Plants growing in nature provide a continuous array of biological efficiencies related to mineral nutrition. Nearly all of the temperate and tropical areas of the earth are covered with species adapted to the unique properties of particular soils. These properties may represent the extremes in element availability from very deficient to toxic levels and may be associated with wide ranges in pH.

Differences found among diverse plant materials must have a genetic basis. Since speciation is a dynamic, continuing process, we can assume that intraspecific differences in response to mineral nutrition should also exist. Adaptation to toxic levels of nutrients would be anticipated since selection pressure eliminates the unadapted. In contrast, inherent efficiencies in the use of mineral elements by agricultural crops would probably remain undetected in a production system where most crops are grown in soils amended by inorganic and organic fertilizers. This type of production system provides little selection pressure for efficiency in utilization per se.

Research workers can mimic the processes of speciation and develop plants adapted to toxic soils. Adaptation to Cu toxicity in only 5 years has been reported in interbreeding populations of *Agrostis stolonifera* by Wu et al (17). Metal-tolerant cultivars for use on heavy-metal-contaminated land (Pb, Zn and/or Cu) have been developed in England in *Festuca rubra* and *Agrostis tenuis* (5). There is also work on cultivar development tolerant to Al in the U.S. and Brazil, particularly in *Triticum aestivum*.

Differential responses to N, P and K have been described (1, 3, 4, 6, 8, 13, 14). Strain differences have been reported for minor elements also (10, 15). With this background information plus personal experiences, we decided about 1960 to set up a research program based on the following assumptions:

1. Genetic variability for the growth of plants under stress levels of an element should exist with every species.
2. Current laboratory techniques in growing plants should permit the detection of single-plant variability in growth within a population of plants growing under nutrient stress.

The remainder of this paper will summarize the data from a series of graduate research programs on this problem.

Several arbitrary decisions were made initially. We chose water cultures in preference to soil culture, modifying a Hoagland's solution as needed. We also restricted our research to the vegetative phase of growth. We chose to study two diploid species with short life cycles, both of which had been used commonly in water culture and genetic research, *Lycopersicon esculentum* and *Phaseolus vulgaris*. Lastly, we concentrated on the macronutrients N, P and K. The use of water cultures prevents the discovery of plants capable of removing a tightly-bound element from the soil, but in a water culture we know the amount of each element available to the plant, we can measure the disappearance of the element from the root environment, and the entire root tissue is readily recovered for experimental analysis. Limiting our studies to the vegetative phase of the growth cycle does not give us any insight into the correlated effects on agricultural yield of seeds and fruits, but the comparative relationships of efficiency in using a limiting element in the early stages of growth must be understood first. Agricultural yield would be a logical subsequent study.

### Literature Cited


### Determining the level of the limiting elements

Preliminary studies were made to determine growth of tomatoes and beans in response to varying levels of the element to be studied. Optimum levels per plant for detecting strain variation in response to N was 35 mg, to P was 2-3 mg and to K was 5 mg for both species. Plants were grown in separate containers without any chance for interplant competition for substrate.

Beans, which contain significant amounts of P and K in their cotyledons, posed a special problem in estimating the proportion of the stored P and K made available to the growing seedling. The P and K contents of seeds to be germinated for plant production are readily estimated by analyzing seeds of varying size for each seed lot and plotting a regression line of P or K content against seed weight from which estimates can be made on other seeds of the same seed lot. P content in particular varies greatly among seed lots of a single cultivar grown under differing conditions. The media were adjusted for each bean plant to the proper level of P and K based on estimated P and K contents of seeds. About 97% of P and K in bean cotyledons is mobilized and utilized by the growing plant (16).

### Measuring plant growth in relation to efficiency

Restriction in plant growth may be classified in several ways. K deficiency symptoms are rather well known, distinct and can be used to quantify differences. N deficiency symptoms are also well known but somewhat less unique. P deficiency symptoms are difficult to use and are absent in many instances.

The primary criterion of efficiency in our studies has been the amount of fresh and/or dry weight produced per plant per unit of N, P and/or K taken up by the plant. Since the stress levels of N, P and K are so low that all of the limiting element is taken up from the nutrient solution, it can be assumed that total fresh and/or dry weight produced is an adequate estimate of efficiency. This assumption assumes uniform patterns of distribution of the element throughout the plant, regardless of genotype. However, our experience has shown that the distribution of an element within the plant and the pattern of mobility within the plant growing at a stress level vary according to strain. Efficiency ratios (ER) which denote the mg dry weight of plant/mg of the stressed element in the "above ground" proportion of the plant appear to be much better estimates of growth in response to a limiting element. Coefficients of variation for ER are uniformly high for total dry weight of plant. Thus variability in distribution of the stressed element appears to be important in determining the maximum amount of growth which can be obtained in our tests.

Care must be taken to avoid substitution of an element for the element studied, e.g., Na for K. Although the phenomenon of substitution tends to be species specific, intraspecific differences have been found recently by Makmur et al (7).

The environment in our tests has varied from greenhouse conditions with differing daylengths, temperatures and light intensities to the highly controlled University of Wisconsin Biotron facilities. Most studies are carried out in an air-conditioned basement room at about 25°C, with a 14 hr day and with light provided by fluorescent lamps (CW/SHO tube) at an intensity of 1.3-1.5 klx. All cultures are aerated using a water trap to exclude impurities. Air is uniformly metered to each container using uniform sized hypodermic needles inserted into a rubber air line hose.

Environmental interactions must also be minimized. This is done in various ways. Differences in growth between strains are usually large enough so that 3-4 replicates (plants) provide meaningful esti-
mates of strain performance for detecting extremes in efficiency. The number of strains evaluated at any one time is then limited to the growing space available.

Differences in environmental parameters are bound to exist from one evaluation to another. The extremes of efficiency from the various screenings are evaluated in a simultaneous test in a common environment with adequate replication to assure reasonable comparative estimates of the potential efficiency of each strain to be used in subsequent physiological and genetic research.

Identifying strain differences

The strains of beans classified as efficient and inefficient by Shea et al. (12) were based on deficiency symptoms and later confirmed on a dry weight basis. Efficient strain 58 produced 47% more dry weight than inefficient strain 63. All 5 strains are plant introductions from foreign countries. Only 66 seed sources were screened to obtain these variants, suggesting the occurrence of efficient germplasm to be rather frequent.

In 156 tomatoes screened for K efficiency, 2 efficient lines were selected which produced an average of 78% more dry weight than 2 inefficient lines and were selected as parents for genetic studies (7). Plants of the 4 strains growing at 200 mg K did not differ in dry weight per plant.

Whitaker et al. (16) isolated 6 strains of beans representing the extremes in P efficiency. Only 54 strains were screened. Phosphorus efficiency ratios (PER) varied from 380 to 671.

Tomato strains also varied in efficiency in N utilization when grown under N stress. Two efficient lines isolated from 146 seed lots produced about 47% more dry weight/plant than did the inefficient isolates (9).

Measuring ion substitution

Plants growing under extreme nutritional stress may differ in growth if ion substitution can occur differentially: Na substitution for K was our main concern. It occurs readily in beets but was not detected in beans. We did not expect Na substitution in tomatoes, but Makmur et al. (7) (Table 1) did detect a significant response to Na at K levels of 5 and 25 mg/plant in his Line No. 42 (43 Oshogbo from Nigeria). Tests for Na substitution over a broad range of K levels for the 2 efficient lines suggest that Na substitution may be a significant but not the sole factor contributing to efficiency at stress K levels.

Genetic tests

As soon as lines were confirmed as efficient and inefficient in preliminary screenings, a series of controlled reciprocal matings were made as follows:

efficient x efficient
efficient x inefficient
inefficient x inefficient

The F1 progeny were each backcrossed to their respective parents and simultaneously allowed to self pollinate. Thus by retaining remnant seed of parents and F1 hybrids, adequate seed can be accumulated for a simultaneous comparative test of the following:

1. Parents:
   - F1 self pollinated
   - P1 x P2
   - P2 x P1

2. Reciprocal F1 hybrids
   - P1 x P2
   - P2 x P1

3. Backcross progeny
   - (P1 x P2) x P1
   - (P2 x P1) x P1
   - (P1 x P2) x P2
   - (P2 x P1) x P2

4. F2 progeny
   - (P1 x P2) self pollinated
   - (P2 x P1) self pollinated

The simultaneous test of these progenies minimizes the possibility of unknown environmental interactions interfering with comparisons between progeny groups.

Segregating backcross and F2 progenies may be self pollinated for purposes of estimating heritability. However, identification of efficient plants in segregating populations to be saved for seed production must be based either on fresh weight of plant or on deficiency symptoms. The method of selecting efficiency must correlate with dry matter production.

The use of reciprocal F1 hybrids provides an estimate of maternal and/or cytoplastic effects. Thus far there has been no indication of maternal control of plant response to mineral nutrients except in a study by Rice (11) which indicated that large seeded strains of beans were significantly more tolerant of excessively high levels (380 mg/plant) of P than small seeded. Since seed size is determined maternally the large x small hybrids were tolerant, the reciprocal hybrids were intolerant and the F2 progeny of the 2 hybrids were similar in response.

The simultaneous test of the parents, hybrids and F2 progeny of the hybrids provides an opportunity to estimate population means. Each of the means can be expressed in terms of the F2 mean (m); and additive (a), dominance (d), additive x additive (aa), additive x dominance (ad) and dominance x dominance (dd) gene effects. The 6 parameters were estimated from the population means using the relationships described by Gamble (2).

The sensitivity of tests to differences in dry matter accumulation per g of stress element (N, P or K) is supported by coefficients of variation that are uniformly smaller for P1, P2 and F1 but generally larger in BC and F2 progeny. The sensitivity of the test also allows us to demonstrate transgressive segregation among progeny whose parents differed in efficiency or were similar in efficiency. Lastly, the isolation of plants differing in efficiency in F2 and BC populations provides an opportunity to produce F3 and BC F2 seed respectively for studies on heritability.

Genetic controls have been of all types. K efficiency in beans was controlled by a single recessive gene, ke ke; in tomatoes control was quantitatively inherited with additive genes the major contributors to variation in K utilization. The genetic controls of P efficiency in beans is rather complex but suggest the F1 to be as efficient or more efficient than the more efficient parent with relatively few genes involved in these differential response. In N efficiency in tomatoes, like P in beans, dominance gene effects made the major contribution to variation in efficiency of N utilization.

Table 1. K-Na interaction affecting KER of efficient tomato lines 42 and 98 grown at 4 levels of K in low (4.8 mg/plant) and high (165.0 mg/plant) Na nutrient solutions (7).

<table>
<thead>
<tr>
<th>K (mg/plant)</th>
<th>Low</th>
<th>High</th>
<th>Low</th>
<th>High</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dry wt (g)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>0.93</td>
<td>1.44</td>
<td>1.31</td>
<td>1.64</td>
</tr>
<tr>
<td>Na %</td>
<td>0.21</td>
<td>1.31</td>
<td>0.22</td>
<td>1.36</td>
</tr>
<tr>
<td>25</td>
<td>2.37</td>
<td>3.97</td>
<td>3.45</td>
<td>3.80</td>
</tr>
<tr>
<td>50</td>
<td>3.57</td>
<td>4.77</td>
<td>5.05</td>
<td>5.63</td>
</tr>
<tr>
<td>KER</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>87</td>
<td>104 ns</td>
<td>115</td>
<td>114 ns</td>
<td>1.47</td>
</tr>
<tr>
<td>Na %</td>
<td>0.09</td>
<td>1.36</td>
<td>0.09</td>
<td>1.47</td>
</tr>
</tbody>
</table>

*Comparison of KER values between low and high Na levels within each strain significant at 1% level.

Unanticipated information

A relatively new approach to studying intraspecific differences of a basic physiological mechanism might be expected to discover unique but related plant responses. This project is no exception. For example, symptoms of K deficiency are not uniform among strains of either beans or tomatoes. Likewise, P deficiency in a few strains of beans is characterized by large necrotic areas on the leaves. Bean plants of Ke ke and Ke Ke genotypes can be identified by rapid reflexing of the leaves prior to the development of chlorosis. This change in leaf position is associated with the onset of an abscission layer, thereby preventing the normal movements of metabolites and water to and from the leaves? In a carefully monitored experiment we found that the K in the unifoliate leaves was reexported quickly to the growing point of the plant, and the abscission layer formed after depletion of all K from the unifoliate leaves. Plants of genotype ke ke did not exhibit this rapid retransport of K; the abscission layer did not form, and typical K deficiency symptoms either did not develop or developed very slowly. P transport is also more rapid from unifoliate leaves of inefficient plants than in plants of efficient strains of beans; associated with P depletion is a rapid correlated drop in net photosynthesis (16).
Table 2. Total dry weight of root of efficient strain 11 compared to mean of 12 experimental strains grown at 3 levels of P (16)

<table>
<thead>
<tr>
<th>Source</th>
<th>Root dry wt (mg/plant)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2 mg P</td>
</tr>
<tr>
<td>Line 11</td>
<td>356</td>
</tr>
<tr>
<td>Mean of 12 lines</td>
<td>180</td>
</tr>
</tbody>
</table>

Strains of beans grown under P stress exhibit different patterns of root growth. In an experiment comparing growth in Hoagland's solution modified to provide a total of 2, 31 (normal) and 62 (2x normal) mg P per bean plant the root growth rate of efficient strain 11 was nearly twice as much at 2 mg P as when grown under normal and twice normal P levels (Table 2).

Do laboratory and field performance correlate?

Our initial objective was to study efficiency at stress levels of a mineral nutrient during the early vegetative phase of growth without regard for ultimate yield of fruit and/or seed. Flowering and fruiting represent a whole set of variables that should be studied using material whose vegetative response is well understood. Studies on agricultural yield have not been done, but data in hand suggest that studies on agricultural yield can now be pursued effectively. We have shown that under field conditions K efficient strains of beans produced more dry matter per plant under low soil K than did K inefficient strains. Thus there is hope, particularly for crops whose economic value resides in vegetative growth, e.g., grasses, lettuce and alfalfa.

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Breeding Vegetable Tolerant to Environmental Stress

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U.S. agriculture has an unmatched record for efficiently producing an abundance of high-quality, modestly priced food. At the 1971 ASHS meeting, and in subsequent articles, Weddleigh eloquently described the impact of agricultural research on the production of vegetables and other horticultural crops since 1920 (35, 36). Scientists of many disciplines have contributed to this success story, but much of the credit goes to plant breeders for the development of improved cultivars.

As great as our crop yields are, we know that most cultivars have the genetic potential to yield far above the current national averages if growing conditions are optimum (37). But farmers are very much aware that environmental stresses pose a constant threat to their crops. Secretary of Agriculture Robert Bergland, speaking from personal experience as a farmer, has said "Science and technology affect yield trends over the long haul. But in a given year, weather is the overriding factor" (3). In addition to weather, stresses caused by man-made air, soil and water pollutants, radiation, and biotic factors such as diseases and insects affect yields and quality of crops. For this discussion, I will restrict my comments to abiotic stresses.

Since we are not yet able to control the weather or eliminate various man made pollutants, farmers depend on plant breeders to provide them with cultivars able to resist the stresses caused by these factors. Breeding plants resistant or tolerant to environmental stress is not a new objective. It has always been a primary one of most vegetable breeding projects. However, it is more common to refer to breeding for wide adaptability rather than for stress tolerance, although we know that a large part of adaptability is tolerance to environmental stress.

Often selection for stress tolerance or resistance is inadvertent.

Selection of a healthy green plant when others in a population are weak, off color or unproductive can be selection for stress tolerance even though the breeder may not understand why the one plant performs better. Selection of high yielding plants may also be an example of unknowingly selecting for resistance to environmental stress. If flowering occurs during periods of high temperature, the breeder may be selecting for the ability to set fruits under this condition.

Plant breeders have unquestionably made significant progress in selecting vegetables tolerant to a range of environmental stresses. Rather than attempt to discuss all vegetables, I will use tomatoes to cite examples of past and present efforts to breed for stress tolerance since I am most familiar with them and the points I wish to make can be readily illustrated with this crop.

Temperature

Horticultural literature contains many references to the differing responses of vegetable accessions to temperature variations. Tomatoes can be used to illustrate the opportunities plant breeders have to select for tolerance to the effects of temperature extremes on several aspects of plant growth.

Several studies have shown that seeds of tomato accessions differ rapidly and uniformly in cool soils.

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