EFFECT OF PLANT GROWTH REGULATORS ON THE NITROGEN FIXING (ACETYLENE REDUCTION) ACTIVITY OF SOYBEAN PLANTS

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Abstract: Two plant growth regulators, kinetin and 2, 4-D, were tes ted on soybean plants to find their relative effects on nitrogen fixation. 2, 4-D gave much more severe effects on both morphologically and physiologically than did the kinetin.

As a result of the treatments with exogenous kinetin and 2, 4-D, the leaf-area, leaf-number, and the height of the plants was reduced. Leaves showed chlorosis, and the chlorophlls in the leaves (mostly the chlorophyll b) was markedly decreased, the accumulation of the dry matter in the tissue fractions diminished, and other apparent changes involved the shoot-root ratio and the chlorophyll-a to chlorophyll-b ratio.

The nitrogen fixing (acetylene reduction) activity of intact plants or of detached nodules was suppressed by kinetin and 2, 4-D. One of the detrimental effects of 2, 4-D or kinetin on nitrogen fixation was a decrease in the root-nodule-number. It is presumed that the nodule-initiation or nodule-degeneration is inhibited or promoted by the stimulation with kinetin or 2, 4-D.

INTRODUCTION

It has previously been reported that the nitrogen fixing activity of soybean plants was predominantly controlled by photosynthetic activity[10,11]. Therefore, it was assumed that if the photosynthetic activity of soybean plants could be promoted (a) by increasing their leaf-area, and/or, (b) by enhancing the number of chloroplasts or concentration of chlorophylls in leaves, and/or (c) by delaying or preventing leaf senscence; with treatments by anyone of the plant growth regulators, that the capability of nitrogen fixation could be promoted, and the seed yield of soybean plants could be increased.

Kinetin is known to produce various effects on the growth and development of plants. In addition to the promotion of seed germination, kinetin participates in stimulating leaf enlargement[15], enhancing flowering and fruit development[17], and one of the most studied regulatory actions of kinetin is its action in delaying leaf senescence[25]. Another control exerted by kinetin is its effect on chloroplast development[27].

2, 4-D (2, 4-dichlorophenoxyacetic acid) is known as one of the most widely used synthetic auxins. It has been found that the incorporation of labelled nucleotides into the nucleic acids is stimulated by either IAA or 2, 4-D[12]. These experiments imply that 2, 4-D or IAA may be involved in the regulation of RNA synthesis, and hence of RNA-directed protein synthesis[18]. In studies of 2, 4-D stimulated RNA synthesis in soybean tissues, O'Brien *et al.* (1969) found that the major effect of 2, 4-D on RNA-directed protein synthesis might be through an enhancement of RNA polymerase activity[19].

It has been reported that the role of phytohormones involved in the nitrogen fixing activity of leguminous plants is due to the activity of IAA, kinetin or 2, 4-D acting as triggers for

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the development of root nodules[4,14]. However, others have reported that exogenous kinetin or 2, 4-D severely reduces or prevents noudle initiation or nodule development[3,5,16,28]. Our study presents ample data to show that the application of exogenous kinetin or 2, 4-D effects the soybean plants adversely and changes their morphological features and physiochemical activities which consequently result in reduction of nitrogen fixation.

MATERIALS AND METHODS

Soybean (Glycine max, Kaoshiung #3) seeds were germinated for 3 days in vermiculite, and single seedlings were transplanted after inoculation with a commercial preparation of *Rhizobium japonicum*, to a mixture of sand, vermiculite (2:1, v/v) in clay pots having a 15 cm top diameter. The sand had previously been washed a couple of times with tap water and deionized water. The seedlings were grown in a controlled environment chamber (day/night irradiance, 0.4 cal/cm^2-minute, cool white fluorescent; day/night temperature, 29/25°C; day/night relative humidity 75%; photoperiod, 16 hours). During the first 10 days after planting, the seedlings were watered with Hoagland's solution containing a very low concentration of nitrate every three days. It has been reported that the small amount of N supplied in the solution provides a source of N which enhanced nodulation and N_2 fixation[8,9]. After 10 days, plants were watered once daily by using a nitrogen-free nutrient solution (1.6 mM $MgSO_4$, 0.8 mM KH_2PO_4, 4.0 mM KCl, 6.3 mM $CaCl_2$, 0.1 mM $MnCl_2$, 0.4 μM Na_2MoO_4, 0.3 μM $CuSO_4$, 0.7 μM $ZnSO_4$, 46.2 μM H_3BO_3, 4.9 μM $FeC_6H_5O_7$, adjusted to pH 7.0 with 1 N KOH) or water on alternate days. Treatment of the plants was also started on the 10th day by addition to the nutrient solution of a concentration of 20 ppm or 40 ppm of kinetin, or concentrations of 20 ppm, 40 ppm, or 2×10^{-8} M, 4×10^{-8} M of 2, 4-D twice a week, respectively. As a precaution against the accumulation of kinetin or 2, 4-D in the soil, all plants were excessively irrigated with deionized water every 7th day. During the growing period, all lateral branches were excised. Plants receiving each treatment were harvested at indicated intervals for measuring the nitrogen fixation rate (acetylene reduction) and nodular nitrogenase activity. The concentration of ammonia, soluble carbohydrate, and soluble protein in nodules was determined by assaying the 20,000 g supernatant solution of thoroughly homogenized nodules.

The soluble carbohydrate extracted from the detached nodules was determined by the Anthrone reagent[28].

The ammonia were estimated by the Nesslerization method[28].

The soluble protein was determined by the Lowry-Folin Method[29].

The chlorophylls in the leaves were extracted and assayed by the method described by Arnon[1].

Dry weight (after over night drying at 85°C) of nodules, roots, stems were measured on the terminal dates of the experiments indicated.

Acetylene reduction was used to assay the nitrogen fixing activity by the soil-plant system. The undisturbed pot-soil-root system of an intact plant was sealed in an acetylene reduction apparatus[10]. Acetylene equivalent to 10% of the net air volume of the chamber was injected. Gas samples (0.5 ml) were withdrawn every 30 minutes and chromatographed immediately. Acetylene and ethylene were separated with a gas chromatograph having a hydrogen ionization detector at an oven temperature of 65°C. A glass column 0.8 m long and having a 3 mm inside diameter packed with Porapak R was used for the separation. The carrier gas was nitrogen flowing at 30 ml per minute.

The nitrogen fixation (acetylene reduction) of detached nodules was also assayed as above, except that the detached nodules were incubated in 25 ml bottles instead of in acetylene reduction chambers.

RESULTS

The nitrogen fixing (acetylene reduction) activity of the intact plants increased significantly during the plant growth period. The maximum rate of nitrogen fixing of soybean plants occurred around the 9th week after transplanting, after that the nitrogen fixing activity declined as the plants grew older[7]. Table I shows that the untreated plants maintained a higher nitrogen fixing activity than those treated with kinetin. The plants that were treated with kinetin with a concentration of 20 ppm or 40 ppm had only about 50% or 40% of the nitrogen fixing activity as compared with untreated ones on the terminal date, i. e. at the 9th week of the experiment. This confirms that exogenous kinetin had an adverse effect on the nitrogen fixing activity of soybean plants. It had been reported that infection of Trifolium glomeration seedlings was severely restricted by exogenous kinetin[3].

Table I. Changes of the acetylene reduction (N_2-fixation) activity of intact soybean plants treated with two different concentrations of kinetin (CK).

Treatment	Control		CK, 20 ppm		CK, 40 ppm	
unit / duration of plant growth	μ mole C_2H_4 1 ml sample-hr ($\times 10^{-4}$)	Total μ mole C_2H_4 plant-hr	μ mole C_2H_4 1 ml sample-hr ($\times 10^{-4}$)	Total μ mole C_2H_4 plant-hr	μ mole C_2H_4 1 ml sample-hr ($\times 10^{-4}$)	Total μ mole C_2H_4 plant-hr
6th week	25.82	22.72	8.92	8	9.12	7.4
7th week	9.84	23.48	18.12	13.48	11.2	12.84
8th week	31.12	31.24	28.6	22.24	22.28	14.28
9th week	49.88	48.4	29.84	23.36	29.68	19.2

Remark: Each value was the mean of triplicates.

Previous studies have reported that rates of nitrogen fixation of soybean plants paralleled the rate of photosynthesis[10,11]. The leaf number as well as the leaf area is involved in controlling the magnitude of photosynthesis, because photosynthesis is associated with leaf number and leaf area. The data shown on Table II indicates that plants which were treated with kinetin had a smaller leaf area than the control plants. Besides, the leaf number was also reduced by the application of exogenous kinetin. However, plants showed different levels of response to the various concentrations of kinetin. The plants treated with a low concentration, 20 ppm, of kinetin were like the untreated ones in that their leaf area and leaf number

Table II. Changes in both leaf-area and leaf-number of soybean plants treated with two different concentrations, 20 and 40 ppm of kinetin (CK).

Treatment	Control		CK, 20 ppm		CK, 40 ppm	
measurement / duration of plant growth	leaf-number	leaf-area, cm^2	leaf-number	leaf-area, cm^2	leaf-number	leaf-area, cm^2
	plant	plant	plant	plant	plant	plant
6th week	9.00	612	5.6	254	5.2	227
7th week	10.33	855	6.8	339	7.0	348
8th week	13.50	960	7.8	400	7.0	341
9th week	21.00	1794	9.8	523	7.5	304

Each value was the mean of triplicates.

progressively increased with the extension of the growth period, even though their increases were comparatively smaller and slower than in the corresponding controls. On the contrary, at a high concentration, 40 ppm, of kinetin caused plants to reduce both their leaf area and leaf number retrogressively as the growth periods were extended. This suggested that the lower activity of acetylene reduction in plants treated with kinetin was associoted with inhibitory effects of kinetin on leaf enlargement and leaf development, which correspondingly reduced the total levels of chlorophylls in leaves. Consequently, the photosynthetic activity was diminished.

It is well been known that chlorophyll a and chlorophyll b play an important role in the photochemical reactions of photosynthesis, and so carbon dioxide assimilation is dependent upon the photosynthetic processes[18]. As a result of the treatments with kinetin, the plants became yellow. Consequently, the plants showed a decrease in both chlorophyll a and chlorophyll b, but particularly chlorophyll b. Kinetin also increased the chlorophyll a to b ratio. It was found that the levels of chlorophyll b were drastically reduced by the kinetin at a concentration of 40 ppm, but chlorophyll a unlike the chlorophyll b was less sensitive to the treatments of kinetin. Therefore, the plants treated with a high concentration of kinetin had a higher chlorophyll a to b ratio than those of controlled plants. However, no significant differences were found in the ratio between the treated and untreated plants when kinetin was applied at 20 ppm (Table III). The detrimental effects of kinetin on the nitrogen fixing activity of soybean plants apparently resulted from the decreasing of the levels of the chlorophylls, and the disturbing the balance between chlorophyll a to b, which may have caused the reduction of photosynthesis. Chlorophyll a and chlorophyll b are known to be indispensable pigments involved in photosynthesis. The efficiency of photoacts of photosynthesis is promoted by the presence of chlorophyll b in both photosynthetic unit I and II, which is so-called Emerson-Enhancement[18]. Thus, the concentration of chlorophylls or the chlorophyll a to b ratio in leaf may act as an index of nitrogen fixing activity of plants.

Table III. Changes of concentration of chlorophyll a and chlorophyll b in soyban plants treated by kinetin* (CK).

measurement / treatment	chlorophyll a		chlorophyll b		total chlorophyll		chl a / chl b ratio
	mg chl a / gm leaf	% of the control	mg chl b / gm leaf	% of the control	mg (chl a+chl b) / gm leaf	% of the control	
Control	0.17	100	0.14	100	0.31	100	1.15
CK, 20 ppm	0.14	87	0.13	80	0.27	87	1.15
CK, 40 ppm	0.15	88	0.11	77	0.26	83	1.32

* plants were harvested at the 9th week.

A visible plant response to the application of kinetin, in addition to the decrease in chlorophylls, was that the plants were reduced in height. The plants treated with kinetin were shorter than the untreated ones. It is presumed that kinetin, which functioned as an anti-auxin, altered the metabolic activity of the apical meristem or released the apical dominance that induced the reduction of cell enlongation. In addition, plants were not so succulent as the corresponding controls. The total fresh weight of a treated plant was reduced on the average by 25% when treated with 20 ppm of kinetin but was reduced by 40% at with 40 ppm. This indicates that water absorption by the plant root was retarded by the kinetin. The value of the shoot-to-root ratio confirms this indication, because the ratios were found lower in

Table IV. Harvests of leaves, stems, roots, and changes of shoot height of soybean plants treated kinetin (=CK).

plant fraction	Leaf						Stem						Root						Total fresh weight			Shoot/Root ratio			plant height		
measurement	Fresh weight (gm)			dry weight* (gm)			Fresh weight (gm)			dry weight (gm)			Fresh weight (gm)			dry weight (gm)			(gm)						(above the ground, cm)		
growth duration / treatment	7th wk	8th wk	9th wk	7th wk	8th wk	9th wk	7th wk	8th wk	9th wk	7th wk	8th wk	9th wk	7th wk	8th wk	9th wk	7th wk	8th wk	9th wk	7th wk	8th wk	9th wk	7th wk	8th wk	9th wk	7th wk	8th wk	9th wk
Control	15.13	16.76	19.18	—	—	—	9.7	18.25	21.95	2.49	4.2	6.02	19.12	27.62	33.94	2.32	2.90	5.07	43.95	62.63	75.07	1.298	1.267	1.218	36	40	57.6
CK, 20 ppm	5.86	6.53	8.30	—	—	—	4.59	7.16	7.58	1.00	1.74	2.16	12.58	12.89	13.74	1.39	1.83	2.05	23.03	26.40	29.62	0.831	1.048	1.155	30.7	34.0	51.7
CK, 40 ppm	5.64	5.72	5.26	—	—	—	6.93	5.84	5.54	1.77	1.63	1.55	14.58	10.98	8.48	2.19	1.86	1.55	27.55	22.54	19.28	0.839	1.052	1.293	23	26.7	44

* For extracting the chlorophylls from the fresh leaves, no dry weight was taken.
wk: week.

Table V. Effect of kinetin on soybean nodule acetylene reduction, ammonia content, protein content, carbohydrate content, fresh weight ant dry weight. (kinetin=CK.)

measurement	nodule acetylene reduction, μ mole C_2H_4 / plant-hr			nodule NH^+_4, μg/plant			nodule protein, μg/plant			nodule CHO, μg/plant			nodule weight/plant (gm)					
													fresh weight			dry weight		
duration of plant growth / treatment	7th wk	8th wk	9th wk	7th wk	8th wk	9th wk	7th wk	8th wk	9th wk	7th wk	8th wk	9th wk	7th wk	8th wk	9th wk	7th wk	8th wk	9th wk
Control	9.96	15.8	18.6	166	332	435	0.07	0.09	0.12	4346	6448	6746	2.19	3.75	5.28	0.52	0.70	1.10
CK, 20 ppm	8.2	13.4	17.08	115	134	144	0.09	0.08	0.09	5056	4576	4596	0.94	1.30	1.57	0.23	0.33	0.40
CK, 40 ppm	7.08	9.72	11.52	160	122	115	0.08	0.08	0.07	4096	3360	3360	1.03	1.21	1.07	0.33	0.30	0.30

wk: week.

treated plants than in the untreated (Table IV). It was observable by the naked eye that the roots of treated plants had a shortage of root hairs. Apparently, the growth and development of root hairs were very sensitive to kinetin, so that the absorptive surfaces of roots of treated plants, consequently, was reduced. Furthermore, the treated plants had a highly significant decrease in dry weight of their tissue fractions (Table IV). These results are consistent with the notion that the accumulation of dry matter of plant tissues is regulated by both the photosynthetic (carbohydrate synthesis)- and nitrogen fixing (nitrogenous compounds synthesis)- activity, since these two assimilatory functions have been shown to have been decreased by kinetin (Table III and I).

The influences of kinetin treatment on symbiotic nitrogen fixation of nodules, nodular protein-N, and other forms of nitrogen are shown in Table V. The higher the concentration of the kinetin applied to a plant, the lower was the nitrogen fixing activity of the nodules, and so the less amount of nodule-NH_4, and nodule protein was formed. Since the primary product of nodular nitrogen fixation is ammonia, the smallest quantity of nodular ammonia obtained from nodules which possessed the lowest nitrogen fixing activity was due to the high concentration of kinetin. However, tiny amounts of protein were found distributed at more or

Table VI. Effect of 2, 4-D on the various aspects of soybean metabolism. plants were irrigated daily with N_2-free nutrient solution, and treated with two different concentrations of 2, 4-D twice a week. All plants were excessively irrigated with deionized water every 7th day to prevent the accumulation of 2, 4-D in the soil. On the 8th week the plants were harvested and assayed.

Assay (measurement) \ Treatment	Control	2, 4-D, 2×10^{-8}M	2, 4-D, 4×10^{-8}M
N_2fixation (acetylene reduction) by intact plant, μ mole C_2H_4/plant-hr	40.08	18.52	13.92
Nodule N_2fixation (acetylene reduction), μ mole C_2H_4/plant-hr	35.04	15.36	13.52
Nodule ammonia, μg/plant	3780	5120	4100
Nodule carbohydrate, μg/plant	6880	8100	7140
Nodule protein, μg/plant	0.09	0.08	0.09
Nodule number, number/plant	199	87	83
Nodule fresh weight, gm/plant	2.85	1.56	1.43
Nodule dry weight, gm/plant	0.68	0.40	0.37
Leaf-area, cm^2/plant	847	809	692
Leaf-fresh weight, gm/plant	15.74	11.15	10.00
Chlorophyll a, mg/gm leaf	0.14	0.19	0.11
Chlorophyll b, mg/gm leaf	0.11	0.16	0.09
chl a/ chl b ratio	1.24	1.21	1.27
Root fresh weight, gm/plant	24.39	14.11	13.72
Root dry weight, gm/plant	2.81	1.86	1.80
Stem fresh weight, gm/plant	13.34	8.76	7.96
Stem dry weight, gm/plant	3.41	2.43	2.16
Shoot/root ratio,	1.20	1.41	1.31
plant height (above the ground), cm	68.7	64	60

less the same level in nodules of both treated- or untreated- plants. It has been estimated that more than 90% of the nitrogenous compounds, mostly amino acids, (glutamic acid and aspartic acid), and amines, (glutamine and asparagine), are derived from nitrogen fixation, and nitrogen assimilates are actively transported out of nodules into other parts of host plant[20,21]. Therefore, only small amounts of amino acids or amines are left in the nodules to build up protein. There is a relationship, which is known as "source and sink", for suppling- and receiving- carbohydrates occuring between the leaves and root nodules[11,20]. The carbohydrate in the nodules for nitrogen fixation comes from a continuous supply of carbohydrate, and photosynthesis is that source The nodular dry matter was very significantly lower in nodules of treated plants than in nodules of corresponding controls. This suggests that the accumulation of nodule dry matter was affected by both the nodule nitrogen fixing activity and the supply of carbohydrate from the leaves.

The indications of detrimental effects of 2, 4-D (2, 4-dichlorophenoxy acetic acid) on soybeans are listed in Tables VI and VII. 2, 4-D, like kinetin, caused plants to have both morphological and physiological modification. The application of 2, 4-D caused the stem tips of plants to become twisted. The leaf number and leaf size were markedly reduced, and the leaves showed chlorosis, some of them were wilted, evidently photosynthesis and the accumulation of dry matter in tissue fractions had sharply declined. In addition, plant height was reduced. All these morphological responses of soybean plants to 2, 4-D are in agreement with previous studies[2,5,29]. The reduction of symbiotic nitrogen fixation of intact plants is closely correlated with the decreasing in levels of chlorophylls in leaves and the number of root nodules (Table VI and VII). The plants treated with 2, 4-D had less than 50% of the normal number of nodules- and 76% of chlorophylls in their leaves- as compared with the controls (Table VI). Consequently, the nitrogen fixation (acetylene reduction) of treated plants had declined to 36.56% of that of the untreated plants. This confirms the reports that pod number and seed yields of soybeans are decreased by the application of 2, 4-D[2,5,29]. By comparing the morphological and physiological responses of plants to treatment of 2, 4-D or kinetin, the plants were apparently much more sensitive to 2, 4-D than to kinetin (Table VII). This may well support the notion that 2, 4-D has a herbicidal function. In our preliminary studies, it was found that seeds which had been treated with 2, 4-D did not germinate.

Table VII. Comparisons of the effects of kinetin and 2, 4-D on the various aspects of soybean metabolism. Plants were treated either with kinetin or with 2, 4-D twice a week. As a precautin against the accumulation of kinetin or 2, 4-D in the soil, all plants were excessively irrigated with deionized water every 7th day. Plants were harvested at the 9th week for assaying. (kinetin≒CK).

measurement \ treatment	Control	20 ppm		40 ppm	
		CK	2, 4-D	CK	2, 4-D
acetylene reduction N_2 fixation, $\frac{\mu \text{ mole } C_2H_4}{\text{plant-hr}}$	48.08	23.48	11.76	19.16	5.32
leaf area, cm^2	1794	523	289	304	298
chlorophyll (a+b), mg/gm	0.31	0.27	0.50	0.26	0.34
leaf chl a/chl b ratio,	1.147	1.146	1.148	1.320	1.299
height, cm	58	52	36	44	31
nodule acetylene reduction, $\frac{\mu \text{ mole } C_2H_4}{\text{plant-hr}}$	18.44	17.2	11.64	11.64	9.8

DISCUSSION

The application of an exogenous plant growth regulator, such as kinetin or 2, 4-D, to soybean plants significantly decreased their symbiotic nitrogen fixation ability. Several hypotheses may help explain this reduction:

(1st). Nodule initiation is inhibited by exogenous kinetin or 2, 4-D. The commercial preparation of the inoculant, *Rhizobium japonicum*, may be so sensitive to kinetin or 2, 4-D that the process of nodule initiation is suppressed. Although kinetin has been known to induce nodulation[22,23], the levels of kinetin used in our experiments may have been too concentrated or too low to initiate nodulation, therefore, fewer nodules were formed.

(2nd). Normal nodule development may have been interfered with by kinetin or 2, 4-D, thus, the treatments with kinetin or 2, 4-D caused a decrease in nodule formation.

(3rd). Nodule degeneration may have been hastened by treatments with kinetin or 2, 4-D. Consequently, the number of nodules disintegrating or decaying may have been higher than those being formed, thus the total number of nodules, over a period of time, would decrease.

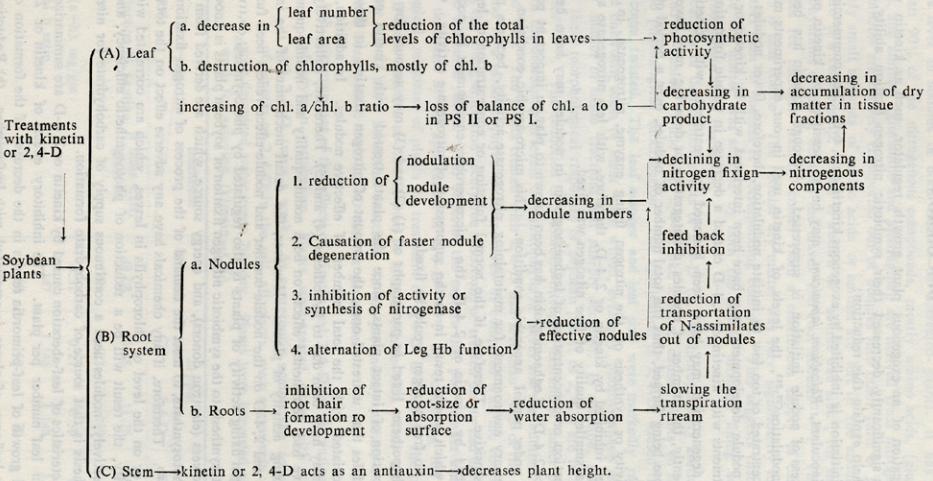
(4th). The activity or synthesis of the nitrogen fixing enzyme, nitrogenase, in nodules may have been inhibited by kinetin or 2, 4-D, therefore, with less nitrogenase activity in the nodule, a smaller quality of free nitrogen was fixed.

(5th). The function or concentration of leghaemoglobin in nodules may have been altered by kinetin or 2, 4-D. Leghaemoglobin has been known to play an important role in keeping nodules under partial anaerobic condition. This micro-aerobic condition in nodules is the necessary environment for maintaining nitrogenase activity, because nitrogenase is oxygen sensitive. Therefore, if the normal function or concentration of leghaemoglobin is disturbed by kinetin or 2, 4-D, the micro-aerobic environment in nodules may be altered, which would result in th the suppression of nitrogenase activity. Consequently, little or no free nitrogen would be fixed.

All these hypotheses listed in paragraphs of (1) (2) and (3) may account for the reduction of nitrogen fixation of intact plants, since the plants treated with kinetin or 2, 4-D had fewer root nodules than untreated ones, the amount of nitrogen fixation in these plants was apparently associated with the total number of effective nodules. In addition, on a nodule basis, activity may have been due to hypotheses (4) and (5). Therefore, collectively, kinetin and 2, 4-D may have had detrimental effects on both nodule-quality and nodule-quantity, and thus the nitrogen fixing activity of plants. However, the detail mechanism of the inhibitory control of kinetin and 2, 4-D on the nodule-number and nodule-effeceiveness need further study.

The nitrogen fixing activity of plants is also regulated by photosynthesis[10,11]. The contribtiuon of photosynthesis to the symbiotic nitrogen fixation of soybean plants is known to provide the reducing powers (electron donors), and energy source, which are derived from the catabolic reactions of photosynthate, to meet the demand of the process of nitrogen fixation and nitrogen assimilation[11,20]. Therefore, if any chemicals have any adverse effect on the total leaf-number and leaf area, or on the level of chlorophylls in leaves, which are correlated with the photosynthetic activity, the result will be a reduction of photosynthetic efficiency, and of nitrogen fixation. Collectively, nodules need a continuous supply of carbohydrate for nitrogen fixation, and photosynthesis is that source of carbohydrate formation.

The characteristics of leaf-aberation caused by kinetin or 2, 4-D are summarized as follows: (a) Decrease in leaf number per plant. The inhibitory effects of kinetin or 2, 4-D on development and growth of leaf-primordia results in the decrease of the formation of new leaves. Obviously, the leaf-area of plants is associated with leaf-number. (b) Reduction in leaf area (on the individual leaf basis). It seems that kinetin or 2, 4-D may cause the compact arrange-


Treatments with kinetin or 2, 4-D
Soybean plants
(A) Leaf
a. decrease in leaf number / leaf area
reduction of the total levels of chlorophylls in leaves
reduction of photosynthetic activity
b. destruction of chlorophylls, mostly of chl. b
increasing of chl. a/chl. b ratio
loss of balance of chl. a to b in PS II or PS I.
decreasing in carbohydrate product
decreasing in accumulation of dry matter in tissue fractions
declining in nitrogen fixign activity
decreasing in nitrogenous components
(B) Root system
a. Nodules
1. reduction of nodulation / nodule development
decreasing in nodule numbers
2. Causation of faster nodule degeneration
feed back inhibition
3. inhibition of activity or synthesis of nitrogenase
4. alternation of Leg Hb function
reduction of effective nodules
reduction of transportation of N-assimilates out of nodules
b. Roots
inhibition of root hair formation ro development
reduction of root-size or absorption surface
reduction of water absorption
slowing the transpiration rtream
(C) Stem
kinetin or 2, 4-D acts as an antiauxin
decreases plant height.

ment of mesophyll cells or the loss of intercellular spaces in the leaf, Thus, the leaves of treated plants remain smaller in size. (c) The lowering of chlorophyll content in leaves. Plants treated with kinetin or 2, 4-D became chlorotic. Stems and leaves appeared light green in color. This suggests that kinetin or 2, 4-D has caused a destruction of chlorophylls, particularly chlorophyll b, so the photosynthetic activity of plants was negatively modified, It can be concluded that the reduction of nitrogen fixation was associated with the decrease in the concentration of chlorophylls in leaves, and the levels of the chlorophylls was directly or indirectly damaged by treatment with kinetin or 2, 4-D.

The plants treated with kinetin or 2, 4-D had less accumulation of dry weight in their tissue fractions. These results are in agreement with studies previously reported[2,5,20], and suggest a decrease in both the carbohydrate- and protein-synthesis, which are related to photosynthetic- and nitrogen fixing-activities. Besides, the catabolic activities (breakdown reactions) were hastened by kinetin or 2, 4-D. It has been reported that 2, 4-D stimulates the respiratory activity of plants and thereby enhances the breakdown of carbohydrates or proteins from the tissues of treated plants, which results in a reduction of the accumulation of dry weight[5]. It has also been found that red kidney beans treated with 2, 4-D induces greater proteinase and peptidase activity in leaves[5] than in untreated plants.

CONCLUSION

The interpretation of changes in the morphological features and physiological activities, resulting in the reduction of nitrogen fixation, caused by the application of exogenous kinetin or 2, 4-D are summarized as shown on page 90.

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