TRANSPIRATION AND SOME OTHER RESPONSES OF ADZUKI BEANS (PHASEOLUS SP.) TO GIBBERELLIC ACID[1]

by

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INTRODUCTION

The gibberellins are well recognized as being able to regulate the growth and development of a great variety of plants.(1,3,11,12) Some responses of Adzuki (Azuki) beans to these substances were reported by Shimada(10) and recently by Chiang and Chiang.(5) It was suggested by Chiang and Chiang(5) that the acceleration of the conduction of water and translocation of other substances from the root to the shoot occurred in gibberellic acid (GA)-treated Adzuki beans because the formation of secondary xylem was greatly stimulated. The effect of GA on transpiration rate of this plant was, therefore, investigated. More recently Linck and Sudia(9) reported that GA accelerated the absorption and translocation of phosphorus-32 in bean plants (*Phaseolus vulgaris*).

MATERIALS AND METHODS

Adzuki (Azuki) beans (*Phaseolus radiatus* L. var. *aurea*, Prain), a commercial dwarf bean chiefly cultivated in Japan and China, was used. Lanolin containing 1% potassium salt of gibberellic acid, "Gibrel" of Merck and Co., Inc., was used in the present investigation. The plants were grown in a greenhouse at National Taiwan University under natural daylengths and temperatures.

For the experiment on transpiration, seeds were planted in moist sand on September 1, 1958. Five days after planting, seedlings of uniform size were selected and transplanted to tap water in 600 ml. glass beakers. Each beaker was covered with a thin wooden plate containing three holes. A seedling was supported in each hold by a cotton plug at the lower part of the first internode. Hoagland's complete nutrient solution was used for culture after September 7. Culture solutions were renewed every third day during first week, and every second day in the second week. GA-lanolin paste was smeared around the upper portion of the first internode in a 2 mm. band one week after seeding (September 3). Control plants were smeared with plain

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lanolin. The loss in weight of each culture beaker within 24 hours was measured on a sensitive balance. The water evaporated from another sets of beakers without plants growing in them served as a control. All measurements of water loss were taken at night when the transpiration rates were less in order to minimize the experimental errors. The leaf area was obtained by tracing paper method. Plant height and dry weight of tops and roots of these plants were also measured. Measurements were made at the end of first and second weeks after treatment with GA.

For the experiment on internode extension, internode diameter, and flowering, Adzuki bean seeds were soaked in tap water for one day and then planted in garden soil in 25 cm. diameter unglazed flower pots on September 1, 1958. After germination of the seeds, each pot was thinned to four seedlings of uniform size. Ten pots of seedlings were used in this experiment. Plants in five pots were treated with GA-lanolin. Those in the remainder were treated with lanolin and served as controls. GA-lanolin paste was applied twice during this experiment. The time and position of these applications are indicated in figure 2. Each pot was watered twice daily with tap water, once in every two or three days with 100 ml. of four times Hoagland's solution. Internode measurements were made daily up until three weeks after first treatment (*i.e.*, two days before flowering). Diameter of internode of the same plants was taken at weekly intervals. The number of open flowers were counted each day at the flowering stage.

In another investigation on the effect of GA on petiole elongation, uniform Adzuki bean plants which had three trifoliate leaves were used. The elongation of various parts of petioles was determined by marking the petioles with Indian ink at millimeter intervals. GA-lanolin paste was smeared around three petioles of each treated plant in a narrow band near the blades as indicated in figure 5. Petioles of control plants were treated with plain lanolin. The elongation of petioles was determined two weeks after treatment (April 24, 1958).

For determination of development of roots and observation on nodulation, Adzuki beans were planted in 25 cm. unglazed pots containing rich garden soil. Each pot was obliquely fitted with a transparent glass plate on one side of the pot in order to make observation and determination on development of roots (figure 7, upper). The glass surface was covered with black paper. After germination, the seedlings were thinned to a uniform stand of one plant per pot. Particular attention was paid to uniformity in root length. One week after planting (April 27, 1959), plants were smeared with GA paste at the upper portion of the 1st internode. Controls were smeared with plain lanolin. All plants were autoirrigated to maintain a constant soil moisture. All roots appearing on the glass plate were traced on cellophane paper daily up to the seventh day after treatment. Two weeks after treatment, typical plants of the control and treated series were photographed.

EXPERIMENTAL RESULTS

Transpiration, Plant Height, Leaf Area, and Dry Weight.

The results of the study on the effect of GA on transpiration in Adzuki beans are presented in table 1 and figure 1. Plant height, leaf area, and dry weight of those plants are also presented in table 1. Plants treated with GA showed a remarkable greater height than controls. Total leaf surface area was, however, notable less. The dry weight of roots of treated plants showed a significant less than controls. The dry weight of tops of treated plants, on the other hand, did not show any remarkable change. Therefore, the tops to roots ratios of GA-treated plants were larger than those of controls. Transpiration rates were computed by weight of water loss per plant, unit leaf area, dry weight of tops, dry weight of roots, and total dry weight. As shown in table 1, the rates computed on different bases give different information about the effect of GA on transpiration. The transpiration rates of GA-treated plants were smaller than that of controls if the rates were

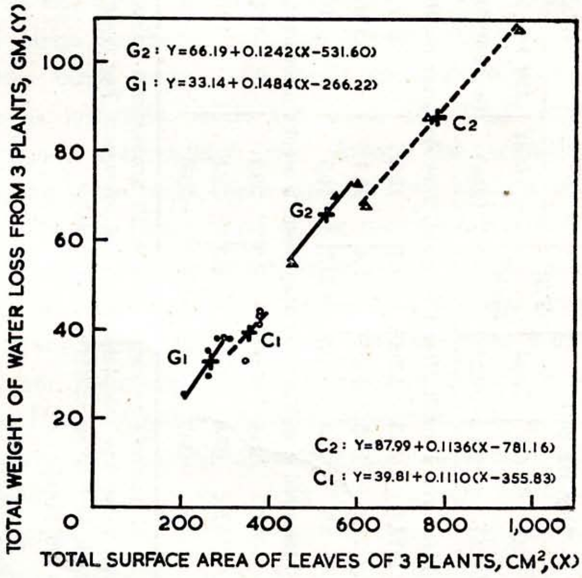

Fig. 1. Graph showing the relationship between leaf area (X) and weight of water loss (Y) in Adzuki beans. Solid lines and broken lines are the regression lines of treated (1% GA-lanolin) and control (plain lanolin) respectively. The results obtained at the end of first week after treatment are indicated by black dots (treated) and circles (control); black triangles (treated) and white triangles (control) are the results obtained at two weeks after treatment. + indicates the average value. This figure was made according to the same data as presented in table 1.

calculated per plant, per dry weight of tops or per total dry weight. If leaf area and dry weight of roots were used as bases for expressing rates of transpiration, the rates of treated plants were greater than that of the controls. It can be seen in figure 1 that there is a close correlation between leaf area and water loss. The leaf

Table 1. Effects of Gibberellic Acid on Plant Height, Leaf Area, Dry Wieght, T/R Ratio, and Transpiratiration Rate in Adzuki Beans[a]

	Plant Height (cm.)	Leaf Area[b] (cm².)	Dry Weight[c]			T/R Ratio[d]	Water Loss (or Water Consumption)				
			Tops (mg.)	Roots (mg.)	Total (mg.)		Per plant (gm.)	Per leaf area (mg/cm²/24 nr.)	Per DW[e] of tops (mg/mg/24 hr.)	Per DW[e] of roots (mg/mg/24 hr.)	Per DW[e] of total (mg/mg/24 hr.)
Measured 1 week after treatment, 2 weeks after seeding											
Control	22.3	118.6	177.7	43.3	221.0	4.1	13.3	112.1	74.7	306.9	60.1
Treated	44.6	88.7	171.4	31.5	202.9	5.4	11.1	124.1	64.5	349.2	54.5
% Increase[f]	+100.0	−25.2	−3.5	−27.3	−8.2	+31.7	−16.5	+10.7	−13.7	+13.8	−9.3
Measured 2 weeks after treatment, 3 weeks after seeding											
Control	28.7	260.4	389.0	93.6	482.6	4.3	29.3	112.6	75.4	317.4	64.2
Treated	58.8	177.2	355.2	56.8	412.0	6.2	22.1	124.5	62.1	387.1	52.7
% Increase[f]	+104.9	−32.0	−8.7	−39.3	−14.6	+30.6	−24.6	+10.6	−17.6	+22.0	−17.9

[a] Plants were cultured in Hoagland's nutrient solution in transparent glass beakers. 1% GA-lanolin paste was applied to 1st internode on Sept. 7, 1958. Each value was calculated from the average of five (measured one week after treatment) and three (measured two weeks after treatment) replicate beakers of three plants each.

[b] Total leaf surface area per plant.

[c] Dry weight per plant.

[d] Tops to roots ratio in dry weight.

[e] DW means dry weight.

[f] Percent increase or decrease over control.

area was reduced by the application with GA and the total amount of water loss was accordingly less. The regression coefficients between leaf area and water loss are slightly greater when GA was applied.

Internode Extension, Internode Diameter, and Flowering.

The effect of GA on the rate of extension of internode of Adzuki beans is shown in figure 2. It can be seen in this figure that the extension rate of internodes of traeted plants was greater than that of controls. The cessation of internode extension, however, took place slightly earlier (one to two days) in GA-treated plants. The appearance of new internodes of GA-treated plants was earlier than that of controls. The number of internode was not affected by application with GA though the appearance of internode was hastened. It also can be seen in figure 2, that the accelerating effect of GA on stem elongation was very remarkable during the first week after the first treatment but not at the second week. After the second application with GA, the promoting effect on stem elongation was slight.

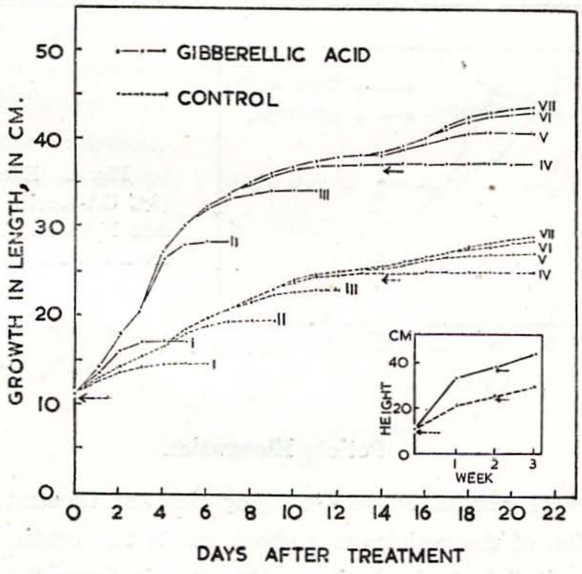

Fig. 2. Effect of gibberellic acid (1% GA-lanolin) on internode extension in Adzuki bean plants grown in soil in pots. Roman numerals indicate internode number. Arrows indicate position of application. First application was made on September 7, 1958, one week after planting. Each dot is the average of 20 plants.

The growth in diameter of internodes was illustrated in figure 3. The diameter of the first internode of treated plants was greater than that of controls. The diameter of second and third internode were, however, less in GA-treated plants. The promoting effect of GA on growth in diameter occurred during the first week after treatment. No growth promoting effect could be found after the first week (figure 3).

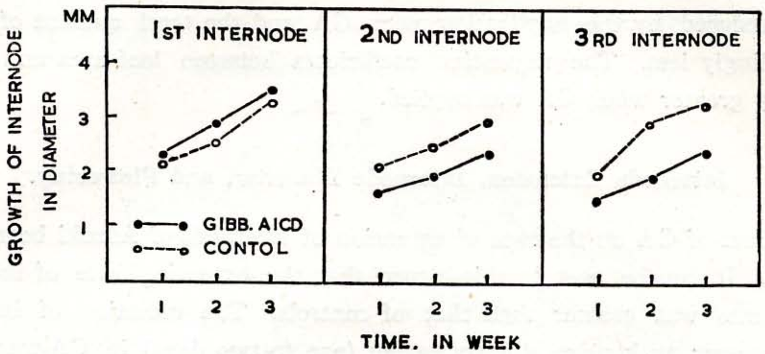

Fig. 3. Effect of gibberellic acid (1% GA-lanolin paste) on growth of internode diameter of Adzuki bean. The data were obtained from the same plants as in figure 2.

Flower buds of treated and control plants appeared at the same time on October 1. Number of open flowers counted at daily interval are illustrated in figure 4 by denoting the number of opened flowers at two days intervals. The flower numbers were not significantly affected by GA-treatment nor did GA hasten flowering. All flowers of both control and treated plants opened from October 2 to October 16.

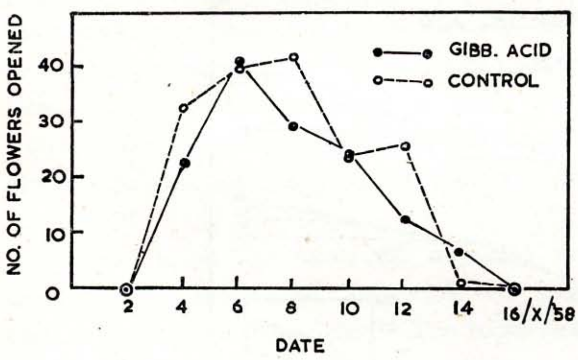

Fig. 4. Effect of gibberellic acid (1% GA-lanolin paste) on flowering of Adzuki beans. The data were obtained from the same plants as in figure 2.

Petiole Elongation

The experimental results on petiole elongation are illustrated in figure 5. The region of elongation of the petioles was about one to two centimeters in length back from the base of trifoliate leaf. As it can be seen in figure 5, the promoting effect of GA on petiole elongation closely coincides with growing region of the petiole. The greatest accelerating effect of GA on elongation was noticed from younger petiole at the most rapid growing region. The less growing petiole or part of petiole was less affected with GA treatment. No effect of GA on elongation of petiole could be found at the region where growth had already ceased. A length of one millimeter in the most rapidly growing region on the intact petiole treated with GA elongated to a length of about 35 millimeters. On the other hand, one millimeter of the most rapid growing region of control petiole elongated to only about 15 millimeters.

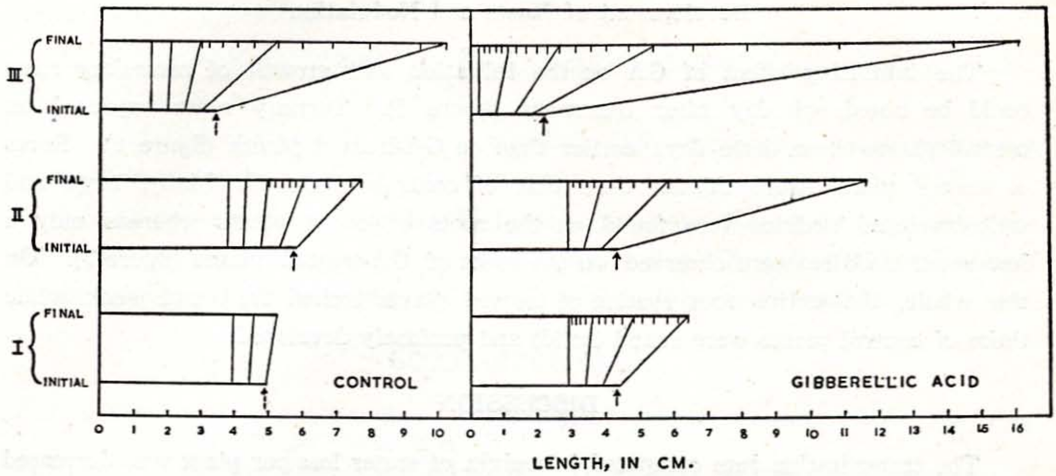

Fig. 5. Effect of gibberellic acid (1% GA-lanolin paste) on elongation of Adzuki bean petiole. Roman numerals indicate the petioles of trifoliate compound leaves on a plant in the numerical order from base to tip. INITIAL: initial length of petioles; FINAL: two weeks after treatment. Arrows indicate sites of application with GA (solid) and plain lanolin (broken). Short lines were marked on initial petioles (INITIAL) with Indian ink every one millimeter. The elongation of those 1 mm. zones at the end of two weeks after treatment was determined (FINAL).

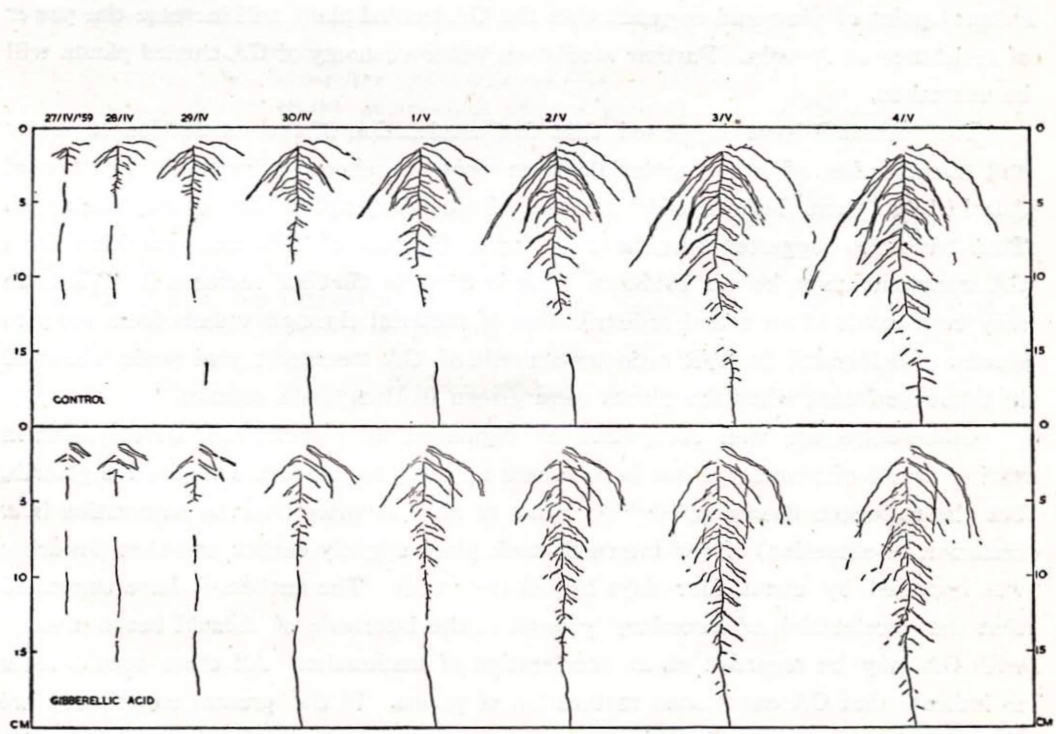

Fig. 6. Effects of gibberellic acid on development of roots in Adzuki beans. 1% GA-lanolin paste waste was applied to the first internode one week after seeding on April 27, 1959. Growth of roots was traced every 24 hours up to seventh day after treatment.

Development of Roots and Nodulation

The inhibiting effect of GA on the initiation and growth of secondary roots could be noted one day after treatment (figure 6). Tertiary roots appeared on control plants about three days earlier than on GA-treated plants (figure 6). Roots of treated plants were thinner than that of controls (figure 7). Many, large and well developed nodules were found on the roots of cotrol plants whereas only a few small nodules were observed on the roots of GA-treated plants (figure 7). On the whole, the entire root system of treated plants looked frail and weak while those of control plants were sound (solid) and profusely developed.

DISCUSSION

The transpiration rate computed in weight of water loss per plant was decreased by the application with GA. This is a natural results in that both transpiring (leaf area) and absorbing organs (roots) were greatly reduced by GA treatment. The transpiration rate expressed by the weight of water loss per total dry weight of a plant showed a decrease by GA-treatment. This indicates that the GA-treated plants do not use more water than control plants. This is especially interesting from an agricultural point of view and suggests that the GA-treated plant will increase the power of resistance to drought. Further studies on water economy of GA-treated plants will be unertaken.

The authors[5] have suggested that the acceleration of the conduction of water and translocation of other materials from roots to shoots occurred in GA-treated Adzuki bean plant, because the formation of secondary xylem was greatly promoted. They have also suggested that the remarkable increase of T/R ratio resulting from GA treatment may be an evidence as it is thought that an increase in T/R ratio may be a result of an actual redistribution of material through vessels from roots to shoots. An increase in T/R ratio as a result of GA treatment was again observed in this experiment when the plants were grown in Hoagland's solution.

Gibberellins are well recognized as regulators of growth and development in various kinds of plants. It has been shown not only to accelerate vegetative growth, but also to hasten flowering.[1,3,11,12] Brian *et al.*[4] reported that the maturation (*i.e.* cessation of extension) of pea internode took place slightly earlier and that flowering was hastened by about four days by GA-treatment. The authors[5] have suggested that the acceleration of secondary growth in the internode of Adzuki beans treated with GA may be regarded as an acceleration of maturation. All these results seem to indicate that GA can hasten maturation of plants. In the present experiment we observed that the maturation (*i.e.* cessation of extension) of Adzuki bean internode treated with GA took place earlier than that of control but the flowering of this plant was not affected. This indicates that the acceleration of internode maturation and

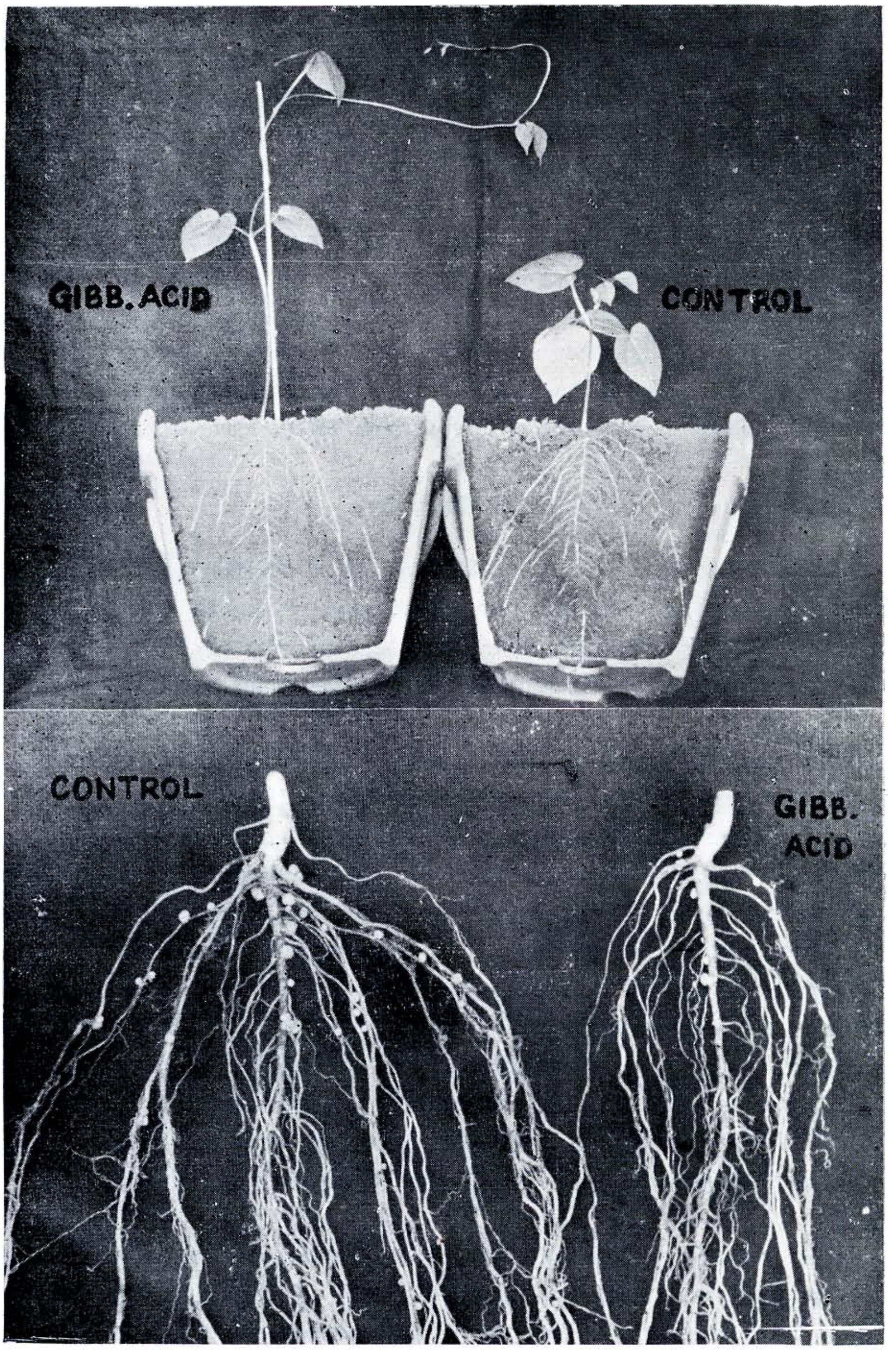

Fig. 7. Effects of gibberellic acid on development of roots and nodulation in Adzuki beans. 1% GA-lanolin paste was applied to the first internode one week after seeding on April 27, 1959. At the end of two weeks after treatment, plants were photographed. GIBB. ACID: treated with gibberellic acid; CONTROL: control plant. The plants are the same plants as in figure 6.

secondary growth by GA-treatment are not always accompanied by an acceleration of flowering.

Shimada[10] reported that the growth in mean length of roots of Azuki (Adzuki) beans was promoted by treatment with fungal extract of *Gibberella fujikuroi*. Our present observation showed that both initiation and growth of secondary and tertiary roots were remarkable inhibited by GA treatment. It was noted by Brian *et al.*[2] that GA inhibited the growth of cress root. Kato[8] also reported that gibberellin A inhibited the root formation of pea stem cuttings.

In the present experiment we observed that the nodulation of Adzuki beans was greatly depressed by the application with GA. The effect of GA on nodulation was recently discussed by Galston.[7] Thurber *et al.*[13] found an inhibiting effect while Fletcher *et al.*[6] reported no effect of GA on nodulation.

SUMMARY

1. Effect of gibberellic acid (1% K-salt of GA in lanolin) on transpiration rate, plant height, leaf area, dry weight of tops and roots, internode extension, internode diameter, flowering, development of roots, and nodulation in Adzuki (Azuki) bean (*Phaseolus* sp., a commercial dwarf bean cultivated in Japan and China) were investigated.

2. Young seedlings grown in Hoagland's nutrient solution in glass beakers were treated with GA-paste at the first internode. Water loss (*i. e.* water consumption) experienced by culture solutions during a period of 24 hours were measured one and two weeks after treatment. Plant height, leaf area, and dry weight were measured at the same time. Height of treated plants was remarkable greater; total leaf surface area of them, however, was notable less. Dry weight of both tops and roots of treated plants was less; dry weight of roots of treated plants was notable less while that tops was not significantly affected. T/R ratio of treated plants was greater than that of controls. Transpiration rates of GA-treated plants were less than that of controls if the rates were calculated per plant, per dry weight of tops or per total dry weight. If leaf area or dry weight of roots were used as a basis for expressing rates of transpiration, the rates of GA-treated plants were greater than that of controls.

3. Young plants grown in soil in pots were treated with GA-paste at the first internode one week after seeding. The appearance of new internode of GA-treated plants was earlier than that of controls. The cessation of internode extension took place earlier in treated plants. Number of internode was not affected by GA application. Diameter of the first internode of treated plants was greater than that of controls whereas the diameters of the second and third internodes were less. Flowering of these plants was not hastened. Flower numbers were not significantly af-

fected by GA application.

4. The growth promoting effect of GA on petiole elongation was closely coincided with the growth region of the petiole.

5. Initiation and growth rate of secondary and tertiary roots, and nodulation were inhibited by the application of GA-paste.

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