd	697 e	73
76	2371 cd	843 e	64
160	2287 d	880 e	62

*Values not followed by a common letter in a column are significantly different at the 5% level.

SCREENING FOR DROUGHT RESISTANCE WITH THE IRRIGATION GRADIENT SYSTEM

The line-source irrigation gradient system was used by Watts et al. (1980) to screen drought tolerance of 20 S_2 progenies from population NP9BR (Ross, 1974) at the Sandhills Agricultural Laboratory, Tyron, Nebraska in 1977 and 1978. This site has a high probability of drought. Plots were irrigated ca weekly to replace the estimated ET during the previous week. Grain yields were recorded in four selections of each plot where ET replacement levels were 95, 65, 35, and 5%. Grain yields and percentages of yield reduction (i.e., yield at 5% ET replacement level: yield at 95% ET replacement level) due to drought stress for the 20 progenies and Martin cultivar (check) are presented in Table 4. The rankings and relative performances of the lines were similar over the two seasons, even though drought stress was less in 1977 than in 1978. Lines 121, 142, and 272, for example, were high yielding and stable to drought, whereas lines 103 and 160 were poor yielding and drought susceptible.

Changes in yield with increasing drought stress are shown for some of these selected sorghum lines and Martin cultivar in Figure 5. Martin gave stable yields from 95 to 35% ET replacement, but at 5% replacement, its yield dropped sharply. Line 22 had a high yield potential when well irrigated, but its yield declined rapidly under drought stress until productivity was reduced by 70% at 5% ET replacement. Garrity et al. (1982c) analyzed the yields of these lines across a series of nurseries characterized by varying drought stresses using the model of Eberhart and Russell (1966). They found that all lines showed linear responses. The slopes of the regression for the higher yielding lines were lower than those of the lower yielding lines. Thus, the drought responses of the lower yielding lines were reflected in greater relative yield reductions than the higher yielding lines.

The line-source irrigation system presents some difficulty with respect to statistical analyses, but it permits the evaluation of many drought stress treatments in close proximity to each other.

HIGH TEMPERATURE INTERACTIONS

Often, drought is accompanied by high atmospheric temperature and desiccating wind, in which situation leaf temperature may increase well above that of the ambient air (Tanner, 1963). In Nebraska, leaf temperature of sorghum measured in the field during drought has been found as high as temperature that caused a marked reduction in photosynthesis of leaf sections under controlled conditions (Sullivan and Ross, 1979). At this high heat stress (leaf temperatures of 42-43C), heads of the Redlan cultivar were blasted on the south facing sides of panicles (Sullivan, unpublished results). Redlan subsequently was found to be more susceptible to reduction of photosynthesis at 43C than several other genotypes measured (Sullivan et al., 1977).

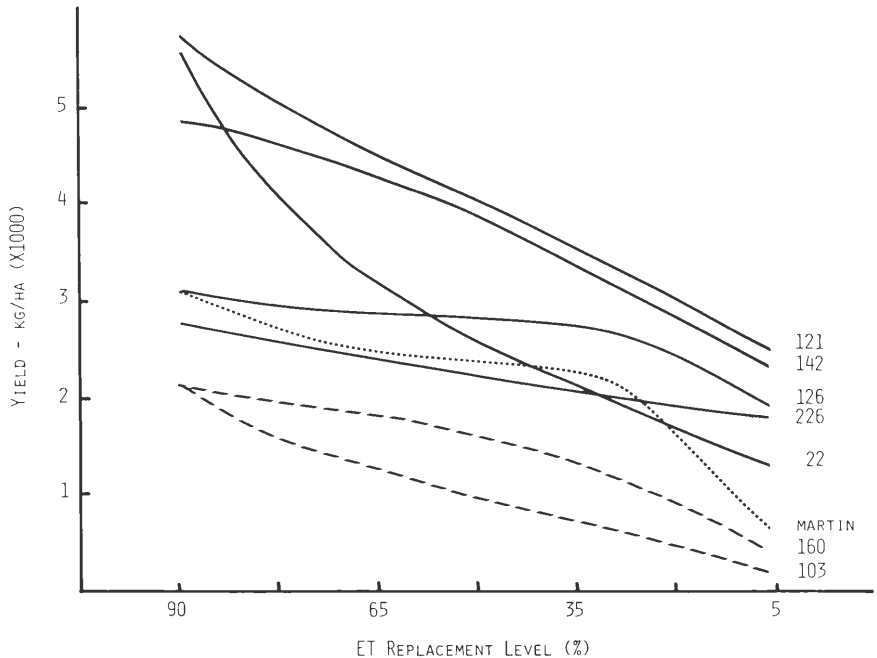

Figure 5. Yield responses across an irrigation gradient of S_2 sorghums selected from population NP9BR (from Watts et al., 1980).

OTHER MECHANISMS OF DROUGHT RESISTANCE

Stomatal closure, to reduce transpirational loss of water when plants experience water deficits, is a drought-resistance mechanism. Blum (1974) reported differences in stomatal response to water deficits for sorghum genotypes subjected to drought stress in the field. Henzell et al. (1975) found stomata of Shallu (*Sorghum bicolor*) were more sensitive to decreasing soil-water potentials than were those of sorghum M.35-1. However, selecting for stomatal response to drought has not greatly improved drought resistance of sorghum.

Leaf rolling, a visual symptom of drought stress, differs among genotypes of upland rice (*Oryza sativa*) (Chang et al., 1974). Maintenance of unrolled leaves during drought indicates drought-avoidance mechanisms.

Variations in leaf epicuticular wax may cause differences in drought resistance (Jordan and Monk, 1980), and proline accumulation during drought stress may be a factor in drought resistance of sorghums (Blum and Ebercon, 1976).

Numerous other physiological mechanisms associated with drought resistance have been reported; however, it is not clear how many of them are related to crop drought resistance.

CONCLUSIONS

Many mechanisms relate to drought resistance of plants, but no single one can account for drought resistance as observed in the field. The relative contributions of various mechanisms to the expression of drought resistance differ with specific conditions of drought occurrence; e.g., a specific drought-resistance mechanism may function only if the stress occurs during a specific stage of plant development.

One approach to utilizing known mechanisms of drought resistance in plant breeding is to select genotypes that are very different from the mean of the mechanism under study, and introduce them into random mating populations, with subsequent selection in naturally droughty environments. This should result in selection of lines with the mechanisms of drought resistance that are useful in the natural environments.

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BREEDING FOR DROUGHT RESISTANCE IN RANGE GRASSES[1]

K. H. Asay and D. A. Johnson[2]

ABSTRACT. Mechanisms conditioning resistance of plants to drought stress traditionally have been grouped into three categories: (1) escape, (2) maintenance of high water potential under stress (avoidance), and (3) tolerance of low tissue water potentials. Although substantial progress has been made in characterizing factors associated with drought resistance, relatively few effective screening procedures or selection criteria have been developed for breeding programs. Limited successes have primarily related to improving the capacity of the plant to escape or avoid the effects of drought. Development of cultivars that mature earlier and have more extensive root systems are notable examples. In laboratory trials conducted by the USDA-ARS at Logan, Utah, significant genetic variability was detected among crested wheatgrass [*Agropyron cristatum* (L.) Gaertn. and *A. desertorum* (Fisch. ex Link)] Schult. and Russian wildrye [*Psathyrostachys juncea* (Fisch.) Nevskij] progeny lines in seedling emergence under drought stress, and seedling recovery after exposure to drought. However, no consistent relationship was found between these two laboratory parameters and stand establishment under semiarid conditions in the field. Laboratory determination of seedling emergence from deep plantings and subsequent seedling vigor and seed size were much more closely related to field results. Until the plant responses to environmental stresses are better understood, final selections for drought resistance in range grasses should be based primarily on field performance.

Index Descriptors: drought stress; plant selection; genetic variability; *Agropyron; Psathyrostachys*; crested wheatgrass; Russian wildrye; seedling emergence; and seed size.

INTRODUCTION

Inadequate water is a more serious deterrent to the world's crop production than any other single factor (Kozlowski, 1968). Western rangelands in the U. S. are particularly susceptible in that substantial periods of water deficit are expected each year. In addition, poor grazing management has compounded the effects of drought, leaving many areas badly depleted of productive vegetation. The most viable means of reclaiming this valuable resource is the development of grasses and other forage plants that have been bred for improved resistance to drought and are better adapted to the multiple demands imposed on western range.

Breeding for drought resistance in forage plants has been reviewed by Carlson and Ditterline (1981), Johnson (1980), Johnson et al. (1981), and

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[1] Cooperative investigations of the USDA-ARS and the Utah Agricultural Experiment Station, Logan, Utah 84322. Approved as Journal Paper No. 2763.

[2] Research Geneticist and Plant Physiologist, respectively, USDA-ARS, Utah State University, UMC 63, Logan, Utah 84322.

Wright (1975). Similarly, breeding to alter plant responses to drought was reviewed by Blum (1979) and Sullivan and Ross (1979) for grain sorghum, *Sorghum bicolor* (L.) Moench; by Hurd (1976) and Townley-Smith and Hurd (1979) for wheat, *Triticum aestivum* L.; and by O'Toole and Chang (1979) for rice, *Oryza sativa* L. Breeding research to improve the drought resistance of range forages, particularly range grasses, has received comparatively little attention.

MECHANISMS OF DROUGHT RESISTANCE

Drought resistance has been defined as the ability of a plant to grow and yield satisfactorily in areas subjected to periodic water deficits or, in ecological terms, the ability of a plant to survive when its water supply is limited (Turner, 1979). Mechanisms conditioning resistance of plants to drought stress have been reviewed by several workers (Blum, 1979; Carlson and Ditterline, 1981; Hall et al., 1979; Hurd, 1976; Jones, 1979; Levitt, 1980; May and Milthorpe, 1962; Moss et al., 1974; Parsons, 1979; Townley-Smith and Hurd, 1979; Turner, 1979; and Wright, 1971. In their reviews, May and Milthorpe (1962) and Turner (1979) considered drought as a meteorological term or a period of deficient water supply in contrast to a plant water deficit (Levitt, 1980). They described three mechanisms that condition the resistance of plants to drought stress:

1. *Drought escape*—the capacity of a plant to complete its life cycle before water stress becomes a serious limiting factor. This is accomplished through rapid phenological development or developmental plasticity (Turner, 1979).
2. *Drought endurance with high internal water content or tissue water potential*—the ability of a plant to maintain a high water status during drought periods. Levitt (1980) described this mechanism as drought avoidance because the effect of drought is excluded from the plant tissues. A high water potential is maintained by: (a) the reduction of water loss by way of stomatal or cuticular resistance, reduced absorption of radiation, or reduced leaf area; and (b) efficient root systems that maintain water extraction from the soil.
3. *Drought tolerance at low water potential*—the ability of the plant to: (a) maintain turgor through osmotic adjustment, increased elasticity, or decreased cell size, and (b) endure and recover from desiccation of its protoplasm.

Most species rely on a combination of escape, avoidance, and tolerance mechanisms at some point in their life cycle to resist drought. Improved range

plants must be particularly flexible to survive periods of severe drought stress and to recover and actively grow during periods when moisture is at more optimum levels (Johnson et al., 1981). Because most range grasses are perennial, mechanisms conditioning survival during stress after annual plants have completed their life cycle are critical adaptations. Survival characters including dormancy often function at the expense of growth rate or forage yield (Bailey, 1940; Mueller and Weaver, 1942).

Drought resistance by maintaining a high internal water content is considered a more powerful mechanism of drought resistance than tolerance (May and Milthorpe, 1962; Wilson et al., 1976). Turner (1979) suggested that shortening the life cycle has done more to improve the productivity of wheat under water-limited environments than any other breeding objective. Derera et al. (1969) found a consistent correlation between grain yield of 15 wheat cultivars and earliness under drought. Their data indicated that from 40 to 90% of the variation among genotypes in drought resistance could be attributed to earliness. However, as Turner (1979) pointed out, early maturity could be a disadvantage in a crop species when grown under more optimum moisture environments. Also, early cultivars would have to be adapted to cooler temperatures.

The advantages of an extensively developed root system in maintaining a high water potential under moisture stress are well documented (Carlson and Ditterline, 1981; Detera et al., 1969; Hurd, 1968, 1971; Hyder, 1974; Salim and Schlehuber, 1965; Sullivan and Ross, 1979; and Wilson et al., 1976). As much as 90% of the total plant dry matter is in the form of roots in some species (Fisher and Turner, 1978; Turner, 1979). Root biomass is associated with both root density and length. Both factors are undoubtedly important, but little information is available regarding the optimum structure of a root system (Carlson and Ditterline, 1981; Turner, 1979). It has been suggested (Johnson, 1980) that root growth at the expense of vegetative development may actually be a disadvantage in some forage situations, particularly when drought is not a limiting factor.

Morphogenesis of the root system can be a major factor in the establishment of grass seedlings on arid and semiarid range sites (Hyder, 1974; Tischler, 1980). The grass root system consists of seminal and adventitious roots. Because the seminal roots are usually short-lived and have limited water extraction capabilities, ultimate establishment of the seedling is dependent on the rate and extent to which the adventitious roots are developed. In most cool-season grasses, the coleoptile elongates during seedling emergence. The coleoptilar node, from which the adventitious roots originate, remains essentially at the planting depth where moisture and other microclimatic effects are less variable than at the soil surface. In contrast, the subcoleoptile internode elongates during seedling emergence in most warm-season grasses. This elevates the coleoptile to or near the soil surface where development of the adventitious

roots is often delayed by inadequate moisture and cool temperatures. Until the adventitious roots are functional, moisture must be supplied to the seedlings by the seminal roots through the subcoleoptile internode. This has been a serious limiting factor in the establishment of blue grama [*Bouteloua gracilis* (Willd. ex H.B.K.) Lag. ex Griffiths] in the Central Great Plains. Seedlings often die 6 to 10 weeks after emergence because the adventitious roots fail to develop and insufficient moisture is supplied to the expanding leaves by the seminal roots (Wilson, 1981; Wilson and Briske, 1979; Wilson et al., 1976).

Jones (1979) discussed the role of stomatal characteristics to regulate the loss of water from plant tissues, especially during intermittent and unpredictable periods of drought. Although stomatal closure can impede photosynthetic rate, changes in aperture of the stomata are reversible, permitting high conductivity during optimum conditions and more efficient utilization of water during drought. Heritability of stomatal characters including size and frequency per unit leaf area are of sufficient magnitude in a number of species to permit progress through breeding (Jones, 1979). Other avoidance mechanisms such as thick cuticles, pubescence, and decreased leaf area act to maintain the water status of plant tissues (Kramer, 1959; Ehleringer, 1980; Chatterton et al., 1975; Turner, 1979).

Several reviews have appeared of plant mechanisms that condition drought resistance at low tissue water potentials (Levitt, 1980; Sullivan and Eastin, 1974; Turner, 1979). Turner (1979) discussed the importance of distinguishing between mechanisms in which adaptation to lower water potentials are maintained without marked sacrifices in productivity and those primarily involved with survival. Growth processes such as leaf expansion are dependent on the plant's ability to maintain turgor at low water potentials. This is accomplished by increased elasticity or smaller cells and by lowering the osmotic potential of the cellular protoplasm (Quarrie and Jones, 1977).

Although much remains to be learned about desiccation tolerance, it is known that plants of most species can endure very low water potentials at some stages during their life cycle (Turner, 1979). Several mechanisms have been suggested that may protect the protoplasm from mechanical and physiological effects of dehydration. Protoplasmic proteins are considered resistant to denaturation, coagulation, or hydrolysis in drought-tolerant plants (Gates 1964; Sullivan and Eastin, 1974).

Permeability of the cellular membranes, viscosity of the protoplasm, and small cell size also have been related to desiccation tolerance (Lee-Stadelmann and Stadelmann, 1976; Levitt, 1956, 1980). It is well established that mechanisms conditioning tolerance to desiccation are affected by pretreatment or degree of hardening. Consequently, several cycles of stress may be necessary before plants can realistically be evaluated for their tolerance to desiccation.

SCREENING PROCEDURES

The recent acceleration of research into mechanisms of drought tolerance has failed to generate a substantial number of successful screening or breeding procedures. It has become increasingly evident that resistance of plants to drought is governed by complex interactions among several factors. Consequently, effective screening procedures must be based on plant response or a combination of plant characters.

Seedling Emergence

On western range, drought stress is of particular concern during the seedling development phase. Not only are seedlings more susceptible to drought than established plants, but seedlings must develop rapidly to take advantage of moisture during the spring and fall when moisture conditions are usually most optimum. Wright and co-workers have successfully used artifically controlled environments to screen large populations of warm-season grasses for seedling drought resistance (Wright, 1964, 1971, 1975; Wright and Brauen, 1971; Wright and Jordan, 1970).

Osmotic solutions including mannitol, polyethylene glycol, and sodium chloride have been extensively used to artifically impose drought stress on germinating seeds and young seedlings. However, direct contact with the osmotica may have toxic effects on seedling development independent of water potential. These confounding effects are serious considerations in the interpretation of results from screening trials (Carlson and Ditterline, 1981; Johnson, 1980). In light of these limitations, Johnson and Asay (1978) modified a procedure proposed by Kaufmann (1969) to screen 120 crested wheatgrass [*Agropyron cristatum* (L.) Gaertn. and *A. desertorum* (Fisch. ex Link) Schult.] and 134 Russian wildrye [*Psathyrostachys juncea* (Fisch.) Nevski] progeny lines for seedling emergence under drought stress. Soil in germination vessels was separated from a polyethylene glycol-6000 (PEG-6000) osmoticum with a cellulose acetate membrane. The movement of water across the semipermeable membrane into the soil was controlled by the water potential of the PEG-6000 solution. After soil had equilibrated to the desired moisture level, seeds were uniformly distributed over the soil surface and covered with approximately 0.5 cm of air-dry soil. Vessels were then sealed and transferred to an incubator at 25 C with no light. Trials were replicated from three to four times and emergence counts were made after 7 to 11 days.

The procedure effectively detected significant ($P < 0.01$) differences among progeny lines in both species and under both stress levels (Table 1). It was somewhat discouraging that relative differences among progenies were not consistent over the two stress levels as indicated by the highly significant progeny X stress level interaction and low correlation between the two stress

Table 1. Seedling emergence data from 120 crested wheatgrass and 134 Russian wildrye progeny lines under two levels of moisture stress in the laboratory.[a]

	Crested Wheatgrass			Russian Wildrye		
	Soil Moist. Pot. (MPa)		Pooled	Soil Moist. Pot. (MPa)		Pooled
Parameter	-0.35	-0.55	Data	-0.58	-0.84	Data
Emergence (%)						
Max.	94.6	87.8	91.2	99.0	78.0	85.5
Min.	14.2	1.1	13.5	71.0	14.0	45.4
Mean	58.7	37.3	48.0	88.8	40.8	64.8
$S\bar{x}$	6.7	8.6	5.4	4.0	9.8	5.3
Significance level						
Progenies (P)	**	**	**	**	**	**
Stress Level (S)	—	—	**	—	—	**
PxS	—	—	**	—	—	**
Gen. Var. (%)	87	83	51	51	61	63

**Significant at 0.01 level of probability.

[a] From Asay and Johnson (1980) and Johnson and Asay (1978).

levels (r = 0.14 and 0.40 for Russian wildrye and crested wheatgrass, respectively). In all cases, well over 50% of the phenotypic variation among progeny lines was due to genetic differences. This, along with the magnitude of ranges, suggests that emergence under drought can be altered through selection. However, the inconsistency of differences over stress levels indicates that selection should be made over the range of moisture regimes to which the resultant germplasm is most likely to be subjected.

Seedling Recovery After Exposure to Drought

Asay and Johnson (1980, in press) used procedures similar to those proposed by Wright (1964) to screen crested wheatgrass and Russian wildrye progenies for seedling recovery after exposure to drought stress. Seedlings were established in cone-shaped plastic containers with a 3.8-cm diameter top tapering over the 21 cm length to a 2.5 cm bottom. After three weeks, the containers were transferred from the greenhouse to a growth chamber programmed to provide a 12-hr daylength, 30/10 C day/night temperature, 20/60%

day/night relative humidity, and 900 $\mu E/m^2$/sec of irradiation. The experimental design varied somewhat over the course of the trials. In general, plots (which consisted of seven containers each) were arranged as randomized complete blocks from three to four replications. After a 7-day equilibration period, water was withheld for 17 days. The plants were then returned to the greenhouse and watered daily for three weeks. Each group of seven containers was then rated for degree of recovery by three independent observers using a scale of 1 to 9 (1 = no living plants and 9 = maximum recovery).

The crested wheatgrass progeny lines differed significantly ($P < 0.01$) in seedling recovery after drought in three of the four trials conducted (Table 2). Where significant differences were obtained, the genetic variance comprised over 50% of the phenotypic variance among progenies. It is evident that sufficient genetic variability is available in our breeding population of crested wheatgrass for selection. In contrast, differences in seedling recovery after drought among the Russian wildrye progenies were not significant. The small genetic variance and relatively high experimental error were probably influenced by the timing of seedling removal from the growth chamber. A critical period of just a few hours near the end of the stress period could mean the difference between essentially complete survival and total mortality of the stressed seedlings. This period, in which differential survival can be obtained, may be shorter for Russian wildrye than for crested wheatgrass. Also, to compensate for temperature variation within the chamber, trays of plants should be removed individually over a period of a day or more. In future trials, we propose that plants of a check entry be included in each tray and that removal of the tray be determined by the appearance of the check.

Seedling Emergence from Deep Seedlings

Lawrence (1963) concluded that breeding for improved seedling vigor in Russian wildrye could best be accomplished by first selecting for large seeded lines followed by evaluation of seedling emergence from deep seedings in the greenhouse or field. These procedures were applied in the development of 'Swift,' an improved cultivar recently released from that research program (Lawrence, 1979). Asay and Johnson (1980) screened their breeding population of Russian wildrye for rate of emergence from a 7.6-cm planting depth and subsequent seedling vigor. Significant differences ($P < 0.01$) were found among the breeding lines in all attributes studied (Table 3) suggesting that excellent opportunities are available for improving seedling emergence and vigor under stress imposed by excessive planting depth.

Field Screening Trials

The merit of laboratory screening procedures must ultimately be based on comparisons with actual field data. Accordingly, Asay and Johnson (1980,

Table 2. Seedling recovery of 118 crested wheatgrass and 144 Russian wildrye progeny lines after exposure to drought in the laboratory.

Parameter	Crested Wheatgrass[a] Trial 1	Trial 2	Trial 3	Trial 4	Russian Wildrye
	Ratings				
Max.	7.2	7.2	7.2	6.3	6.7
Min.	1.0	0.8	0.2	0.0	0.8
Mean	3.1	4.4	3.7	3.0	3.1
$S\bar{x}$	0.9	1.0	1.4	1.1	1.5
Sign. Level	**	**	N.S.	**	N.S.
Gen. Var. (%)	66	54	12	59	11

**Significant at 0.01 level of probability.

[a] Each crested wheatgrass trial consisted of a different set of entries replicated four times. All 144 Russian wildrye entries were included in each of four trials (trials = replications).

Table 3. Seedling emergence and vigor of 134 Russian wildrye progeny lines using a 7.6 cm planting depth in the laboratory.[a]

Parameter	Emergence 10-day (%)	10-day ht (cm)	DM Yield mg/plot
Max.	32.0	6.7	118
Min.	0.5	0.5	1
Mean	12.2	3.8	31
$S\bar{x}$	3.7	0.9	11.1
Sign. Level	**	**	**
Gen. Var. (%)	66	52	70

** Significant at 0.01 level of probability.

[a] From Asay and Johnson (1980).

in press) conducted field studies to evaluate the stand establishment characteristics of the same crested wheatgrass and Russian wildrye breeding lines included in their laboratory trials. The crested wheatgrass breeding lines were evaluated at two locations, Site 1 in northwestern Utah and Site 2 in south central Idaho. These areas, which were selected to represent typical semiarid range environments, receive an average annual precipitation of 24.4 cm (Site 1) and 28.5 cm (Site 2). Soil in both areas is silty loam of the aridisol order. All plots were seeded during early November so that germination did not occur until the following spring. Plots consisted of a 3-m row in which 500 seeds were uniformly distributed at a 3.5 cm depth. Plots were arranged in a randomized complete block with from two to four replications. Seedling emergence, seedling vigor, and fall stand were determined from the center 1.8 m section of the plot.

As in the laboratory trials, significant genetic differences were found for most characters associated with stand establishment in the field (Tables 4 and 5). The genetic variance accounted for approximately 50% of the phenotypic variance in most instances. Although some consideration must be given to the genotype X location interactions, it appears that ample opportunities exist to improve stand establishment of the two breeding populations on the basis of screening trials conducted in the field.

Laboratory-field Correlations

In general, Asay and Johnson (1980; in press) found a low correlation between laboratory and field determinations of seedling characteristics associated with stand establishment (Table 6). The poor correlation between seedling emergence under drought stress in the laboratory and emergence in the field was a major disappointment. However, this poor relationship may be related to moisture conditions in the field at the time of seedling emergence. Field plantings were made in the fall and soil moisture did not become limiting until after emergence. Under these conditions, emergence from a deep seeding or seedling vigor per se with no attempt to impose drought stress would give a more realistic prediction of field emergence. The correlations involving the Russian wildrye data would support this approach (Table 6). It is noteworthy that seed weight was significantly ($P < 0.01$) and positively correlated with all characters measured in the field for crested wheatgrass ($r = 0.46$ to 0.57, 118 d.f.) and with data from laboratory trials involving deep seedings with Russian wildrye ($r = 0.47$ to 0.57, 118 d.f.).

These data suggest that seed wieght would be a useful criterion in a crested wheatgrass or Russian wildrye breeding program to develop germplasm with improved seedling establishment characteristics. Screening in the laboratory for emergence under drought stress and seedling recovery after exposure to drought would have questionable merit. Seedling emergence from deep seedings

Table 4. Seedling vigor and stand establishment of crested wheatgrass progeny lines on two range sites.[a]

Location	Parameter	May		July	October
		Seedling Emergence	Seedling Ht.	DM Yield	Stand
		No./plot	cm	g/plot	%
1	Max.	82.0	5.0	7.6	87.7
	Min.	13.3	1.7	0.1	40.7
	Mean	43.2	3.6	2.0	71.9
	$S\bar{x}$	9.0	0.4	1.1	6.5
	Sign. Level	**	**	**	**
	Gen. Var. (%)	46	62	48	37
2	Max.	70.0	10.0	31.3	94.5
	Min.	9.0	2.0	1.3	44.0
	Mean	32.7	5.6	10.7	69.6
	$S\bar{x}$	8.9	1.0	4.3	8.2
	Sign. Level	**	**	*	N.S.
	Gen. Var. (%)	36	49	27	16
Pooled	Max.	66.4	6.6	14.8	86.8
Data	Min.	12.2	1.8	0.5	43.6
	Mean	39.0	4.4	5.5	71.0
	$S\bar{x}$	6.5	0.5	1.7	5.1
	Sign. Level				
	Prog. (P)	**	**	**	**
	Loc. (L)	**	**	**	**
	PxL	N.S.	**	**	N.S.
	Gen. Var. (%)	52	70	53	44

*,**Significant at 0.05 and 0.01 probability levels, respectively.

[a]From Asay and Johnson (in press).

Table 5. Seedling vigor and stand establishment of Russian wildrye progeny lines in the field.[a]

Parameter	May		August	October
	Seedling Emergence	Seedling Height	DM Yield	Stand
	No./Plot	cm	g/plot	%
Max.	120	14.3	30.5	86
Min.	8	8.5	1.5	42
Mean	63.2	10.7	10.9	70.4
$S\bar{x}$	8.7	0.8	4.1	6.0
Sign. Level	**	**	**	**
Gen. Var. (%)	56	56	45	35

**Significant at 0.01 level of probability.

[a] From Asay and Johnson (1980).

Table 6. Correlations (r) between laboratory and field determinations of seedling vigor in crested wheatgrass and Russian wildrye.[a]

Field Criteria	7.6 cm planting		Emerg. under drought[b]		Seedling Recovery
	Emerg.	Dry Wt.	Mod. Stress	High Stress	
			———Crested Wheatgrass———		
Emergence (May)	—	—	-0.02	-0.08	0.05
Dry Wt. (July)	—	—	-0.23*	0.12	0.15
% Stand (Oct.)	—	—	-0.01	-0.04	0.22*
			———Russian Wildrye———		
Emergence (May)	0.24**	0.23**	0.14	0.07	0.01
Dry Wt. (Aug.)	0.33**	0.22**	0.00	-0.15	-0.09
% Stand (Oct.)	0.21*	0.18*	-0.05	0.01	-0.02

*,** Significant at 0.05 and 0.01 probability levels, respectively.

[a] From Asay and Johnson (1980; in press).

[b] Moderate Stress = -0.35 MPa for crested wheatgrass and -0.58 MPa for Russian wildrye. High Stress = -0.55 MPa for crested wheatgrass and -0.84 MPa for Russian wildrye.

in the laboratory demonstrated promise as a selection criterion in Russian wildrye and should be evaluated in other range grasses. It would appear, however, that until a better understanding of plant responses to environmental stresses is developed, final decisions should be based on seedling establishment under semiarid conditions in the field.

Root Development

Kittock and Patterson (1959) measured the relative root penetration of 10 range grass species in 5 X 122-cm pyrex tubes that had been uniformly filled with fine unwashed sand. Root penetration after 7 weeks in the tubes was positively correlated ($r = 0.87$) with survival data of the range grasses in a spring seeded trial under range conditions. Rate of growth during the later weeks of the trial was most closely related to seedling survival. They concluded that evaluation of root penetration in pyrex tubes would be a valuable procedure to estimate survival of species in range areas where most of the precipitation occurs during the winter.

A fast-growing extensive root system has been associated with drought resistance in wheat (Hurd, 1968), and root characteristics were of concern in the development of the cultivar 'Wascana' (Hurd et al., 1972). Jordan and Miller (1980) discussed the relationship between root development and drought resistance and the genetic potential of breeding for a more efficient root system in sorghum. Sullivan and Ross (1979) described procedures used in their research program to screen breeding lines and individual plants of sorghum for root development. Plants grown in hydroponic culture were evaluated for main root numbers, secondary root branching, maximum root length, root volume, dry weight, root to shoot ratio, and possibly root activity. Hydroponic culture allowed them to accurately control mineral nutrition and water stress by the addition of carbowax 600. They did not detect any uptake of the carbowax or secondary effects from the direct contact of the roots with the solution. Sullivan and Ross considered the hydroponic system to be superior to the evaluation of seedling responses to drought in pots or flats because the level of stress was easier to control and conditions were more uniform near the individual plant roots.

Other Screening Procedures

Johnson and Brown (1977) measured the turgor pressure of five grass species over a range of decreasing levels of leaf water potential. They found significant differences among the grasses in the regression of turgor pressure on leaf water potential and turgor pressure at zero water potential. Differences among species were related to variation expected on the basis of field performance. However, because of the time required for the assay and unexplained

variability, turgor evaluation by psychrometry did not appear to be of immediate value as a breeding tool for detecting the genetic variability within species.

Other criteria proposed to screen plants for resistance to drought include: chlorophyl stability (Kaloyereas, 1958), desiccation and heat tolerance of leaf discs, photosynthetic rate of stressed plants (Sullivan and Ross, 1979), and proline content (Singh et al., 1972).

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MECHANISMS FOR SALINITY TOLERANCE IN PLANTS[1]

Suzan J. Stavarek and D. William Rains[2]

ABSTRACT. Salinity poses a major threat to agriculture and may be the ultimate limitation to irrigated farming. Salinity can be managed by either physical manipulation of the environment (leaching, drainage, water quality) or genetic manipulation of plants to enhance biological tolerance to salinity. A combination of these two approaches will provide an integrated method of adapting the best crop to the best agronomic procedures as attempts are made to extend crop production into saline environments.

Biological management of plants involves selection of plants with genetic traits related to salt tolerance. Identification of specific characteristics related to salt tolerance will provide biological markers useful in selecting salt tolerant crops.

Salinity exposes plants to an environment with toxic levels of ions and with reduced water availability. A number of mechanisms are related to these stresses. Plants exclude ions and selectively absorb ions to regulate internal ionic environment. Sodium chloride selected plant cells were found to maintain higher levels of intracellular potassium than nonselected plant cells, when the two populations were exposed to saline environments. This maintenance of intracellular potassium was related to the ability of the selected cells to take up potassium at high levels of osmolality in the media. The nonselected cells showed decreasing potassium uptake with increasing osmolality. Water availability of plant tissues is regulated by osmotic adjustment through organic and inorganic osmoticum. Energy costs related to adjustment to stress may also influence adaptation of plants to salinity.

Preliminary investigations demonstrated a higher energy efficiency of salt selected cells than nonselected cells when these populations were exposed to increasing levels of salinity.

Index Descriptors: cell culture, alfalfa, salt tolerance, and salinity.

INTRODUCTION

Accumulation of high levels of salt affect a significant portion of the terrestrial environment. Besides the typically saline nature of the major deserts, there are about 400 million hectares of salt-affected land in the world. Losses of land to salts accumulated through irrigated agriculture are estimated to amount to at least several tens of thousands hectares a year (Flowers et al., 1977). There are 2.2 million hectares of saline soil in the United States, and one-fourth of the irrigated acreage is salt-affected. In California one-fifth of the irrigated agricultural land (approximately 360 thousand hectares) is affected to some extent by salt (Croughan and Rains, 1982).

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[1] Research support in part from a grant from California Crop Improvement Association.

[2] Staff Research Associate and Professor, Department of Agronomy and Range Science, University of California, Davis, California 95616, respectively.

Salinity thus may eventually become the ultimate limitation to irrigated agriculture (Rains, 1979). Such a possibility is based on both the deleterious effects of sodium salts on the physical structure of soils and on the chemical toxicity of high levels of salts on biological systems, particularly those of higher plants.

The impact of salinity on plant productivity can be reduced by physical manipulation of the environment in which the plant grows or (and) by biologically manipulating the plant (Epstein, 1980; Rains, 1981). Physical manipulation of the soil environment entails procedures that reduce the level of salt in the soil, leaching salts from the soil, or altering the level of salts in the irrigation water. Biological manipulation of the plant involves identification of plant genotypes capable of increased tolerance to salt and incorporation of such traits into economically useful crop plants. Epstein and Norlyn (1977) and Norlyn (1980) discussed the current success of breeding for salt tolerant plants using whole plant breeding techniques. Another source of potential germplasm for development of salt tolerant plants is through the use of cell culture techniques. By selecting for cells with increased tolerance to salt, and regeneration of plants from these cells, new germplasm of salt tolerant plants would be developed.

Understanding of the mechanisms by which plants and plant cells adjust to a saline environment is important to all programs attempting to establish salt tolerant crop species. The identification of specific characteristics related to salt tolerance will provide potential biological markers useful in the identification of salt tolerant plants and plant cells.

REVIEW OF LITERATURE

This section reviews information on mechanisms related to salt tolerance and provides current data on selected processes.

Mechanisms of Salt Tolerance

Plants in nature have evolved several adaptive mechanisms to cope with the presence of salt in their environment (Rains, 1979). Three strategies are possible and have been identified among plants growing in saline environments: (1) avoidance, (2) exclusion, and (3) physiological tolerance, involving compartmentation and osmotic adjustment using inorganic and organic constituent.

Avoidance of Salinity

Little or no evidence supports avoidance as a significant mechanism of salt tolerance for crop plants. Basically, it allows a glycophyte, by growing at a

particular time and/or place, to survive where one would normally expect only halophytes.

Exclusion of Salts

Exclusion of salts in plants can either occur at a whole plant or cellular level. In some plants, a low internal salt concentration is maintained despite high levels present in the external environment. Plants may accomplish this via a low permeability of external toxic salts in the roots or by actively extruding entering salts back into the external environment. Plants can also extrude salts that reach the shoot through the use of salt glands located on the surface of leaves.

Exclusion at the cellular level can mean either not permitting ions into the cell in the first place or pumping them out once they are in. Rains (1972) pointed out that "outpumps" would be energetically more expensive than simple exclusion at the plasmalemma. Exclusion is then seen as preferential absorption of K^+, for example, over Na^+, and salt tolerance could indicate higher than usual K^+ specificity for the transport mechanism. Nevertheless, Na^+ outpumps have been postulated (Pitman and Sadler, 1967; Jennings, 1968; and Etherton, 1967). Pierce and Higinbotham (1970) found that Na^+ was pumped out of the plasmalemma and suggested that the plasmalemma was the site of ion selectivity. Rains (1972) and Epstein (1980) supported this by giving evidence that halophytes show greater affinity for K^+ at high Na^+ concentrations than do glycophytes. Exclusion of salts, either directly or by outpumps, would certainly be an energetically expensive proposition for a cell bathed in sea water.

Physiological Tolerance

The most significant mechanism for dealing with salt entails actual physiological tolerance by the plant to high levels of salt within the tissue. This "true tolerance" has been found to provide the greatest tolerance of salt and is characteristic of many plants inhabiting extremely saline environments. Possible mechanisms of osmoregulation used by cells in saline environments have been reviewed (Rains, 1972; Hellebust, 1976; Flowers et al., 1977; Greenway and Munns, 1980). It is apparent from these discussions that plant cells exposed to saline environments must contend with three basic problems: (1) maintaining favorable water relations, (2) coping with potentially toxic ions, and (3) obtaining the required nutrient ions despite the predominance of other ions in the external media. The plant cells must be able to cope with these problems efficiently so growth can be maintained. Following are plant processes which have been proposed as mechanisms which enhance salt tolerance.

Organic osmotica. To maintain favorable water relations despite high levels of osmotically active ions within the soil solution, the water potential within the plant must be maintained at a more negative value than that of the external environment. To accomplish this, the plant may produce its own organic osmotica (e.g., organic and amino acids) or accumulate ions from the external medium, thereby acquiring an internal concentration of osmotically active solutes sufficient to maintain water flow into the plant (Rains, 1972; Flowers et al., 1977; Greenway and Munns, 1980). The production or absorption of sufficient osmotica is metabolically expensive and is potentially limiting the plant by consuming significant quantities of carbon that could otherwise be used for growth (Rains, 1979).

Rains et al. (1980) listed several organic compounds, for example, organic acids, nitrogen compounds, and carbohydrates, found in microorganisms and higher plants that were used for osmoregulation in response to increasing osmotic stress. Jefferies (1980) recently discussed the role of organic solutes in osmoregulation in halophytic higher plants. He suggested that certain organic solutes were selected which generate less demand on limited resources such as carbon or nitrogen.

Osmond (1967) believed that oxalate production in *Atriplex* is a response to excess cation accumulation in the vacuole and determined that oxalate production was sufficient to account for 75% of the necessary charge balance.

Numerous workers have reported correlations between altered nitrogen metabolism and increased salt tolerance when plants are exposed to salt. Flowers and Hall (1978) found that the halophyte *Suaeda maritima* would accumulate glycinebetaine while under salt stress. Wyn Jones (1980) discussed evidence showing that glycinebetaine was located in the cytoplasm (Hall et al. 1978) and that it is nontoxic at high concentations to cytoplasmic functions (Pollard and Wyn Jones, 1979). He believed these data support the hypothesis that glycinebetaine can act as nontoxic cytosolic osmotica. Storey and Wyn Jones (1979) suggested that *Atriplex spongiosa* and *Suaeda monoica* utilized glycinebetaine with K^+ salts as major cytoplasmic osmoticum and Na^+ salts as vacuolar osmotica.

Ben-Amotz and Grunwald (1981) found that glycerol was used for osmoregulation in the halotolerant alga *Asteromonas gracilis*, while Kauss (1981) observed that *Poterioochromonas malhamensis* accumlates isofloridoside.

Steward and Lee (1974) found proline to be the most abundant amino acid in halophytes, accounting for as much as 30% of the total amino acid pool. Because this was true only for halophytes, it follows that proline accumulation may be an adaptive response to salinity. Hanson et al. (1979), examining the role of proline in osmoregulation in barley (*Hordeum vulgare*), found the exposure of plants to environmental stress such as drought resulted in an increase in proline levels. When barley varieties with different tolerances to drought were compared, however, it was not apparent that proline levels were

positively correlated with the drought tolerance. In a cell culture system using NaCl-resistant and senstive *Nicotiana sylvestris* (Dix and Pearce, 1981) both cell lines were found to accumulate proline when under salt stress. Other responses of nonhalophytic plants to salinity are discussed by Greenway and Munns (1980).

Inorganic osmotica. The use of organic compounds for osmotic regulation by the cell may help in adjusting the internal water content as well as balancing excess cation levels. However, the energy required to make the organic compounds used for osmoregulation, and also the carbon skeletons required, may limit the growth of the organism.

The alternative to this process is the accumulation of a sufficient concentration of ions from the external medium. This, however, may result in high ionic concentrations that interfere with normal biochemical activities within the cell (Poljakoff-Mayber, 1975).

Halophilic bacteria have adapted to salt by altering the basic structure of their proteins (Hochachka and Somero, 1973). These organisms require salt; low salt levels result in protein denaturation and ribosomal dissociation. High salt levels are required for enzyme activity and ribosomal function. On the contrary, among halophilic higher plants, elevated salt levels disrupt enzyme and ribosomal functioning *in vitro* (Greenway and Osmond, 1972). A salt-stimulated increase in the specific activity of some enzymes from some halophytes (Treichel et al., 1974; Boucaud and Billard, 1981) and glycophytes (Turner et al., 1980), however, has been observed. Flowers (1972a, 1972b) assayed several enzymes from both halophytes and glycophytes for activity in the presence of salt. Only one, an adenoisine triphosphatase (ATP) from halophytes, was stimulated by salt; the others were inhibited. Hawker et al. (1973) investigated the effect of salt on adenosine diphosphate-glucose from salt-tolerance and salt-sensitive species and, without exception, found inhibition. Poljakoff-Mayber and Meiri (1969) reported that salinity reduced the ability of excised pea (*Pisum sativum*) root tips to incorporate amino acids into protein. Likewise, Hall and Flowers (1973) demonstrated that protein synthesis is sensitive to salt in both halophytes and glycophytes.

Kalir and Poljakoff-Mayber (1975) found that mitochondrial malic dehydrogenase (MDH) was more sensitive to salt than was soluble MDH, and Austenfeld (1976) reported that glycolate oxidase activity was undisturbed by up to 1.0 M NaCl or KCl. The enzyme, glutamine synthetase, isolated from plants exposed to elevated levels of NaCl showed enhanced activity (Boucaud and Billard, 1981). But when the enzyme was isolated from the plant and then exposed to salinity, there was no response in the presence of NaCl.

Compartmentation. Most of the evidence suggests that salt tolerance must be due in large part to removal of ions to compartments (i.e., vacuoles)

and is paralleled by production of osmotica to maintain favorable water relations in the cytoplasm. Hall et al. (1974) studied the site of salt accumulation in halophytes by using Rb^+ as an electron-dense tracer and found accumulation of Rb^+ in the vacuole to the point of absence from the cytoplasm. Pitman et al. (1981) showed a higher K/Na ratio in the cytoplasm than the vacuoles of barley (*Hordeum vulgare*). Yeo (1981) found that *Suaeda maritima* accumulates Na in its vacuoles and that the tonoplast permeability to Na is low, compared to K, which may be mechanistically favorable in maintaining the compartmentation. He suggests this to be an energetically inexpensive method of osmoregulation. The idea, then, is that toxic ions are sequestered inside the vacuole and that this accumulation maintains a favorable osmotic balance between the inside of the cell and the external saline environment.

Nutritional Imbalance. Another problem faced by a plant in a saline environment is meeting the plant's nutritional requirement for certain ions (e.g., K^+) that are present at much lower concentrations in the external environment than other chemically similar ions (e.g., Na^+). One adaptive response seems to be that halophytes absorb and tolerate high internal levels of ions and, in contrast to glycophytes, can selectively concentrate considerable quantities of K^+ as well as Na^+, despite the much lower concentrations of K^+ in the soil (Rains and Epstein, 1967; Epstein, 1980).

The absorption of salt by plants exposed to salinity varies from small amounts for the salt excluders to considerable quantities for the halophytes. In general, as the NaCl level in the soil increases, both the Na^+ and Cl^- levels within the plant increase. Concurrently, the levels of Ca^{2+} and K^+ tend to decrease (Waisel, 1972). In *Phaseolus vulgaris*, LaHaye and Epstein (1969) found that Ca^{2+} kept the level of Na in the plants proportional to growth. When Ca^{2+} was present the plants grew as well with salt as the unsalinized control plants. It appears that at both low and high concentrations of NaCl, halophytes not only tend to accumulate more Na^+ and Cl^- than do glycophytes but also manifest little if any interference by Na^+ on the uptake of K^+. Furthermore, in the absence of salt, halophytes tend to accumulate higher levels of NO_3^- and SO_4^{2-}, which may function to substitute for Cl^- in its absence (Flowers et al., 1977).

METABOLIC ENERGY COST OF TOLERANCE

Whatever the mechanism or combination of mechanisms that account for salt tolerance, there is an energy cost. It is important to determine whether plant cells respond to salt with enhanced respiration. If salt respiration is observed, it may represent an extra energy cost to the cells, reducing the potential for cell growth.

The effect of salt on respiration may be manifested in a number of ways. Salt in the environment could divert energy from growth and possibly increase the maintenance costs of cells under stress. The diversion of energy from growth would reduce the potential for plant productivity. The situation will be similar even if respiration is not enhanced in the presence of salt. A greater portion of respiratory energy may be diverted to processes resulting in salt tolerance rather than in growth. But there is little information on the energetics of ion fluxes in halophytes (Flowers et al., 1977). Adding salt, however, clearly presents the possibility of adding an energy burden to the active transport system. Higher requirements for internal salt levels, in turn, require higher energy expenditure at the plasmalemma. Beevers (1961) estimated that the passage across the plasmalemma of one mole of cations required the energy of one mole ATP. If outpumps are extruding ions back into the media (Pitman and Sadler, 1967), that, again, adds to the energy cost. Halophytes in general appear to maintain a cytoplasmic salt concentration at about one-third that of the vacuole, implying an active pumping mechanism at the tonoplast as well. It is thus reasonable to hypothesize that the energy cost of maintaining a favorable osmotic gradient through ion absorption is significant and consequently competitive for the energy resources of a growing cell.

Likewise, energy is required to produce and replace carbon skeletons in the synthesis of cytoplasmic organic osmotica. As with inorganic osmoticum, which requires the expenditure of energy through respiration of carbon compounds during the accumulation process, the formation of organic compounds competes directly for cellular energy currency and reduces the potential for productivity of plants growing in saline environments.

Conceptually, respiration can be divided into growth respiration and maintenance respiration. Growth respiration provides the ATP, reductant and carbon skeletons required for growth processes and is proportional to the rate of growth (Raven, 1976). Maintenance respiration provides energy for such processes as macromolecular turnover and maintenance of solute gradients. Maintenance respiration is proportional to biomass and not related directly to growth (Beevers, 1970; McCree, 1974; Penning de Vries, 1972, 1975). Total respiration is the sum of the two.

A series of mathematical models have been developed (Pirt, 1965; McCree, 1970; Fick et al., 1975) to quantify plant respiration. Penning de Vries et al. (1974) provided theoretical estimates of the maximum efficiency of conversion of glucose to the major components of plant biomass (amino acids, protein, lipid, carbohydrate, nucleic acids, etc.). The weight of the compound manufactured from one gram of glucose was defined as the "production value." Production values ranged from 0.36 for lipids to 1.43 for carboxylic acids. Penning de Vries (1974) further established the cost in glucose units of specific cellular compounds. For example, converting glucose to lysine was given as:

$$1.00 \text{ g glucose} + 0.156 \text{ g } NH_4 + 0.039 \text{ g } O_2 + 0.269 \text{ g } H_2O =$$

$$0.671 \text{ g lysine} + 0.255 \text{ g } CO_2$$

These theoretical calculations show close agreement with the experimental results of Kandler (1953) who grew maize (*Zea mays*) embryos and determined the amount of glucose consumed over a five-day period. He found 75.4 ± 2.4 mg glucose was needed to synthesize 47.5 mg dry matter. Penning de Vries' theoretical calculation showed a requirement of 71.5 to 74.5 mg glucose.

Loomis (1982) presents an elegant method for calculating energy consumption by plants. Following the McDermitt Method, glucose is used as a substrate standard, and Glucose Equivalents or Glucose Values are calculated from the formation of energy carriers (ATP) and reductant ($NADH_2$). We believe this method will be of great value in developing information on metabolic cost of stress.

Cell culture offers a unique opportunity to determine the relation between salinity and plant respiration. Respiration can be determined via oxygen uptake both before and following the treatment of cells with salt. An increase in respiration due to salt would then be directly measurable as an increase in oxygen consumption. Furthermore, since one result of an increase in respiration is an increase in carbohydrate consumption, glucose consumption by cell cultures can provide an additional measurement of the metabolic cost of salt tolerance. All carbon skeletons and energy required for growth are derived from the glucose in the medium, and all the glucose input to the system can be accounted for by measuring glucose consumption and accumulation of dry matter. Thus glucose consumption allows calculation of the energy costs for cells in saline conditions (Hunt and Loomis, 1976). On this basis, Kato and Nagai (1979), using tobacco tissue culture cells, measured growth yields of 0.59 g cell dry matter per g glucose.

Penning de Vries (1972) defined growth as dry weight increase, and quantification of the effect of salinity on this growth is a first step in understanding energy requirements. Changes in growth and growth rate from nonsaline to saline conditions, however, may not be immediately obvious as an increase in cell dry weight or CO_2 evolved. For example, a population of cells may divert carbon skeletons to the production of organic osmotica, thus increasing cell dry weight to maintain turgor, with no corresponding increase in population size and, likewise, with the absorption of inorganic osmotica.

Further information could be gained, therefore, by characterizing the relative amounts of basic biomass constituents (cellulosic materials, proteins, amino acids, lipids, organic acids, sugars, and minerals) (Talmadge et al., 1973). From such data, it is possible to describe basal cellular composition under nonsaline and saline conditions. In both cases it would be possible to

calculate and to compare integrated "production values" of the biomass produced under each condition.

Alterations in the energetics of cells exposed to salt can, therefore, be characterized both in terms of changes in relative amounts of basic biomass constituents and in variation in production values. A third parameter to measure is carbon dioxide production in terms of the weight of glucose needed to produce a specific amount of CO_2. Alterations would indicate an increased cellular effort towards what Penning de Vries et al. (1974) termed "tool maintenance," the respiratory cost of maintaining synthetic systems (i.e. mRNA, enzymes, etc.).

Comparison of CO_2 production factors and productivity values as well as relative amounts of basic biomass constituents of control and salt-treated cells should elucidate the energy cost of tolerance to salt.

USE OF CELL CULTURE SYSTEMS FOR STUDIES ON SALT TOLERANCE

Cell culture has several advantages as a technique for studying salt tolerance. It allows the processes or physiological markers involved in salt tolerance to be characterized at the cellular level. The relative lack of differentiation in the cultured cells eliminates complications arising from the morphological variability of differentiated cells of the various tissues of whole plants. The culturing of plant cells on rigidly defined culture media also permits a relatively uniform and precise treatment of these tissues with salt and uniform control of their environment.

Several researchers have compared the performance of plants and their corresponding callus under salt stress. Tal et al. (1978) and Orton (1980) both found that callus performance paralleled the plant response to salt. On the other hand, Smith and McComb (1981), making similar comparisons among a salt-sensitive glycophyte, a salt-tolerant glycophyte, and two halophytes, found that both the plant and callus of the salt-sensitive glycophyte decreased in growth with increasing salt. The salt-tolerant glycophyte, as to both plant and callus, showed increased growth at intermediate salt levels but a decrease with higher levels. The halophytic plants showed increased growth with higher levels of salt while that of the callus decreased. They suggested that the salt-tolerant glycophyte has cellular level mechanisms for salt tolerance while that of the halophytes depends on the structure of the whole plant.

It is now well established that cell culture techniques can be used to select salt tolerant cell lines from non-tolerant agricultural plants. Nabors et al. (1975) obtained tobacco (*Nicotiana tabacum*) cells that showed superior tolerance to 0.16% NaCl (w/v) and, subsequently, to 0.52% NaCl. Dix and Street (1975) selected lines of tobacco and pepper (*Capsicum frutescens*) cells that grew in liquid medium containing up to 2% NaCl. In both studies, growth was measured as an increase in cell number or change in packed cell volume. Dix and

Street (1975) observed that the packed cell volume tended to decrease after exposure to salt despite an increase in cell number. This was attributed to a decrease in cell size.

In our laboratory, we have isolated two NaCl selected lines of rice (*Oryza sativa*) cells (Croughan, 1981). These lines grow better than the nonselected rice cells on NaCl, with an optimum level of NaCl being 1.5%. Higher levels of K^+ are also observed in the NaCl tolerant cells than the nontolerant. We have also obtained data from a study employing a salt selected line of alfalfa (*Medicago sativa*) callus cells showing superior growth in the presence of NaCl and an unselected cell line from which these variants were selected (Croughan et al., 1978). Cells of the salt selected line grew better than unselected ones at high levels of salt. This suggested that the selection had effectively isolated variant cells with an increased capacity for growth in the presence of high levels of NaCl. The salt tolerant line also displayed other characteristics indicating that the tolerance was the consequence of a shift towards a true halophytic nature.

Besides tolerance of high levels of salinity, halophytes actually require salt, as evidenced by their poor growth in its absence and the growth stimulation which results from the addition of salt (Black, 1960, Brownell, 1965). This same pattern was displayed by the salt selected line of alfalfa cells which grow optimally at 0.50% (85 mM) NaCl and poorly in the absence of salt (Croughan et al., 1978).

EXPERIMENTAL RESULTS

This section reports experiments with salt selected and nonselected plant cells. Comparative studies on ion regulation and energetics of cells exposed to saline environments are discussed.

Cell lines were selected in the presence of NaCl as described by Croughan et al. (1978). These NaCl selected and nonselected cell lines were used in a series of experiments reported in this section.

Ion Regulation

Under the presumption that ionic regulation within plant cells may represent a marker common to many plant species showing enhanced salt tolerance, ionic contents of alfalfa cell lines selected in the presence of NaCl were compared with unselected alfalfa cells. The alfalfa cells were grown in media containing a range of NaCl concentrations for approximately 40 days. The cell lines were harvested and fresh and dry weights measured, and ionic content was determined on the harvested cells. Details of analytical procedures are available in Croughan et al. (1978).

A comparison of the ionic content of the two alfalfa cell lines indicates that in the presence of salt the salt selected line accumulated more Cl^- and K^+

than the unselected line (Figs. 1 and 2). The capacity to maintain higher levels of K^+ in the presence of high levels of Na^+ is noteworthy and consonant with previous findings in both halophytic bacteria and halophytic plants (Rains, 1972, 1979; Rains and Epstein, 1967). The elevated levels of NO_3^- within the salt selected line at the low salt levels are also consistent with observations that halophytic plants tend to accumulate NO_3^- when grown in the absence of Cl^- (Flowers et al., 1977) (Fig. 3).

Preliminary uptake experiments show differences in K^+ uptake between the nonselected and salt selected alfalfa cells (Stavarek and Rains, 1981). With increasing osmotic levels (achieved through the addition of mannitol), the capacity of the non-selected cell line to take K^+ up decreased markedly. The salt selected line, on the other hand, was able to maintain K^+ uptake and accumulate K^+ with increasing osmotic levels (Fig. 4). The ability of plant cells to accumulate K^+ in the presence of high osmotic environments is an important and significant mechanism commonly associated with salt tolerance.

Determination of the Metabolic Energy Cost of Tolerance

As discussed earlier a biological system exposed to stress very likely expends more metabolic energy than the same biological system in the absence of stress. This extra energy is probably consumed in processes involved in osmotic adjustment or could be dissipated as heat from uncoupled reactions. Evaluation of the metabolic energy cost of tolerance can be accomplished directly in cell culture systems. Differential use of energy substrate by cells exposed to stress can be measured directly and related to efficiency. Efficiency of growth is calculated by determining the amount of cell biomass produced with a unit of glucose.

The efficiency of growth of the two alfalfa lines has been compared on increasing levels of salt (Croughan et al., 1982). Growing the salt tolerant and nontolerant alfalfa cells as suspension cultures using glucose as the energy source at various levels of salinity, we determined efficiency values as shown in Fig. 5. The solid line is for the nonselected cells, which show a decrease in efficiency as the salt level rises. The dotted line is for the salt selected lines, which shows less efficiency in the absence of salt but a substantial improvement in efficiency as the salt level is raised. In the absence of salt, the nonselected cells appear to be better adapted, having twice the efficiency as the salt selected cells. Under salt stress, however, the salt selected cells are better adapted to this environment and have approximately three times the efficiency of the nonselected cells. The maximum efficiency for both cell lines is similar, but for the nonselected cells that efficiency is reached in the absence of salt, whereas the salt selected cells, with an apparent requirement for salt, reach maximum efficiency in the presence of salt.

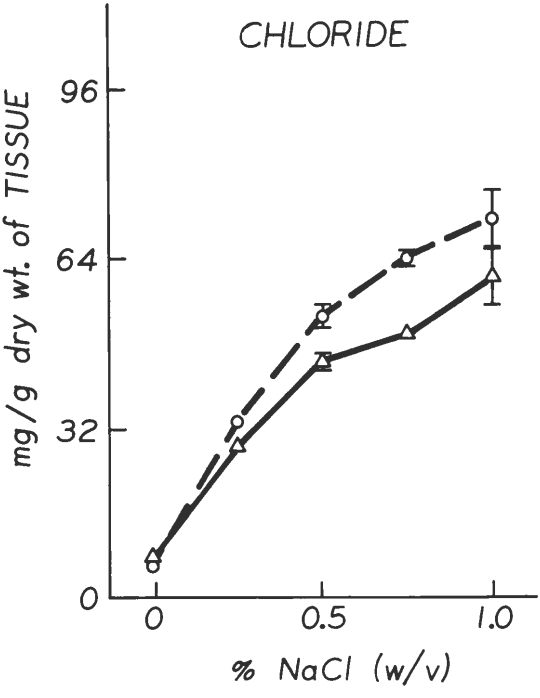

Figure 1. The chloride content of salt-selected (0—0) and nonselected (△—△) alfalfa callus grown on various levels of NaCl. Alfalfa cultivar 'W74-RS' callus was grown for 40 days on modified Blaydes media with levels of NaCl as indicated on figure. The callus was harvested, dried at 70 C for 48 hrs and weighed. Dried material was extracted with water and Cl determined by potentiometric titration. Values are averages of six replicates ± standard error indicated by cross bars.

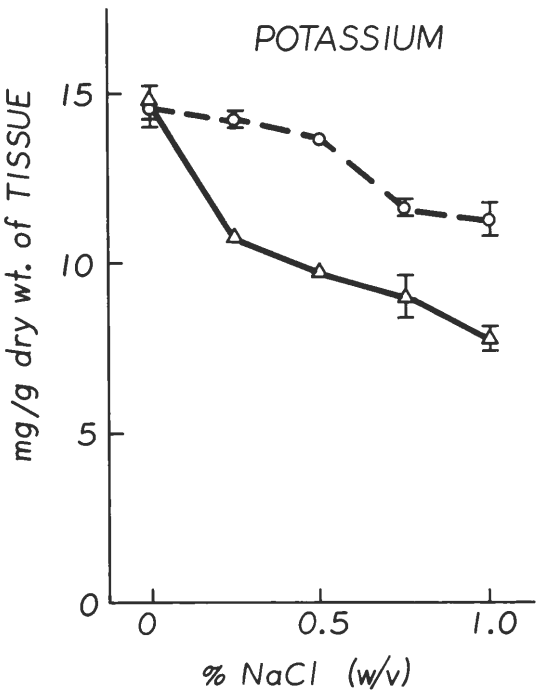

Figure 2. The potassium content of salt-selected (0—0) and nonselected (△—△) alfalfa callus grown on various levels of NaCl. All conditions and conventions as in Figure 1 except potassium determinations were done on acid digested callus by atomic adsorption spectrophotometry.

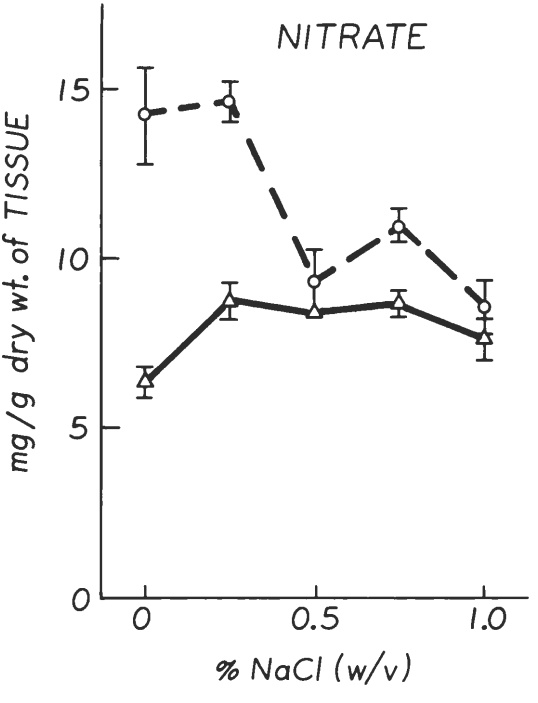

Figure 3. The nitrate content of salt-selected (0—0) and nonselected (△—△) alfalfa callus grown on various levels of NaCl. All conditions and conventions as in Figure 1 except NO_3^- were determined by ion specific electrode.

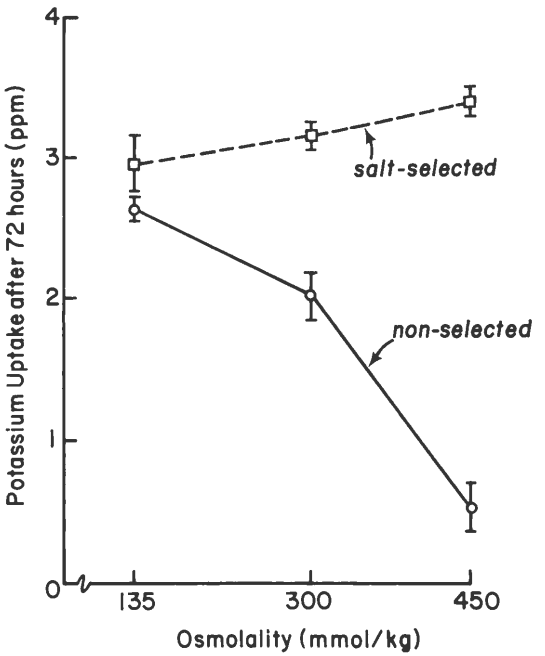

Figure 4. Potassium uptake of salt selected and nonselected alfalfa cells as a function of increasing osmolality. Alfalfa callus was pretreated 4 days in suspension medium lacking K^+ in order to deplete callus of excess ions. Pretreatment suspensions were changed every 12-24 hours. Mannitol was used to adjust the medium to the osmotic levels indicated. After pretreatment cells were filtered and 0.5 grams of tissue was added to a 25 ml flask with a 5 ml solution containing 0.5 mM $CaSO_4$, 0.1 mM KCl, 0.1 mM NaCl, 2% sucrose and the appropriate level of mannitol. Flasks were placed on a rotary shaker. At specific times the suspensions were filtered, cells rinsed, and K^+ determined in the tissue and uptake solution.

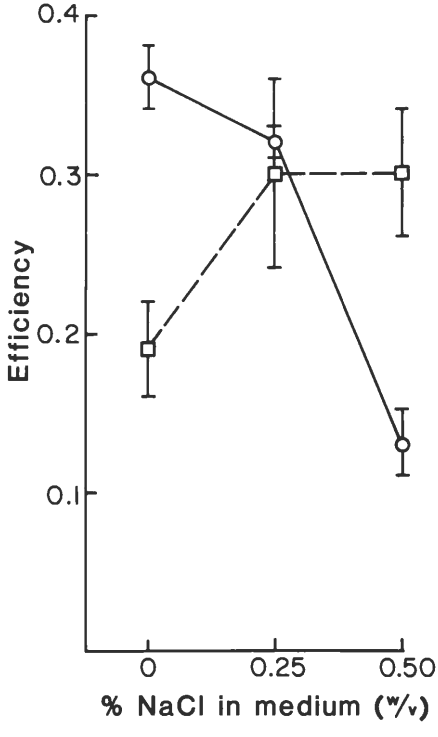

Figure 5. Energy efficiency ($\frac{\text{Increase in dry weight (gm)}}{\text{gm glucose consumed}}$) of salt selected (□—□) and nonselected (0—0) alfalfa cells as a funtion of increasing NaCl. Alfalfa cells were grown for 60 days as suspension cultures in medium with glucose as the carbon source and levels of NaCl as indicated. Suspensions were filtered, cells weighted, and the glucose remaining determined on a high pressure liquid chromatograph.

CONCLUSIONS

Plants exposed to saline environments are subjected to water stress and specific ion toxicities. These effects are manifested in a number of physiological and morphological processes. From a review of the literature and from results presented in this paper, a number of these processes were identified. These include: (a) inorganic ion regulation and maintenance of essential nutrient ions; (b) osmotic adjustment by regulation of organic and inorganic substances; (c) physiological tolerance of cells to toxic ions; and (d) efficient use of metabolic energy.

An understanding of these processes and their involvement in salt tolerance will provide information on the criteria required to select plants for enhanced productivity in saline environments. The study of variant cell lines, which differ in their tolerance of normally lethal levels of salt, could possibly elucidate the mechanisms involved in dealing with salt, and provide possible biochemical markers useful in the selection of salt-tolerant cells and plants. And finally, a major limitation to yield in stress environments such as salinity may be the efficient use of metabolic energy for growth. A better understanding of this efficiency of carbohydrate utilization may lead to improved crop production in stress environments.

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