# Deep placement of lime nitrogen promotes nitrogen fixation and seed yield of soybean with efficient utilization rates.

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## Abstract

Average soybean yield is low compared with the potential yield. N is derived from three sources; $N_2$ fixation, soil N, and fertilizer N. A heavy supply of N fertilizer often depresses nodule development and $N_2$ fixation activity, which sometimes results in the reduction of seed yield. We developed a new fertilization technique for soybean cultivation by deep placement (at 20 cm depth from the soil surface) of slow release N fertilizers, coated urea and lime nitrogen (calcium cyanamide) at the rate of 100kgN/ha. These treatments consistently promoted seed yield compared with conventional cultivation in the field with various types of soils. Fertilizer N was efficiently used to supplement N during seed filling stage without concomitant depression of nitrogen fixation and nodule growth. In this research, the effect of deep placement of lime nitrogen was evaluated using a rhizobox to investigate the distribution of nodules and roots. The result showed that deep placement of lime nitrogen promoted nodulation in the upper layers of the root system at pod filling stage. The fate of lime nitrogen in soil was investigated by incubation test, and it was suggested that cyanamide is rapidly degraded in 2 weeks, but nitrification was depressed for a long period.

## Key Words

Lime nitrogen, deep placement, nitrogen fixation, nodule, soybean, cyanamide.

## Introduction

Annual world production of soybean seeds increased up to 220 million t in 2007. The highest yield in Japan was recorded 7.8 t/ha, and the yield is around 6 t/ha under good climatic conditions (Takahashi *et al.* 1991). However, the world average seed yield is 2.3 t/ha, and Japanese average is only 1.7 t/ha. Soybean seeds contain a large amount of protein N and the total amount of N assimilated in a plant is highly correlated with the soybean seed yield. One t of soybean seed requires about 70-90 kg N, which is about four times more than in the case of one t of rice grain production. Soybean plants assimilate the N from three sources; N derived from symbiotic $N_2$ fixation by root nodules (Ndfa), absorbed N from soil mineralized N (Ndfs), and N from fertilizer when applied (Ndff). For the maximum seed yield of soybean, it is necessary to use both $N_2$ fixation and absorbed N from roots (Harper 1987). Sole $N_2$ fixation is often insufficient to support vigorous growth, which results in the reduction of seed yield. On the other hand, a heavy supply of N fertilizer often depresses nodule development and $N_2$ fixation activity and induces nodule senescence, which sometimes results in the reduction of seed yield. Therefore, no nitrogen fertilizer is applied for soybean cultivation or only a small amount of N fertilizer is applied as a starter N to promote initial growth in Japan.

A polymer coated controlled release N fertilizer (commercial name LP in Japan or MEISTER outside Japan) has been invented by Fujita and co-workers (1999). This type of fertilizer has a spherical shape about 3 mm diameter with 50-60 μm coat thickness, which consists of polyolefin (polyethylene), ethylene vinyl acetate and talc mineral. The N release rate from coated urea is temperature dependent and not affected by other chemicals, physical and biological conditions in soil, and the release pattern can be predicted as a function of temperature and time period after application. Since the release of N from the fertilizer meets the plant N demand, and the fertilizer efficiency (recovery rate of N in plants from fertilizer) is high, the use of coated urea can reduce the environmental problems by decreasing nitrate accumulation and leaching in the soil. Furthermore, the use of coated urea saves the labour of farmers by eliminating a top dressing to supply N during later stage.

Takahashi *et al.* (1991,1992,1993,1994,1999) developed a new fertilization technique for soybean by deep placement of coated urea. They applied coated urea by deep placement at 20 cm depth from a soil surface using a fertilizer injector. They used CU-100 in which 80 % of N is released in 100 days in water at 25 C. Fertilizer experiments were carried out from years 1989 to 1991 in the field, which had been converted from a paddy rice field in the previous year. The seed yield was from 10 to 23 % higher in deep placement than the control treatments. The promotion of leaf growth and retardation of leaf senescence were observed during the maturing stage by deep placement of coated urea. The seed yield was very high about 6 t/ha in deep placement in 1990 due to the favorable climatic conditions compared with years 1989 and 1991. The absorption efficiency of fertilizer N determined by $^{15}N$ labeled fertilizers was calculated from recovery of $^{15}N$ in the shoots at R7 stage. In 1990, the absorption efficiency from the deep placement of CU-100 was 62 %, which was much higher than the top dressing of CU-70 (33 %) and basal application of ammonium sulfate (9 %). Recently, Tewari *et al.* (2002,2003,2004ab, 2005,2006ab) investigated the effects of deep placement of lime nitrogen (calcium cyanamide, $CaCN_2$) in comparison with coated urea (CU-100). The fertilizer experiments were combined with new inoculation method of bradyrhizobia using a paper pot inoculation method. All the experiment was carried out in 2001 in three different sites in Niigata Prefecture: a rotated paddy field in Niigata Agricultural Experiment Station, a first cropping field after reclamation with the dressing of mountain soil without indigenous bradyrhizobia and a sandy dune field of Faculty of Agriculture in Niigata University. The effects of the application of different fertilizers, urea, coated urea and lime nitrogen on the growth, N accumulation and $N_2$ fixation activity of soybean plants were compared.

Lime nitrogen contains about 60 % of calcium cyanamide ($CaCN_2$) with calcium oxide and carbon, and the N content is about 20-23%. It corresponds chemically and physiologically to a basic fertilizer, and it neutralizes the soil acidity. Calcium cyanamide is converted to urea in soil, which is again degraded into $NH_3$ and $CO_2$. Dicyandiamide contained in lime nitrogen or formed during the degradation of calcium cyanamide in soil retards the oxidation of $NH_3$ to $NO_3^-$, since it is a potent nitrification inhibitor. Therefore, the ammonium produced by $CaCN_2$ decomposition persists for a longer period of time and the nitrate concentration remains low in soil. It is expected that the inhibition of nodulation and of the $N_2$ fixation activity may be alleviated by low level of nitrate accumulation in the soil. In addition, this fertilizer exerts some hormonal effects on plants and is used for controlling soil diseases caused by bacteria and fungi.

The effect of deep placement of ammonium sulfate (AS), urea (U), coated urea (CU) and lime nitrogen (LN) on the N origin was investigated by $^{15}N$ dilution method (Tewari *et al.* 2005). Deep placement of $^{15}N$ labeled fertilizers (100kgN/ha) were applied in the converted paddy field in the same field as above (Nagaoka). Soybean cv. Enrei and the non-nodulated isogenic line En1282 were planted. Whole plants were sampled at maturing stage, and $^{15}N$ abundance and N concentration in each part were analyzed. The evaluation of Ndfa, Ndfs and Ndff was conducted by $^{15}N$ dilution method using En1282 as a reference plant. The value of the seed weight per plant of Enrei was highest in LN (73g) followed by CU (63g), U (47g), AS (37g) and control without deep placement (26g). The value of Ndfa estimated by $^{15}N$ dilution method was higher in LN (3.6g) and CU (2.8g) than in U (2.3g) and AS (2.3g) treatments. The recovery rate of fertilizer N was higher in LN (43%) and CU (36%) than U (21%) and AS (21%) treatments. These results confirmed that deep placement of both LN and CU is effective to improve soybean growth and seed yield by promoting nitrogen fixation by root nodules.

To investigate the utilization of N from LN compared with CU, soybean plants were periodically sampled with deep placement of $^{15}N$ labelled LN and CU. The N absorption was initially lower with LN than CU at the R3 and R5 stages, but the absorption was from LN exceeded CU at R7 stage. The recovery rate was 70% in LN and 61% in CU. The daily $N_2$ fixation activity and N absorption rate was calculated by a simple relative ureide method. In all the treatments, the higher $N_2$ fixation activity was found during R3 and R5 stages with LN (630 $mg/m^2.d$), CU (616 $mg/m^2.d$), and Cont (464 $mg/m^2.d$) treatments. The seed yield per plant was 37g (Cont), 67g (CU), and 71g (LN).

The yield increase by deep placement of CU-100 and $CaCN_2$ were mainly due to the increase in the pod number per plant. The deep placement of CU-100 as well as $CaCN_2$ contributed to the increase of the total node number in the branches and the pod number per node, leading to the increase of seed yield. This condition prevents flower and pod shedding due to nutrient competition or stress during the transition from the vegetative to the reproductive growth, which may account for the good yield obtained with both the $CaCN_2$ and CU-100 treatments.

## Methods

*The effect of deep placement of lime nitrogen on soybean nodulation*

The experiment was conducted in a rotated paddy field of Niigata Agricultural Research Institute, Nagaoka. Conventional basal dressing of ammonium sulfate (16 kgN/ha), fused magnesium phosphate (60 kg$P_2O_5$ /ha) and potassium chloride (80 kg$K_2O$/ha) fertilizers were mixed in the plow layer (0-13 cm depth) of the experimental plot. A soybean plant was cultivated in the center of the wooden box (45 cm x 4.5cm x 30 cm Length, Width, Depth) filled with the field soil. The boxes were buried in the field. The soil is a Fine-Textured Gray Lowland soil. Average chemical properties of the soil were as follows: texture; CL, pH($H_2O$); 6.6, CEC; 28.8 (cmol(+)/kg), total carbon content; 10.9 g/kg, total N content; 1.02 g/kg, amount of mineralized N determined by the incubation of air dry soil under upland conditions for 4 weeks at 30 C; 47 mg/kg. Two fertilizer treatments were conducted as follows: Control; no additional fertilizer. Deep placement of lime nitrogen; basal deep placement (20cm depth from a soil surface) of lime nitrogen (1.12gN/plant) in the box. At R1 (initial flowering stage) and R5 (pod filling stage) (Fher 1971), rhizoboxes were sampled. The soil profile was separated by 5 cm x 5cm blocks, and soils were washed out. The roots and nodules were dried and the dry weight was measured.

*Changes in lime nitrogen incubated with field soil*

To investigate the fate of lime nitrogen in soil, 250 mg of lime nitrogen was mixed with 10.3 g of air dry soil and incubated at 25 C for 0, 2, 4, 6, 8 weeks after water concentration was adjusted at 60% of maximum water holding capacity. Then soil was extracted by 2% acetic acid, and cyanamide concentration was determined by amino acid analyzer (Nagumo *et al.* 2009). Dicyandiamide was determined by capillary electrophoresis. Ammonia was analyzed by Indophenol method. Urea was determined by Indophenol analysis of ammona after it was hydrolysed by urease. Nitrate was analysed by Cataldo method (Cataldo *et al.* 1974).

## Results

*The effect of deep placement of lime nitrogen on soybean nodulation*

At R1 stage, total nodule weight of the plants treated with deep placement of lime nitrogen was 0.57g/plant and lower than the control nodule weight 0.73g/plant. The nodule distribution was not different between deep placement of lime nitrogen and control treatment. On the other hand, at R5, the total nodule dry weight of deep placement of lime nitrogen was 1.17g/plant and much higher than that in control plants 0.73g/plant. The nodule weight in the upper layer of soil in lime nitrogen treatment was higher than control treatment. This observation supported the estimation of promotion of nitrogen fixation by deep placement of lime nitrogen by relative ureide method. The dry weight of the shoots was also higher in deep placement of lime nitrogen (37g/plant) compared with control plants (28g/plant) at R5 stage. From these results, it was confirmed that deep placement of lime nitrogen provides the N in the reproductive stage, and it promotes nodule growth and nitrogen fixation through vigorous photosynthetic activity of the leaves.

*Changes in lime nitrogen incubated with field soil*

At the start of incubation, most N was in the form of cyanamide. However, the cyanamide concentration decreased to 0.66% of original concentration after 2 weeks of incubation, and it was very rapidly decomposed. At 4,6,8 weeks of incubation, the concentration of cyanamide was 0.45%, 0.38% and 0.28 % or original concentration. The concentration of urea-N increased at 2 weeks to 18 % of total N, then decreased to 1.4% at 8 weeks. The concentration of ammonia-N increased gradually to 1%, 6%, 12%, 12%, and 18% at 0, 2, 4, 6, 8 weeks. During the incubation period up to 8 weeks, the nitrate-N concentration was 0.2% of total added lime nitrogen, and the nitrification was completely depressed. The concentration of dicyandiamide was very high tup to 60% of lime nitrogen N under these experimental conditions.

## Conclusion

For maintaining agricultural production and protecting the environment, it is crucial important that efficient use of N fertilizer. The forms, rate, time and place of fertilizer application are important to provide N for the demand of various crops grown under different soil and climatic conditions. For soybean cultivation, deep placement of lime nitrogen promoted plant growth and seed yield through promotion of nitrogen fixation, especially after initial flowering stage. In this study, the promotive effect of deep placement of lime nitrogen was confirmed by rhizobox cultivation. Also, by soil incubation test of lime nitrogen, the nitrification was completely depressed until 8 weeks, and it was in association to the accumulation of dicyandiamide.

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