Effects of Application of Slowly Available Nitrogen Fertilizers and Nitrfication Inhibitors on Soybean Growth and Nitrogen Fixation

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ABSTRACT A pot experiment was carried out with soybean plants inoculated with *Bradyrhizobium japonicum*, in order to elucidate the effect of various forms of N fertilizers and nitrification inhibitors on the initial growth and nitrogen fixation of soybean plants. Following six treatments were designed as a basal dressing of N fertilizer; control (weithout N supply), sodium nitrate, ammonium sulfate, ammonium sulfate + DCS, ammonium sulfate + MAN, and CDU + DCS. The initial growth was slightly enhanced by the application of nitrate, ammonium, or ammonium + MAN, compared with control plants. On the other hand, ammonium + DCS and CDU + DCS treatment significantly depressed the plant hight, whereas drastically increased the branch numbers. The senescence of cotyledons was observed to be rapid in the control and nitrate treated plants, and it was retarded by the ammonium + DCS, CDU + DCS, ammonium + MAN application. Regardless of treatments, the application of N fertilizer severely depressed the nodule development (ca. 1/10) and acetylene reduction acxtivity (ca 1/30) compared with the control plants. Seed yields were almost the same level among treatments.

INTRODUCTION

The protein content of soybean seed is extroadinary high about 40% compared with other cereal grains, and soybean plants require much amount of N not only for the reproductive growth but also for the vegetative growth. Therefore it is suggested that N often becomes a limiting factor for the soybean growth and seed yields. Soybean plants utilize both gaseous nitrogen fixed by root nodules and combined nitrogen absorbed by roots. Harper concluded that both symbiotic nitrogen fixation and nitrate utilization appeared essential for maximum soybean yields (1). It is recognized that without any supply of combined nitrogen from soils or fertilizers the initial growth of soybean plants sometimes become poor especially before the initiation of nitrogen fixation (2). On the other hand, the heavy application of N fertilizer is known to depress nodulation and nitrogen fixing activity, and in some cases it causes over-luxuriant growth and less yield (3). Nitrate is considered to be a main form of available N in the soil originated either from fertilizer or soil organic matters, because nitrification activity of upland is high during soybean cultivating season.

So far the mechanism has been not fully undersood concerning to the inhibitory effect of combined N on nodulation and nitrogen fixation of leguminous crops. There is a hypothesis that the absorbed nitrate is converted to nitrite and the produced nitrite inhibits the infection process and nodule initiation (4). Altanative hypothesis is that the assimilation of absorbed combined nitrogen requires much amount of carbohydrate, and the depletion of carbohydrates in nodules is a main reason why combined N depresses nodulation and nitrogen fixation. When high level of inorganic N is supplied, roots consumes much amount of carbohydrate for the absorption and assimilation of combined N and the depression of carbohydrate may easily occure in nodules (5, 6). From the above hypotheses, it may be considered that if low level N is slowly available to the roots, carbohydrate comsumption in roots is not so high as the nodules are starved. Therefore, application of slowly available N fertilizer may not depress the nodulation and nitrogen fixation of soybeans. Futhermore, nitrification inhibitor may be able to evade the nitrate specific inhibitory effect of nitrogen fertilization on nodulation and nitrogen fixation.

In this study, the effect of the application of slowly available N fertilizers and nitrification inhibitors was investigated on the nodule growth, nitrogen fixation activity and the initial vegetative growth.

MATERIALS & METHODS

Soybean (variety Norin No. 2) was surface sterilized by 0.5% sodium hypochlorite solution for 15min, and thoroughly washed with tap water. Then seeds were immersed in a water suspension of *B. japonicum*.

Every two plants were planted at May 26th in the 1/5000a Wagner pot containing 2.5kg of Tanashi soil. Basal dressing was 1.43g KH_2PO_4, 0.43g $Ca(H_2PO_4)_2$ and various kinds of N fertilizers as shown in **Table 1.** CDU (crotonylidene diurea) is a representative of slowly available N fertilizer. DCS (N-2, 5-dichlorophenyl-succinamic acid) strongly inhibits nitrification of soil (7).

Table 1. Experimental design

TREATMENT	
Control	–
$NaNO_3$	$NaNO_3$ (0.5g-N)
$(NH_4)_2SO_4$	$(NH_4)_2SO_4$ (0.5g-N)
$(NH_4)_2SO_4$+DCS	$(NH_4)_2SO_4$ (0.5g-N)+DCS (25mg)
$(NH_4)_2SO_4$+MAN	$(NH_4)_2SO_4$ (0.3g-N)+MAN (0.2g-N)
CDU+DCS	CDU (0.5g-N)+DCS (25mg)

Furthermore, MAN (mathylene-amino acetonitryl) is known to be a slowly available N fertilzer and nitrification inhibitor at the same time (8).

The initial growth of plants were measured during 40 days after planting. Numbers of nodules, nodule weight and nitrogen fixation activity was determined by 40 days old plants. Nitrogen fixing activity was measured by the acetylene redution assay. Immediately after the soils and the shoot was removed, the nodulated roots were put into a 50ml conical flask and sealed with a double stopper. Then air inside the flask was changed by a gas mixture composed of 10% acetylene, 20%oxygen and 70% of helium (V/V). The flasks were incubated at 30℃ for 30min. One ml of gas was sampled with a hypodermic syringe and the ethylene formation was measured by the gas chromatography equipped with FID detecter. Prapak T column was used for separation.

RESULTS & DISCUSSION

Table 2 shows the effect of various N fertilizers and nitrification inhibitors on the shoot length of soybean plants. The shoot length of plants supplied with sodium nitrate, ammonium

Table 2. *The effect of various nitrogen fertilizers and nitrorification inhibitors on the shoot length of soybean plant.*

length, (mm)

Date	Control	$NaNO_3$	$(NH_4)_2SO_4$	$(NH_4)_2SO_4$ +DCS	$(NH_4)_2SO_4$ +MAN	CDU+DCS
Jun. 6	118 (13)	128 (12)	120 (10)	114 (12)	110 (8)	123 (16)
8	140 (13)	148 (9)	136 (12)	134 (9)	131 (9)	146 (13)
10	163 (13)	168 (9)	158 (7)	158 (13)	160 (14)	175 (15)
13	203 (19)	214 (9)	199 (14)	199 (15)	200 (15)	216 (14)
16	233 (19)	240 (15)	229 (19)	223 (14)	217 (10)	231 (22)
20	249 (22)	275 (23)	245 (13)	243 (13)	239 (11)	244 (22)
24	274 (25)	283 (14)	275 (19)	264 (16)	264 (12)	270 (26)
27	326 (29)	333 (13)	330 (21)	320 (17)	319 (16)	318 (29)
30	383 (31)	388 (14)	394 (21)	364 (23)	388 (13)	352 (49)
Jun. 4	443 (38)	455 (17)	458 (31)	418 (20)	458 (13)	404 (42)

*Numbers in parenthesis are standard deviation.

Table 3. *The effect of various nitrogen fertilizers and nitrification inhibitors on the number of soybean branches (40 days after planting)*

The number of plant

Branch number	Control	$NaNO_3$	$(NH_4)_2SO_4$	$(NH_4)_2SO_4$ +DCS	$(NH_4)_2SO_4$ +MAN	CDU+DCS
0	7	5	3	0	1	0
1	1	3	3	0	4	0
2	0	0	2	1	2	0
3	0	0	0	3	1	4
4	0	0	0	3	0	2
5	0	0	0	1	0	2

8 plants were measured.

sulfate and ammonium + MAN was slightly longer than that of the control plants at 40 days after planting.

On the other hand, the shoot length of the plants supplied with CDU + DCS and ammonium + DCS was significantly shorter compared with control plants. This depressive effect might be due to the application of DCS, because the effect seemed to be indipendent of the forms of N fertilizers supplied. Namioka observed that DCS application induced the dwarfing of leguminous crops (9).

Table 3 shows the effect of each treatment on the number of branches of 40 days old plants. In this stage, seven control plants has no branch and only one has a branch. The application of sodium nitrate or ammonium sulfate slightly promoted the branch formation, whereas, ammonium + DCS and CDU +DCS treated plants formed 2-5 brances in one plant.

The senescence of cotyledon was observed to be fairly different among treatments. In **Table 4** the number of cotyledons attached to the stem and still looked green was shown in eight plants at 22 days after planting. The cotyledons of the control and nitrate treated plants soon became yellow and many of them had been fallen off at this stage. The senescence of cotyledone was retarded by the application of ammonium, ammonium + DCS, ammonium + MAN or CDU +

Table 4. *The effect of various nitrogen fertilizers and nitrorification inhibitors on the senescence of cotyledons.*

Number of cotyledons/8 plants

	Control	$NaNO_3$	$(NH_4)_2SO_4$	$(NH_4)_2SO_4$ +DCS	$(NH_4)_2SO_4$ +MAN	CDU+DCS
No. cotyledons remained at stem	5	4	16	15	14	12
No. cotyledons still alive (green colour)	1	1	3	14	7	7

Table 5. *The effect of various nitrogen fertilizers and nitrorification inhibitors on C_2H_2 reduction activity and numbers and dry weights of nodules.*

	Control	$NaNO_3$	$(NH_4)_2SO_4$	$(NH_4)_2SO_4$ +DCS	$(NH_4)_2SO_4$ +MAN	CDU+DCS
μM C_2H_2 red. / hr. plant	36.5 (7.5)	1.7 (1.1)	1.3 (0.4)	0.9 (0.1)	1.5 (0.9)	0.8 (0.4)
Nodule No. / Plant	43 (14)	22 (1)	28 (9)	15 (0)	23 (3)	17 (4)
Nodule Wt. (mg) / Plant	124 (25)	11 (3)	12 (2)	9 (0)	11 (2)	10 (2)

Numbers in parenthesis are standard deviation.

DCS. Especially, most of all cotyledons of the ammonium + DCS treated plants had green cotyledons at 22 days after planting.

The results that ammonium + DCS and CDU + DCS depressed the plant hight as well as promoted branch formation suggests that DCS may play a growth regulater like TIBA (2, 3, 5-triiodobenzoic acid). TIBA is known to be an anti-auxin substance, and the application of TIBA to the soybean plant induced the dwarfing, and sometimes increased the seed yield by suppression of over-luxurient growth (10). It is reported that MAN also promoted tillering of millet (8), however, MAN was not so effective for the soybean branching in the this study. The difference of the senescence of cotyledon may be involved in the hormone balance changes induced by DCS or MAN application, or related to the C/N balance in the cotyledons.

Table 5 shows the acetylene reduction activity, nodule number and nodule dry weight of the 40 days old plants. Acetylene reduction activity and nodule development was strongly depressed by the application of N fertilizers irrespective of their forms. In the case of N application, the nodule number was about half of the control plant, but nodule weight decreased more drastically about 1/10. Furthermore, acetylene reduction activity was depressed most severely about 1/30.

Table 6. *The effect of various nitrogen fertilizers and nitrorification inhibitors on the yied compornents of soybean plant.*

	Control	$NaNO_3$	$(NH_4)_2SO_4$	$(NH_4)_2SO_4$ +DCS	$(NH_4)_2SO_4$ +MAN	CDU+DCS
Pods number / Plant	14 (5)	16 (4)	14 (3)	17 (2)	17 (1)	14 (1)
Seeds number / Plant	24 (5)	27 (9)	23 (4)	28 (4)	28 (2)	22 (2)
Seeds weight(g) / plant	3.1 (1.0)	3.4 (1.2)	2.7 (0.8)	3.5 (0.8)	3.5 (0.3)	2.5 (0.3)
100 seeds weight (g)	14.5 (4.0)	15.5 (2.8)	14.3 (1.5)	16.5 (1.2)	14.1 (1.3)	15.8 (2.3)

*Number in parenthesis are standard devition.

Therefore the specific acetylene reduction activity (activity/gDW of nodules) was declined to 1/3.

These results indicate that nodulation and nitrogen fixing activity is severely depressed not only by nitrate fertilizer, but also by the application of the slowly available N fertilzers or nitrification inhibitors. So, in this experiment the inhibitory effects of combimed N was not by nitrate specific inhibition. It seems that the compatible basal dressing of N fertilizer with nitrogen fixation is not nesesarily obtained by nitrification inhibitors or slowly available N fertilizers.

Pod number, seed number, seed dry weight and yields were not significantly different between treatments (**Table 6**). Therfore, application of either slowly available N fertilizers or nitrification inhibitors could not improve the reproductive growth, though the harmfull effects were not observed.

Hoshi et al. (11) reported that the side dressing of N fertilizer after flowering stage did not cause over luxurient growth and increased the seed yields. Futhermore, it was observed that appropriate basal supply of farmyard manure enhanced the nodulation and nitrogen flxation activity of soybeans (12, 13). The supplemental supply of combined N may improve soybean production, if it is not competitive to nitrogen fixation.

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ダイズの生育ならびに窒素固定に及ぼす緩効性窒素肥料と硝化抑制剤の施用効果

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摘　　要

窒素の施肥形態および硝化抑制剤の施用が，ダイズの初期生育ならびに窒素固定に及ぼす影響を調べる目的でポット試験をおこなった。緩効性窒素肥料としては，CDU（クロトニリデンジウレア）とMAN（メチレンアミノアセトニトリル）を用い，硝化抑制剤としてはDCS（N-2,5-ジクロロフェニルスクシナミン酸）を用いた。ただし，MANは硝化抑制効果も合わせ持つ。田無土壌2.5kgを詰めた1/5000aポットあたり，0.5g−Nを基肥として施用した。処理区は，硝酸区，アンモニア区，アンモニア＋DCS区，アンモニア＋MAN区，CDU＋DCS区の五窒素施用区と無窒素区を設けた。

ダイズの草丈は，無窒素区と比べて硝酸区，アンモニア区，アンモニア＋MAN区で若干高くなった。一方，アンモニア＋DCS区，CDU＋DCS区では草丈が低下し，分枝形成が著しく促進された。また，無窒素区と硝酸区では他の処理区より子葉の老化が早いことが観察された。

窒素固定能（アセチレン還元能）および根粒の発育は窒素施用により著しく低下した。この効果は，窒素の施肥形態によらず，緩効性窒素肥料や硝化抑制剤を用いても，基肥窒素による窒素固定の阻害は回避できなかった。収穫時の莢数，子実数，百粒重，子実収量は，処理区による大きな差は認められなかった。