GENETIC-PHYSIOLOGY OF IRON-INDUCED MANGANESE CHLOROSIS IN BEANS (*PHASEOLUS VULGARIS* L.)

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ABSTRACT

GENETIC-PHYSIOLOGY OF IRON-INDUCED MANGANESE CHLOROSIS IN BEANS (PHASEOLUS VULGARIS L.)

By

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A genetic-physiologic study of iron-induced manganese chlorosis in beans was initiated. The experiments were conducted in a greenhouse in sand-culture. Soil culture was used for crossing and seed multiplication only. Approximately 60 breeding lines of navy beans (Phaseolus vulgaris L.) were planted under two nutrient conditions; normal (control) and screening (5.0 ppm iron and zero manganese). Differential chlorotic symptoms were noted on plants in the screening solution 3-4 weeks after planting. On the basis of the appearance, two extremely chlorotic (50 and 80) and two non-chlorotic lines (40 and 47) were selected for further studies. The analysis of plant parts indicated that the chlorotic and nonchlorotic lines differ in the pattern of distribution of iron and manganese. Chlorotic lines transport relatively more iron and less manganese to the shoot, whereas the converse is true for nonchlorotic lines.

The F_1, F_2, and backcross populations were evaluated for qualitative response to study the mode of inheritance.

The appearance of F_1 combinations indicated phenotypic dominance of the chlorotic type. F_2 populations segregated 9 chlorotic:7 nonchlorotic plants which suggests the involvement of two genes.

Grafts were made in various root/scion combinations to determine possible sites of control. Based on the appearance of chlorotic symptoms, the genotype of rootstock line 50 and the genotype of scion line 80 are responsible for the development of chlorosis. The analysis, however, indicated that roots of both lines 50 and 80 were efficient in retaining iron, but they also retained manganese. On the basis of both appearance and analysis, it is concluded that retention of iron by roots depends upon the genotype of the scion whereas manganese holding-capacity depends mainly on the genotype of the rootstock.

Nonchlorotic lines exuded less sap in the screening solution than chlorotic lines. Although the concentration of iron was greater in nonchlorotic lines, the total amount (conc x g) was less. Both the concentration of manganese and total manganese were greater in the sap of nonchlorotic lines but the pattern did not hold when the total amount of manganese produced under the screening solution was expressed as a percent of manganese in normal solution. Fe/Mn ratio and pH of the sap were lower for nonchlorotic lines.

In order to relate the genetic and physiological mechanisms, it is postulated that the genes function to control the amount of iron and manganese in the shoot by affecting sap flow.

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INTRODUCTION

A sub-optimal or supra-optimal concentration of a nutrient element(s) in the substrate or in plant tissue may disrupt physiological or biochemical processes which require the direct or indirect involvement of the element(s). Foliar manifestations of the disturbances are the deficiency or the toxic symptoms. The relation between the concentration of the element(s), morphological manifestations, and the internal processes is often incomplete and unclear, probably because of elemental interactions occurring either in the medium, at the uptake, at the transport, or at the utilization sites. Different species and different genotypes within species, however, differ qualitatively and quantitatively in their responses to these alterations and interactions, owing their capabilities to genetic differences.

Iron (Fe) and manganese (Mn), two of the essential micronutrients, are antagonistic and when present in an improper balance, toxic concentrations of the one induces symptoms resembling deficiency of the other and vice versa. Researches have revealed that the two elements are functionally related and interfere with each other during uptake and transport. Modes of genetic control and the relation

between the genetic and physiological mechanism(s) which induces the symptoms are not understood.

This investigation was initiated to study and to relate the genetic and physiological mechanism(s) responsible for the iron-induced manganese chlorosis in beans (Phaseolus vulgaris L.).

LITERATURE REVIEW

Fe and Mn Interactions

Twyman (48), in his review, cites that plants were grown by altering the balance between Fe and Mn as far back as 1848 by Salm-Horstmar, who noted the symptoms of the so-called "grey-speck" disease in oats, when the nutrient solution consisted of a high amount of Fe and little or no Mn. The description given by him is regarded as the first recorded symptoms of Mn deficiency. Twyman (48) also referred to Pugliese, who raised cereals in water-culture using Knop's solution with varying amounts of ferrous sulphate and manganese salts. Pugliese's results indicated that plants could tolerate larger doses of Mn in the presence of Fe.

Tottingham and Beck (47), using Knop's solution minus ferric phosphate, observed that high concentrations of Mn depressed the stimulating effect of Fe and vice versa, while at low levels, Mn, in the presence of Fe, caused chlorosis in wheat. They also suggested that Mn interfered with Fe in the formation of chlorophyll.

Somers and Shive (43) considered that symptoms due to excessive Fe were identical with those when Mn was deficient

and vice versa. They found that high concentrations of soluble Mn in the tissue invariably were associated with low concentrations of soluble Fe and vice versa, thus they concluded that Fe and Mn were functionally related. They further suggested that the oxidation of ferrous to ferric ions by active Mn resulted in an inactivation and precipitation of Fe in the form of a ferric-organic complex. Weinstein and Robbins (53), using green and albino scions grafted to green rootstocks in solution culture, similarly noted Fe deficiency symptoms on the plants supplied with a high level of Mn, as well as with a low level of Fe. On the basis of biochemical analysis, they concluded that a high level of Mn in the presence of low Fe induced a true Fe deficiency. Mn deficiency was noted, however, only when Mn in the substrate was low but it was not noted on plants supplied with high Fe.

In contrast, Berger and Gerloff (4) observed Mn toxicity (stem-streak necrosis) in potatoes, when Mn was present in toxic concentrations. However, the Fe in the tissue gradually decreased as the Mn in the tissue increased. Morris and Pierre (28) reported that lespedeza exhibited Mn toxicity if grown in a nutrient solution containing low Fe. An increase of Fe in the culture solution, up to a certain level, markedly reduced the toxicity of a given concentration of Mn. The beneficial effect of Fe in reducing Mn toxicity resulted from a decrease in Mn absorbed by the plant rather than an increase

in Fe absorption. Similarly, Agrawal et al.(1) noted symptoms of Mn toxicity in barley grown in sand culture when an excess supply of Mn was accompanied by a low level of Fe. These toxicity symptoms were distinct from Fe-deficiency chlorosis. Excess Fe did not produce any characteristic effect.

Interference in uptake and transport have been reported by some investigators (12,41,49). According to Chapman (12), Bertrand proposed that by converting Fe to an insoluble ferric form, Mn prevented its transport in or from the leaves. Sideris (41) observed the deposition of Fe in the exodermal root tissue of Ananas comosus, when a high concentration of Mn was supplied. The oxidation of Fe by Mn was suggested as a possible explanation. Vlamis and Williams (49) reported a reduction in Mn content of roots consistent with increased Fe supply in barley. Mn at low levels stimulated the uptake and transport of Fe while at the higher concentrations it exerted an inhibitory effect in soybeans (24), and in tomatoes (39). The inhibition, however was not very effective as compared to zinc (24).

Antagonism between Fe and Mn has been further emphasized by Gerloff et al. (15) in an interaction study between Fe-Mn-Mo. They observed that Mo accentuates Fe chlorosis in tomatoes induced by excess Mn in the nutrient medium. Fe content of the tissue decreased with increasing concentrations of either Mn or Mo in the external medium. In a similar

study, Kirsch et al. (22) reported that at a low Fe level, Mn decreased Mo uptake while at a higher Fe level, Mn increased the uptake of Mo, indicating the antagonism between Fe and Mn.

Genetic Differences In Ion Accumulation

A. Qualitative Responses

Differential visual symptoms in response to differing levels of nutrient elements have often been noted and used as criteria to study the mode of inheritance. Weiss (54) noted that two soybean varieties differed in their iron requirement and reported that the efficiency to utilize Fe was governed by a single dominant gene. Bell et al. (2,3) reported a homozygous yellow stripe mutant (ys_1/ys_1) of corn incapable of utilizing iron efficiently. The inefficiency of the mutant was due to its inability to utilize ferric iron at the root tips.

Brown (7) grew iron-deficient (yellow stripe-1, ys_1/ys_1) and iron-efficient (Pa 4) corn on calcareous soil and found that Fe deficiency was accentuated by phosphate (PO_4). The two corn genotypes differentially absorbed and translocated PO_4 from organic phosphate, a factor that could be associated with Fe-utilization.

Wall and Andrus (51), comparing the uptake and transport of boron in two varieties of tomatoes, found no differences in uptake; but translocation was inefficient in T 3238 as

compared to Rutgers, and this inefficiency was caused by a single recessive gene.

Pope and Munger (35,36) observed that two celery varieties were differentially susceptible to low boron (0.01 ppm) and to low magnesium (2.5 ppm). Efficiency was dominant in both cases and was shown to be conditioned by a single dominant gene.

Bernard and Howell (5) studied the inheritance of differential responses of certain soybean varieties to high P in nutrient solution. Segregation in the F_2 and F_3 generations indicated the existence of a major gene pair, Np and np, responsible for the differences.

Polson (34) reported that two genes are involved in the differential response of two dry bean varieties to a low level of zinc. Differences in the response were also observed at a high level of zinc (5.0 ppm), but the number of genes could not be established for this level because of a limited population sizes.

B. Quantitative Responses

Differences in concentration of elements or the total accumulation within plants has been noted. An approximate mode of inheritance has been reported for a number of cases, establishing that such processes are under genetic control.

Gorsline et al. (16) found differential ear-leaf accumulations of Ca, Mg and K in various single crosses and inbreds of maize. For Ca and Mg, the differences were highly

heritable on an additive basis, but for K, nonadditive elements also were included. Additivity accounted for different levels of calcium and strontium accumulation between genotypes of corn (17). The inheritance of the concentration of a number of elements in the corn leaves and grain by diallel crosses was studied (18). Nonadditive gene action was indicated for ear-leaf concentration of P, Mg, Cu and B and for the grain concentration of K. Probable number of loci and the effect of each gene pair have been established (19) for the ear-leaf concentration of Sr-Ca, Mg, K, P, Zn, Cu, B, Al-Fe, and Mn.

Massey and Loeffel (26) determined zinc content (38.4-15.5 ppm) of grain from inbred lines of corn and proposed that genes control the ability of the plant to absorb zinc or to translocate it to the kernels. They (27) related the high concentrations in the kernels to the net transfer of zinc from stalk and leaves to the grains, the effectiveness of which depended on the general zinc content of the plant.

Kleese (23) established that ^{89}Sr and ^{45}Ca accumulation in soybean seeds was largely controlled by the genotype of the stem. In the terminal 12-15 Cm, however, ^{89}Sr and ^{45}Ca accumulation depended on both the genotype of the root, and the stem, with the stem being relatively more important.

Rasmusson et al. (38) found large and significant genotypic differences for strontium accumulation in wheat and barley. The pattern of inheritance was not reported.

By including two pairs of isogenic lines in their studies, they established that the genes or gene responsible for ^{89}Sr accumulation was not associated with the gene for the condition of starch (normal starch and waxed starch), but the gene controlling row number or genes closely linked to it played an important role. Substantial genetic control of the process of ^{89}Sr accumulation in the grains of barley was indicated by a heritability study involving F_1, F_2, and backcross populations (37). With few exceptions, however, concentration in the grain was accounted for by additive gene effects.

Possible Mechanisms for the Observed Differences

In a number of cases, the observed differential responses have been accounted for by differences in anatomical or physiological mechanisms. It is possible, therefore, that the genes exert their effects on ion-accumulation via affecting the physiological mechanisms involved.

Differential responses of inbred lines and crosses of maize to variations in P and N (42), and to P (25) were attributed to variations in root-morphology. Plants with greater proportions of secondary to primary roots were more efficient.

Vose (50) in his review, emphasized that the factors of nutrient absorption, translocation, and metabolism could account for the varietal differences in mineral nutrition.

Recently, strains of snapbeans have been shown to differ in K utilization (40). Since differences were not caused by variations in seed size or root absorption capacity, they may be associated with transport or utilization at the cellular level.

Numerous investigators have utilized root/scion grafts to differentiate between the root and the shoot as possible sites of control for the observed differences (23,8,14,34).

Kleese (23) established that the genotype of the soybean stem was more effective in controlling strontium accumulation in the seed and in the apical 12-15 Cm region than the genotype of the root. Brown et al. (8) associated the genotype of the rootstock with efficiency of Fe absorption in soybeans, where Hawkeye rootstocks were more efficient than PI-54619-5-1. They (9) also reported that the efficient genotype was more capable of reducing Fe^{+++} to Fe^{++} at the root surface and therefore the reductive capacity of the roots, their capacity to absorb Fe, and the susceptibility of these plants to Fe chlorosis were all related. Foote and Howell (14) observed the differential response of soybean varieties Chief (tolerant) and Lincoln (sensitive) to high P. By grafting tolerant shoot on sensitive root, the tolerant shoot was found to develop P-toxicity. Conversely, sensitive shoots did not develop symptoms when grafted on the tolerant root. Polson (34) noted that the genotype of the scion was responsible for the differential tolerance of two navy bean varieties to high

zinc (5.0 ppm). He also found that the variety, Saginaw, tolerant to low zinc, transferred a greater volume of plant sap in a given time than Sanilac. The concentration of zinc was approximately three times greater in the exudate of tolerant than in the sensitive variety.

Brown et al. (11) used the split-root technique to demonstrate the internal inactivation of Fe in PI-54619-5-1 soybeans, principally from the combined effects of P and Ca. Ca stimulated root growth, increased absorption and translocation of P and Ca to the above ground parts, but decreased the absorption and translocation of Fe in the presence of P. Hawkeye remained green under conditions which induced chlorosis in PI-54619-5-1.

Wallace et al. (52) reported that the PI soybean variety inhibited ^{59}Fe uptake by Hawkeye when both were grown in the same nutrient solution but Hawkeye had no effect on ^{59}Fe in PI. This may be considered as an indication that PI exudes an inhibitory factor into the medium which decreases the absorption of Fe by Hawkeye.

The carrier concept of ion uptake, originally proposed by Epstein and Hagen (13), has gained further support by the work of Pardee (32) and Pardee et al. (33), who isolated a sulphate binding protein from Salmonella. Differential affinity of carrier sites for different elements and the competition between elements for the available sites accounted for the different proportions of various elements entering the plant root.

Hiatt (20) proposed that organic and amino acids play an important role in ion accumulation by providing nondiffusible charges which may bind or retain inorganic ions within the cell, also the nonadditive component of ion uptake which becomes important at salt concentrations higher than 1 mM is a result of diffusion of neutral salts according to Donnan phenomena. Ion uptake by this mechanism would not necessarily involve the action of carriers.

Fe has been shown to exist as Fe-malate, Fe-malonate and Fe-citrate in stem exudate (44,45,46). On this basis, these natural chelating agents have been postulated to act as transport carriers. The quality and the quantity of the chelating agents, the involvement of similar carriers for various elements, or the competition between ions for the same carrier also might affect differential transport.

MATERIALS AND METHODS

The genetic and physiological studies were conducted in sand-culture in the greenhouse. Soil-culture was used for crossing and seed multiplication only. The general procedure of raising plants in sand involved the following.

Polyethylene bags were placed inside clay pots to avoid contamination from the pot, and a hole was made at the bottom to facilitate drainage. Acid washed sand was put in the pots. Seeds were then planted at a depth of one-half to one inch. Sand was moistened with deionized water and deionized water was supplied till germination, if necessary to prevent desiccation. Following germination, a routine of nutrient application was initiated which consisted of applying the nutrient solution every second day and deionized water on alternate days. All the culture pots were flushed with deionized water once a week.

Two types of nutrient solutions were prepared, i.e., normal (control), and high iron minus manganese (screening). Slightly modified Hoagland's (21) macronutrient solution was used as the basal solution. Chemicals and their quantities are listed below:

Chemical	Quantity (g/liter)
$Ca(NO_3)_2 \cdot 4H_2O$	1.401
KH_2PO_4	0.0793
KNO_3	0.5284
$MgSO_4 \cdot 7H_2O$	0.4323
$NH_4H_2PO_4$	0.670
KCl	0.0434

Composition of the micronutrient solution also was based on Hoagland's (21) formula but slight variation was adapted in accordance with the objective. The proportion and sources of various elements are presented for both the solutions (Table 1).

Table 1. Proportion and sources of micronutrient elements in nutrient solutions.

Element	Source	Concentration (ppm)	
		Normal	Screening
Boron	H_3BO_3	0.5	0.5
Manganese	$MnCl_2 \cdot 4H_2O$	0.5	None
Zinc	$ZnSO_4 \cdot 7H_2O$	0.05	0.05
Copper	$CuSO_4 \cdot 5H_2O$	0.02	0.02
Molybdenum	$H_2MoO_4 \cdot H_2O$	0.01	0.01
Iron	Fe-citrate	0.5	5.0

Reagent grade chemicals were used to make the nutrient solutions. The pH of the final solution varied between 5.3 and 5.7. Temperature and light conditions were those of the greenhouse environment.

Screening for Genotypes Differing in Their Responses

Approximately 60 breeding lines of navy beans (Phaseolus vulgaris L.) were grown in sand with either normal or screening solution. The entire experiment was replicated twice. Three to four weeks after planting, differential chlorotic symptoms developed on plants where screening solution was provided. In this study, no attempt was made to determine whether the chlorosis was due to Fe toxicity or due to Mn deficiency. The term chlorosis in this thesis refers to the appearance of chlorotic lesions on the foliage of certain genotypes when supplied with screening nutrient solution. On the basis of appearance, two extremely chlorotic lines 50 and 80 and two nonchlorotic lines 40 and 47 were selected for further studies.

Plants were harvested and divided into roots, stems and leaves. Samples were rinsed in 0.5N HCl and deionized water and left for drying. Dried plant samples were ground in a 20-mesh Wiley mill and then analyzed for Fe and Mn contents. Both of the replications were analyzed separately. Selected lines were replanted, harvested and analyzed again. Thus the results reported in the initial screening section are the average of three analyses.

Analytical Procedure

Known amounts of the ground material were placed in porcelain crucibles and ashed at 600 C for about 10 hours. Temperature was raised gradually to prevent ignition of the samples. Ash was moistened with deionized water and 5 ml of 2N HCl was added. The solution was filtered, using Whatman number 2 filter paper. Deionized water was poured through the filter paper in aliquots, the latter of which rinsed the crucible, and adjusted to a given volume. In some samples, sand remained on the filter paper. These were preserved and weighed. The weight of sand was subtracted from the previously recorded weight to determine the actual weight of the plant material.

The solutions were analyzed for Fe and Mn content in a Perkin-Elmer model 290 atomic absorption spectrophotometer. Standards of known concentrations were used and since readings (percent absorption) were linear, it was possible to calculate a constant (K) by dividing the reading by ppm concentration. The percents of absorption were converted to μg/g dry wt, according to the following formula:

$$\mu g/g \text{ dry wt.} = \frac{K \times \text{Percent absorption} \times \text{Volume of solution}}{\text{Weight of the sample}}$$

Part of the investigation was completed using the Perkin-Elmer model 303. The procedure was the same, except that the readings (percent absorption) were converted to absorbance

first, according to the table published by Perkin-Elmer Corporation, Connecticut, U. S. A. Conversion to μg/g dry wt was accomplished by the same formula.

Genetic Study

Selected lines were planted in soil in the greenhouse. Crosses were made in all possible combinations. Seeds of reciprocals were not kept separately but pooled together. Resulting F_1 seeds were planted in sand in two replications for each nutrient solutions, i.e., normal and screening. Two seeds of each parent and their F_1 were planted in the same pot. At the same time, some F_1 seeds were planted in soil to produce F_2 seeds and to make backcrosses. As the chlorosis appeared in parents, phenotype of the F_1 was recorded and photographs were taken.

The F_2 seeds were collected from 40 x 50 and 47 x 80 combinations only, thus four backcrosses involving the F_1 and their respective parents, i.e., (40 x 50) x 40, (40 x 50) x 50, (47 x 80) x 47, (47 x 80) x 80, were made. Seeds of F_2 and backcrosses were planted in sand in screening nutrient solution only along with the parents. After 3-4 weeks, when the parents exhibited differences, individuals of segregating populations were evaluated and categorized as chlorotic or nonchlorotic, based on phenotypic appearance. A Chi-square test was used to evaluate the differences statistically.

Grafting Experiment

Grafts in different scion/root combinations were made in an attempt to localize possible sites of control. Seeds of the chlorotic and nonchlorotic lines were planted side by side in sand in the same pot. After germination and before the primary leaves had fully expanded, grafts were made in the following scion/root combinations: 40/40, 40/50, 50/40, 50/50, 47/47, 47/80, 80/47 and 80/80.

The stems were cut diagonally between the cotyledonary node and the sand surface. A small piece of toothpick was inserted into the pith which supported the scion of the desired combination. After putting the stock and scion together, a small piece of latex bandage was placed around the graft to facilitate reunion and to prevent desiccation. Deionized water was supplied and the pots were covered with plastic bags. Subsequently all the grafts were transferred to a moist chamber, where they remained for about 5 days. During this period, they received deionized water as needed. After 5 days, the bags were removed and grafts of each combination were divided into two groups for nutrient application. After about 4-5 weeks, chlorotic symptoms appeared; photographs were then taken, and the plants harvested immediately for analysis. Plant parts from all the grafts of one combination were ground together and two analyses were made.

Exudate Experiment

Seeds of chlorotic and nonchlorotic lines were planted in the same pot in sand in two replications. Following emergence, the number was reduced to 3 plants of each line in one pot. Partial shading was provided during germination and the week following to elongate the hypocotyl. Nutrient conditions and routine of application were as previously described. A week before collecting the exudate, however, the set which had been receiving screening solution was supplied with the normal concentrations of Mn. Four weeks after planting, leaves were removed, cut surfaces were covered with vaseline and stems were cut at a height of approximately nine inches from the sand surface. Cut stems were bent over and placed into small plastic vials. Exudate was collected for a period of 24 hours. The weight of exudate, and the Fe and Mn contents were used to determine differences in rate of transport. The pH of each sample also was recorded.

RESULTS AND DISCUSSION

1. Initial Screening

Genotypes of beans consistently differed in their visual symptoms in response to screening solution; lines 50 and 80 developed chlorosis, while 40 and 47 did not (Figure 1). The onset of chlorosis occurred about 3 weeks after planting, although distinct differences were evident until the end of the fourth week; therefore, this was considered a critical period in the present investigation.

The primary purpose of analyzing plant parts for Fe and Mn contents was to establish a relation between the visual symptoms and concentration of the elements. Thus the mode of inheritance could be studied by basing the evaluation of progeny performance both on the appearance and the analysis of plant parts. Since the F_2 and backcross populations were grown in screening solution only, the initial problem centered around identifying genetic differences in the screening solution alone without involving the comparison with normal. Analytical data are summarized in Tables 2 and 3.

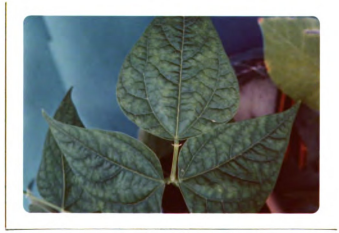
Figure 1. The appearance of navy bean lines grown in screening solution.

A. Line 40

B. Line 47

Figure 1 (cont'd)

C. Line 50



D. Line 80

Table 2. The concentration of Fe and Mn (μg/g dry wt)[1] in the roots, stems, and leaves of navy bean lines grown in normal and screening solutions.

Element	Lines	Root			Stem			Leaf		
		Normal	Screening	Screening as % of normal	Normal	Screening	Screening as % of normal	Normal	Screening	Screening as % of normal
Fe	40	88.2	216.6	243.8	41.0	61.1	149.1	164.6	199.7	121.3
	47	90.9	310.9	342.0	36.3	40.9	112.3	147.9	210.8	142.5
	50	97.8	171.4	175.2	42.7	70.0	163.7	131.7	166.0	126.0
	80	148.3	400.2	269.8	36.5	46.4	127.1	121.6	311.0	256.5
Mn	40	68.1	34.0	49.8	65.7	61.1	93.0	191.5	36.9	19.2
	47	63.9	30.4	47.7	42.9	32.4	75.6	138.2	37.3	26.9
	50	72.2	38.0	52.6	51.5	14.4	28.0	124.4	34.5	27.7
	80	59.2	54.5	91.9	73.1	34.4	47.0	118.7	43.0	36.2

[1]Average of three analyses (see page 15).

Table 2 shows the Fe concentration in roots, stems, and leaves. It is clear that the genotypes growing in screening solution had more Fe in various plant parts than the same genotypes cultured in normal solution. Although the concentration of Fe was 10 times greater in the screening solution (5.0 ppm) than in the normal (0.5 ppm), concentration in the plant parts did not vary in same proportion. This may be the result of preferential exclusion of ions at the root surfaces. In normal solution, leaves usually contain a greater concentration of Fe than roots with the exception of line 80 which has more in the root and the least amounts are in the stems. In the screening solution, roots always have greater concentrations followed by leaves and stems. This may indicate that uptake increases with supply to a certain extent, but transport does not keep pace. Uptake and transport are two separate components of the accumulation process. Part of uptake may be accounted for by diffusion, whereas transport is largely active. Disproportionate uptake and transport of Fe may then result during metabolic disturbances, which, in this case was induced by altering the composition of the nutrient solution. The comparison between chlorotic and nonchlorotic lines does not reveal a pattern for Fe neither in the screening solution nor in comparison with normal. It is possible that the Fe concentration <u>per se</u> is not directly associated with the development of chlorosis.

Table 2 also contains the Mn concentrations in roots, stems, and leaves. Mn is higher in plants grown in normal solutions than those grown in screening solution. In spite of the fact that cultures grown in screening solutions were not provided with Mn, Mn was always detected in plant tissue. This may be the residual Mn from seeds and/or contamination from various chemicals used in preparing the nutrient solutions. Although chlorotic lines had more Mn in roots, the concentration in line 50 (38.0 ppm) was slightly greater than the nonchlorotic lines (30.4 and 34.0 ppm). Stems and leaves do not exhibit significant differences. If the Mn content of plants grown in screening solution is expressed as percent of normal, nonchlorotic lines tend to have lower values in the roots and leaves and higher values in the stems as compared to chlorotic lines. Apparently, nonchlorotic lines have the capacity of transporting Mn to the foliar parts efficiently especially when exogenous Mn is very low, whereas the chlorotic lines lack this capability or are not equally efficient. If the distribution of Mn is associated with chlorosis, as has been assumed, leaves, being the utilization site, should have a higher value in nonchlorotic lines, but actually they had less. This discrepancy may result from the mobile nature of Mn. In such a situation the Mn of the stem should not be considered separate from the Mn of the leaf. It may be reserve and/or transitional, capable of being moved to the organ where needed. It is not possible, however, to

draw a firm conclusion on the basis of this study since total uptake is not available, yet this may be considered as an indication that the distribution of Mn is different in the two sets of lines.

If the distribution is different for Fe and Mn in chlorotic and nonchlorotic lines, it should be expressed in the Fe/Mn ratios. This would emphasize the importance of the relative balance of the two elements and at the same time could be used as a reliable guide for evaluating intergenotypic differences.

In the screening solution, the comparison of Fe/Mn ratios between the chlorotic and nonchlorotic lines does not reveal a pattern (Table 3). When the ratios from screening solution are expressed as percent of ratios in normal solution, roots of nonchlorotic lines show higher values and stems the lower than the chlorotic lines, whereas the leaves do not show a consistent pattern. It seems in agreement with the previous assumption and findings, i.e., that higher Fe/Mn ratios in the roots in screening solution indicate that the nonchlorotic lines retain relatively more Fe and less Mn in the roots than the chlorotic lines.

The development of chlorosis is an end result of a series of internal disorders occurring in response to the screening nutrient solution. An ionic imbalance will affect various processes requiring direct or indirect involvement of Fe and/or Mn. This may also affect other processes via affecting

Table 3. Fe/Mn ratios[1] in the roots, stems, and leaves of navy bean lines grown in normal and screening solutions.

Lines	Root			Stem			Leaf		
	Normal	Screening	Screening as % of normal	Normal	Screening	Screening as % of normal	Normal	Screening	Screening as % of normal
40	1.3	6.3	490.0	0.6	1.0	166.6	0.8	5.6	658.8
47	1.4	10.1	717.6	0.8	1.2	150.0	1.0	5.6	528.2
50	1.3	4.5	334.7	0.8	5.0	625.0	1.0	5.0	476.2
80	2.5	7.3	293.6	0.5	1.2	240.0	1.0	7.0	686.2

[1]Fe (μg/g dry wt)/Mn (μg/g dry wt).

either the availability or the utilization of other elements due to interactions with Fe and/or Mn. Since it is possible to make only limited qualitative observations, a reliable quantitative guide should be established. Tissue concentrations of Fe and Mn were chosen for this purpose.

On the basis of the composition of nutrient solution, both Fe toxicity and Mn deficiency may be expected to develop. Although attempts were not made to identify real cause, similar symptoms were noted throughout the investigation with little variation in the intensity of the chlorosis that developed. Visually observed chlorosis may be correlated with distribution of the two elements within the plant. Studies involving single elements have revealed that the capacity of certain genotypes to tolerate toxicity under high levels is a result of their inability to transport the element to the shoot (31), while the capability to escape the deficiency at low supply is due to efficient transport (34). The concentrations of Fe and Mn and Fe/Mn ratios may indicate that the differences observed in this study are of a combined nature; an efficient Fe transport and an inefficient Mn transport in the chlorotic lines and the converse in nonchlorotic lines. In conclusion, therefore, the appearance of chlorotic and nonchlorotic conditions may be related to the differential distribution of Fe and Mn within the plant; relatively high Fe and low Mn in the foliar parts is conducive to chlorosis and the converse for nonchlorosis. Differences in Fe

transport were noted by Brown (6) in two soybean varieties and the involvement of oxidative phosphorylation was postulated as a likely cause. The involvement of natural organic acid chelates (10,44,45) often has been associated with Fe stress. The differences in the quality or the quantity of these chelates have been postulated to account for the differences in Fe transport. Mn has been shown by Munns et al. (29,30) to exist in various forms in the roots. The size and the turn-over rate of labile forms has been shown to account for the differences in shoot-Mn among oat varieties. Similar systems may be operative in bean plant for Fe and Mn, but it is not possible to elucidate the nature of the mechanism(s) involved. An inverse relation between Fe and Mn may be a result of direct antagonism occurring during transport, as has been reported (12,41). This may also arise due to a greater uptake of interfering ions, e.g., phosphorus, which might render Fe inactive in the root system (11) and promote Mn transport. It is also possible that Fe and Mn are transported independent of each other, in a manner depending on the genetic constitution. Then it should be possible to identify recombinant segregates with transport combinations different from the parents in segregating populations, and also, certain genetic combinations should tolerate the antagonistic effects of Fe and Mn.

It is apparent from the results presented that the chlorotic and nonchlorotic lines exhibit quantitative

differences. In most instances these differences are not expressed in data from the screening solution alone, but require a comparison with data from normal solutions. Since it was not possible to establish an index to reveal genotypic differences in screening solution alone, genetic studies were based on visual symptoms.

2. Genetic Studies

Plants in F_1, F_2, and backcross populations were evaluated for qualitative responses in order to determine an approximate mode of inheritance. All the F_1 combinations were planted in screening, as well as normal, solution. Phenotypic appearance of various hybrids in the screening solution is presented in Table 4 and Figure 2.

Table 4. Phenotypic appearance of F_1 combinations of navy bean lines grown in screening solution.

Cross	Phenotype
40 x 47 (nonchlorotic x nonchlorotic)	nonchlorotic
40 x 50 (nonchlorotic x chlorotic)	chlorotic
40 x 80 (nonchlorotic x chlorotic)	chlorotic
47 x 50 (nonchlorotic x chlorotic)	chlorotic
47 x 80 (nonchlorotic x chlorotic)	chlorotic
50 x 80 (chlorotic x chlorotic)	chlorotic

Figure 2. Phenotypic appearance of F_1 combinations of navy bean lines grown in screening solution.

A. Cross 40 x 47

B. Cross 40 x 50

Figure 2 (cont'd)

C. Cross 47 x 80

D. Cross 50 x 80

The results (Table 4) indicated phenotypic dominance of the chlorotic condition, since crosses involving at least one chlorotic parent exhibited chlorosis and a nonchlorotic hybrid arose only if both the parents were nonchlorotic (40 x 47). The two chlorotic lines behaved identically as did the two nonchlorotic lines, judged by the appearance of different F_1 combinations. This may indicate that the genetic constitutions of both chlorotic lines, 50 and 80, are identical, and similarly for the two nonchlorotic lines, 40 and 47.

Individual plants in each of the two F_2 and four backcross populations were examined visually and categorized as chlorotic or nonchlorotic. Chi-square values for goodness of fit to theoretical ratios are presented in Table 5. Both of the F_2 populations fit a 9 chlorotic:7 nonchlorotic segregation pattern, indicating the involvement of two genes. If the genotypes of chlorotic and nonchlorotic parents are assumed to be AA BB and aa bb respectively, the genetic constitution of the F_1 plants will be Aa Bb, giving a chlorotic phenotype. The backcross of a F_1 plant to a nonchlorotic parent (Aa Bb x aa bb) will produce four genotypes (Aa Bb, Aa bb, aa Bb and aa bb), giving an expected phenotypic ratio of 1 chlorotic:3 nonchlorotic. The chi-square values and probabilities were compatible with the theoretical expectations. With this genetic hypothesis, the backcross involving the F_1 and the chlorotic parent (Aa Bb x AA BB) should produce all chlorotic plants with the following genotypes: AA BB,

Table 5. Observed and expected numbers of chlorotic and nonchlorotic plants and Chi-square values for goodness of fit for the F_2 and backcross populations of navy bean lines grown in screening solution.

Population	Number of Plants				Assumed ratio	Chi-square value	Proba-bility
	Observed		Expected				
	Chlorotic	Non-chlorotic	Chlorotic	Non-chlorotic			
(40 x 50) F_2	34	21	30.9	24.1	9:7	0.70	0.50-0.30
(40 x 50) F_1 x 40	12	20	8	24	1:3	2.66	0.20-0.10
(40 x 50) F_1 x 50	24	10	34	0	All chlorotic	--	--
(47 x 80) F_2	39	31	39.4	30.6	9:7	00.0082	0.95-0.90
(47 x 80) F_1 x 47	13	23	9	27	1:3	2.36	0.20-0.10
(47 x 80) F_1 x 80	28	6	34	0	All chlorotic	--	--

AA Bb, Aa BB and Aa Bb. Chi-square values could not be calculated because of the unexpected appearance of nonchlorotic plants.

Since the nonchlorotic plants were noted in both the backcross populations involving F_1 and their respective chlorotic parents in considerable numbers, the assumed genetic constitution(s) or the mechanism(s) may be an oversimplification of the more complex mechanism really involved. An alternate hypothesis is being proposed.

The analysis of plant parts in the initial screening section indicated that the distribution of Fe and Mn differs in the two sets of lines and also, the relative balance of the two elements is more important than the absolute concentration of either one. Although, total amount of Fe and Mn is not known, nevertheless, the ratio of total Fe/total Mn in the foliar parts may be a reliable index for the intergenotypic differences. Thus, the characteristics of the chlorotic and nonchlorotic lines may be as follows:

Chlorotic	High Fe and Low Mn transport	High Fe/Mn ratio
Nonchlorotic	Low Fe and High Mn transport	Low Fe/Mn ratio

Genotypes were initially assumed to be AA BB and aa bb for chlorotic and nonchlorotic parents respectively. This assumption is not supported by the backcross populations derived from the F_1 and the chlorotic parents. It is then postulated that the genotype of a chlorotic parent is AA bb

(AA- High Fe transport, bb- Low Mn transport), and of a non-chlorotic parent is aa BB (aa- Low Fe transport, BB- High Mn transport). Heterozygosity at the A locus (Aa) results in the transport of more Fe than AA, and BB is completely dominant over bb. An additional assumption is being made, i.e., Aa with BB or Bb and aa bb produce nonchlorotic plants since the Fe/Mn ratio would not be too high. The implications of this postulation are discussed.

An F_1 with genetic constitution Aa Bb would be chlorotic. The Fe/Mn ratio would be higher than the chlorotic parent because Fe transport is accentuated in the hybrid more so than Mn transport. The range of ratios in the F_2 population should exceed the lower and higher limits imposed by the parental genotypes because of different transport recombinants. The proposed genetic constitutions, their proportions, pattern of Fe and Mn transport, Fe/Mn ratios and the phenotypes in the F_2 and backcross populations are given in Table 6.

Chi-square values were calculated for backcross populations according to the expected ratios (Table 7). The values are non-significant indicating that the assumed ratios and the genetic constitutions can be accepted.

On this assumption, genotypes of F_2 could be arranged on the basis of the Fe/Mn ratio in shoots in ascending order (classes 1-6) as follows:

Table 6. The genetic constitutions, their proportions, pattern of Fe and Mn transport, Fe/Mn ratios and the phenotypes in the F_2 and backcross populations according to "heterozygote superiority" hypothesis.

Population	Genotypes	Proportion	Transport pattern		Expected Fe/Mn ratio	Appearance
			Iron	Manganese		
F_2	AABB	1	High	High	Intermediate	Nonchlorotic
	AABb	2	High	High	Intermediate	Nonchlorotic
	aaBB	1	Low	High	Low	Nonchlorotic
	aaBb	2	Low	High	Low	Nonchlorotic
	aabb	1	Low	Low	Intermediate	Nonchlorotic
	AaBB	2	Higher	Low	High	Chlorotic
	AaBb	4	Higher	Low	High	Chlorotic
	AAbb	1	High	Low	High	Chlorotic
	Aabb	2	Higher	Low	High	Chlorotic
	Phenotypic ratio 9 chlorotic:7 nonchlorotic					
BC_1*	AABb	1	High	High	Intermediate	Nonchlorotic
	AAbb	1	High	Low	High	Chlorotic
	AaBb	1	Higher	High	High	Chlorotic
	Aabb	1	Higher	Low	High	Chlorotic
	Phenotypic ratio 1 nonchlorotic:3 chlorotic					
BC_2**	aaBB	1	Low	High	Low	Nonchlorotic
	aaBb	1	Low	High	Low	Nonchlorotic
	AaBB	1	Higher	High	High	Chlorotic
	AaBb	1	Higher	High	High	Chlorotic
	Phenotypic ratio 1 chlorotic:1 nonchlorotic					

* = Backcross to the chlorotic parent (Aa Bb x AA bb).

** = Backcross to the nonchlorotic parent (Aa Bb x aa BB).

Table 7. Observed and expected numbers of chlorotic and nonchlorotic plants and Chi-square values for goodness of fit for backcross populations of navy bean lines grown in screening solution, according to "heterozygote superiority" hypothesis.

Population	Number of plants				Assumed ratio	Chi-square values	Probability
	Observed		Expected				
	Chlorotic	Non-chlorotic	Chlorotic	Non-chlorotic			
(40 x 50)							
F_1 x 40	12	20	16	16	1:1	2.00	0.20-0.10
F_1 x 50	24	10	25.5	8.5	3:1	0.34	0.70-0.50
(47 x 80)							
F_1 x 47	13	23	18	18	1:1	2.78	0.10-0.05
F_1 x 80	28	6	25.5	8.5	3:1	0.97	0.50-0.30

aa BB aa Bb	aa bb	AA BB AA Bb	AA bb	Aa BB Aa Bb	Aa bb
1	2	3	4	5	6

Classes 1, 2, and 3 are expected to be nonchlorotic (7/16) and classes 4, 5, and 6 chlorotic (9/16). Furthermore, 8/16 of the F_2 population (classes 5 and 6) should have a higher Fe/Mn ratio than the chlorotic parent and 2/16 (class 6) higher than the F_1. The backcross to the chlorotic parent should produce plants in a 1 : 1 : 1 : 1 proportion in classes 3, 4, 5, and 6, respectively. Backcross with nonchlorotic parents should have one-half of the population similar to the nonchlorotic parent and the other half like the F_1 (chlorotic).

The alternate hypothesis proposed here seems plausible and explains the appearance of nonchlorotic plants in backcross populations with chlorotic parents.

3. Grafting Experiment

Grafts in different root/scion combinations were made to localize possible sites of control. Established grafts were divided into two groups for nutrient application, i.e., normal and screening solutions. The appearance of grafted plants after five weeks was recorded and the observations are presented in Table 8 and Figure 3.

It is clear (Table 8) that the genotype of the rootstock is responsible for the appearance of chlorosis in line 50, since on this root even the scion of nonchlorotic line 40

Table 8. The appearance of grafted navy bean plants grown in screening solution.

Graft combination (scion/rootstock)	Appearance
40/40 (nonchlorotic/nonchlorotic)	nonchlorotic
40/50 (nonchlorotic/chlorotic)	chlorotic
50/40 (chlorotic/nonchlorotic)	nonchlorotic
50/50 (chlorotic/chlorotic)	chlorotic
47/47 (nonchlorotic/nonchlorotic)	nonchlorotic
47/80 (nonchlorotic/chlorotic)	nonchlorotic
80/47 (chlorotic/nonchlorotic)	chlorotic
80/80 (chlorotic/chlorotic)	chlorotic

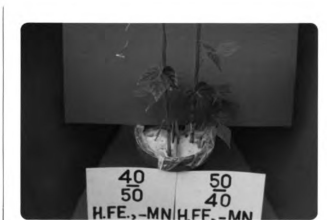
develops chlorosis while the scion of line 50 grafted on the rootstock of line 40 does not. Conversely, susceptibility in line 80 seems to be determined by the genotype of the scion because it always developed chlorosis whether grafted on a chlorotic or a nonchlorotic rootstock. In addition, the rootstock of line 80 does not seem to be inefficient since chlorosis does not develop when a scion of line 47 is grafted onto a rootstock of line 80.

The concentrations of Fe and Mn in roots, stems and leaves are presented in Table 9.

Table 9 contains the concentrations of Fe and Mn (μg/g) in roots, stems and leaves. The values for plants grown in screening solution are also expressed as percent of normal.

Figure 3. The appearance of grafted navy bean plants grown in screening solution.

A. 40/40 and 50/50



B. 40/50 and 50/40

Figure 3 (cont'd)

C. 47/80

D. 80/47

Table 9. The concentrations of Fe and Mn (μg/g dry wt)[1] in the roots, stems and leaves of grafted navy bean plants grown in normal and screening solutions.

Graft	Plant part	Iron			Manganese		
		Normal	Screening	Screening as % of normal	Normal	Screening	Screening as % of normal
40/40	Root	308.8	517.7	167.6	119.1	23.4	19.6
	Stem	190.0	58.2	30.6	72.4	5.3	7.3
	Leaf	140.2	155.7	111.0	143.4	41.0	28.5
40/50	Root	60.4	731.2	1210.0	26.9	45.5	168.8
	Stem	103.1	57.8	56.0	76.4	14.8	19.3
	Leaf	228.8	131.4	57.4	149.4	18.4	12.3
50/40	Root	141.8	693.6	489.0	150.7	21.1	14.0
	Stem	85.6	78.3	91.4	77.2	14.1	18.2
	Leaf	212.0	200.0	94.3	138.8	36.8	26.5
50/50	Root	64.2	542.5	843.9	139.6	21.3	15.2
	Stem	195.5	81.6	41.7	57.9	7.2	12.4
	Leaf	194.6	160.7	82.5	130.1	24.6	18.9

47/47	Root	100.7	900.0	893.3	70.6	9.2	13.0
	Stem	35.6	119.9	336.7	8.9	25.1	28.2
	Leaf	155.0	108.8	70.1	152.2	24.6	16.1
47/80	Root	110.6	734.6	663.8	138.3	30.8	22.2
	Stem	114.1	99.9	87.5	84.6	28.7	33.9
	Leaf	158.5	125.3	79.0	80.5	32.7	40.6
80/47	Root	157.0	317.2	201.9	131.2	8.6	6.6
	Stem	109.1	78.1	71.5	80.9	19.0	23.4
	Leaf	210.0	171.4	81.6	127.4	17.8	13.9
80/80	Root	185.8	838.7	451.3	140.7	50.9	36.2
	Stem	65.8	101.7	154.5	55.5	25.4	45.7
	Leaf	315.9	187.9	59.4	206.1	32.7	15.8

[1]Average of two analyses (see page 18).

The concentration of Fe in the roots in screening solution does not reveal a pattern for root/scion combination. If the concentration of Fe in roots in the screening solution is expressed as percent of Fe in roots in the normal solution, lines 40 and 50 seem to behave oppositely than what was noted in the screening experiment. Yet, however, the capacity of both the chlorotic lines 50 and 80 to retain Fe in the root is enhanced when grafted in combination with the nonchlorotic scions, 40 and 47. This suggests that probably the capacity of roots to retain Fe or to transport it to the shoot is governed by the genotype of the scion, and the nonchlorotic scions, 40 and 47 are more efficient for Fe retention by the roots than the chlorotic scions, 50 and 80.

Considering Fe concentration in the leaves, all the combinations having nonchlorotic scions, e.g., 40/40, 40/50, 47/47 and 47/80, have less Fe than when the chlorotic scion is involved. The pattern is not clear, however, if the concentration in screening solution is expressed as percent of normal. This again tends to show that if a nonchlorotic scion is involved in the graft, the concentration of Fe is lower in the leaves than when the scion is of chlorotic line. Also, the concentration of Fe <u>per se</u> does not seem to be the sole factor in the development of chlorosis.

Mn in roots does not reveal a pattern. The combination 40/50 has the greatest concentration in the root. Rootstock of line 80 always has high concentration of Mn, showing the

efficiency of line 80 rootstock in retaining Mn. However, the pattern is not clear for the chlorotic symptom.

In the leaves, Mn concentration does not reveal a consistent pattern. In the grafts of line 40 and 50 combinations, both the nonchlorotic grafts have more Mn than the chlorotic combinations. In the graft combinations of line 47 and 80, when the rootstock 80 is involved, leaves have more Mn than when the rootstock is line 47. If the values of plants grown in screening solution are expressed as percent of normal, the nonchlorotic combinations tend to have higher values than the chlorotic combinations with the exception of combination 47/47. It seems that the concentration of Mn is an important factor in the development of chlorosis, though it may not be the sole factor.

Fe/Mn ratios are presented in Table 10. If the scion is line 40, the ratio is lower in the roots than if the scion is of line 50 in the graft combinations of line 40 and 50. In line 47 and 80 graft combinations, if the rootstock is line 47, the ratio is higher than if the rootstock is line 80, but the pattern fails to hold if the ratios of screening solutions are expressed as percent of ratios in the normal solution. In comparison with normal, however, both the nonchlorotic combinations, 47/47 and 47/80 have higher values than the chlorotic combinations.

In the leaves, all the nonchlorotic graft combinations have relatively lower Fe/Mn ratios than the chlorotic

Table 10. Fe/Mn ratios[1] in the roots, stems and leaves of grafted navy bean plants grown in normal and screening solutions.

Graft	Root			Stem			Leaf		
	Normal	Screening	Screening as % of normal	Normal	Screening	Screening as % of normal	Normal	Screening	Screening as % of normal
40/40	2.59	22.0	851.0	2.6	10.9	419.2	0.9	3.8	422.2
40/50	2.24	16.0	717.0	1.3	3.9	300.0	1.5	7.1	473.3
50/40	0.9	32.7	3481.1	1.1	5.5	500.0	1.5	5.4	360.0
50/50	0.4	25.4	5532.6	3.3	11.3	342.4	1.5	6.5	433.3
47/47	1.4	97.9	6894.3	4.0	4.7	117.5	1.0	4.4	440.0
47/80	0.8	23.8	3010.1	1.3	3.4	261.5	1.9	3.8	200.0
80/47	1.1	36.5	2330.0	1.3	4.1	315.3	1.6	9.6	600.0
80/80	1.3	16.4	1245.0	1.1	4.0	363.6	1.5	5.7	380.0

[1]Fe (μg/g dry wt)/Mn (μg/g dry wt).

combination, though the pattern is not clear if compared with normal. This agrees with the previous assumption that the relative balance between Fe and Mn is more important in the development of chlorosis than the absolute concentration of either one. High ratios are always associated with the chlorotic condition. However, it is not possible to state a critical ratio which separates the chlorotic and nonchlorotic condition, according to this study.

The results of initial screening indicated that the nonchlorotic lines (40 and 47) are efficient in transporting Mn to the shoot and inefficient in transporting Fe. The converse is true for chlorotic lines which upsets the relative balance between Fe and Mn. Results of the grafting experiment show little deviation from what was noted earlier in the screening experiment. It should be re-emphasized, however, that due to grafting the appearance of chlorosis was delayed about a week and plants were harvested only after the symptoms were noted. According to the observations in the screening experiment, this was beyond the critical period. It is possible that the visual appearance of chlorosis is delayed because of the effect of grafting. Extreme deviations are shown by lines 40 and 50 and they seem to behave oppositely in some respects. It may be that the degree of resistance in line 40 is relatively low or the differences between line 40 and 50 are not of large magnitude.

Results indicate that rootstock line 50 is capable of holding Fe but it is particularly efficient in retaining Mn. The graft of 40/50 develops chlorosis because line 50 rootstock not only retains Fe but at the same time it also retains Mn, and therefore, the balance in the foliar parts is upset. The reciprocal graft of 50/40 does not develop chlorosis because of the inefficiency of line 40 rootstock in retaining Mn and its efficiency in holding Fe. Similarly, rootstock of chlorotic line 80 seems to have the capacity to retain Fe but it also holds Mn, behaving in this respect like line 50.

It seems probable that the Fe holding capacity of both lines 50 and 80 rootstocks is not inherently inefficient but that Fe retention is governed by the genotype of the scion. Both lines 40 and 47 scions are efficient in retaining Fe in the roots of any genotype to which they serve as scions. They probably are deficient in some process involved in the transport of Fe. It is difficult, however, to describe the nature of the mechanism. The results are not very clear for the lines 40 and 50, yet the efficiency of line 50 rootstock in retaining Fe is greatly enhanced if the scion is of line 40. Also, if a line 47 scion is placed on a line 80 rootstock, this root can retain Fe equally well. Conversely, a line 47 rootstock does not hold Fe if the scion is line 80. At the same time, Mn holding capacity is largely dependent upon the genotype of the rootstock; both lines 50 and 80 are

efficient. The development of chlorosis then seems to depend upon the genotypes of both the rootstock and the scion. If the rootstock holds Mn, chlorosis develops depending upon the Fe status of the shoot, and this is governed by the genotype of the scion.

On the basis of chlorotic symptoms on plants of various graft combinations, lines 50 and 80 are different. The analytical data, however, suggests that the physiological mechanism(s) involved is similar in both the chlorotic lines, i.e., both of them retain Mn and are efficient in retaining Fe. This supports the conclusions of the initial screening experiment.

4. Exudate Experiment

Results in the previous sections suggested that the chlorotic and nonchlorotic lines differed in transporting Fe and Mn to the shoot. In order to determine the differences in the rate of transport, exudate was collected and analyzed. The data are presented in the following Tables 11-15.

Table 11 contains data on the total weight of exudate. The values indicate that in normal solution excised plants of nonchlorotic lines deliver more sap in a given time, whereas in screening solution they deliver less. If the values in screening solution are expressed as percent of normal, plants of chlorotic lines have higher values than the nonchlorotic lines. In nonchlorotic plants, a more efficient root system,

Table 11. Total wt of exudate in g per bean plant[1] grown in normal and screening solutions.

Lines	Normal solution	Screening solution	Screening as % of normal
40	1.8	0.6	33.3
47	1.7	0.8	47.0
50	0.8	1.0	125.0
80	1.5	1.1	73.3

[1]Average of 4 plants.

greater capacity of xylem system and differential osmotic pressures in the steler tissue, than in chlorotic plants which have been postulated (34), may account for the different rates of sap flow of plants grown in normal solutions. Reduced sap flow by plants of nonchlorotic lines in screening solution may be a physiological mechanism for adapting to the altered composition of the nutrient solution. It is possible that the over all salt accumulation in these lines is low, and this, by affecting water uptake and transport, also affects the volume of sap exuded.

Fe and Mn content (ppm) are given in Table 12.

The Fe concentration of plants grown in screening solution does not reveal any pattern. When the values are expressed as percent of normal, however, nonchlorotic lines

Table 12. Fe and Mn concentrations (ppm) in the exudate of bean plants grown in normal and screening solutions.

Lines	Fe			Mn		
	Normal	Screening	Screening as % of normal	Normal	Screening	Screening as % of normal
40	0.43	0.72	166.60	0.60	0.16	26.60
47	0.86	1.43	166.60	0.60	0.20	33.30
50	0.57	0.86	150.00	0.90	0.08	8.88
80	0.86	1.14	133.30	1.10	0.06	5.45

show higher values than chlorotic lines. The Mn concentration is higher in plants of nonchlorotic lines grown in screening solution as well as in comparison with plants grown in normal solution than chlorotic lines. High Fe concentration in nonchlorotic lines is in disagreement with the earlier results. Total Fe and Mn were then calculated by multiplying ppm concentration by the weight of exudate. These values are given in Table 13.

Total Fe does not show distinct differences in screening solution alone, but if expressed as percent of normal, chlorotic lines have higher values than nonchlorotic lines. Mn is high in nonchlorotic lines grown in screening solution. If the values are expressed as percent of normal, however, differences are not clear.

Table 13. Total Fe and Mn (μg) in exudate of bean plants grown in normal and screening solutions.

Lines	Fe			Mn		
	Normal	Screen-ing	Screening as % of normal	Normal	Screen-ing	Screening as % of normal
40	0.78	0.48	64.10	1.10	0.11	10.00
47	1.51	1.17	77.48	1.06	1.16	15.44
50	0.45	0.89	197.77	0.71	0.08	11.70
80	1.35	1.27	94.07	1.74	0.07	3.83

The Fe/Mn ratios in the exudate were calculated and are given in Table 14.

Table 14. Fe/Mn ratios in the exudate of bean plants grown in normal and screening solutions.

Lines	Normal	Screening	Screening as % of normal
40	0.71	4.40	615.30
47	1.40	7.15	510.71
50	0.63	10.70	1698.00
80	0.78	19.00	2435.00

Chlorotic lines have high ratios in screening solution. They also show high values if the ratios of screening solution

are expressed as percent of normal. This supports the findings reported previously.

The pH of the exudate was measured. Table 15 contains these values.

Table 15. pH of the exudate of bean plants grown in normal and screening solutions.

Lines	Normal	Screening	Screening as % of normal
40	5.50	6.00	109.10
47	6.30	6.00	95.23
50	5.85	6.15	105.13
80	5.90	6.55	111.01

In screening solution the chlorotic lines have a higher pH, but there is no pattern if compared with plants cultured in normal solution. Both the nonchlorotic lines tend to have a pH around 6.00, and this may be considered a best pH for physiological adaptation to the altered composition of nutrient solution.

The findings from the exudate experiment are consistent with the view that the reduction in the amount of sap being delivered by nonchlorotic lines is a physiological adaptation to ionic imbalance. The Fe content (ppm) of nonchlorotic lines is higher than chlorotic lines, but when total Fe

(conc x g of exudate) is calculated, chlorotic lines have more Fe, i.e., more Fe is transported in a given time. Mn is higher in nonchlorotic lines than chlorotic lines, but the differences are not clear when total Mn in screening solution is expressed as percent of Mn in normal. This discrepancy may be because a normal amount of Mn was provided one week prior to exudation and during exudation and it is possible that the lines differ in their abilities to recuperate after stress. The Fe/Mn ratio was highest for the chlorotic lines and this agrees with the previous findings. It is difficult to relate pH with the differences in Fe and Mn transport due to limited data. It seems then that the nonchlorotic lines adjust to the ionic imbalance by reducing total Fe transport via reducing the sap delivery. At the same time sap is relatively more concentrated for Mn and therefore the balance between Fe and Mn is not upset.

With respect to the relation between the genetic and physiological mechanisms, it is suggested that the genes do not control the concentration *per se* but the accumulation or the total amount of an element via affecting the rate of sap flow. The differences are not always clear for Mn, but the amount of Fe shows a distinct pattern. Higher Fe transport is associated with a greater amount of exudate, which results in a higher Fe/Mn ratio which eventually induces chlorosis.

CONCLUSIONS

The findings may be summarized as follows:

1. Initial Screening

Lines of beans responded differentially to the screening solution; lines 40 and 47 were nonchlorotic whereas lines 50 and 80 developed chlorosis. The analysis of plant parts indicated that the chlorotic and nonchlorotic lines differed in the pattern of distribution of Fe and Mn in root and shoot--relatively low Fe and high Mn transport to the shoot in the nonchlorotic lines and the converse in chlorotic lines. The quantitative differences could not be used for genetic study, since they involved comparisons with plants grown in a normal solution, and such comparisons were not possible on individual plants of segregating populations.

2. Genetic Studies

The appearance of the F_1 combinations indicated phenotypic dominance of the chlorotic type. Both the chlorotic lines behaved identically as did the two nonchlorotic lines. Both F_2 populations segregated 9 chlorotic:7 nonchlorotic plants indicating the involvement of two genes.

3. Grafting Experiment

Visual appearance of the graft combinations indicated that the genotype of the rootstock line 50 and the genotype of the scion line 80 were responsible for the development of chlorosis. Tissue analysis, however, suggested that the roots of both chlorotic lines were efficient in retaining Fe but at the same time they also retain Mn. On the basis of the appearance and the analysis, it was postulated that the capacity of roots to retain Fe or to transport it to the shoot is dependent on the genotype of the scion, whereas the capacity to retain Mn is due mainly to the genotype of the rootstock.

4. Exudate Experiment

The nonchlorotic lines had lower rate of sap flow when grown in screening solution than chlorotic lines. Although the concentration of Fe was greater in the exudate of nonchlorotic lines, yet the total amount delivered was less. The Mn concentration of nonchlorotic lines was greater than that of chlorotic lines, but the pattern was not distinct for the amount. The Fe/Mn ratios and pH_s were greatest in the exudate of chlorotic lines.

Genes were postulated to control the amount of Fe and Mn via affecting the rate of sap delivery.

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