THE UPTAKE OF CALCIUM BY ISO LINES AND KENTUCKY VARIETIES OF *NICOTIANA TABACUM* L.

A Thesis 540

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Master of Science in Biology

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ABSTRACT OF THESIS

THE UPTAKE OF CALCIUM BY ISO LINES AND KENTUCKY VARIETIES OF NICOTIANA TABACUM L.

Plant-to-plant variations have been observed in the uptake of calcium in several varieties of tobacco. Iso lines, because of their homozygous condition were expected to exhibit a minimum plant-to-plant variation. This study involved an analysis of the uptake of calcium by Iso lines and normal heterozygous lines of burley tobacco. It was found that the Iso lines exhibit the same degree of plant-to-plant variation in calcium uptake as the Kentucky varieties.

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Accepted by the faculty of the School of Sciences and Mathematics, Morehead State University, in partial fulfillment of the requirements for the Master of Science degree.

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INTRODUCTION

For many years, quantitative variations have been recognized in the uptake of the essential elements by higher plants (16). Several varieties of burley tobacco (*Nicotiana tabacum* L.) have shown no consistent differences in their rate of uptake of calcium. However, it has been found that within these varieties, individual plants varied considerably in their rate of calcium uptake (3).

In a recent review of mineral nutrition, Gerloff has stated that a great deal of caution must be used in evaluating data on the quantitative differences in the nutritional requirements of organisms, due to the individual variation within varieties and strains. He also states that comparisons of this nature should involve tissues of nearly the same age, protoplasmic and non-protoplasmic content (16). Theoretically, if organisms of the same genotype could be used the problem of an adequate control would be surmounted.

Stokes recently developed complete homozygotes of tobacco by using colchicine to double the chromosome number of haploid plants. These double haploid plants were then selfed to develop homozygous diploid varieties called Iso lines. Stokes hypothesized that these Iso lines would be extremely useful in any study that demanded a constant genotype, or one in which plant-to-plant variation must be limited to micro-environmental response (31).

This study was undertaken to determine if plants of an Iso line will take up calcium from nutrient solution at a more consistent rate than plants of the diploid varieties.

REVIEW OF LITERATURE

Nicotiana tabacum was first described by Linnaeus in 1754. According to Goodspeed, *Nicotiana tabacum* (N=24) is a natural amphidiploid of *Nicotiana sylvestris* (N=12) and either *Nicotiana tomentosoformis* (N=12) or *Nicotiana otophora* (N=12) (19). Goodspeed, Cameron and Clausen stated that *Nicotiana tabacum* may be thought of as a normal diploid from a cytogenetic point of view (19, 8).

In 1922, Blakeslee et al, noted the occurrence of haploids in Jimsonweed (*Datura stramonium*) (1). Shortly thereafter haploids were reported to occur in cotton, tobacco, primrose, maize, wheat, rice, tomato, pepper, and potato (21, 6, 15, 29, 14, 28, 25, 7, 23). In 1950, Maheswari reported that haploid-diploid twins occurred in nine genera and ten species, one of which was *Nicotiana tabacum* (26). In the same year, Kehr published an extensive study on the production of haploids in *Nicotiana repanda*, *Nicotiana glutinosa*, and *Nicotiana tabacum* (24).

According to Eames, haploid-diploid twins may arise in several ways (10). The diploid may come from the integument, nucellus, or zygote, while the haploid may come from the synergids, antipodals, sperm, or an unfertilized egg. Campos and Morgan found androgenic haploids developed from sperm nuclei (4). Because of the amphidiploid origin of

Nicotiana tabacum, one might expect the haploid to survive. In 1960, de Nettancourt and Stokes found haploid-diploid twins occur in tobacco seedlings at a rate of 1:10,000 (9). Two years later Burk reported a method for isolating haploids by using genetic markers (2). In 1967, a more rapid technique for the isolation of haploids based on stomatal size was developed by Flowers and Stokes (13).

In 1949, Chase suggested that if corn haploids were treated with colchicine and then selfed, the resulting progeny should be homozygous diploid in nature (5). In 1963, Stokes used this technique to develop complete homozygotes of tobacco. These homozygous diploid lines of haploid origin were called Iso lines. Stokes developed three Iso lines; Iso line "One" was derived from a Kentucky 16 haploid, Iso line "Three" was derived from a Burley 37 haploid, and Iso line "Four" from a Kentucky 151 haploid (31). Burk developed Iso line "Two" from a Hicks haploid (2). Stokes has hypothesized that these Iso lines could be useful in experimentation in which plant-to-plant variation must be limited to micro-environmental response (31).

In 1963, Rao and Stokes observed that calcium deficiency symptoms were found more frequently in some varieties than in others. They demonstrated that this condition is in some way associated with the H chromosomes which carry the mosaic resistance factor. It was also shown that the mosaic resistance factor, per se, was not the cause of calcium deficiency symptoms, and that some mosaic resistant varieties did not show deficiency symptoms (30).

Recently, Brumagen and Hiatt found no consistent differences in the rate of calcium uptake between calcium deficient and non-calcium deficient varieties of tobacco. However, they did find that individual plants of the same variety varied considerably in their rate of calcium uptake (2).

Epstein and Leggett found that calcium uptake in barley roots occurred by both exchange absorption (non-metabolic) and active transport (metabolic) modes of uptake (11). Epstein has also suggested that the mechanism of metabolic uptake is similar to enzymatic reactions (12). In 1960, Moore, Jacobson, and Overstreet, found calcium uptake to be largely non-metabolic in excised barley roots. They postulated that since calcium actively influences the absorption of other cations, it is localized on the cell surface of the roots (27). Jacobson et al., found that calcium drastically altered the ratio of absorption of sodium and potassium. This controlling behavior of calcium was found in six different species (27). Handley and Overstreet, found that the uptake of calcium in the meristematic portion of corn root tips was non-metabolic, however calcium uptake in vacuolated portions of the root was strongly temperature dependent and, thus largely metabolic (20).

MATERIALS AND METHODS

I. Growth and Harvest of Plants

Experiment I

Seeds of Ky 16, Ky 12, Iso 1, and Iso 3, were planted in vermiculite filled plant-growth tubes. Each tube had a hole in the bottom to allow the entry of water and nutrient solution. Two foot square plywood boards with 49 one-and-one-half inch holes were used to support the plant growth tubes. A collar at the top of the growth tubes prevented them from passing completely through the plywood flats. The flats were constructed to fit into growth chambers which contained a florescent light source. The flats were easily removed from the growth chamber for watering purposes.

Three to five seeds were planted in each growth tube. After germination the plants were thinned to one plant per tube. The plants were randomized by variety on each flat. The plants were irrigated for twenty minutes each day with Hoaglands solution No. 1a (22).

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1. Throughout this thesis the following abbreviations will be used for the varieties of tobacco used: Ky 16 (Kentucky 16), Ky 12 (Kentucky 12), Iso 1 (Iso line 1, plant 14, Kentucky 16), Iso 3 (Iso line 3, plant C-1, Burley 37).

Plants were grown in this manner until they were about four inches tall. At this time they were removed from the growth tubes, and the vermiculite was carefully washed from the roots. Ten plants of each variety were assigned random positions on another flat (18). The flat was covered with saran wrap, and small holes were made in the saran wrap to allow the roots of the plants to hang completely immersed in the nutrient solution. The plants placed on this flat were allowed to equilibrate in Hoagland's solution for one week. Two aquarium pumps and an electric stirrer were used to aerate the nutrient solution.

After equilibrating the flat of plants was placed on another watering box containing 32 liters of nutrient solution. This solution was modified by the addition of 0.448 mg of $Ca^{45}Cl$ with a specific activity of 4,680 mc/gm of Ca^{45}. The isotope dilution factor was 14,680 Ca^{40} atoms to 1 Ca^{45} atom. After twenty-four hours, the plants were removed from the experimental solution for analysis of calcium uptake.

<u>Experiment II</u>

In experiment II, seeds of Iso 1_{P-14}, Iso 1_{P-15}, Iso 1_{P-16}, Iso 1_{P-17}, Iso 1_{P-18}, were grown in the same manner as the plants of experiment I. Eight plants of each variety were used in this experiment.

II. Determination of Plant Calcium Uptake

The procedure to determine the uptake of calcium was the same for experiments I and II.

The harvested plants were placed in paper bags and were numbered to correspond to their positions on the flat. The bags containing the samples were placed in a drying oven and dried at 100° to 110° C for twenty-four hours. The samples were then placed in numbered crucibles and ashed in a muffle furnace at 600° C for twenty-four hours. The ash weight of the plants was then determined to the nearest 0.01mg.

Twenty ml of 0.1 N HCl was added to each crucible to dissolve the ash. Four one ml samples were then taken from each crucible and placed on a correspondingly numbered planchet. The planchets were then dried under a heat lamp. The Ca^{45} activity of each sample was counted by a Nuclear Chicago two pi gas flow partition counter. The four samples from each crucible were used to determine an average activity per ml for each plant. The average activity per ml for each plant was then multiplied by 20 to determine the total Ca^{45} uptake for each plant. The total uptake of calcium for each plant was then determined by the isotope dilution method. The results were expressed in mg Ca/g ash wt. x 10^3.

EXPERIMENTAL RESULTS

Experiment I

This experiment was conducted to determine if there is less plant-to-plant variation in the total calcium uptake in Iso lines than there is in Ky varieties of tobacco. In this study, experiment I was repeated four times. The results (Table I, page 12) indicate that the Iso lines exhibit as much variability in total calcium uptake as the Ky varieties of tobacco.

An analysis of variance for each repetition of the experiment, (Table II, page 14) revealed no significant differences in total calcium uptake between the Iso lines and Ky varieties used.

Figure 1 (page, 20) illustrates the plant-to-plant variation in total calcium uptake that was found in experiments I-A thru I-D. Figure 1 also illustrates that the reason there is no significant difference between the Iso lines and Ky varieties is due to the individual plant-to-plant variation within these lines.

Experiment II

Experiment II was conducted to determine if there is less plant-to-plant variation in total calcium uptake in the Iso lines when plants from the same seed pod are

TABLE I

UPTAKE OF CALCIUM BY FOUR VARIETIES OF TOBACCO

Experiment 1a			
Iso 1	Iso 3	Ky 12	Ky 16
(mg. Ca/g. ash wt. x 10^3)			
66.0	100.1	84.0	84.0
100.6	71.2	108.2	76.3
102.0	114.0	92.8	90.8
62.8	120.0	107.0	106.0
77.6	74.0	115.0	87.2
82.8	92.0	102.0	95.6
65.6	80.8	96.8	80.4
96.0	83.5	65.0	67.0
53.0	46.7	73.0	51.3
105.6	53.2	62.5	78.5
Av. = 81.2	Av. = 83.5	Av. = 90.6	Av. = 81.7

Experiment 1b			
Iso 1	Iso 3	Ky 12	Ky 16
(mg. Ca/g. ash wt. x 10^3)			
102.0	47.6	74.4	49.2
86.8	137.0	90.0	96.4
51.2	82.4	71.2	70.4
90.8	57.2	94.8	81.2
71.2	72.4	86.0	86.4
82.4	87.2	78.0	73.2
76.8	67.6	98.8	109.0
79.2	82.0	72.4	52.9
102.0	46.0	116.0	104.0
114.5	62.6	129.0	95.0
Av. = 85.7	Av. = 74.2	Av. = 91.0	Av. = 81.8

TABLE I (continued)

Experiment 1c			
Iso 1	Iso 3	Ky 12	Ky 16
(mg. Ca/g. ash wt. x 10^3)			
87.6	116.0	57.8	80.0
107.6	77.4	95.4	94.2
66.7	48.0	76.0	53.0
78.7	54.1	110.2	76.7
103.4	95.1	82.1	78.1
83.1	84.1	79.1	57.0
72.3	94.6	87.5	59.0
52.4	107.0	108.3	107.3
94.5	68.1	98.0	88.6
92.1	58.3	100.6	86.3
Av. = 83.8	Av. = 80.3	Av. = 89.5	Av. = 78.0

Experiment 1d			
Iso 1	Iso 3	Ky 12	Ky 16
(mg. Ca/g. ash wt. x 10^3)			
64.1	82.2	109.3	84.7
96.6	56.2	99.0	99.0
109.8	57.6	60.3	48.7
54.6	109.0	118.1	79.8
74.3	47.8	84.9	72.1
77.1	74.1	78.6	98.1
90.4	98.7	87.2	96.3
101.9	73.0	62.4	89.2
86.7	101.8	93.4	63.4
81.5	93.5	104.7	83.5
Av. = 83.4	Av. = 79.4	Av. = 89.8	Av. = 81.4

TABLE II

ANALYSIS OF VARIANCE OF CALCIUM UPTAKE BY FOUR VARIETIES OF BURLEY TOBACCO

Experiment 1a

Source	Degrees of Freedom	Sum of Squares	Mean Square	F Value
Between Varieties	3	569.47		
Iso vs. Ky	1	144.02	144.02	0.38 N.S.*
Iso 1 vs. Iso 3	1	27.67	27.62	0.07 N.S.
Ky 12 vs. Ky 16	1	397.83	397.83	1.05 N.S.
Within Varieties	36	13,607.55	377.99	
Total	39	14,177.02		

Experiment 1b

Source	Degrees of Freedom	Sum of Squares	Mean Square	F Value
Between Varieties	3	1,510.23		
Iso vs. Ky	1	418.60	418.60	0.93 N.S.
Iso 1 vs. Iso 3	1	660.10	660.10	1.46 N.S.
Ky 12 vs. Ky 16	1	431.53	431.53	0.96 N.S.
Within Varieties	36	16,231.25	450.87	
Total	39	17,741.48		

$F_{.05}$ (1,36) = 4.13

* This F value is not significant

TABLE II (continued)

Experiment 1c				
Source	Degrees of Freedom	Sum of Squares	Mean Square	F Value
Between Varieties	3	751.75		
Iso vs. Ky	1	29.07	29.07	0.08 N.S.
Iso 1 vs. Iso 3	1	63.73	63.73	0.18 N.S.
Ky 12 vs. Ky 16	1	658.95	658.95	1.90 N.S.
Within Varieties	36	12,482.98	346.75	
Total	39	13,234.73		

Experiment 1d				
Source	Degrees of Freedom	Sum of Squares	Mean Square	F Value
Between Varieties	3	605.40		
Iso vs. Ky	1	178.50	178.50	0.52 N.S.
Iso 1 vs. Iso 3	1	81.61	81.61	0.24 N.S.
Ky 12 vs. Ky 16	1	345.28	345.28	1.00 N.S.
Within Varieties	36	12,398.07	344.39	
Total	39	13,003.47		

$F_{.05}(1,36) = 4.13$

used. This experiment was repeated three times. The results (Table III, page 17) indicate that the plant-to-plant variation is just as extreme in plants from the same seed pod as it is when random plants of an Iso line are used.

A statistical analysis (Table IV, page 19) for each repetition of the experiment, revealed no significant differences between the plants from different pods. Figure 2 (page, 22) illustrates the plant-to-plant variation in samples from the same seed pod.

TABLE III

UPTAKE OF CALCIUM BY PLANTS FROM FIVE DIFFERENT PODS OF ISO LINE 1

Iso 1_{p-14}	Iso 1_{p-15}	Iso 1_{p-16}	Iso 1_{p-17}	Iso 1_{p-18}
		Experiment II a		
		(mg. Ca/g. ash wt. x 10^3)		
58.9	68.5	83.3	88.2	53.2
119.6	117.5	84.1	60.6	100.8
93.8	79.1	62.2	82.3	72.3
76.8	82.5	86.8	106.5	83.1
124.6	75.5	72.3	56.8	60.8
67.6	69.7	74.4	94.8	75.2
90.9	84.5	82.0	66.4	71.8
77.9	83.0	108.6	103.9	75.4
Av. = 88.7	Av. = 82.6	Av. = 81.7	Av. = 82.4	Av. = 74.0
		Experiment II b		
Iso 1_{p-14}	Iso 1_{p-15}	Iso 1_{p-16}	Iso 1_{p-17}	Iso 1_{p-18}
		(mg. Ca/g. ash wt. x 10^3)		
111.0	61.2	63.8	76.5	116.0
120.0	77.8	74.0	89.9	57.7
74.3	70.6	75.2	88.7	85.7
68.7	89.9	88.5	66.8	61.5
59.6	111.0	86.8	112.0	86.1
76.0	70.3	107.0	67.9	79.7
73.1	96.2	76.0	57.7	80.8
92.2	80.1	103.0	96.4	68.5
Av. = 84.2	Av. = 82.1	Av. = 84.2	Av. = 81.9	Av. = 79.5

TABLE III (continued)

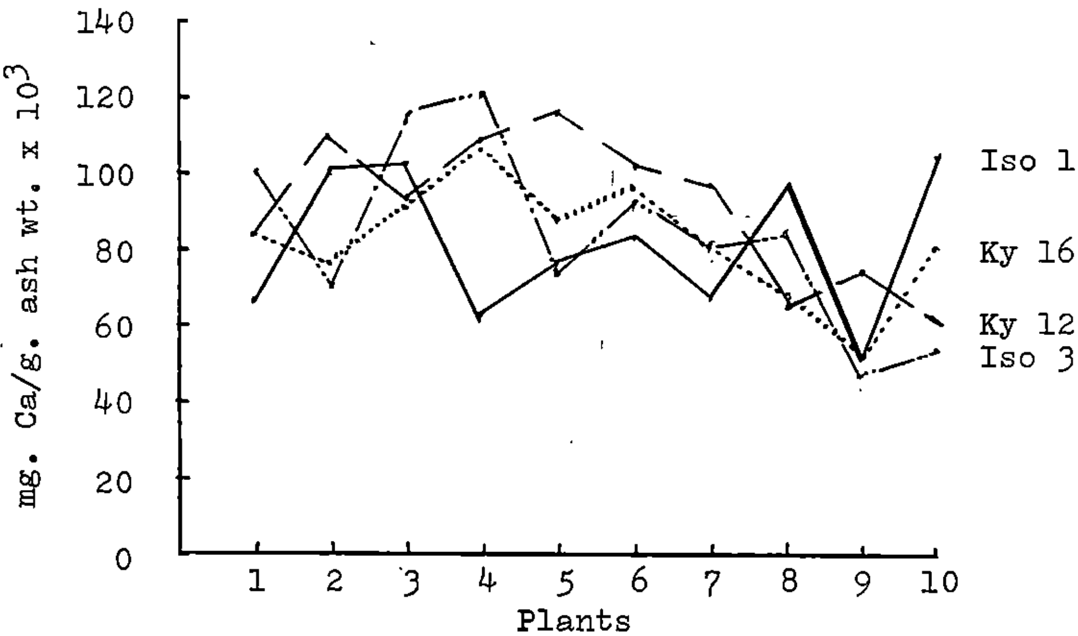
Experiment IIc				
Iso 1_{P-14}	Iso 1_{P-15}	Iso 1_{P-16}	Iso 1_{P-17}	Iso 1_{P-18}
(mg. Ca/g. ash wt. x 10^3)				
90.4	94.6	110.0	83.4	70.2
97.2	97.5	63.8	71.1	68.6
61.3	75.0	88.4	88.7	72.9
113.0	85.8	73.8	79.9	87.0
97.2	104.0	95.2	74.7	113.0
79.8	85.8	80.6	107.0	83.3
64.3	83.1	74.7	86.4	74.6
71.4	60.9	55.2	68.9	79.1
Av. = 84.3	Av. = 85.8	Av. = 80.2	Av. = 82.5	Av. = 81.1

TABLE IV

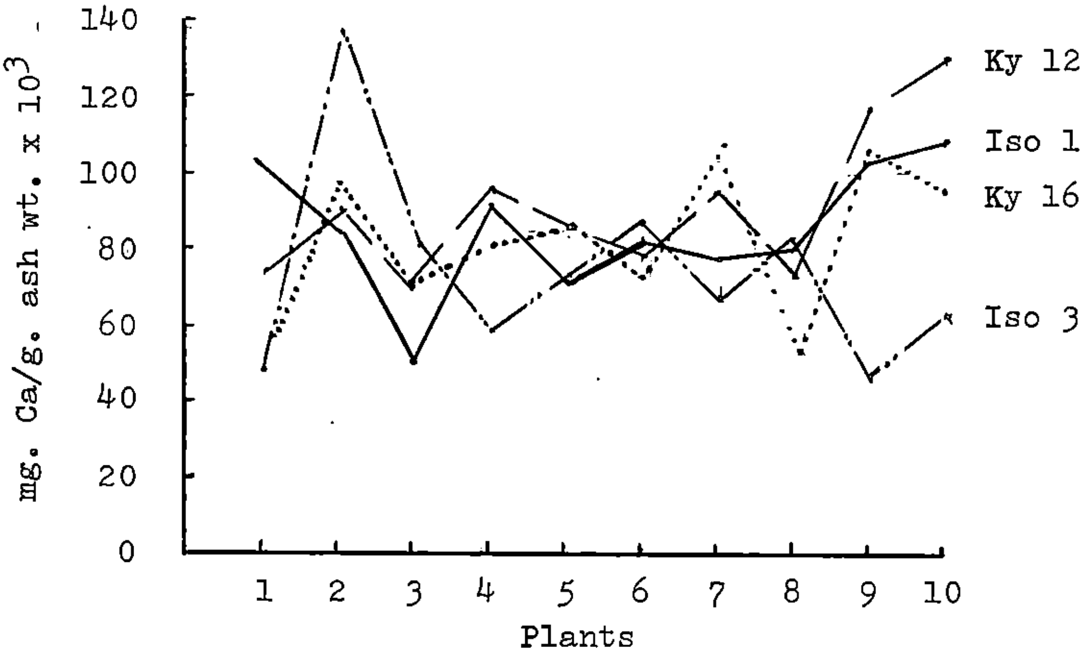
ANALYSIS OF VARIANCE OF CALCIUM UPTAKE BY PLANTS FROM FIVE DIFFERENT PODS OF ISO LINE 1

Source	Degrees of Freedom	Sum of Squares	Mean Square	F Value
		Experiment IIa		
Between Pods	4	872.44	218.11	0.70 N.S.
Within Pods	35	10,831.58	309.47	
Total	39	11,704.02		
		Experiment IIb		
Between Pods	4	158.01	39.50	0.12 N.S.
Within Pods	35	11,187.48	319.64	
Total	39	11,345.49		
		Experiment IIc		
Between Pods	4	170.10	42.53	0.18 N.S.
Within Pods	35	8,227.52	235.07	
Total	39	8,397.62		

$F_{.05}(4,35) = 2.65$



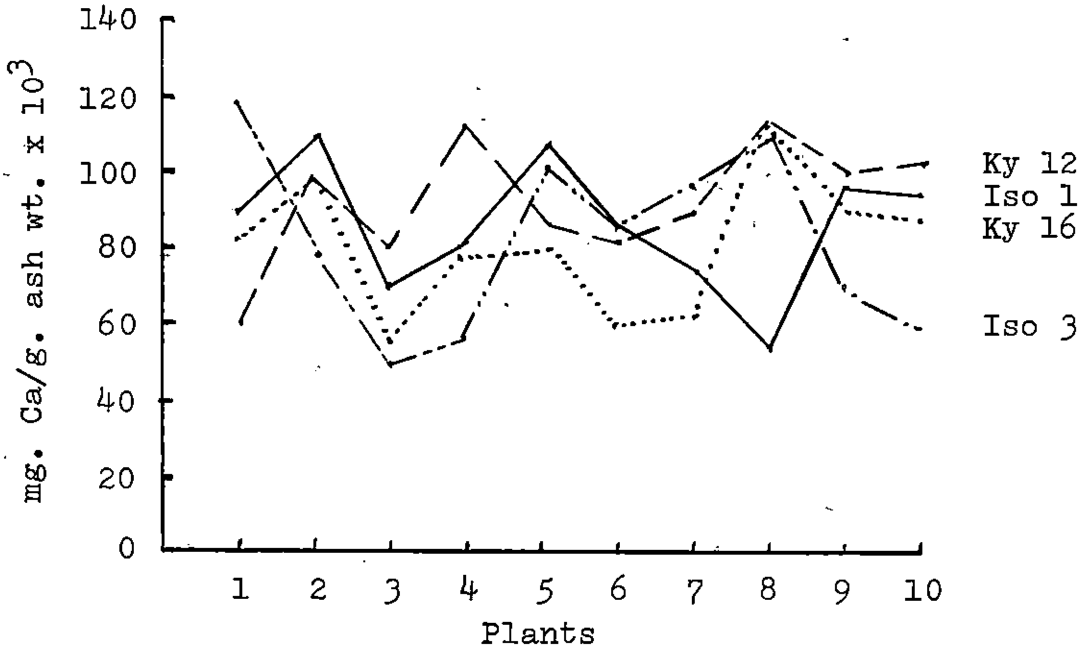
Experiment I-a



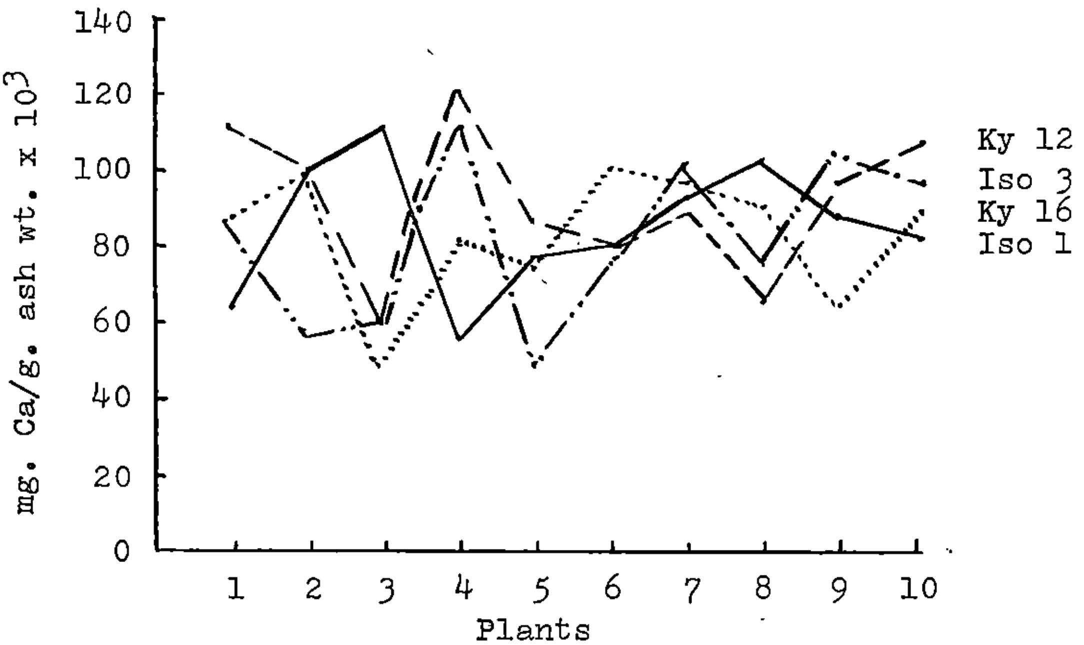
Experiment I-b

FIGURE 1

Comparison of the Uptake of Calcium by Four Varieties of Burley Tobacco



Experiment I-c



Experiment I-d

FIGURE 1 (continued)

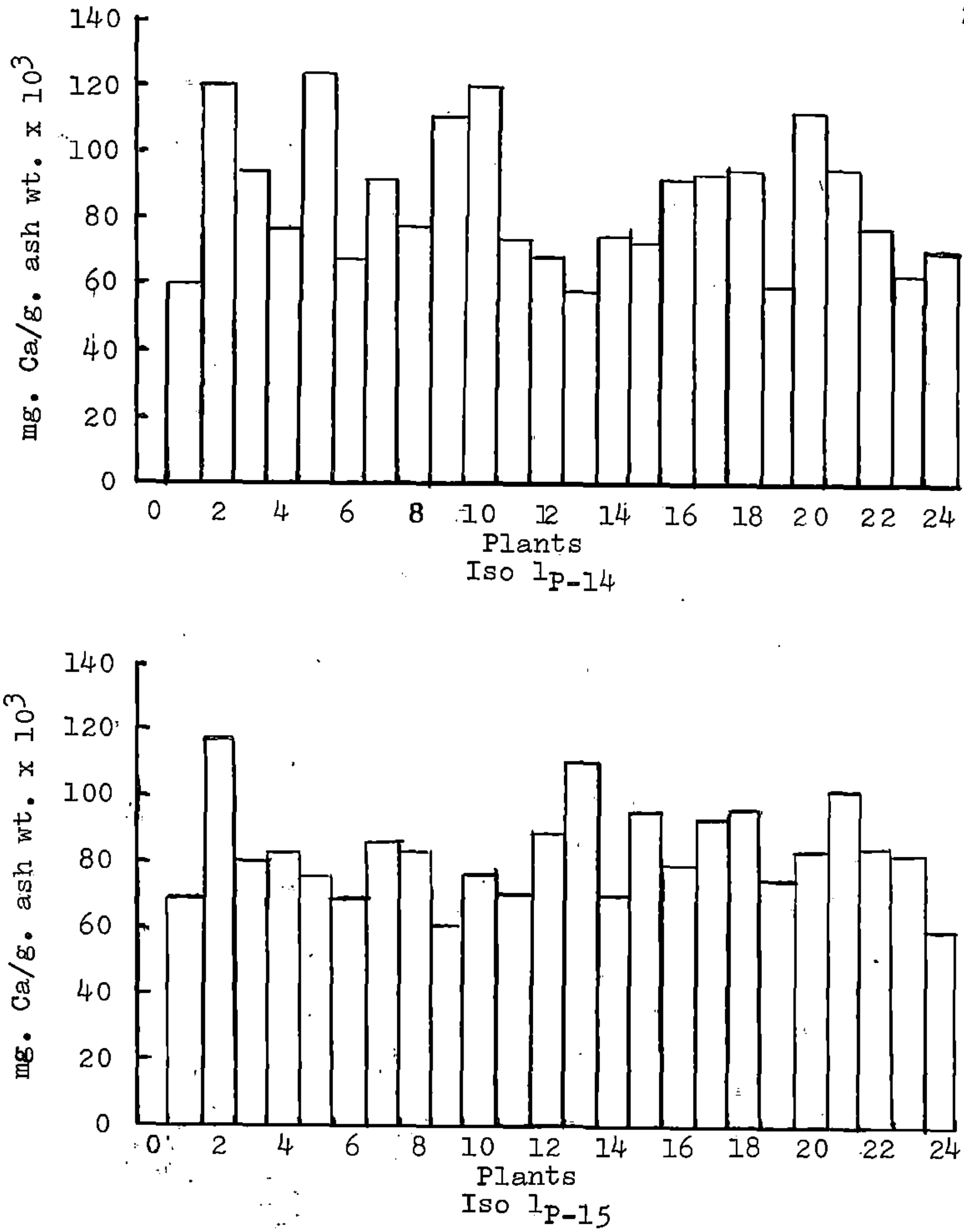
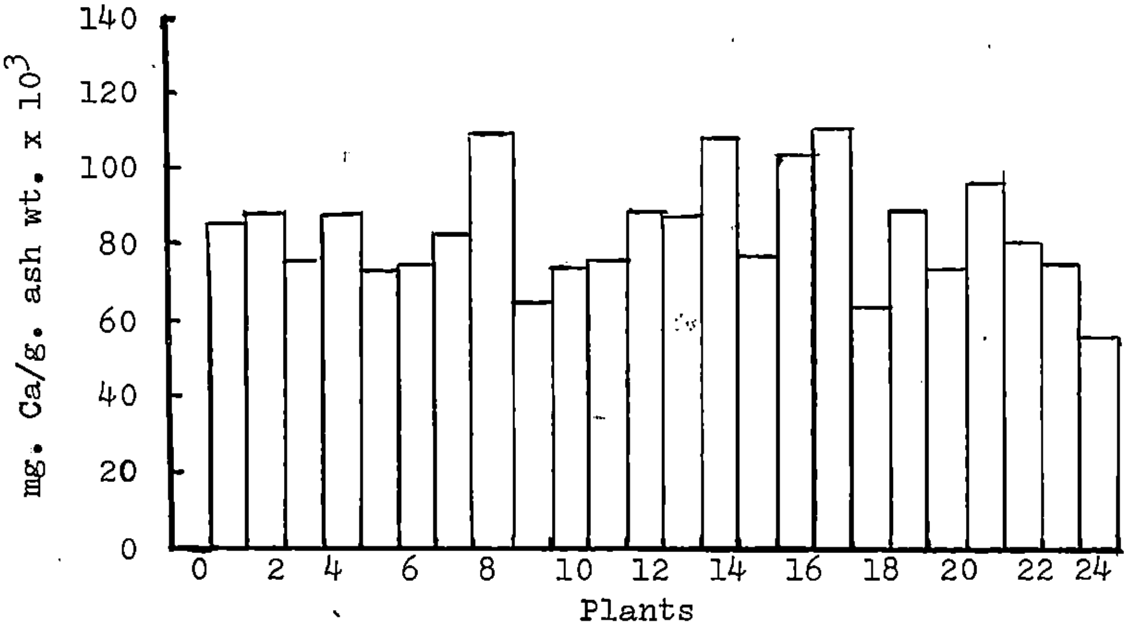
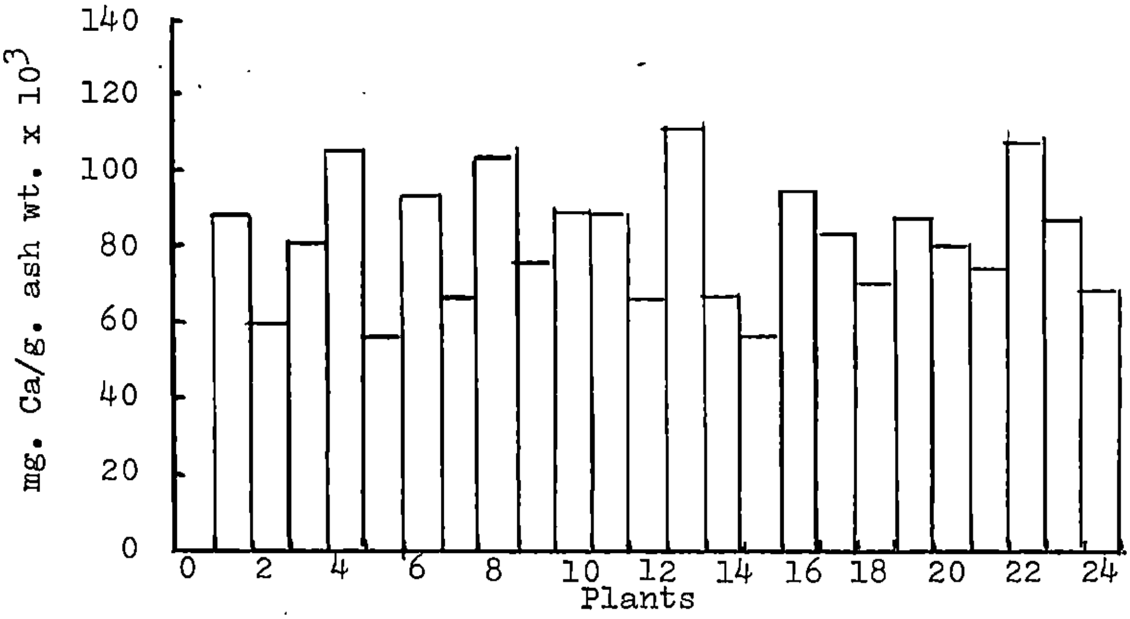


Figure 2

Comparison of the Uptake of Calcium by Individual Plants from Five Different Pods of Iso Line 1



Iso 1_{P-16}



Iso 1_{P-17}

FIGURE 2 (continued)

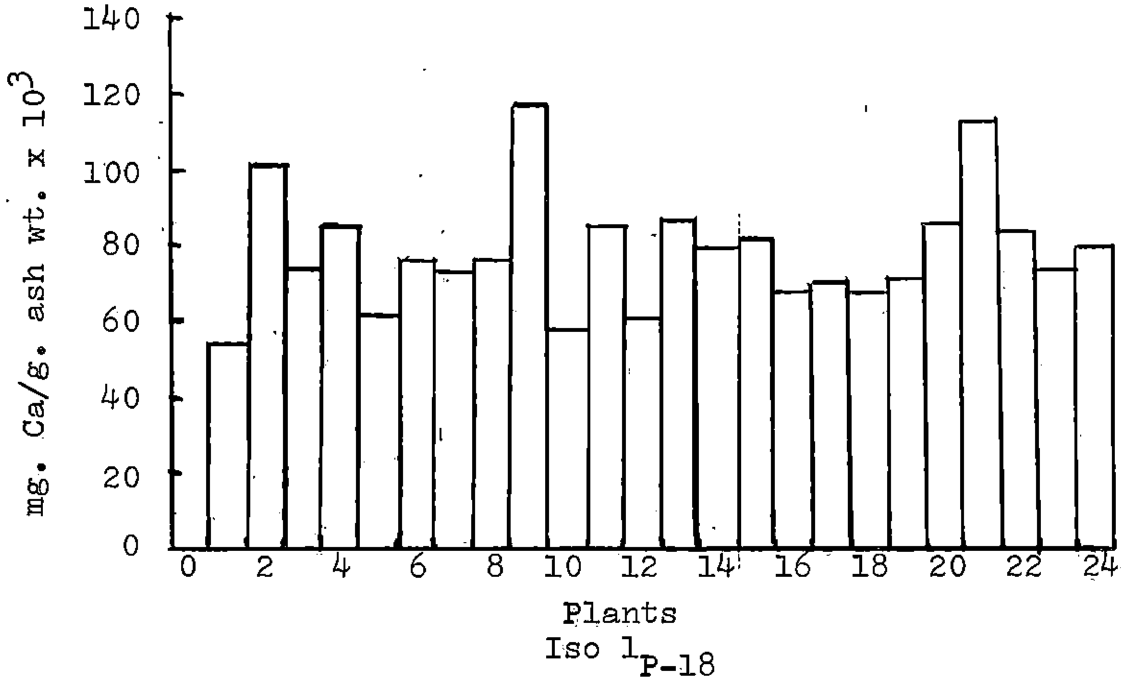


FIGURE 2 (continued)

DISCUSSION

Studies dealing with the mineral nutrition of plants have been greatly hindered by the individual variations found in strains and species (16). These plant-to-plant variations have been observed on many occasions. It has been suggested that these differences could be diminished by using organisms of closer genetic composition. The Iso lines are homozygous diploids derived from a single plant and thus it was thought that they presented an organism in which plant-to-plant variations in the uptake of nutrient elements would be minimal.

In this study a statistical analysis, Table II, of the uptake of calcium revealed no significant differences between the Iso lines and heterozygous Kentucky varieties. It was also found that even when plants from the same seed pod were used, plant-to-plant variations still existed. Therefore these observations indicate that the Iso lines, even though they are genetically alike, are no more consistent in calcium uptake than heterozygous varieties.

During this study indirect measurements on the rate of calcium uptake for twenty-four and fourty-eight hour periods were conducted. The results indicated that during the first twenty-four hours there is a high rate of uptake which greatly decreases during the second twenty-four hours. This is in agreement with the observations of Epstein. Epstein indicates that the initial uptake of calcium is largely non-metabolic, and as the concentration of the ion decreases the uptake becomes metabolic (11).

Rao and Stokes found that calcium uptake was in some way associated with the H chromosome; however, attempts to identify a single factor failed (30). In view of this, the variations in the homozygous Iso lines may be due to quantitative genetic factors. If the calcium uptake were controlled directly or indirectly by a series of genes, then slight differences in the micro-environment of the individual plants during growth would then cause observable differences in the uptake of calcium. The plants used in this study were not grown in an environment in which the temperature and humidity were controlled. Since two plants can not occupy the same position in time and space, the micro-environment may differ, and thus this would probably account for a large degree of the differences observed in both the Iso lines and the heterozygous varieties of tobacco.

SUMMARY

Iso lines and Kentucky varieties of tobacco were analyzed for total calcium uptake. The results indicate the following:

1. Statistical analysis revealed no significant differences in the uptake of calcium in Iso lines and Kentucky varieties.
2. Plants from the same seed pod of an Iso line exhibit as much variation in calcium uptake as random plants of an Iso line.

LITERATURE CITED

1. Blakeslee, A. F., J. Belling, M. E. Farnham, and A. D. Bergner. 1922. A haploid mutant in the Jimsonweed, _Datura stramonium_.

2. Brumagen, D. M., and A. J. Hiatt. 1966. The relationship of oxalic acid to the translocation and utilization of calcium in _Nicotiana tabacum_. Plant and Soil 24:239-249.

3. Burk, L. G. 1962. Haploids in genetically marked progenies of tobacco. Jour. Hered. 51:102-104.

4. Campos, F., and D. T. Morgan. 1958. Haploid pepper from a sperm. Jour. Hered. 49:135-137.

5. Chase, S. S. 1949. Monoploid frequencies in a commercial double cross hybrid maize, and in its component single cross hybrid and inbred lines. Genetics 34:328-332.

6. Chipman, R.H., and T.H. Goodspeed. 1927. Inheritance in _Nicotiana tabacum_. VII. Cyto logical features of purpurea haploid. Univ. Calif. Publ. Bot. 11:141-158.

7. Christensen, H. M., and R. Bamford. 1943. Haploids in twin seedlings of peppers, _Capsicum annum_ L. Jour. Hered. 34:99-104

8. Clausen, R. E., and D. R. Cameron. 1944. Inheritance in _Nicotiana tabacum_. XVIII. Monosomic analysis. Genetics 29:447-477.

9. de Nettancourt, D., and G. W. Stokes. 1960. Haploidy in tobacco. Jour. Hered. 51:102-104.

10. Eames, A. J. 1961. Morphology of Angiosperms. McGraw-Hill Book Co., New York.

11. Epstein, E., and J. E. Leggett. 1954. The absorption of alkaline earth cations by barley roots: kinetics and mechanism. Am. Jour. Bot. 41:758-791.

12. Epstein, E., and R. L. Jefferies. 1964. The genetic basis of selective ion transport in plants. Ann. Rev. of Plant Physiol. 15:169-181.

13. Flowers, R. A., G. W. Stokes, and J. H. Smiley. Identification of tobacco haploids by stomatal size. In press.

14. Gaines, E. F., and H. C. Aase. 1926. A haploid wheat plant. Amer. Jour. Bot. 13:373-385.

15. Gates, R. R. 1929. A haploid Oenothera. Nature (London) 124:948.

16. Gerloff, G. C. 1963. Mineral nutrition in plants. Ann. Rev. of Plant Physiol. 14:107-123.

17. Gerstel, D. U. 1955. Essay on the origin of tobacco. Sci. 5:15-17.

18. Goldstein, A. 1964. Biostatistics. Macmillon Co., New York.

19. Goodspeed, T. H. 1954. The Genus Nicotiana. Chronica Botanica Co., Waltham, Mass.

20. Handley, R., and R. Overstreet. 1961. Uptake of calcium chlorine in roots of Zea mays. Plant Physiol. 36:766-769.

21. Harland, S. C. 1920. A note on a peculiar type of "rogue" in Sea Island cotton. Agric. News Barbados 19:29.

22. Hoagland, D. R. and, D. J. Arnon. 1950. The water culture method for growing plants without soil. Calif. Agr. Ex. Sta. Cir. 347.

23. Hougas, R. W., S. J. Peloquin, and R. W. Ross. 1958. Haploids of the common potato. Jour. Hered. 49:103-106.

24. Kehr, A. E. 1951. Monoploidy in Nicotiana. Jour. Hered. 42 :107-112.

25. Lindstrom, E. W. 1929. A haploid mutant in tomato. Jour. Hered. 20:23-30.

26. Maheswari, P. 1950. The embryology of Angiosperms. Mc Graw-Hill Book Co., New York.

27. Moore, D. P., L. Jacobson, and R. Overstreet. 1961. Uptake of calcium by excised barley roots. Plant Physiol. 36:53-57.

28. Ramiah, K., N. Parthasarathi, and S. Ramanujami. 1933. A haploid plant in rice, (*Oryza sativa*). Current Sci. 1:277-278.

29. Randolf, L. F. 1932. Some effects of high temperature on polyploidy and other variations in maize. Proc. Nation. Acad. Sci. U. S. A. 18:222-229.

30. Rao, P. N., and G. W. Stokes. 1963. Role of chromosome H in the development of calcium deficiency symptoms in Burley Tobacco. Crop Sci. 3:265-266.

31. Stokes, G. W. 1963. Development of complete homozygotes of tobacco. Sci. 141:1185-1186.

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