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Ikeda, Motoki

Plant Nutrition Laboratory, Faculty of Agriculture, Kyushu University

Ikeda, Noriyuki

Plant Nutrition Laboratory, Faculty of Agriculture, Kyushu University

Shimizu, Kouichi

Plant Nutrition Laboratory, Faculty of Agriculture, Kyushu University

Ishizuka, Junji

Plant Nutrition Laboratory, Faculty of Agriculture, Kyushu University

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Effect of Irradiance on Soybean Utilization of Nitrate During Pod Fill

Motoki Ikeda, Noriyuki Ikeda, Kouichi Shimizu and Junji Ishizuka

Plant Nutrition Laboratory, Faculty of Agriculture,
Kyushu University, Fukuoka 812, Japan
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Pot experiments were conducted in sand culture to evaluate the effect of irradiance on the utilization of nitrate by soybean (*Glycine max* (L.) Merr. cv Orihime) during the pod-filling period. Different irradiant conditions were obtained by shading the plant stands with a cheesecloth. At 55%-ambient light the dry matter, the seed yield, and the nitrogen utilization were reduced in a nitrate nutrition but those were only slightly affected in an ammonium nutrition. When 0 and 2 milimolar nitrate were applied, the reduction in carbon exchange rates, leaf nitrate reductase activity and leaf nitrogen concentration took place earlier in high irradiance than in low irradiance, but the irradiant conditions did not affect the final dry matter production, the seed yield and the nitrate utilization. An increase in irradiance was required for a greater dry matter and seed production and higher nitrate utilization when 10 milimolar nitrate was applied. A decrease in irradiance lowered the nitrate uptake rather than its assimilation, which ultimately led to a lower utilization of a high level of nitrate applied.

INTRODUCTION

Solar radiation is a primary determinant in dry matter production of plants through photosynthetic carbon fixation. In general, the plant material has a C/N ratio of 5-15 which indicates great acquisition of nitrogen. Irradiance enhances nitrogen absorption and it regulates the nitrogen assimilating enzyme activities. Therefore, there is no doubt that nitrogen absorption and assimilation also interact with photosynthesis. Compared to ammonia assimilation nitrate assimilation requires more energy because of the reducing equivalents necessary in reducing the nitrate to ammonia (Guerrero *et al.* 1981). This leads us to a hypothesis that the growth of plants which utilize large amounts of nitrate would be greatly influenced under irradiant conditions.

The soybean plant acquires more nitrogen than other crops, and after flowering it accumulates the nitrogen in pods (Mengel *et al.* 1987). In nodulated soybeans most of the nitrogen is derived through nitrogen fixation in nodules whereas in non-nodulated soybeans the nitrogen is absorbed as nitrate. In field-grown non-nodulating soybean isolate A62-2, the shading effect on yields and nitrogen accumulation was more evident under conditions of nitrogen application than under those of no nitrogen application (Tanaka *et al.* 1980). This indicates that irradiance can strongly affect nitrogen accumulation in soybean under nitrogen sufficient conditions while in the nitrogen deficient soybean plants nitrogen is a limiting factor. Since the soybean plant has a capacity to rapidly assimilate absorbed nitrogen during reproductive growth, it is considered that the soybean plant at this growth stage is a suitable material to

investigate the effects of irradiance on the utilization of nitrate in plants.

In the present study, we first compared the effect of shading on the utilization of nitrogen which soybean plants absorbed as ammonium or nitrate during pod fill, and then investigated the utilization of nitrate in soybean plants which was supplied at different levels during pod fill under shading and nonshading conditions.

MATERIALS AND METHODS

Plant culture *Experiment 1* Seeds of soybean (*Glycine max* (L.) Merr. cv Orihime) were germinated in a sterilized vermiculite medium on May 27, 1986. Sixteen-day-old seedlings (one plant per pot) were transplanted in sand in Wagner pots (200 cm² x 19 cm(H)) which were placed on a cart. The cart was placed in a greenhouse during rainy days. A one-half strength modified Hoagland solution (1 L) containing 7.5 mM NaNO₃ as the nitrogen source was poured into the pot every morning until July 31. This nitrogen supply completely inhibited the nodulation. The flowering started on July 4. On August 1, treatments were initiated and continued until August 28. The triplicate treatment included three levels of irradiance, such as no shading (ambient light), 30% shading with a white cheesecloth (70%-ambient light), and 45% shading with a black cheesecloth (55%-ambient light), and two nitrogen sources were also used; i. e. 7.5 mM NaNO₃ and NH₄Cl labelled with ¹⁵N (5.1 atom %). A nitrification inhibitor AM (2-amino-4-chloro-6-methyl-pyrimidine) was added at the rate of 5 mg L⁻¹ into the nutrient solution containing NH₄Cl. During the treatment period 300 mL of a nutrient solution was daily poured into each pot. To prevent a water deficit some pots received deionized water in the afternoon. The plants were harvested on August 29.

Experiment 2 Twelve-day-old soybean (cv Orihime) plants were transplanted in Wagner pots with a drain hole containing 3 L of 1 : 1 (v/v) vermiculite/sand media on June 11, 1988. Before treatment plants daily received a one-fourth strength modified Hoagland solution containing 2 mM NaNO₃. The flowering started on July 8. The treatment was carried out during 14 days after flowering (DAF) and 24 DAF, during the 24 and 34 DAF, and during the 24 and 44 DAF. Three levels of nitrogen (no nitrogen, 2 mM NO₃⁻, 10 mM NO₃⁻) and two levels of shaded irradiance (ambient and 55%-ambient light) were used during the treatments. The nitrogen sources were 1 mM Ca (¹⁵NO₃)₂ for 2 mM NO₃⁻ (10.6 atom %) and a combination of 1 mM Ca (¹⁵NO₃)₂ and 8 mM Na¹⁵NO₃ for 10 mM NO₃⁻ (10.2 atom %). All treatments were triplicated. The plant tops were harvested on July 22 (14 DAF), August 1 (24 DAF), Aug. 11 (34 DAF), and Aug. 21 (44 DAF).

Assay of *in vivo* nitrate reductase activity The *in vivo* nitrate reductase activity was determined with a method of Hageman and Hucklesby (1971) at 21, 31, and 41 DAF. Twelve discs (diameter; 12 mm) were prepared from the trifoliate leaf below the flag leaf. The activity was calculated using the amount of nitrite produced during 45 and 75 min.

Carbon exchange rate measurements The carbon exchange rates were measured at 22, 32, and 42 DAF with an open gas exchange system (Ikeda et al. 1993). An attached trifoliate flag leaf was placed in a temperature-controlled chamber. The light inten-

sity was $650 \mu\text{mol m}^{-2} \text{s}^{-1}$ (400 nm-700 nm) and the leaf temperature was 25°C . The humidified ambient air was passed through the chamber.

Harvest and chemical analysis Plants were separated into root, stem plus petiole, leaf, pod shell and seed. Fallen leaves were added to a leaf fraction. Freeze-dried plant parts were weighed, ground with a Willy mill and stored in a desiccator. Plant samples were digested by the Eastin's method (Eastin 1978). The nitrogen in the digest was determined with a distillation and titration method. An abundance of ^{15}N of ammonium in the distillate was quantified by a combination of the Dumas method and emission spectrometry with a JASCO ^{15}N analyzer NIA-1 (Kumazawa 1975 ; Fukutoku *et al.* 1987).

RESULTS

Experiment 1 In the daily supply of the new nutrient solution, the effluent pH from the pots was about 7.5 both in the ammonium and nitrate solutions. The total dry weight was lowest at 55%-ambient light, followed by 70%-ambient and ambient light, and it was higher when the nitrate was supplied than when the ammonium was supplied, regardless of the irradiant conditions (Table 1). At ambient and 70%-ambient light, the dry weight of seeds was higher in the nitrate nutrition than in the ammonium nutrition. The dry weight of seeds was significantly reduced at 55%-ambient light in the nitrate nutrition, but in the ammonium nutrition the reduction of seed weight at 55%-ambient light was only slight. Hence, there was only a little difference in the dry weight of seeds between the ammonium and nitrate nutrition under poor light conditions. This result suggests that the dry matter production required a large supply of energy when the nitrogen source was nitrate.

When plants were grown at low light intensities the nitrogen concentrations in dry plant parts were high except for the root (data not shown). During the maturity stage the total nitrogen accumulation from ammonium nitrogen was not affected by the irradiant conditions and it was smaller at ambient and 70%-ambient light than that of nitrate nitrogen, which was smaller at 55%-ambient light than at 70%-ambient and ambient light (Table 1).

Table 1. Effect of nitrogen sources and irradiance on dry matter production and nitrogen accumulation in uninoculated soybeans.

Nitrogen source	Irradiance	Dry weight		Nitrogen	
		Seed	Total	Seed	Total
	% of ambient light	g per plant		mg per plant	
nitrate	100	12.5	33.3	650	822
	70	12.1	32.0	655	846
	55	8.5	26.3	490	688
ammonium	100	10.6	30.1	560	736
	70	9.6	29.3	526	727
	55	9.0	23.5	549	709

Total accumulation of the labelled nitrogen from ammonium was little affected by the irradiant conditions while the total accumulation of the labelled nitrogen from nitrate was at the lowest point at 55%-ambient light (Fig. 1). This is the reason for the low accumulation in seeds of labelled nitrogen in plants supplied with nitrate at 55%-ambient light. The labelled nitrogen occupied 40 to 49% of the total nitrogen accumulated, and it was partitioned to seed more under a higher light intensity (Table 1 and Fig. 1). At the beginning of the treatment the amount of unlabelled nitrogen which accumulated in a plant was 390 mg. At the maturity stage the amount of unlabelled nitrogen was at most 15% greater compared to the initial amount, suggesting that nitrogen fixation had no or very slight, if any, contribution to the nitrogen accumulation during the treatment period of the experiment.

Experiment 2 In plants given 0 and 2 mM nitrate the leaf nitrate reductase activity decreased very rapidly at ambient light and slowly at 55%-ambient light during pod filling (Fig. 2 A, B). The high nitrate reductase activity was maintained at 55%-ambient and ambient light when there was a supply of 10 mM nitrate.

Regardless of the nitrogen nutrition and irradiant conditions, the carbon exchange rates of the flag leaf decreased with elapsed time (Fig. 2 C, D). The highest carbon exchange rates were maintained in plants when 10 mM nitrate given. The carbon exchange rates were higher at 55%-ambient light than at ambient light.

The leaf nitrogen concentration was higher in plants which were grown on high levels of nitrogen, but the leaf nitrogen concentration decreased during pod filling (Fig. 2 E, F). The decrease was more remarkable at ambient light than at 55%-ambient light. The trend in leaf nitrogen concentration changes was similar to the trend in those of the carbon exchange rates.

The dry matter and seed production during pod filling are shown in Table 2.

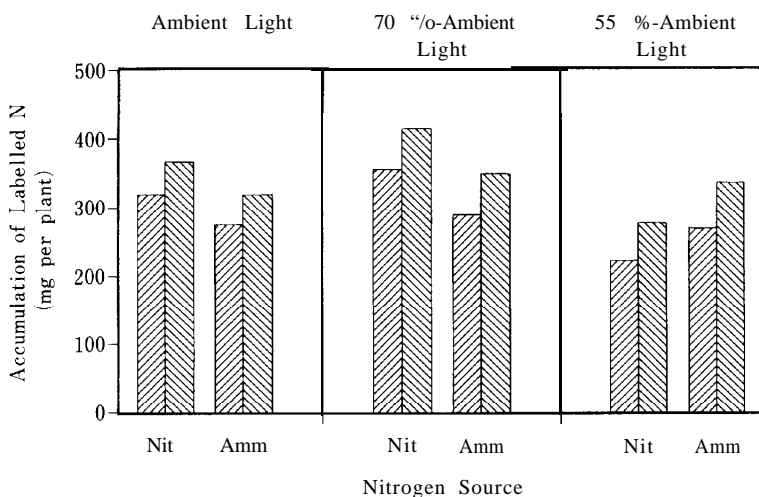


Fig. 1. Effect of irradiance on the accumulation of nitrogen from labelled nitrate and ammonium in whole plants (▨) and seeds (▩).

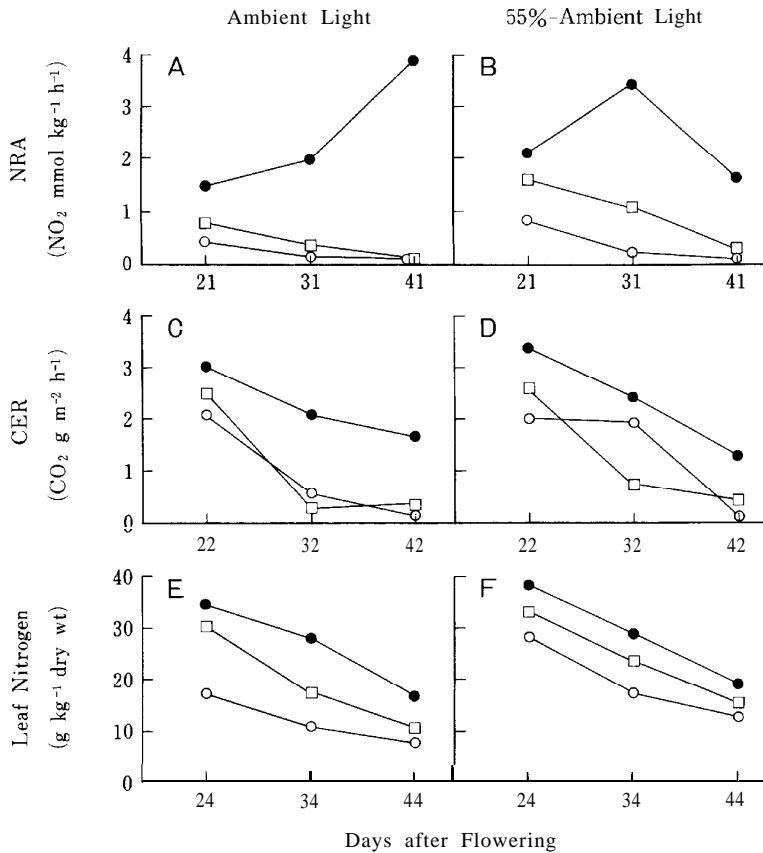


Fig. 2. Changes in the *in vivo* nitrate reductase activity (NRA ; A, B), carbon exchange rates (CER; C, D) and leaf nitrogen content (E, F) during the pod-filling period of soybean plants grown on three levels of nitrate at ambient and 55%-ambient light.
 ● : 10 mM NO_3^- , □ : 2 mM NO_3^- , ○ : no NO_3^- .

When nitrogen was not supplied, the dry weight of plant top did not increase at ambient light after 24 DAF, but it did increase at 55%-ambient light until 44 DAF. The dry weight of the plants with no nitrogen given was larger at 55%-ambient light than at ambient light. Although at 24 DAF there was more dry matter produced in high irradiance, which was given 2 mM nitrate, than in low irradiance, there was also little difference in the dry weight of plants at ambient and 55%-ambient light because of the small increases in the dry weight of the plants at ambient light during the latter pod-filling period. When 10 mM nitrate was given to the plant, the dry weight was consistently larger at ambient light than at 55%-ambient light. Increases in dry weight linearly continued until 44 DAF at ambient light, but dry matter production at 55%-ambient light was slight after 34 DAF.

During the early pod-filling period the seed weight similarly increased, regardless

of the irradiant conditions and nitrogen supply (Table 2). After 24 DAF, the high irradiant conditions brought about more seed production only in plants which were given 10 mM nitrate, whereas the irradiant conditions did not affect seed production in plants which were given 0 and 2 mM nitrate.

The total nitrogen accumulation increased throughout the pod-filling period when large amounts of nitrogen were applied (Table 2). In plants to which no nitrogen was given the nitrogen accumulation was larger at ambient light than at 55%-ambient light, and the nitrogen accumulation in seed was similar between the two irradiant conditions. When 2 mM nitrate was applied, the high irradiance rather than low irradiance caused larger nitrogen accumulation in plant top and seed during the early pod-filling period, but the reverse was true during the late pod-filling period. The nitrogen accumulation in seed was larger at ambient light than at 55%-ambient light in plants which were given 10 mM nitrate, especially during the late pod-filling period although the total nitrogen accumulation was larger only during the late pod-filling period at ambient light than at 55%-ambient light.

The irradiant conditions only slightly affected the accumulation of labelled nitrogen in top and seed of plants which were given 2 mM nitrate (Fig. 3). When 10 mM nitrate was given to the plants the accumulation of labelled nitrogen in plant top and seed was greater at ambient light than at 55%-ambient light, and this difference was more evident during the late pod-filling period. The proportion of labelled

Table 2. Effect of nitrate levels and irradiance on dry matter production and nitrogen accumulation during pod filling in soybeans.

Days after flowering	Irradiance	Nitrate level	Dry weight		Nitrogen	
			Seed	Total	Seed	Total
	% of ambient light	mM	g per plant		mg per plant	
24	100	0	3.7	37.7	177	532
		2	5.2	36.4	271	698
		10	5.2	37.6	289	866
	55	0	3.0	31.4	157	591
		2	3.0	28.1	166	616
		10	4.0	32.2	232	820
34	100	0	9.6	38.4	426	603
		2	13.2	44.2	629	878
		10	14.3	45.3	768	1142
	55	0	10.6	38.5	434	656
		2	11.1	42.1	566	861
		10	13.4	44.3	751	1109
44	100	0	11.5	38.4	555	666
		2	15.9	45.4	736	869
		10	21.4	55.3	1238	1487
	55	0	13.5	42.9	613	781
		2	15.8	44.2	856	1055
		10	18.6	46.3	1057	1256

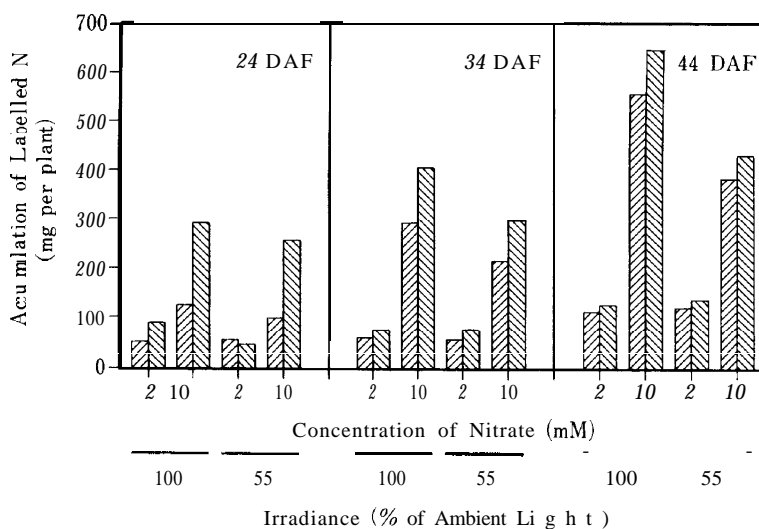


Fig. 3. Effect of irradiance on the accumulation of nitrogen from labelled nitrate (2 mM or 10 mM NO_3^-) in whole plants (▨) and seeds (▧).

nitrogen in seed increased with seed filling. It reached 86–88% for labelled nitrogen absorbed between 22 and 44 DAF, but it was not affected by irradiant conditions and nitrogen levels.

DISCUSSION

It was reported that soybeans in vegetative stages similarly absorbed and utilized ammonium and nitrate when the rhizosphere pH was kept in the proper ranges (Ruffy Jr. et al. 1983). Hoshi et al. (1978) reported that after flowering soybeans in community conditions absorbed ammonium more rapidly than nitrate. Rabie et al. (1980) showed that although the seed nitrogen concentration was higher in a soybean plant which was grown on an ammonium solution the incorporation of ^{15}N into its seeds was greater from nitrate than from ammonium which was supplied in pod-setting and initial pod-filling stages. In the present experiment the nitrate nutrition for 30 days provided greater seed production and nitrogen accumulation compared to the ammonium nutrition except under extremely limited irradiant conditions in which those were similar between the ammonium and nitrate nutrition. Because the pH of leachate was not low in the pots which were given ammonium, the high medium acidity which would be caused by the absorption of ammonium did not take place. It is considered that the nitrate absorption levels were regulated by the irradiant conditions when high levels of nitrogen were supplied, but ammonium absorption levels were slightly affected by them.

When low levels of nitrate were supplied the nitrate absorption rate gradually

decreased during the pod-filling period. The plants which were given high nitrate levels showed a maximum absorption rate during the mid pod-filling period (Fig. 3). In addition the total amount of labelled nitrogen indicated that the nitrate absorption rate was consistently high under high irradiant conditions. Kato (1981) reported that nitrate absorption continued in nodulated soybeans until the late seed development stage whereas Imsande (1986) and Imsande and Edwards (1988) stated that the utilization of nitrate was impaired during the late stages of reproductive growth. Senescence is rapidly progressing with pod filling so that the abilities of absorption and assimilation of nitrate in soybean plants would decrease to different extents in each experiment. Hence, these discrepancies may be attributed to the nitrate amount supplied and irradiant conditions as shown in the present experiment, and/or the continuation of nitrogen fixation. It is conceivable that the irradiant conditions primarily affect the uptake of nitrate rather than nitrate assimilation, because the proportion of labelled nitrogen accumulated in seeds was not large in plants with a high seed yield (Fig. 3).

The high nitrate reductase activity in leaves requires a continuous flow of nitrate into leaves (Shaner and Boyer 1976). The leaf nitrate reductase activity was high in the late pod-filling stage in plants which were given 10 mM nitrate (Fig. 2 A, B). This shows that the absorption and distribution of nitrate were continued during the late pod-filling period, which led to a great accumulation of labelled nitrogen. The higher nitrate reductase activity at ambient light, than at 55%-ambient light, at 41 DAF might be related to the greater accumulation of labelled nitrogen in plants which were given 10 mM nitrate at ambient light.

It is noted that there is a close relationship between seed yield and photosynthesis when photosynthesis is measured after flowering (Shibles *et al.* 1987). When 0 or 2 mM nitrate was supplied, the carbon exchange rates, nitrate reductase activity and leaf nitrogen concentration decreased more rapidly at higher irradiance. This can be explained as a reason why the dry matter and seed production were not always large in plants receiving no nitrogen or small amounts of nitrogen even under higher light conditions. Carbon acquisition is large due to active photosynthesis under strong irradiance. If there is a low or a lack of exogenous nitrogen supply in soybeans, then most of the nitrogen necessary for seed production must depend upon the mobilization of endogenous nitrogen, which causes accelerated senescence under strong light conditions.

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