TOXIC AND BENEFICIAL EFFECTS OF CHLORINE ON THE EARLY GROWTH OF ADZUKI BEAN PLANTS IN SOLUTION CULTURES[2]

by

YOU-LONG CHIANG[1], CHEN-KUEI SHIU, and CHI-PIN LIN

ABSTRACT

CHIANG, Y. L., CHEN-KUEI SHIU, and CHI-PIN LIN. (National Taiwan University, Taipei) Toxic and beneficial effects of chlorine on the early growth of Adzuki bean plants in solution cultures. Taiwania 10: 21-27 1965.

Chlorine shows some of the characteristics which qualify it as a micronutrient element for the early growth of Adzuki bean plants in solution cultures: 1. Chlorine was required only in a minute amount in the medium; increased growth was observed when some micronutrient elements were supplied as chlorides instead of sulfates. 2. As is true in all the currently known micronutrient elements, chlorine is toxic to Adzuki bean plants when present in a large amount in the medium. 3. The absence of chlorine in the medium results in growth depression.

The chlorine content of both shoots and roots, especially of the shoots, increases with the increase of th chlorine concentration of the medium. The growth depression in high chlorine media was due to the chlorine accumulated in the plant body.

The chlorine content of Adzuki bean seeds (bought on the market) is in the order of 0.65 mg per gm dry weight (=650 ppm). This value was nearly in agreement with that of shoots and roots of the Adzuki bean plants which showed the best growth in this experiment. From this, it is thought that "seed analysis" will provide more reliable information concerning mineral nutrition of certain plants, especially in their early growth, than "leaf analysis".

INTRODUCTION

Many higher plants are known to contain chlorine in their leaves usually in considerable quantity (DeKock, 1961). Studies concerning the nutritional effect of chlorine on plants have been carried on for many years. The classic works show that chlorine is beneficial rather than essential for plant growth and development (Tottingham, 1919; Lomanitz, 1924; Garner *et al.* 1930; Lipman, 1938). Some evidences that chlorine is a qualified micronutrient element for plant growth can be found in a paper published by Eaton (1942) who showed that chlorine was beneficial for plant growth in a minute amount and that it was toxic if present in more than a minute amount. Quite recently it has been shown by Broyer *et al.* (1954) and Ulrich *et al.* (1956) that chlorine is more definitely a micronutrient element for growth of some higher plants. Machlis and Torrey (1955) take a cautious attitude on this problem, stating that "the claim that chlorine is essential must be substantiated in several different research laboratories for a larger number of plants before chlorine can be included in the list of essential elements without question". The object of

---

[1] Associate professor of botany at the National Taiwan University.

[2] Based upon a thesis submitted by the second and third authors in partial fulfillment of their B. S. in Horticulture.

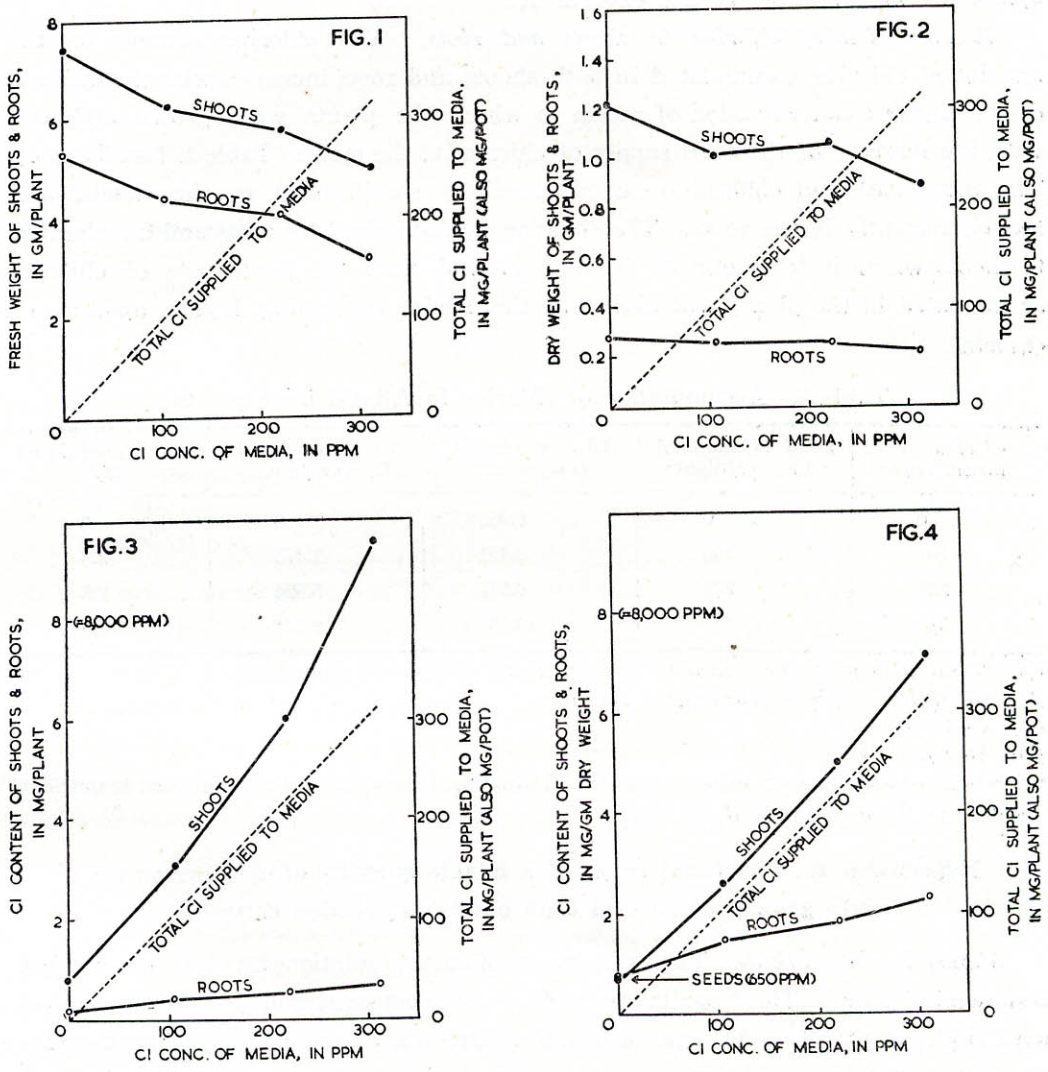
this report is to present some evidences of chlorine as a micronutrient for the early growth of Adzuki bean plants.

Experiment 1. Acccmulation and toxicity of chlorine in young Adzuki bean plants grown in water culture.

Materials and methods. The seedlings of Adzuki bean (*Phaseolus radiatus* L. var. *aurea*, Prain) used in this experiment were started from seeds in sand cultures. Although the sands (black river sands) were washed several times with running tap water before use, the sands still contained considerable amounts of impurities, including chlorides. At the end of the second week after seeding, when the seedlings had a pair of simple, opposite, leaves, they were transplanted into distilled water in 250 ml capacity brownish glass containers on October 21, 1964, with one plant per container. The single plant was supported by a cork stopper which was held by a 2 mm thick dark red plastic lid in the container (see figure 1 in Chiang, 1962). A total of 40 plants were used in this experiment. One week after the transplanting of the seedlings to distilled water in the containers, the distilled water was replaced with Hoagland's solution and with different amounts of chlorine, supplied as potassium chloride, added in ppm as follows: 106, 222, 312. The basic culture solution contains the following macronutrients in gm per liter: $Ca(NO_3)4H_2O$, 1.18; KNO_3, 0.51; $MgSO_47H_2O$, 0.49; KH_2PO_4, 0.14 and micronutrients in mg per liter: FeEDTA, 5.00; $MnSO_44H_2O$, 0.516; H_3BO_3, 2.86; $ZnSO_47H_2O$, 0.22; $CuSO_46H_2O$, 0.08; $(NH_4)_6Mo_7O_{24}4H_2O$, 0.086. The nutrient media was renewed three times at weekly intervals during this experiment. All plants (started from seeds) were grown in the greenhouse of the Botany Department of National Taiwan University under natural light condition. The plants were harvested on November 25, 1964 (*i. e.*, at the end of the fourth week after transplanting to nutrient solution) for various studies. The chlorine content of the plant material was determined by Conway's microdiffusion method as described by Ulrich and Ohki (1956).

Results. Chlorine content of seeds. It was determined that the chlorine content of one gm dry seeds was 0.650 mg (=0.065 %; =650 ppm). Since the average weight of one well developed dry seed is 0.165 gm (based on the average of 20 seeds), the chlorine content of one well developed dry seed is 0.108 mg. As can be seen in figs. 1-4, the chlorine content of the seed was nearly in agreement with that of shoots and roots of the plant which showed the best growth in this experiment.

Fresh and dry weight of shoots and roots. The growth in both fresh and dry weight of shoots and roots decreases with the increase of chlorine concentration of media or with the increase of total chlorine supplied to media (figs. 1 and 2). The plants grown in the media without chlorine being added, showed the best growth in this experiment. This result does not mean that chlorine is non-essential, but means that growth is better in the media without chlorine added rather than in



Figures 1—4. Fresh weight (fig. 1), dry weight (fig. 2) and chlorine content of shoots and roots (figs. 3 and 4) of Adzuki bean plants grown for 4 weeks in the nutrient solution without and with different amounts of chlorine added as KCl.

the media with chlorine added in larger or toxic amounts. The plants grown in the solution without chlorine added contain about 700 ppm Cl (see fig. 4) originating in the transplanted seedlings and from the nutrient salts used for preparation of the media as a contaminant (see Table 1). Neither deficiency nor excess visible symptoms were induced in this experiment.

Accumulation of chlorine in shoots and roots. The chlorine content or the amount of chlorine accumulated in both shoots and roots increases with the increase of the chlorine concentration of media in which the plants were grown and also with the increase of the total supply of chlorine to the media (Table 1. figs. 3 and 4). The accumulation of chlorine occurred especially significantly in the shoots, and less significantly in the roots. The chlorine was absorbed by roots and translocated to shoots where it accumulated. Table 1 showed that the percentage of chlorine accumulated in the plants was higher in the media containing higher amounts of chlorine.

Table 1. Accumulation of chlorine in Adzuki bean plants

Cl conc. of media (ppm)	Total Cl supplied (A) (mg/plant)	Cl content (B) (mg/plant)	Cl accumulated (C) (mg/plant)	%Cl accumulated (D)
0	0	0.953*	0	0
106	106	3.431	2.478	2.3
222	222	6.517	5.564	2.5
312	312	10.187	9.234	3.0

(A) Total amount of Cl supplied to nutrient media during this experiment.
(B) Cl content of plants (shoots plus roots)
(C) C=B−0.953
(D) D=C/A×100
* 0.953=Cl content of seed (0.108 mg/seed) +Cl absorbed from sands+Cl contaminated in nutrient salts used for preparation of the media.

Experiment 2. Beneficial effect of a minute quantity of chlorine on the early growth of Adzuki bean plants in solution cultures.

Materials and methods. The composition of culture solution for this experiment is shown in Table 1. These media are based on the composition of Hoagland's solution with slight modification as regards to micronutrients. The minus chlorine medium contains only a trace of chlorine if any originating as a contaminant in the salts used for the preparation of the medium. A small quantity of chlorine (0.511 mg/1) was added to the plus chlorine medium, as chlorides instead of sulfates of some micronutrient elements. Since sulfate is a macronutrient, the additional presence of this small amount of sulfate in minus chlorine solutions is considered to be negligible in influencing the purpose of this experiment. All salts used as nutrients were of analytical reagent grade, not being recrystalized. Well developed Adzuki bean seeds were sterilized with 1% formalin solution by soaking them in the

Table 2. Composition of nutrient media for studying beneficial effects of chlorine on the early growth of Adzuki bean plants in solution cultures.

Nutrient salts	Minus Cl	Plus Cl
Macronutrients (gm/1)		
$Ca(NO_3)_2$ $4H_2O$	1.18	1.18
KNO_3	0 51	0.51
$MgSO_4$ $7H_2O$	0.49	0.49
KH_2PO_4	0.14	0.14
Micranutrients (mg/1)		
Fe EDTA	5.00	5.00
$MnSO_4$ $4H_2O$	2 04	—
$MnCl_2$ $4H_2O$	—	1.18 (0.421 mg Cl/1)
H_3BO_3	2.86	2.86
$ZnSO_4$ $7H_2O$	0.22	—
$ZnCl_2$	—	0.105 (0.055 mg Cl/1)
$CuSO_4$ $6H_2O$	0.08	—
$CuCl_2$ $2H_2O$	—	0.054 (0.035 mg Cl/1)
Na_2MoO_4 $2H_2O$	0.025	0.025
Cl concentration of media	0*	0.511 mg Cl/1 (also ppm)
Total Cl supplied**	0*	0.383

* Trace of Cl originating as an impurity in the nutrient salts of the analytical reagent grade.
** Total Cl supplied, in mg/pot (also mg/plant), during this experiment.

solution for 5 minutes. The seeds were then washed seven times with deionized water and seeded immediately on pulpified filter paper in large Petri-dishes (20 cm in diameter) on May 23, 1965. These Petri-dishes were placed on the desk near the north window in the laboratory. Four days after the soaking of seeds, *i. e.*, May 27, the Petri-dishes were moved to the greenhouse of the Botany Department of NTU under natural light condition. Seedlings were transplanted in nutrient solutions in the brownish glass containers (250 ml capacity) on June 10, 1965. The average fresh weight of one seedling at this time was 1.125 gm. Nurient media were renewed twice, on June 14 and June 19, 1965. All cultures were maintained in the greenhouse, and were covered with a transparent vinyl cloth box during this experiment. Plants were harvested for measurements on June 25, 1965.

Results. Growth in plant height, fresh weight and dry weight is less in the minus chlorine culture (Table 4), but no diagnostic deficiency symptoms of chlorine were induced. Failure to observe the deficiency symptoms of chlorine on the plants in their early growth was due to the chlorine originating in the transplanted seedlings and in the salts used to prepare the media as a contaminant. The plants

with small amount of chlorine (0.511 ppm) added showed increased growth. Although no visible deficiency symptoms were observed, the plants grown in the minus chlorine media tended to result in weak plants with stems which bent down and break off easily. This tendency can be seen in Table 3. Only one plant was found growing upright among 12 plants which were grown in the minus chlorine media. Of the plants grown in minus chlorine media, 7 had bending and 4 had broken stems.

Table 3. Beneficial effect of a small quantity of chlorine added to the media on the supporting tissues of Adzuki bean stems*

Number of plant with:	Minus Cl (trace**)	Plus Cl (0.511 ppm)
Upright stems	1	4
Bending stems	7	6
Broken stems	4	2

* Observation was made on June 19, 1965, 9 days after transplanting to nutrient media.
** Trace of Cl if any originating as an impurity in the nutrient salts of analytical reagent grade.

Table 4. Beneficial effect of a small amount of chlorine added to the media on the early growth of Adzuki plants*

		Minus Cl (trace**)	Plus Cl (0.511 ppm)	% Increase***
Plant height (cm)		40.90	48.70	19.07
Fresh weight (gm)	Shoots	2.40	2.52	5.00
	Roots	1.11	1.13	1.80
	Shoots+Roots	3.51	3.65	3.98
Dry weight (mg)	Shoots	284.51	293.4	3.14
	Roots	64.57	66.90	3.61
	Shoots+Roots	349.08	360.32	3.22

* The total Cl supplied to plus Cl medium was 0.383 mg/plant (also mg/pot). The results are the averages of 10 plants, harvested on June 25, 1965.
** See Table 3.
*** % Increase of plus Cl culture over minus Cl culture.

DISCUSSION

Determination of the content of chlorine and of other mineral elements in plants has been frequently done with leaves and this is called "leaf analysis", but the determination of mineral elements in seeds has not been so frequently done. Leaf analysis as a basic research tool in determining the nutritional status of plants, especially orchard crops, has often been emphasized (Reuther *et al*, 1958). But as to chlorine, "seed analysis" seems more valid than "leaf analysis", because the chlorine content of the seed has been found to be in close agreement with

that of the plants which show the best growth in the present experiment. The seed seems to possess an ability to control the accumulation of chlorine and perhaps other constituents in suitable proportions and amounts for their best growth, especially for their early growth. Further more, as mineral constituents in plant leaves are highly variable depending on edaphic and climatic factors (De Kock, 1961), and as the absence of visible leaf symptoms of diagnostic significance in the plant whose growth was depressed by chlorine deficiency and excess which has been noted by Eaton (1942) and also by us in the present experiment, seed analysis is considered to provide more helpful information concerning the nutritional status of plants than leaf analysis.

ACKNOWLEDGEMENT

The authors wish to thank Professor Tsan-tao Yen (顏滄濤) of the Department of Agricultural Chemistry for loaning us a platinum crucible and Dr. Charles E. De Vol for reading the manuscript.

This work was supported in part by a fund made available to the senior author through the National Council of the Long Range Planning for Science Development in Republic of China.

LITERATURE CITED

(1) BROYER, T. C., A. B. CARLTON, C. M. JOHNSON, and P. R. STOUT.: Chlorine—a micronutrient element for higher plants. Plant Physiol. 29: 526-532. 1954.

(2) CHIANG, Y. L.: Symptoms of mineral deficiencies (major elements) in Adzuki bean plants (*Phaseolus radiatus* L. *aurea* Prain). Taiwania 8: 181-187. 1962.

(3) DEKOCK, P. C.: Mineral content of plant leaves. Biochemists' Handbook, ed. by C. Long. pp. 1039-1042. Princeton: Van Nostrand Co. 1961.

(4) EATON, F. M.: Toxicity and accumulation of chloride and sulfate salts in plants. Jour. Agr. Res. 64: 357-399. 1942.

(5) GARNER, W. W., MCMURTREY, J. E.. BOWLING, J. D., and MOSS, E. G.: Rôle of chlorine in nutrition and growth of the tobacco plant and its effect on the quality of the cured leaf. Jour. Agr. Res. 40: 627-648. 1930.

(6) LIPMAN, C. B.: Importance of silicon, aluminum and chlorine for higher plants. Soil Sci. 45: 189-198. 1938.

(7) LOMANITZ, S.: The influence of sodium chloride upon alfalfa grown in solution cultures. Soil Sci. 18: 353-368. 1924.

(8) MACHLIS, L. and J. G. TORREY: Plants in action, a laboratory manual of plant physiology. San Francisco: W. H. Freeman and Co.. 1959.

(9) REITHER, W., T. W. EMBLETON, and W. W. JONES.: Mineral nutrition of tree crops. Ann. Rev. Plant Physiol. 9: 175-206. 1958.

(10) TOTTINGHAM, M. E.: A preliminary study of the influence of chlorides on the growth of certain agricultural plants. Amer. Soc. Agron. Jour. 11: 1-32. 1919.

(11) ULRICH, A. and K. Ohki.: Chlorine, bromine and sodium as nutrients for sugar beet plants. Plant Physiol. 31: 171-181. 1956.