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COMPARATIVE CHLORINE REQUIREMENTS OF DIFFERENT PLANT SPECIES

by C. M. JOHNSON, P. R. STOUT, T. C. BROYER, and A. B. CARLTON

Department of Soils and Plant Nutrition and The Kearney Foundation of Soil Science, University of California, Berkeley

INTRODUCTION

Since our earlier report [1] that chlorine is a micronutrient element as shown in experiments with the tomato plant, ten other species have been grown under similar cultural conditions. Within the series tested, during a winter season, severe chlorine-deficiency symptoms were produced with lettuce, tomato, cabbage, carrot, sugar beet, barley, and alfalfa. Yield effects, but not obvious symptoms of disease, were obtained with buckwheat, corn, and beans, whereas squash showed no effect in yield and only suggestions of nutritional deficiency symptoms. These wide differences in effect of low chlorine supplies are characteristic of the individual species since replicated causal conditions within a species have always resulted in equivalent plant effects.

Of the species tested thus far, the lettuce plant has proven to be the most sensitive to low chlorine supply. By contrast, squash plants cultivated at the same time under the same conditions were scarcely affected. Chemical analyses of plant materials cultured on low-chlorine solutions have shown the presence of considerably more chlorine than the amounts which were known to be provided in their culture solutions. Moreover, species least affected by low chlorine supplies appeared to be favored in their abilities to acquire chlorine from extrinsic * sources — undoubtedly the atmosphere.

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* In the work, two types of contamination are recognized. The first, *intrinsic* to the plant—culture system, includes known contamination from seed, in the repurified salts and water, and from aeration. The second, *extrinsic* to the system, includes that from airborne or other sources unknown or uncontrolled.

Mechanisms by which some species are able to acquire more extrinsic chlorine than others are the subject of further study. It is suspected that leaf structures of different types of plants are involved and that these organs capture airborne chlorine. Our greenhouses are located in a heavily populated area near the sea. For these reasons, aerosols from sea spray and a variety of chlorine compounds originating from industrial and domestic furnaces are constantly in the atmosphere. The amount of leaf spread characteristic of different species is suggestive though inadequate to explain the great differences in the deficiency susceptibility of different species to the same levels of low chlorine in culture solutions. For example, the highly affected lettuce plant has leaves formed into a compact head whereas leaves of squash plants spread into an open canopy.

From the present experiments, it appears that chlorine is a requirement for the nutrition of higher plants in general, but its demonstration presents greater experimental difficulties for some species than for others.

MATERIALS AND METHODS

The composition of the culture solutions is given in Table I. Amounts of specific salts supplied are given in micromoles per liter. The thirteen elements (now including chlorine) which must be added to solution cultures are listed in microgram atoms per liter in descending order of concentration on the right hand side of the table. The complete culture solution containing the thirteen elements is designated M6 + m7. Concentrations of the macronutrient salts (Solution M6) are little different from those of other standard culture solutions. Concentrations of the micronutrients in Solution m7 are reported in micromoles per liter because, in laboratory practice, all stock solutions are prepared on a chemical equivalent basis. Among the micronutrients, chlorine is placed first in order, since it seems to be required in greater amounts than any of the others. Cultures from which chlorine is withheld are designated M6 + (m7 − Cl).

Stock solutions of the four salts supplying the macronutrients were stored individually at molar concentrations. Of the micronutrients, iron was added separately as 0.002 *M* $FeSO_4$ adjusted with H_2SO_4 to pH 3.5. The iron solution was added twice weekly to each culture at the rate of two ml per litre. The six remaining micronutrients were kept in one solution, each at one thousand times the strength shown for the final culture. Thus, addition of one ml per liter of culture solution provided a single strength micronutrient supply.

At transplanting, to 4-liter pyrex culture vessels, solution salt concentrations were brought up to one-half strength. Additional one-half strength levels were given at the end of one week, and single-strength levels at three weeks, five weeks, *etc.*, as required by crop species, seasonal vigor, or other need for additional mineral elements. All chemical compounds used to prepare the culture solutions, except H_2MoO_4 and H_2SO_4, were recrystallized twice. Our tap distilled water was passed through an activated charcoal filter to aid in removal of oxidized chlorine which might have been distilled from the chlorinated city water suppy. Following filtration it was distilled once more into an all-pyrex condensing and storage system. Plant stems were supported with fibrous non-absorbent cotton in the holes of the lids of the culture vessels. Recently, fibrous Dacron has been substituted for cotton because the latter is rather free of chlorides. Dacron wool may contribute a maximum

TABLE I

Macronutrient solution medium — M6 *				
Specific salts used		Concentration of individual elements		
Compounds	Single strength μ moles per liter	Elements	Single strength μg atoms per liter	ppm
KNO_3	6000	N **	16000	224
$Ca(NO_3)_2.4H_2O$	4000	K	6000	235
$NH_4H_2PO_4$	2000	Ca	4000	160
$MgSO_4.7H_2O$	1000	P	2000	62
		S	1000	32
		Mg	1000	24

Micronutrient solution medium — m7				
Specific salts used		Concentration of individual elements		
Compounds	Single strength μ moles per liter	Elements	Single strength μg atoms per liter	ppm
KCl	50	Cl	50	1.77
H_3BO_3	25	B	25	0.27
$MnSO_4.H_2O$	5.0	Mn	5.0	0.274
$FeSO_4.7H_2O$***	4.0	Fe***	4.0	0.22
$ZnSO_4.7H_2O$	2.0	Zn	2.0	0.131
$CuSO_4.5H_2O$	0.5	Cu	0.5	0.032
H_2MoO_4	0.1	Mo	0.1	0.0096

* Solution pH at single strength = 4.7.

** Total-N includes 14% NH_4-N and 86% NO_3-N which maintains pH near to 5. For control near pH 6 use:

1000 μ moles/liter $(NH_4)H_2PO_4$ plus 1000 μ moles/liter $(NH_4)_2HPO_4$.

With the latter selection of ammonium phosphate salts the total-P level remains constant, but the ratio of NH_4 to NO_3 changes from 14% to 21.4% and the total nitrogen in the culture is increased to 17,000 μg atoms per liter.

*** Iron is added separately, twice weekly (2 × weekly) at the 1S (single strength) level indicated. Stock $FeSO_4$ prepared as 0.002 *M* $FeSO_4$ in H_2SO_4 to pH 3.5 for solution stability in storage; take 2.0 ml stock per liter culture medium.

approximating 2 μg atoms of chlorine per culture. Non-absorbent cotton has been found to contain variable amounts of chloride. Cotton wool may contribute a maximum of approximately 20 μg atoms of chlorine per culture. When it is used to support seedlings in the cover of the culture solution, this chlorine source is reflected in better growth of plants. It is presumed that spray from aeration leaches chlorides contained in the cotton, into the culture solutions. All vessels and equipment were washed with nitric acid and rinsed with redistilled water.

Seeds were germinated in pyrex vessels in a $^1/_{80}$-strength M6 culture solution. Five plants were transplanted to each 4-liter pyrex culture vessel as soon after germination as their size and mechanical strength allowed. After one week, one of the five plants was removed, leaving the four most similar plants.

The macronutrient salts were recrystallized to reduce their contents of chlorine since recrystallization was found more effective than our former method of precipitating halides first with silver and subsequent removal of excess silver with hydrogen sulfide. Analyses of these salts for chlorine, showed that 2.5 μg atoms per 4-liter culture (at the single-strength level) was the degree of purification attained. The distilled water was reduced to 0.3 μg atoms of chlorine per liter. Since 10 to 20 liters of water were required by the plants over the six weeks growth period, 2 to 4 μg atoms of chlorine were supplied from the water. Gas scrubbing chains gave estimates of 3×10^{-6} μg atoms per liter of air used for aeration. Filtration of 45,000 liters of air from the atmosphere through dry Whatman No. 1 filter papers showed an average of 7×10^{-6} μg atoms per liter capturable in this way, but there was no way to ascertain how much of this might be made available to growing plants. Other tests with filter papers exposed on greenhouse tables, without air passing through them, showed the possibility of airborne particulate matter settling at the rate of 0.14 μg atoms per square inch per week. Variable amounts of chlorine were found between species of seeds. These are reported in Table II. The maximum chlorine contamination in the culture medium which could be accounted for from all except unrestricted airborne sources was 12μg atoms per culture. Subsequent experiments have been designed to reduce the extrinsic contamination.

All plants, except the tomato and alfalfa, were harvested seven weeks from

TABLE II

The chlorine content of seeds, μg atoms per seed

Lettuce	0.01	Barley	1.0
Tomato	0.03	Alfalfa	0.04
Cabbage	0.02	Buckwheat	0.05
Carrot	0.16	Corn	2.7
Carrot (washed)	0.03 *	Beans	2.3
Sugar beet	0.33	Squash	0.04

* Carrot seeds were the only ones showing measurable losses of chlorine on washing with water.

transplanting. Alfalfa plants were cultivated for 14 weeks, during which time two cuttings were made before the final harvest.

Plant species used: The following commercial species were used: Compositae, *Lactuca sativa* var. capitata (New York Iceberg Lettuce); Solanaceae, *Lycopersicum esculentum* var. Marglobe (Tomato); Cruciferae, *Brassica oleracea* var. capitata (Copenhagen Market Cabbage); *Umbelliferae, Daucus carota* (Red Cored Chantenay Carrot); Chenopodiaceae, *Beta vulgaris* var. Crassa (Strain No. 22 U.S., Sugar Beet); Graminae, *Hordeum vulgare* var. atlas (Barley); *Zea mays* (Sweet Corn); Leguminosae, *Medicago sativa* (Alfalfa); Polygonaceae, *Fagopyrum esculentum* (Buckwheat); and Fabaceae, *Phaseolus vulgaris* (Dwarf Bush Bean).

Chlorine additions: Since the object of the experiment was to compare only the effect of chlorine in additions to culture solutions, no chlorine was added to the "minus chlorine" group, M6 + (m7 − Cl). At the time of transplanting, 100 μg atoms of chlorine per liter were added as ammonium chloride to the "plus chlorine" group, M6 + m7, making 3.5 ppm of chlorine in solution.

To preclude any chlorine deficiency in the "plus chlorine" control plants, on the fifth week one millimole per liter of potassium chloride was included with the periodic additions of nutrient salts. Thus, for the last two weeks the "plus chlorine" cultures had about 14 per cent more potassium in them than the "minus chlorine" cultures. Potassium chloride was selected because the cultures were already more than adequately supplied with potassium and it was felt that addition of chlorine through the medium of this salt would cause least disturbance to the experimental arrangement.

OBSERVATIONS AND RESULTS

Comparative growth and development

Marked differences in yields and chlorine contents were observed; these are summarized in Table III and Figure 1.

Lettuce: Chlorine-deficient lettuce plants first showed symptoms as wilting. Root growth was restricted, with the first evidence of unusual root development appearing as a many-branched habit of new lateral roots. These root laterals were stubby and developed club tips. Throughout the growth period the difference in rate of growth of roots was one of the most obvious features of the two chlorine levels. No destructive symptoms of mottling developed in the lettuce leaf until about four weeks after transplanting the seedlings. Because of the severe wilting during bright days, it was surprising that the leaves of the chlorine-deficient plants should have remained as free from secondary injuries as they did. Later,

TABLE III

Species	Plant part	Average yield g dry weight per culture		Chlorine in deficient plant material, μg atoms per g dry matter	Yield of chlorine-deficient plants, % of normal	Total chlorine per culture		Ratio of chlorine recovered to known chlorine contaminants
		No Cl added	Plus Cl			From known sources of contamination, μg atoms	Recovered from deficient plants at harvest, μg atoms	
Lettuce	Shoots	11.6	40.6	4.0	29	7.5	60.0	8.0
	Roots	2.4	6.7	5.6				
Tomato	Leaf blades	4.9	13.4	6.5	36	12.0	66.0	5.5
	Stems, petioles	4.1	11.7	4.7				
	Roots	1.6	4.8	9.4				
Cabbage	Shoots	26.6	66.8	1.5	42	7.5	66.6	8.9
	Roots	4.1	7.1	5.9				
Carrot	Shoots	6.2	14.6	2.5	47	7.5	40.0	5.3
	Roots	4.9	8.9	5.1				
Sugar beet	Leaf blades	10.9	23.6	1.7	49	7.5	60.0	8.0
	Petioles	7.2	17.9	2.3				
	Roots	11.1	18.6	2.1				
Barley	Shoots	35.6	55.2	4.0	65	7.5	190	25.3
	Roots	15.0	22.3	3.2				
Alfalfa 1st:	Leaf blades	5.8	12.4	3.4	70	10.0	173	17.3
	Stems, petioles	6.3	11.6	2.4				
2nd:	Leaf blades	5.5	8.7	2.9				
	Stems, petioles	5.6	9.1	2.7				
3rd:	Leaf blades	8.4	10.0	3.4				
	Stems, petioles	14.1	18.4	2.3				
	Roots	18.1	20.9	2.4				
Buckwheat	Leaf blades	3.6	4.6	2.5	73	7.5	25.0	3.3
	Stems	2.9	4.4	2.8				
	Roots	2.1	2.8	3.6				
Corn	Leaf blades	27.2	31.8	3.0	78	7.5	243	32.4
	Stalks	76.8	97.0	1.4				
	Fruit	18.7	26.2	1.5				
	Roots	14.2	20.6	1.8				
Beans	Leaf blades	33.5	33.2	5.1	92	7.5	333	44.4
	Stems	24.5	27.4	2.3				
	Fruit	25.2	30.6	2.8				
	Roots	14.8	15.6	2.3				
Squash	Leaf blades	22.6	21.3	2.9	102	7.5	203	27.1
	Stems, petioles	44.1	41.8	1.2				
	Fruit	31.5	31.5	1.9				
	Roots	5.9	4.4	4.6				

Plate I. Lettuce cultures with deficient (left) and adequate (right) chlorine supply.

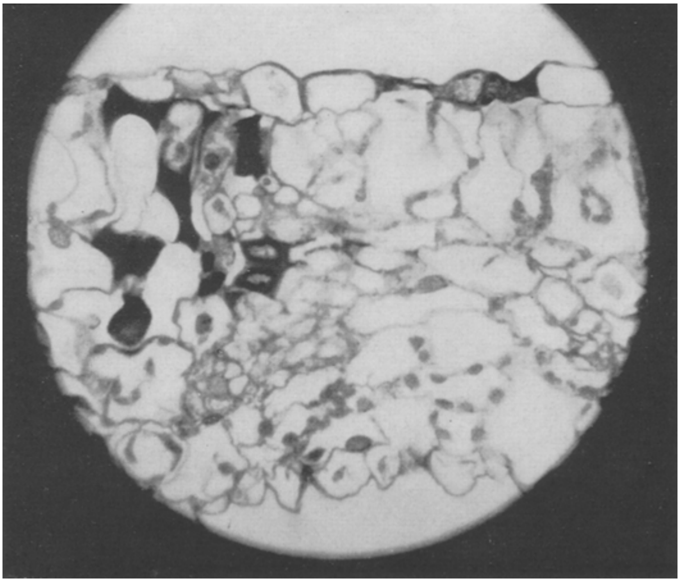

Plate II. Cytological disintegration in bronzing of tomato leaf from chlorine deficiency supply; the darkly stained areas in the tissue section are components of the bronzed areas in the leaf.

Plate III. Cabbage cultures with deficient (left) and adequate (right) chlorine supply.

Plate IV. Detail of wilting and cupping of cabbage under conditions of inadequate chlorine supply.

necrosis occurred on the more severely wilted leaves. Growth was restricted from the time wilting first appeared in the "minus chlorine" lettuce plants. The differential growth was cumulative. These differences in vigor are expressed best by Plate I which gives a direct comparison of lettuce plants grown on cultures having no chlorine other than that supplied in the M6 + (m7 — Cl) solutions and those which received M6 + m7 solutions which provided 100 μg atoms of chlorine per liter (3.5 ppm) at the time of transplanting. The three replicates within each treatment were so much alike that selection of any single culture was proper for photographic comparison.

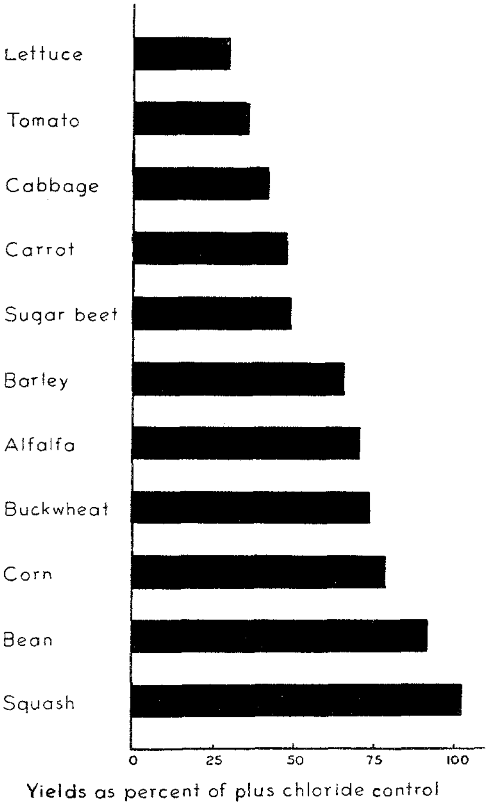

Fig. 1. Plant yields from chlorine-deficient cultures as per cent of those from adequately supplied chloride controls.

The total dry weight yield of the "minus chlorine" lettuce plants was 30% of the "plus chlorine" plants. However, yield alone is an inadequate expression of the real differences between the two treatments. Quality considerations would permit rating the latter plants as good. Their heads had begun to fill and were in every way indicative of an acceptable product by commercial standards. By contrast, the "minus chlorine" plants did not head and would be considered complete failures when invoking standards of market acceptance. There is no question that chlorine is required for the satisfactory growth and development of the lettuce plant.

Tomato: There is no need to review at length here the effects with the tomato plant since an account of the symptoms of chlorine deficiency is given in the earlier report [1]. Comparative root development between "plus" and "minus chlorine" cultures is shown in Figure 2. Again, as was noted previously for lettuce, the lateral roots of chlorine-deficient tomato plants were many branched and stubby, with club tips. This is in contrast with the usual fibrous type of root growth of the tomato plant.

In a special study, J. T. Woolley has partially characterized the chlorine-deficiency bronzing through histological observations. A photomicrograph of a tomato leaflet cross section through a bronzed area is reproduced as Plate II. The tissue thickness was $2\,\mu$, the field of view about $130\,\mu$. The tissue breaks down within individual cells. The destruction has no particular relationship to specific leaf structures, occurring in palisade, spongy tissue, or epidermis. It is sometimes adjacent to veins and at other times apparently includes xylem and phloem. The bronze-bodies take up safranin stain markedly (the basic stain used was fast green — not localized in bodies). The bodies are insoluble in water, xylol, ethanol, methanol, tertiary butanol, methyl cellosolve, weak (5%) HCl or weak (5%) NaOH. Seemingly, an entire cell breaks down into some form of proteinaceous coagulum which appears to fill some intercellular spaces between healthy cells. There is no evidence for a "secretion" from cells. This coagulum is amber in color when unstained and probably gives the bronze color to the leaf tissue affected. Further, chlorine-deficient and chlorine-sufficient leaves of the same age, size, and position had the same general histological structure at the time of onset of chlorine-deficiency symptoms in the "minus chlorine" leaves.

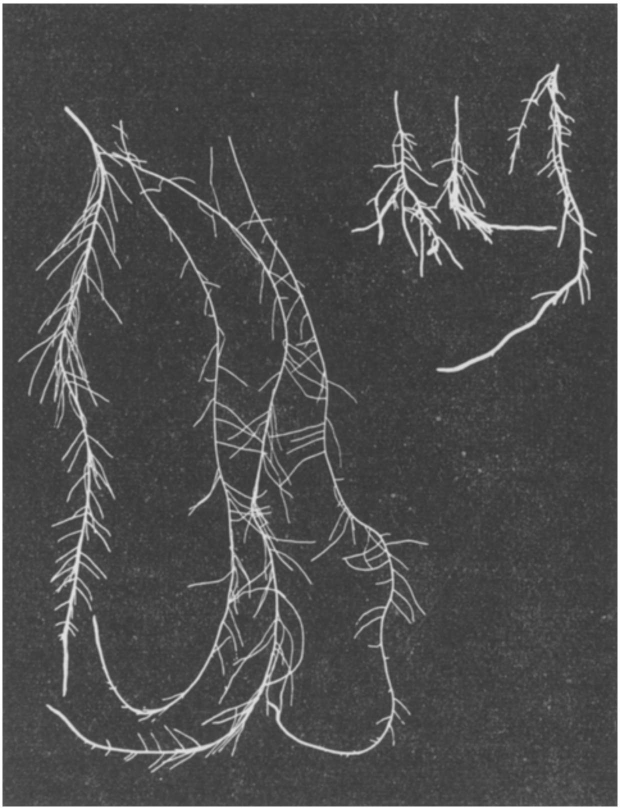

Fig. 2. Comparative lateral root development of tomatoes in inorganic solution cultures with (left) and without (right) chlorine additions.

Cabbage: As with the lettuce plants, there was marked restriction in the growth of cabbage cultured on the M6 + (m7 — Cl) solutions, best exemplified by the comparison of the two cultures shown in Plate III. Over-all growth and quality differences are as striking as with the lettuce plant. Cabbage plants were more variable between individuals than with lettuce. The culture with chlorine-deficient cabbage plants on the left of Plate III shows one of the plants less affected than the others. Even so, this particular plant was smaller than any single plant of the culture shown on the right which was supplied with 3.5 ppm chlorine at the beginning of the experiment. A curious point of comparison between cabbage and lettuce is the habit of root growth. With the "minus chlorine" lettuce plants, inhibition of root growth was obvious very early in the growth period, but with cabbage no such visual difference was observed. Actually, the roots of the cabbage plants were longer in cultures not receiving added chlorine than in cultures which did. However, yield data given in Table III show that the weights of roots from the chlorine-deficient cabbages were 57% of those from the "plus chlorine" cultures. Like all other plants grown with limited supplies of chlorine, cabbage showed obvious water stress. Whenever a leaf was exposed to full sunlight, wilting took place at the tips and margins. The type of leaf wilting of cabbage can be seen in Plate IV. It appears that the wilted outer parts of the leaves do not expand as rapidly as the inner sections, which causes cupping of the cabbage leaves to take place. Sometimes the wilted leaf margins droop downward, leaving the cup shape inverted; on other occasions leaves wilt with margins turning upward in which event the cup shape is upright. Both types of cupping can be seen in Plate IV. Later in the growth period the most newly formed leaves of the chlorine-deficient cabbage plants became chlorotic. As with lettuce plants, cabbages inadequately supplied with chlorine were of such poor quality that they would not be considered acceptable from the point of view of quality. A second point of curious behaviour in cabbage was that the chlorine-deficient plants had no detectable cabbage odor whereas those receiving chlorine additions did. No reasons for this peculiarity are offered except to suggest that under conditions of water stress, transfer of gases from the interior of the leaf might have been lessened. Another possibility is that under conditions of severe growth restriction, unsaturated aldehydic constituents responsible for the characteristic cabbage odor are synthesized in smaller quantity.

Carrot: The carrot plant was restricted in growth of tops and roots as may be seen in Plate V. The root habit of the "minus chlorine" carrot plants tended toward many-branched laterals, a characteristic already noted for the "minus chlorine" lettuce or tomato roots. Carrots displayed the wilting symptom of chlorine deficiency less than any other species. However, about six weeks after transplanting a number of small leaflets turned brown and died. Storage-root development was greatly depressed on the

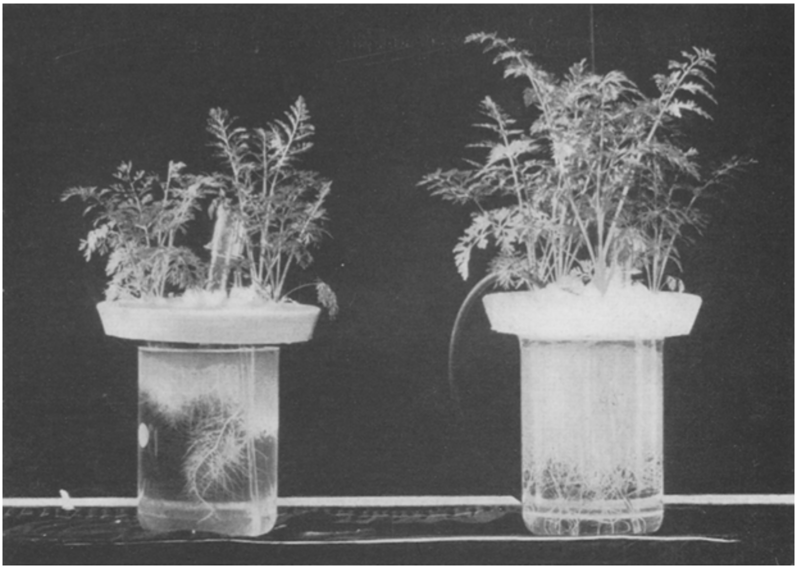

Plate V. Carrot cultures with deficient (left) and adequate (right) chlorine supply.

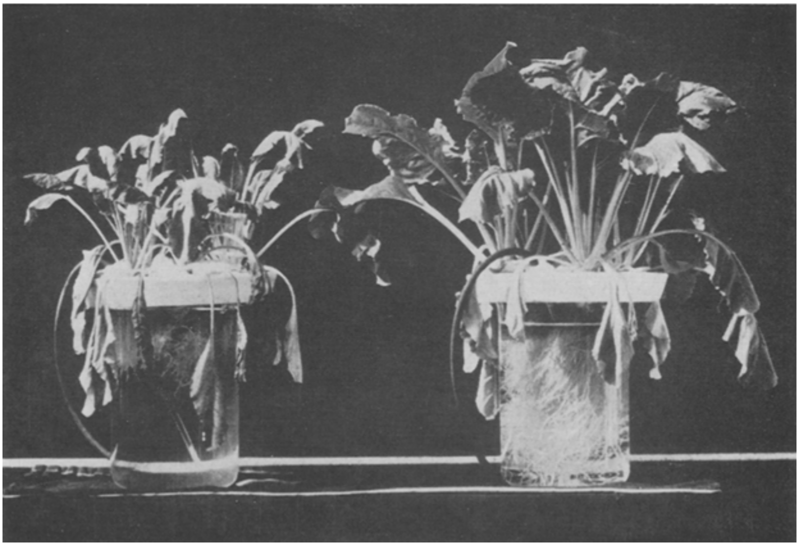

Plate VI. Sugar beet cultures with deficient (left) and adequate (right) chlorine supply.

displayed prominent chlorine-deficiency symptoms as shown in the comparative photograph, Plate VII. It is of especial interest that the roots of these alfalfa plants did not seem to be affected adversely. Some alfalfa leaflets, though expanded before the intrinsic chlorine contaminants had become diluted low enough through plant growth to become limiting, appeared to retrogress, and decreased in leaf area perhaps by virtue of leaf thickening at the expense of lateral expansion. Plate VIII shows the contrast in size and shape of leaves of the same age which developed on alfalfa plants of the M6 + (m7 — Cl) and M6 + m7 culture solutions. These leaflets werep hotographed while on the plant with the same plate-to-lens setting. Consequently both photographs are in the same scale. Tips of the chlorine-deficient leaflets had split along the midrib and irregular chlorotic blotches appeared in the central portions.

Strangely, the second cutting of alfalfa showed less percentage yield depression of the "minus chlorine" plants than for the first cutting. The dry weight of the tops from the first cutting of these plants was 50% of the "plus chlorine" controls; and for the second cutting, 62%. At the third cutting, the yield from the M6 + (m7—Cl) culture group was 80% of that of the controls and the severity of symptoms had considerably diminished. At final harvest (14 weeks after transplanting) weights of the combined cuttings and roots of the "minus chlorine" group were 70% of the controls. The total chlorine recovered in the "minus chlorine" plants was 173 μg atoms per 4-liter culture — about 17 times more than could be accounted for from the chlorine known to have been in the nutrient salts and supplied by water during the growth period. For the alfalfa plant, it can be inferred that chlorine is necessary for its growth and development. Most curious, however, were the following: *a*) the healthy appearance of "minus chlorine" alfalfa roots at all times, and *b*) the large amounts of chlorine acquired by the plants above that which could be accounted for in seed, culture solutions, and added water.

Buckwheat: From the point of view of an experimental plant, buckwheat was the least satisfactory in the series. Under the experimental conditions, it proved to be slow growing and low yielding. At no time during the seven weeks did the M6 + (m7 — Cl) cultured buckwheat plants display symptoms of sufficient prominence to classify them with assurance as being chlorine deficient. At the final harvest the yield of the "minus chlorine" plants was 72% of the "plus chlorine" plants. These yield differences were significant at the 5% level. Buckwheat, like all the other species of the experiment, acquired more chlorine than could be accounted for in the culture solution and added water.

Corn (maize): Corn plants showed excellent growth in these cultures, yielding considerably more dry weight of plant material than any other species within the series. The "minus chlorine" plants displayed the smaller yields, but with the exception of wilting, no leaf symptoms could be seen at any time during growth. Shortly after transplanting corn seedlings to the M6 + (m7 — Cl) culture solutions, they were noted to wilt during hot, bright periods of the day while those on M6 + m7 cultures did not. This species thus gave suggestions of chlorine-deficiency symptoms earlier than any of the others. Consequently, close observations were made for further expressions of abnormality. However, throughout the growth period no other signs of distress or nutritional disease were evident even though the wilting symptom persisted. Only younger leaves of the "minus chlorine" plants wilted and conditions of high light intensity were required to produce this symptom. During intervals of reduced light intensity they regained turgor. This difference in tendency to wilt between plants in M6 + (m7 — Cl) and M6 + m7 cultures, continued throughout the weeks, but otherwise they did not seem to be affected nor was it possible to judge whether or not differential yields could be expected. However, dry weights taken after harvest revealed that the "minus chlorine" plants were 78% of the "plus chlorine" controls, with the difference being significant at the 10% level.

Beans: The only significant difference noted with bean plants from the M6 + (m7 — Cl) and M6 + m7 cultures was found in their yields at the time of harvest. Bean plants cultured on these two solutions resembled each other seven weeks after transplanting. Comparison of plant yield data of Figure 1 clearly demonstrates the inherent differences in species effect under similar, inadequate chlorine environments. All of these plants were cultured on solutions from the same salt source and grew at the same time in the same greenhouse. The been plants acquired very much more chlorine during their growth than the amount originally made available through culture solutions. The total chlorine recovered at harvest was over forty times the amount known to have come from salts, water, and seed. It is not known how much of the extrinsic chlorine having been captured was actually incorporated into the plant in a way to contribute to its physiological processes, but it is suggestive that a high proportion of the chlorine found by analysis was indeed incorporated into the plant in physiologically effective locations. The chlorine contents of 4.0 and 5.1 μg atoms per gram respectively for lettuce and bean leaves (Table III) show that the spreading habit of bean leaves did not give rise to much higher chlorine contamination. We can assume advisedly that the greater advantage in leaf spread captured a greater amount of chlorine, but that this added amount of chlorine resulted in correspondingly more plant growth so that the concentration of chlorine was increased very little. These experiments are to be repeated in a special section of our greenhouses which is being equipped with air-scrubbing devices.

The only symptom exhibited, which was distinctive as between the two treatments, was increased nastic movement of the leaves of the "minus chlorine" plants. For example, it was observed on several occasions that at sunset all the bean plants of the M6 + + (m7 — Cl) treatments could be recognized visibly by reason of their leaves being turned toward the sun, whereas their counterparts, having been supplied with chlorine, were distinctly less oriented. It is suggested that the general tendency of the "minus chlorine" plants to be under stress for water might have allowed greater freedom of leaf movement since the cells would be under less turgor.

Squash: The only suggestion of difference between squash plants with or without chlorine application was a chlorosis of the younger leaves of plants growing on the M6 + (m7 — Cl) cultures. These symptoms were rather transitory. Yields were not decreased by restricted chlorine supply. Actually, the average yield of the "minus chlorine" squash plants was 2% greater than those receiving added chlorine; although this difference was not significant at the 50% level.

DISCUSSION

Status of chlorine nutrition of crop plants

With the realization of the kinds of difficulties besetting experimental demonstrations of chlorine as an essential element for higher plants, it becomes understandable why the subject of chlorine in plant nutrition has remained uncertain for so many years. In retrospect, further strength is given to the suggestions of other investigators that chlorine is required for growth of higher plants. Of the work successfully demonstrating lower yields from "minus chlorine" plants cultured under controlled salt environments, several other species must be considered. Lipman[3] used buckwheat and peas; Eaton[2] tomato and cotton; and Raleigh[5] table beets. For each of these species, lowered plant yields resulted from attempts to exclude chlorine from the culture solutions. There have been many suggestions by agronomists and others that chlorine (usually added as KCl) may influence favorably the turgor of field crops. However, we do not feel that the latter experiments had been refined sufficiently to warrant more than the suggestions. Since chlorine-deficient plants have been produced and chemical analyses have been made of them, we are in position to deduce certain possibilities of economic implication.

Since the plant requirement for chlorine was found to be higher than for any of the other micronutrient elements, the reason for chlorine having escaped notice for so many years must have been its ubiquitous distribution in nature and many uses in industry. As a result, it has been an ever present source of contamination during the manufacture of chemicals needed for the compounding of culture solutions as well as in the growth environment of plants.

In the leaves of tomato plants suffering from acute chlorine deficiency, chlorine is present in the order of 1–3 microgram atoms (35–105 ppm) per gram dry weight of leaf tissue. By contrast, the micronutrient presently recognized as required in least amounts is molybdenum. Molybdenum-deficient tomato plants with equal visual stress will contain 0.001 of a microgram atom per gram (0.1 ppm). Thus, on an atomic-equivalent basis, the chlorine requirement is in the neighborhood of 1,000 times greater than that for molybdenum.

Chlorine needs of higher plants in relation to bromine[4 6] have shown that bromine exercises a pronounced "sparing effect" for chlorine. Other evidence secured in our laboratories shows that fluorine does not, nor does iodine where supplied at lower levels (up to 2 μg atoms per culture). At levels approximating those effective in Cl "sparing action" with bromine (500 μg atoms per culture), there is the possibility that iodine may participate in chlorine functions, but toxicity symptoms from the former interfere with observations. Plants having very low amounts of chlorine cannot be brought up to full yield irrespective of bromine added [4] although moderate deficiencies can be corrected with bromine [6]. In nature, the amounts of bromine are so much less than chlorine, that it is doubtful whether bromine is at all significant in providing supplements to the chlorine available for plant use.

From a soil-fertility point of view it is estimated that plants require one pound of chlorine for each 10,000 pounds of dry matter produced. Large crops would therefore need 2 or more pounds of chlorine per acre. Chlorine, unlike other nutrient elements contained in native rocks, is not fixed by soil colloids. It may be assumed that shortages of chlorine would have been observed long ago unless some process has been replacing regularly the chlorine leached away in drainage waters. A source of continued supply may be found in the chlorine content of rain waters. Many analyses of annual chlorine acquisitions from the atmosphere have been made in many parts of the world. Lower recorded values are in the order of 10 pounds of chlorine per acre per annum with many coastal stations reporting deposition of hundreds of pounds of chlorine per acre.

SUMMARY

Recognition of chlorine as a plant micronutrient has been extended to include ten species. Acute chlorine deficiencies or decreased yields were produced with lettuce, tomato, cabbage, carrot, sugar beet, barley, alfalfa, buckwheat, corn, and beans. Squash plants showed neither loss in yield nor other deficiency symptoms when cultured at the same time and under the same conditions as the aforementioned species. All plants acquired more chlorine during their growth than can be accounted for from seeds, inorganic salts, or water used in the experiments. Plant species least susceptible to injury when cultured upon low chlorine salt solutions were also the ones most capable of acquiring extrinsic chlorine. Of the species studied, lettuce was the most senstitive to "minus chlorine" culture solutions and squash, the least sensi-

tive. However, the concentration of chlorine in all of the species cultured under limited chlorine supply was not greatly different. It is inferred that plants such as corn, beans, and squash survived the "minus chlorine" cultures by reason of greater accretion of extrinsic chlorine from the atmosphere. The form of the atmospherically borne chlorine is not known.

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