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Growth of Kidney Beans after Cotyledon Pruning

By B. E. MICHEL

INTRODUCTION

The determination of the relation of seed weight to the growth, development, and yield of plants has been the purpose of many investigations. These have been adequately reviewed elsewhere (7, 9, 11).

Two sets of observations stimulated this study. In 1951 (10) the author suggested that one possible explanation of inhibition of epicotyl growth as a result of indoleacetic acid application to the hypocotyl might be the diversion of food stored in the cotyledons from the epicotyl to the hypocotyl coupled with an inability of the cotyledons to increase their rate of supply. While a critical examination of this question was not attempted, certain of the results obtained can be interpreted as evidence against the above hypothesis. This will be discussed.

The second set of observations was made by the author as a graduate student attempting, in a course problem, to determine the importance of cotyledons for the growth of bean plants. Various portions of the cotyledons were pruned seven days after planting with the harvest following at fifteen days. This limited experiment indicated a striking correlation between the final weights and sizes of the plants and the weights of material obtained by the plants from the cotyledons or parts thereof.

This work was an attempt to determine quantitatively the effect of cotyledon pruning on subsequent growth of kidney bean plants, especially considering any amelioration of injury with time. The importance of the amount of material removed versus the method of removal received critical attention. Finally, several quantitative measures of growth were compared and various calculations made in an effort to learn as much as possible about the growth of kidney bean plants from the data obtained.

MATERIAL AND METHODS

One lot of seeds of *Phaseolus vulgaris* L. var. Dark Red Kidney grown in California and obtained from Associated Seed Growers, Inc. was used throughout. Selected and weighed seeds in the range, 570-630 mg. (air dry), were soaked in pairs in tilted 8 dram shell vials in 2 ml. of water for 6-10 hours. This procedure was chosen, following preliminary study, to facilitate identification of beans, seed coat removal, and cotyledon pruning. It was also an attempt to circumvent any injurious effects from soaking. Several workers have reported detrimental effects of soaking on the germination

and subsequent growth of bean seeds (2, 3, 4, 6, 8). It is not the purpose of this paper to review this work, although it should be noted that the beans soaked for this experiment received equal soaking and germinated and grew well.

After soaking, the seed coats were removed, the cotyledons were pruned as desired, and the embryos or portions thereof were planted (June 29, 1951) in four inch porous clay pots filled with concrete sand and randomized on greenhouse benches. Following each harvest of plants the pots were rearranged evenly over the same bench space.

Four lots of seeds were planted, consisting of embryos as follows: 1, intact cotyledons; 2, one entire cotyledon removed; 3, the terminal one-half of each cotyledon removed by cutting at right angles to the main axis of the cotyledons; and 4, a portion from the side opposite the hilum approximately equal to one-half of each cotyledon (the upper half) removed by cutting at the smallest possible angle to the main axis of the cotyledons sufficient to miss the epicotyl. All parts of the cotyledons removed and the unused portions of the cotyledons collected from each plant—by the 15th day after planting nearly all of the cotyledons had dropped from the plants and any which had not were removed—were dried in a forced draft oven at 98° C. for a minimum of 24 hours, cooled, and weighed. Previous determination indicated that the oven dry weight of the entire embryo was 80.8 ± 0.4 per cent of the air dry weight of the intact seed. With this information and disregarding changes in weight resulting from the metabolism of the cotyledons, it was possible to calculate the actual weight of material, presumably mostly food, supplied by the cotyledons to the growing plant. This is subsequently referred to as weight of cotyledons utilized.

For the first eight days after planting watering was with tap water; thereafter the culture-solution of Arnon (1) plus 1 ml. per liter of micronutrient solution A4 (1) was used three times a week; iron, as 0.67 ml. of 0.5 per cent ferric tartrate per liter, was added twice a week; and other necessary watering was with tap water. This program was followed in an attempt to prevent any limitation of growth from lack of nutrient elements.

Starting about three weeks after planting occasional leaflets or sections thereof on a few of the plants wilted severely and then died. The cause was never determined although mineral imbalance in the sand of the pots was suspected; therefore the sand was flushed several times with tap water before nutrient solution was given again. All plants showing the above symptoms were discarded; but, because of this difficulty, the experiment was terminated with the fourth harvest.

Ten plants from each lot were harvested at weekly intervals after planting for four weeks. Harvests were started at or after 8

p.m. to reduce variations in turgor during the several hours of each harvest period. As each plant was cut, it was divided into parts, fresh weights were determined immediately, and the material placed in a refrigerator. Lengths of internodes were measured and outlines of leaves were traced as soon as possible after all plants had been harvested. The parts were then dried as indicated previously and dry weights determined. Areas of leaves were determined at leisure from the leaf tracings with a planimeter.

RESULTS AND DISCUSSION

Table 1 indicates that the average weights of the original embryos used for each lot were comparable, as were the amounts of material pruned from the cotyledons by the various methods used. It also shows that there was no significant difference in the rate or amount of cotyledon utilization after removing one entire cotyledon (lot 2) or the terminal half of each (lot 3). The greater area of wounding as a result of the removal of the upper half of each cotyledon (lot 4) seems to have reduced somewhat both rate and amount; however this small difference could not be expected to nor did it have a noticeable effect on growth.

Table 1
Average Dry Weights of Embryos, Cotyledons Removed, and Cotyledons Recovered of Kidney Beans Used

Lot	1	2	3	4
Calc. original dry Wt. embryos (mg.)	482±14*	490±13	485±14	485±11
Wt. cotyl. removed (mg.)	0	235±15	235±13	234±18
Wt. cotyl. recovered from plants harvested at 1 wk. (mg.)	166±23	64± 8	64± 8	69±24
Wt. cotyl. rec. from plants harvested at 2, 3, & 4 wks. (mg.)	37± 5	20± 3	21± 2	26± 3

*All ± values equal to one standard deviation.

The most important fact revealed by Table 1 is that the average amount of material recovered from the single intact cotyledons of lot 2 after 1 week was 23 per cent less than that recovered from each of the two intact cotyledons per plant of lot 1. This indicates that it is possible for cotyledons to increase their rate of supply of material to the seedling. If this is the result of more rapid translocation along an increased gradient, the author's earlier suggestion (10), referred to in the introduction, is of doubtful validity. The flow of material from the cotyledons might be, however, at least partially in response to a stimulus originating in a part of the seedling away from the cotyledons. The intensity of such a

Table 2

Effects of Cotyledon Pruning on Weights and Weight Relationships of Kidney Bean Plants

Weeks after planting	Lot	Cotyledons utilized Dry wt. (mg.)	Tops Fresh wt. (g.)	Leaves Fresh wt. (% tops)	Tops Dry wt. (g.)	Leaves Dry wt. (% tops)	Tops % dry wt.	Dry wt.* tops dry wt. cot. util.
1	1	318±37†	1.60±0.27	46.9±6.0	0.17±0.03	64.2±5.4	10.8±0.5	0.54±0.04
	2	193±10	1.10±0.08	53.3±2.6	0.12±0.01	68.9±1.9	10.6±0.3	0.60±0.03
	3	195±14	0.99±0.09	50.8±3.7	0.11±0.01	68.6±2.9	10.9±0.4	0.55±0.02
	4	182±22	1.08±0.12	50.4±3.9	0.11±0.02	66.8±2.9	10.1±0.5	0.60±0.03
2	1	446±12	9.65±0.75	75.7±1.6	0.91±0.05	77.3±1.5	9.5±0.3	2.04±0.12
	2	231±10	5.88±0.45	76.5±0.9	0.56±0.05	78.5±0.9	9.6±0.3	2.44±0.14
	3	231±12	6.17±0.50	76.8±1.1	0.60±0.06	78.5±1.0	9.7±0.2	2.59±0.22
	4	225±14	5.67±0.56	75.8±1.0	0.54±0.06	78.3±1.1	9.5±0.2	2.39±0.27
3	1	447±15	27.5 ±4.0	75.6±3.3	3.22±0.38	73.7±3.2	11.8±0.6	7.2 ±0.8
	2	236±21	20.4 ±2.4	75.9±2.5	2.32±0.28	74.4±2.5	11.4±0.6	9.8 ±1.1
	3	228±10	19.5 ±2.9	74.9±3.1	2.28±0.31	73.8±3.1	11.7±0.4	10.0 ±1.5
	4‡	228±12	17.6 ±1.7	73.8±2.9	2.03±0.21	72.8±2.8	11.5±0.5	8.9 ±1.1
4	1	439± 8	49.6 ±4.3	74.7±1.5	7.17±0.79	72.1±1.8	14.5±0.7	16.3 ±1.7
	2	236± 5	39.0 ±3.8	75.4±1.9	5.36±0.62	72.9±1.2	13.8±0.9	22.7 ±2.6
	3	222±12	37.4 ±3.6	75.4±1.3	5.42±0.37	73.7±1.0	14.5±1.0	24.4 ±1.7
	4‡	222±16	5.39±0.51	72.8±1.8	23.8 ±1.3

* Average of ratios rather than ratio of averages.

† All ± values equal to one standard deviation, N equals 10 except as noted otherwise.

‡ N equals 8.

stimulus could limit the rate of flow and its concentration as a result of partial cotyledon removal could account for the increase in flow per unit cotyledon. Such a mechanism is not incompatible with the author's earlier suggestion. Regardless of the rate of utilization, the final average weight of each expended cotyledon of lot 1 was slightly less, though insignificantly so, than that of each in lot 2. This was probably the result of a tendency for the cotyledons of the intact seedlings to remain attached a little longer.

It is readily apparent from Tables 2 and 3 that the growth of plants in lots 2, 3, and 4 following different methods of reducing the cotyledons by approximately one half was without significant difference; consequently further comparisons need be only between plants with intact cotyledons and those with pruned cotyledons. The effect of pruning was to reduce growth in almost every instance measured; however the ratio of the amount of reduction in growth to the reduction in weight of cotyledons utilized was always less than one and usually decreased with time. A measure of the efficiency of the use of the material from the pruned cotyledons as compared to that from intact cotyledons can be obtained by comparing the ratio, amount of growth: weight of cotyledons utilized, for the plants with pruned cotyledons with this same ratio for those with intact cotyledons (Table 4). The data indicate that the early growth in weight of the tops was nearly proportional to the weight of cotyledons utilized but became disproportionately greater with time. Pruning had less early effect on leaf area than it did at two weeks, with recovery from the effects of pruning somewhat as for weight thereafter. Pruning had least early effect on stem length, but there was no amelioration of this early effect.

Most workers comparing the growth of plants from seeds of differing weights, occurring either naturally or from mutilation, also report a disproportionately large amount of growth from the light seeds which becomes even more pronounced with time (7, 9, 11, 12). This is usually attributed to growth by photosynthesis overcoming much of the initial stunting; however a similar growth pattern was reported (5) for cantaloupe seedlings grown in the dark and was attributed mainly to the efficiency of use and conservation of stored material. Oexemann (11) reported what might be considered two anomalous results. Although he found mutilated Biloxi soybeans growing in the above pattern, both Mandarin soybean and cucumber plants were proportionately smaller than the initial weights of their mutilated seeds. This was true for weight, leaf area, and height. These results point up the difficulty of making generalizations about the growth responses of different kinds of plants, even on a varietal level.

The weights of leaves expressed as the per cents of tops (Table

Table 3
 Effects of Cotyledon Pruning on Leaf Areas, Dry Weight-Leaf Area Relationships,
 and Stem Lengths of Kidney Bean Plants

Weeks after planting	Lot	Leaves* Area (Sq. cm.)	Dry wt. † tops Area of Leaves	Entire stem Length ‡ (mm.)	Hypocotyl Length ‡ (mm.)	1st inter-node Length ‡ (mm.)	3rd inter-node Length ‡ (mm.)	5th inter-node Length ‡ (mm.)
1	1	30 ± 9**	6.00 ± 0.99	80 ± 6	64 ± 4	15 ± 2		
	2	27 ± 3	4.36 ± 0.18	73 ± 6	55 ± 4	17 ± 3		
	3	23 ± 4	4.82 ± 0.43	69 ± 3	54 ± 3	14 ± 2		
	4	26 ± 4	4.18 ± 0.39	77 ± 6	61 ± 6	16 ± 2		
2	1	295 ± 25	3.09 ± 0.12	262 ± 37	62 ± 5	53 ± 4	42 ± 13	
	2	186 ± 15	3.03 ± 0.09	202 ± 17	49 ± 6	55 ± 8	24 ± 14	
	3	198 ± 21	3.03 ± 0.03	200 ± 25	59 ± 6	50 ± 5	20 ± 6	
	4	177 ± 11	3.03 ± 0.05	197 ± 20	56 ± 6	53 ± 5	18 ± 5	
3	1	1033 ± 51	3.12 ± 0.05	751 ± 120	58 ± 5	54 ± 5	96 ± 8	209 ± 23
	2	799 ± 20	2.91 ± 0.06	645 ± 73	49 ± 3	47 ± 12	86 ± 15	196 ± 29
	3	715 ± 25	3.19 ± 0.14	625 ± 28	56 ± 6	50 ± 4	86 ± 8	200 ± 9
	4	642 ± 29	3.15 ± 0.20	631 ± 27	52 ± 4	49 ± 4	77 ± 7	213 ± 6
4	1	1679 ± 95	4.28 ± 0.10	828 ± 68	56 ± 7	52 ± 5	92 ± 9	220 ± 20
	2	692 ± 106	42 ± 6	54 ± 9	78 ± 9	197 ± 40
	3	1323 ± 54	4.10 ± 0.26	664 ± 78	51 ± 7	48 ± 3	80 ± 13	216 ± 16
	4	634 ± 77	51 ± 6	52 ± 5	82 ± 9	193 ± 23

* N equals 10, first five lines; N equals 5, next three lines; and N equals 4, last six lines. Areas corrected by multiplying by ratio, dry wt. of tops of plants measured for leaf areas: dry wt. of tops of entire sample. This correction was small in all instances.

† N values as above (*), but no corrections needed or made. Dry wt. in mg., area in sq. cm.

‡ N equals 10 for all length measurements excepting lot 4, harvests 3 and 4 where N equals 8.

** All ± values equal to one standard deviation.

Table 4
Relative Efficiency* in Utilization of
Material in Cotyledons for Growth

Weeks after planting	1†	2‡	3‡	4‡
Relative efficiency of dry wt. production	108	121	133	145
Relative efficiency of leaf area production	140	125	135	155
Relative efficiency of total stem length production	155	150	165	155

* Av. meas. of growth, e.g. dry wt., for lots 2, 3, & 4 \div av. dry wt. cotyledons utilized for lots 2, 3, & 4

X 100

av. meas. of growth for lot 1 \div av. dry wt. cotyledons utilized for lot 1.

† Had there been no reduction in growth as a result of pruning, all values in this column would be approximately 167 (equal to $318/190$ —reflecting the weights of cotyledons utilized in 1 wk.).

‡ As above (†) except the value would be 194. This reflects the final weights of cotyledons utilized.

2) are remarkably similar not only within but also among harvests—except for the first week when the first foliage leaves had only partially expanded, and except for the reflection of the more rapid loss of succulence by stems than by leaves in the dry weight column. The values of the ratio, dry weight of tops: area of leaves (Table 3), within each harvest are nearly identical—again excepting the first harvest. This relative constancy of certain of the ratios would seem to indicate a minimal altering by pruning of the fundamental growth patterns of the plants.

A similar constancy of values for the weight of tops: area of leaves ratios in Japanese buckwheat grown from seeds of different weight grades was found by Schmidt (12). Comparisons of the same ratios calculated from Oexemann's (11) data show them to be less alike. Data obtained by others could be compared also, but one is reminded again of the difficulty of comparing the growth responses of different kinds of plants.

SUMMARY

1. Weekly weight, length, and area measurements were made of kidney bean plants grown from intact seeds and seeds from which one half the cotyledonary material had been removed in each of three different ways.

2. The results obtained for the three methods of cotyledonary pruning were not significantly different.

3. On a unit cotyledon basis removal of material from the cotyledons by the plants was more rapid following pruning; however the final amounts removed were not significantly different.

4. The interpretation of the facts in 3., as related to an earlier suggestion by the author was discussed.

5. The ratios of growth to amounts of cotyledonary material utilized were greater in all instances for plants with pruned cotyledons than for those with intact cotyledons, and the relative values of these increased with time except for stem length.

6. The relative constancy of certain of the data was noted as indicating minimal changes from pruning of fundamental growth patterns.

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