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Inheritance of Tolerance to Mineral Element-induced Chlorosis in Rabbiteye Blueberry

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Abstract. **A study of leaf chlorosis in rabbiteye blueberries *(Vaccinium ashei* Reade) grown in soil containing 300 to 400 ppm diethylenetriaminepentaacetic acid (DTPA)-extracted Mn revealed no relationship between leaf Mn content and chlorosis. A second study was conducted to estimate heritability of the content of Mn, Fe, and certain other mineral elements that have been associated with leaf chlorosis and to determine the genetic relationships among shoot dry weight, visual rating, and the mineral elements in rabbiteye blueberry. Heritability estimates were high for all variables except Fe, suggesting that changes in Mn, Zn, Ca, Mg, or K contents could be expected from phenotypic recurrent selection. However, manipulation of mineral content probably would not ameliorate the Fe chlorosis. The high heritability of shoot dry weight and visual rating and the high genetic correlation between these variables suggest that plants resistant to mineral effects on Fe metabolism can be selected on the basis of visual rating.**

Iron chlorosis of blueberry has been observed on mineral soils since the 1930s (Bailey, 1936), and it remains a major problem today (Korcak, 1989). Iron concentrations in chlorotic leaves are often equal to or higher than those found in green leaves (Cain, 1952; Korcak, 1989). Factors, such as form of N (Cain, 1954; Spiers, 1979), P content (Holmes, 1960; Korcak, 1989), K content (Cain, 1954), Ca content (Bailey, 1941), high micronutrient levels (Korcak, 1989), and tis-

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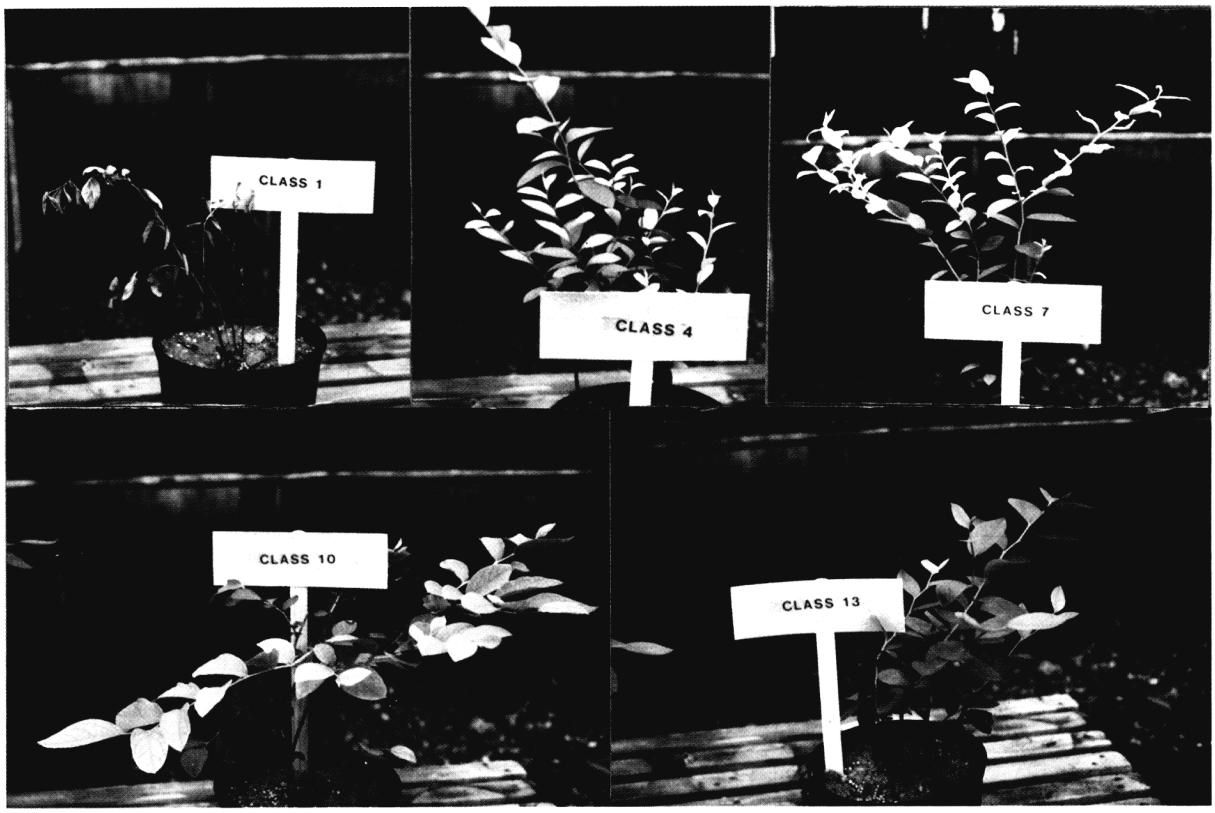

Fig. 1. Typical blueberry plant for five of the 13 classes into which 10 plants from each cross derived from a lo-parent diallel set of crosses and fertilized with a 250-ppm Mn solution were classified after 6 weeks of growth.

Table 1. Characteristics of certain rabbiteye blueberry clones grown in soil containing 300 to 400 ppm diethylenetriaminepentaacetic acid-extracted Mn.

Clone	Visual rating[z,y]	Mn content: Leaf mean[y] (ppm)	Mn content: Ratio basal : tip leaves	Soil pH[y]
T102[x]	4.5 a	245 ab	1.3:1	4.4 ab
T172[x]	4.5 a	394 a	4.5:1	4.4 ab
Climax	1.5 b	303 ab	4.4:1	4.4 ab
Tifblue	2.5 b	200 b	4.8:1	4.5 a
T129[x]	1.0 c	311 ab	4.8:1	4.3 b

[z]Subjective rating, 1 = least plant vigor, most leaf chlorosis or necrosis; 5 = most plant vigor, least chlorosis.
[y]Means within a column are separated by Wailer-Duncan K-ratio *t* test.
[x]Selection from the Georgia Agricultural Experiment Station-U.S. Dept. of Agriculture breeding program.

sue pH (Cain, 1954), have been associated with Fe chlorosis in blueberry. In a review of other crops, Foy et al. (1978) stated, "chlorosis from excess Zn, Cu, Ni, and Cd appears to be due to direct or an indirect interaction with foliar Fe." White et al. (1974) hypothesized that Zn and Mn interfere with Fe use for chlorophyll synthesis in the leaves. Many enzyme systems of plants that are activated by Mg also respond to Mn (Woolhouse, 1983). Blueberries accumulate large amounts of Mn, which has been associated with Fe chlorosis in other crops (Foy et al., 1978). Foy et al. (1978) concluded that metal toxicities in plants are often not clearly identifiable but may result from complex interaction of the major toxic ions in question with other essential or nonessential ions and with environmental factors.

Investigation of chlorosis in research plots at Poplarville, Miss., revealed a rather high (300 to 400 ppm) soil Mn content (D. Creech, personal communication). The objective of our field experiment was to determine the relationship of soil pH and leaf Mn content to observed variability in leaf chlorosis and plant vigor. A study with pots in a shadehouse was designed to estimate heritability (h^2) of the content of Mn, Fe, and some other mineral elements that have been associated with leaf chlorosis and to determine the genetic relationships among shoot dry weight, visual rating, and mineral element content.

Four single-plant replicates of three selections and two cultivars (Table 1) were evaluated for plant vigor, leaf chlorosis and Mn content, and pH of soil near the roots. A visual rating was recorded for each plant where 1 = least plant vigor and most leaf chlorosis and 5 = most plant vigor and least chlorosis. Terminal leaf samples consisted of the last three fully expanded leaves, and basal leaf samples consisted of the three basal-most leaves collected from several shoots on each plant. The leaves were dry-ashed using the *procedures of Jones and Case (1990) with the following modifications: 1) One gram of tissue was weighed and ashed at 450C; 2) the ash was dissolved in 5 ml of 20% HCl on a hot plate; and 3) the solution was transferred to a volumetric flask and brought to 100 ml with deionized water. Manganese content was determined on an Instrumentation Laboratory (Wilmington, Mass.) 157 AA/AE Spectrophotometer. Soil cores were taken to a 15-cm depth underneath the canopy of each plant and the pH of a 1 soil : 2 distilled water (w/v) soil solution was determined on an Orion (Boston) SA 520 pH meter.

Ten plants from each progeny of a lo-parent diallel set of crosses, excluding selfs and reciprocals, were arranged in a randomized complete-block design with five two-plant replicates. Parents included the five clones evaluated in the field (Table 1) plus 'Aliceblue', 'Beckyblue', 'Brightwell', 'Briteblue', and 'Premier'. The roots of 1-year-old seedlings were washed before being planted in 2-liter pots containing washed sand in a shadehouse. A 250-ppm Mn solution derived from $MnSO_4 \cdot H_2O$ and adjusted to a pH of 4.5 was applied 5 daysweek^{-1} at 200 ml/pot followed by leaching the next 2 days

Table 2. Range of progeny values (mean of 10 plants) and heritability estimates from analyses of a lo-parent diallel set of crosses of blueberries.

Variable	Range of value	Heritability (h^2)	SE
Shoot wt (g)	1.0–9.1	0.37	0.21
Visual rating[z]	4.3–9.6	0.43	0.24
Mn content (ppm)	888–1941	0.60	0.35
Zn content (ppm)	22–52	0.48	0.30
Ca content (%)	0.14–0.28	0.67	0.34
Mg content (%)	0.06–0.10	0.59	0.36
Fe content (ppm)	28–63	0.13	0.16
K content (%)	1.1–3.4	0.58	0.32
Ca + Mg + K (%)	1.4—3.7	0.56	0.31

[z]Subjective rating, 1 = dead plant; 13 = most plant vigor, least chlorosis.

Table 3. Genetic correlations among shoot dry weight, visual rating, and mineral content of plants from a lo-parent diallel set of crosses.

Variable	Visual rating	Mineral element content					
		Zn	Ca	Mg	Mn	K	Ca + Mg + K
Shoot wt	0.99	−0.60	0.11	−0.78	−0.07	−0.51	−0.49
Visual rating		−0.42	0.11	−0.78	−0.28	−0.38	−0.38
Zn content			0.87	0.88	0.65	0.63	0.74
Ca content				0.61	0.98	0.23	0.37
Mg content					0.97	0.20	0.29
Mn content						0.56	0.69
K content							0.99

with tap water. A solution of (15N-9P-12K) Peters soluble fertilizer with trace elements (W.R. Grace, Foglesville, Pa.) at 200 ml/pot was applied at the beginning and weekly following leaching during the first 5 weeks. Because plants began to require water in addition to the fertilizer solutions at this time, Osmocote (14N-6P-12K) (Sierra Chemical, Milpitas, Calif.) was applied to ensure adequate macronutrients, and the pots were uniformly irrigated with tapwater as needed.

After 6 weeks each plant was classified on a scale of 1 = dead plant to 13 = vigorous plant with no chlorosis (Fig. 1). Ten weeks after the experiment was initiated, all new shoots grown during that time were collected and dried in an oven at 70C. Total shoot weight from each plant was recorded. The leaves were then stripped, and Mn, Zn, and Fe concentrations were determined by the method described for Mn in the field experiment. The solution resulting from the dry-ashing procedure described above was diluted 1: 10 (v/v) with a 0.5% La_2O_3 solution to determine Ca, Mg, and K on the spectrophotometer. Visual rating, shoot weight, and Mn, Fe, Zn, Ca, Mg, and K content were analyzed by the general least squares diallel analysis of Schaffer and Usanis (1969). Estimates of narrow sense heritability (h^2) for each variable and genetic correlations among all variables were computed.

Visual ratings of five clones in the field revealed three significantly different levels of leaf chlorosis and plant growth (Table 1). Average Mn content of the leaves was high and variable among clones, but none were above the published toxicity level of 450 ppm (Eck, 1988). The Pearson correlation coefficient between visual rating and Mn content was only 0.06, suggesting that Mn content per se does not account for the variation in plant vigor and chlorosis. T172, one of the most vigorous and least chlorotic clones, had the highest Mn content. This phenomenon has been observed in other species (Smith, 1979). The ratio of Mn content in the basal to that in the tip leaves was lowest in T102 and similar in the other clones. Tolerance to excess Mn probably was not related to its entrapment in nonmetabolic centers in older leaves (Foy et al., 1978), since there was no consistent relationship between visual ratings and ratio of Mn content in basal to that in terminal leaves. Any effect of Mn on chlorosis and plant growth was probably caused by its interaction with Fe or other mineral elements. The pH of soil near roots varied only slightly among clones, although that for T129, which had the lowest visual rating, was significantly lower than that for 'Tifblue', which had a significantly higher visual rating and the lowest Mn content (Table 1). 'Tifblue' has been found to accumulate less Mn than other clones in a previous study (Korcak, 1988).

The Mn content of all plants in pots was higher than the reported toxicity level (Table 2). Potassium content exceeded the maximum sufficiency range (0.60%) in all plants. Zinc content was near or above the maximum sufficiency value (30 ppm), Ca and Fe content were near the minimum sufficiency value (0.20% and 60 ppm, respectively), and Mg content was near or below the deficiency level (0.08%). Sufficiency range for each of the above mineral elements appears in Eck (1988).

An almost perfect genetic correlation between shoot dry weight and visual rating suggests that subjective evaluation characterizes plant growth equally as well as shoot dry weight (Table 3). A negative correlation existed between Zn, Mg, and K content and shoot weight or visual rating, but no genetic relationship was found between either Ca or Mn and these traits. The negative value for Mg is puzzling because Mg content was near the deficiency level. Perhaps Mg had an additive effect with the excess Mn since Woolhouse (1983) has observed that many enzyme systems of plants that are activated by Mg also respond to Mn. A high correlation existed among Zn, Ca, Mg, and Mn content. Potassium content was positively correlated with Zn, Ca, Mg, and Mn content. A negative relationship was found between total basic cations (Ca + Mg + K) and visual rating or shoot dry weight. This result supports the postulate by Cain (1954) that these cations might be a regulating factor in the function of Fe through their effect on the plant buffer system and might also explain the negative effect of Mg. Potassium comprised most of the basic cation content, however, and its high content could have had an effect independently of that on the buffer system. The correlation between K and total basic cations was 0.99.

Heritability estimates were high for all variables, except Fe (Table 2). Little or no genetic variability existed for Fe content, which could have been affected by excess Mn and K, total cation content, or interactions with metals. Under the conditions of this experiment, it would be difficult to select for increased Fe content. Considerable change in Mn, Zn, Ca, Mg, or K content could be expected from phenotypic recurrent selection. However, manipulation of mineral content probably would not ameliorate the Fe chlorosis. The high heritability of shoot dry weight and visual rating suggests that plants resistant to the effects of other minerals on Fe metabolism can be selected on the basis of visual rating. In making selections, mineral content can be disregarded, thereby saving the cost of plant analyses and simplifying breeding methodology to develop plant populations resistant to Fe chlorosis. For practical purposes, it is unnecessary to identify the causes of Fe chlorosis or the nature of resistance because plants with resistant enzyme systems or other resistance mechanisms can be identified subjectively. Although the causes of Fe chlorosis in blueberry are evidently many and complex, resistant genotypes can be selected from the germplasm we studied.

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