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Variability in Nitrogen Fixation Activity among Soybean Cultivars Grown under Field Conditions*

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Abstract : Variability in nitrogen fixation activity among soybean cultivars was examined under well-managed field conditions. Four and 15 soybean cultivars were grown in 1989 and 1991, respectively, along with the non-nodulating cultivar T201. Top dry weight, total top nitrogen content and nodule dry weight (1991 only) were determined on three or four dates. Nitrogen fixed by each cultivar was estimated with the difference method. In both years, daily nitrogen fixation rate (DNF) increased until roughly stage R5, showing a close linear relationship with top dry weight. *d*DNF/*d*TOPDW calculated combining all cultivars was 1.62 mg $day^{-1}g^{-1}$ ($r^2=0.91$) in 1989 and 1.69 mg $day^{-1}g^{-1}$ ($r^2=0.92$) in 1991. At the same time, calculation of *d*DNF/*d*TOPDW for individual cultivars before R5 showed a substantial difference among cultivars ranging from 1.29 mg $day^{-1}g^{-1}$ ($r^2=0.47$) of Harosoy in 1991 to 2.23 mg $day^{-1}g^{-1}$ ($r^2=0.93$) of Fusanari in 1991. Calculated nodule specific activity (DNF/NDW, mg $day^{-1}g^{-1}$) in 1991 increased for all cultivars during vegetative growth, while the ratio of module dry weight to top dry weight (NDW/TOPDW, g g^{-1}) decreased. The highest DNF of cultivars which occurred near stage R5 ranged approximately from 0.3 g $m^{-2}day^{-1}$ to 0.5 g $m^{-2}day^{-1}$. During pod filling, DNF was observed generally to decrease, and old cultivars showed rapid declines in DNF just after R5 compared to more modern cultivars.
Key words : Cultivar difference, Dry matter accumulation, Nitrogen fixation, Nodule dry weight, Non-nodulating cultivar, Soybean.

圃場条件におけるダイズの窒素固定活性の品種間差異：白岩立彦，Thomas R. SINCLAIR**，橋川　潮（滋賀県立短期大学，**米国フロリダ大学）

要　旨：ダイズ個体群の窒素固定活性における品種間差異を圃場条件のもとで検討した．1989年と1991年にそれぞれ4および15のダイズ品種ならびに根粒非着生品種 T 201 を栽培した．生育期間中3ないし4回にわたって地上部全乾物重，地上部全窒素含量および根粒重（1991年のみ）を測定した．窒素固定量は差し引き法によって求めた．1989年には堆肥を連用した畑圃場を用いたが，その土壌窒素供給は，1991年に用いた堆肥施用前歴のない水田転換畑と比べて大きかった．1日当たり窒素固定速度（DNF）は両年とも，地上部全乾物重との間に密接な直線関係を示しながら，およそ R 5（子実肥大始）まで増加した．直線の傾き（*d*DNF/*d*TOPDW）を全品種こみで計算した結果は，1989年が 1.62 mg $day^{-1}g^{-1}$（r=0.91），1991年が 1.69 mg $day^{-1}g^{-1}$（$r^2=0.92$）であった．また一方，R 5 までの *d*DNF/*d*TOPDW を各品種ごとに計算したところ，1991年 Harosoy の 1.29 mg $day^{-1}g^{-1}$（$r^2=0.47$）から 1991年房成の 2.23 mg $day^{-1}g^{-1}$（$r^2=0.93$）まで実質的な品種間差異がみられた．栄養生長期間を通じ，根粒重の地上部全乾物重に対する比（NDW/TOPDW, g g-1，1991年のみ）は全品種で低下したが，根粒重当たりの DNF（DNF/NDW, mg $day^{-1}g^{-1}$）は増加した．R 5 頃における DNFの最大値には，品種によっておよそ 0.3 g $m^{-2}day^{-1}$ から 0.5 g $m^{-2}day^{-1}$ の変異が認められた．子実肥大期では一般に DNF が低下したが，特に4つの旧品種が，新しい品種と比較して，早くから急速に DNF を低下させた．
キーワード：乾物生産，根粒乾物重，根粒非着生品種，ダイズ，窒素固定，品種間差異．

The amount of nitrogen in plants has been considered theoretically to be one of the most important features for the improvement of soybean potential yield, because of crucial restrictions of nitrogen on both the duration[10] and efficiency[11] of crop dry matter production. Experimentally also, Shiraiwa and Hashikawa[12] found a significant correlation between mean specific leaf nitrogen content and crop radiation use efficiency (dry matter accumulated per unit radiation energy intercepted) among various cultivars.

In general, symbiotic nitrogen fixation contributes more to nitrogen accumulation in soybean than soil nitrogen absorption, especially in high yielding soybeans[7]. Thus there have been many attempts to characterize the

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field performance of soybean nitrogen fixation[1,3,5,8,14]. But the identification of variability in nitrogen fixation traits among genotypes which is essential for the genetic improvement of soybean potential productivity, has not been fully examined[9].

The objective of this study was to examine variability in nitrogen fixation activity of soybean crop among various cultivars grown under field condition.

Materials and methods

In 1989 and 1991, the seasonal nitrogen fixation of various soybean cultivars was evaluated under two well managed field conditions. In both years, neither drought nor wet stress was observed during the growing period because of sufficient rainfall (1989) or irrigation (1991), and good drainage.

In this study, nitrogen fixed by cultivars was estimated by the difference method ; the nitrogen amount accumulated by a non-nodulating line was subtracted from the amount of nitrogen accumulated by each of the tested cultivars.

The difference method for determining nitrogen fixation activity assumes that there is no difference in soil nitrogen uptake between the non-nodulating line and the test plants, Under favorable soil conditions with moderate nitrate levels, fairly good agreemetns and correlations between estimates by the difference method and ^{15}N dilution method have been shown in the previous reports[9,14]. In one of these studies, the estimates of nitrogen fixation activity for cultivars were derived with a single non-nodulating line[9]. To estimate the accumulated nitrogen fixation activity using the acetylene reduction technique requires frequent measurements because the nodule activity fluctuates with the weather and plant conditions. Even so, integration of acetylene reduction measurements for estimation of seasonal nitrogen fixation is problematical. Consequently, the difference method was adopted for these initial observations of nitrogen fixation activity of soybean cultivars because this technique allows a large number of cultivars to be tested and enables the evaluation of the integrated nitrogen fixation activity for a defined growth period.

In 1989, four nodulating cultivars and a non-nodulating soybean T201 were sown on 13 June and grown at 8.3 pl.m^{-2} of plant density with three replications, on an upland field (silty clay loam soil) at Shiga Prefectural Junior College Farm (Kusatsu). Previous to sowing, P_2O_5 and K_2O were incorporated at 100 kg per ha. The field had been fertilized with about 20 ton per ha per year of manure for more than a decade under successive grass cropping. On 24 Jul., 7 Aug. and 16 Aug., plant materials above the cotyledonary node were harvested from 0.74 m^2 of land area (six plants). The total dry weight of the plants was measured and their nitrogen contents were analyzed by the Kjeldahl method.

In 1991, 15 nodulating cultivars and T201 were sown on 29 June and grown at 7.7 pl. m^{-2} with two replications. The experimental field was a drained paddy field (clay loam soil) converted in use from successive paddy rice production at the Shiga Agricultural Experiment Station at Azuchi. The soil was fertilized with 100 kg per ha of P_2O_5 and K_2O. No manure had been applied to this field, but

Table 1. Nitrogen accumulation in the top of non-nodulating cultivar T201 and the contribution of nitrogen fixation to the nitrogen accumulated in the top of nodulating cultivars grown in 1989 on an upland field and in 1991 on a drained paddy field.

	1989 (upland field)			1991 (drained paddy field)			
DATE	JUL. 24 (34)*	AUG. 7 (48)	AUG. 16 (57)	AUG. 4 (33)	AUG. 14 (43)	AUG. 26 (55)	SEPT. 9 (69)
N amount accumulated by T201 g m^{-2}	0.88	3.22	3.79	0.48	1.24	2.68	5.77
Contribution of N fixation to total top N.**	0.29	0.47	0.63	0.71	0.72	0.74	0.67

* : Days after emergence. ** : Averaged values of all the nodulating cultivars.

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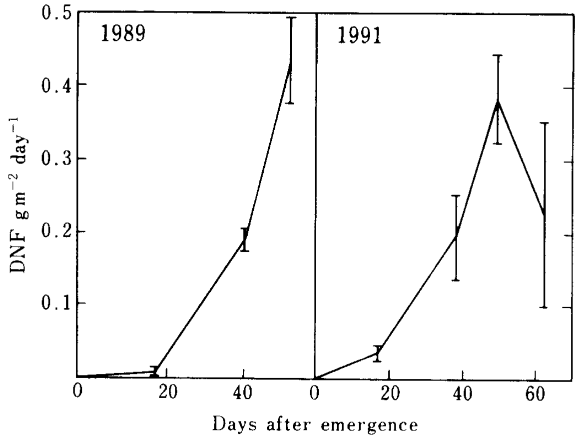

Fig. 1. Seasonal change in daily nitrogen fixation rate (DNF) of soybean grown in 1989 and 1991.
The values for DNF are the averages of all the nodulating cultivars. Days after emergence is for the middle day of the two dates on which measurements occurred and between which DNF was calculated. The vertical line in the figure indicates the standard deviation of DNF among cultivars.

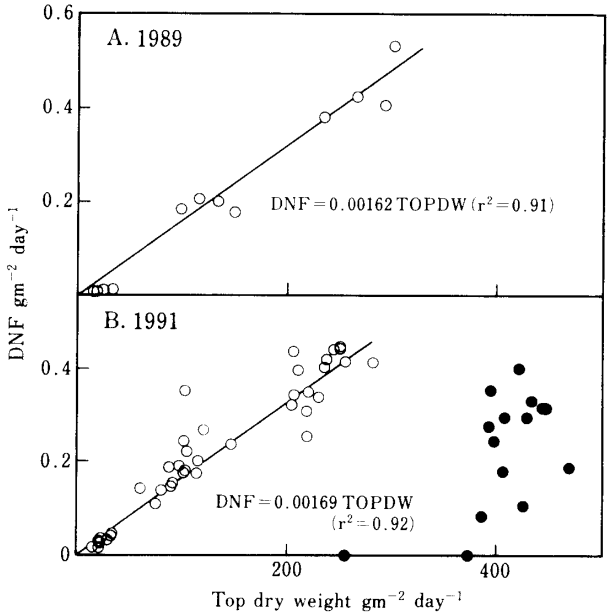

Fig. 2. DNF as related to top dry matter weight.
Top dry weight is the average of top dry weight on two successive sampling dates between which DNF was calculated. (A) : Four cultivars in 1989. (B) : 15 cultivars in 1991. o : before R5 ● : after R5.

the residue rice straw had been incorporated in previous years. Total top dry matter weight and total top nitrogen content were determined on 4 Aug., 14 Aug., 26 Aug. and 9 Sept., for plant materials harvested from 0.52 m^2 land area (four plants). At the first three measurements, below ground parts also were harvested and the nodule dry weight was determined for two plants to a depth of 0.25 m.

The amount of nitrogen fixed by each cultivar was calculated by subtracting total top nitrogen content of non-nodulating T201 from that of each cultivar at each harvest in both experiments.

The daily nitrogen fixation rate (DNF, mg day^{-1}m^{-2}) of each cultivar was calculated for each sampling interval, with the following equation.

$$[\{(N_{cultivar})_n - (N_{T201})_n\} - \{(N_{cultivar})_{n-1} - (N_{T201})_{n-1}\}]/(T_n - T_{n-1}),$$

where n : the number of sampling time from 1 to 4, and T : days after emergence.

Results

1. Nitrogen uptake

Total top nitrogen content in T201 (Table 1) indicated that soil nitrogen availability in the 1989 experiment was greater than in the 1991 experiment during the early growing period. Nitrogen amounts in T201 at approximately growth stage R5[2] were 3.8 g m^{-2} on 16 Aug., 1989 and 2.7 g m^{-2} on 26 Aug., 1991. Preceding manure applications to the field used in 1989 experiment apparently resulted in greater soil nitrogen release than in the 1991 experiment. On the other hand, nitrogen uptake after 26 Aug. in 1991 was as much as 3.1 g m^{-2} and greater than the total nitrogen uptake before the period. The reason for this result is not fully understood, but it might be consequence of the nature of the drained paddy soil in 1991 which was higher in clay content and relative fertility. In addition cool temperature in early August in 1991 (2°C less than the average year) also might have caused a further delay of the peak of soil nitrogen release.

In 1989, because of the relatively faster nitrogen uptake in early period, the symbiotically fixed nitrogen by the nodulated cultivars at the first measurement (Table 1) averaged only 29% of the total accumulated nitrogen. Thereafter the symbiotically fixed fraction in 1989 increased up to 63% of the

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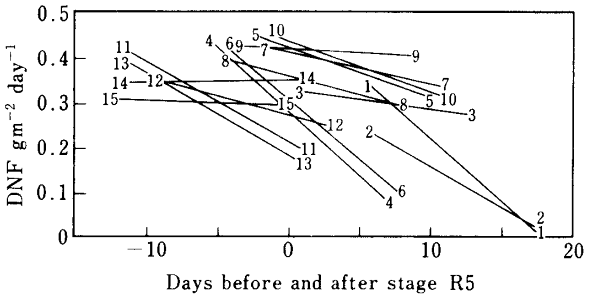

Fig. 3. DNF change from the third estimate period to the fourth period of 15 cultivars grown in 1991, as related to the days before and after stage R5.
The numeral in the figure indicates the cultivar number defined in Table 2.

accumulated nitrogen on 16 Aug., In 1991, the average symbiotically fixed fraction was quite stable at high values around 70% of the accumulated nitrogen.

2. Seasonal change in daily nitrogen fixation rate

The seasonal change in daily nitrogen fixation rate (DNF) is summarized in Fig. 1, in which the averaged values of all the cultivars at three (1989) and four (1991) sampling intervals are presented against days after emergence of the middle date of the interval. DNF increased exponentially for about 50 days after emergence (the third estimate) and decreased at the fourth interval in 1991. The

Table 2. The date of stage R5 (beginning of grain filling), nodule dry weight per top dry weight (NDW/TOPDW), DNF per nodule dry weight (DNF/NDW), and the cofficient of daily nitrogen fixation rate against top dry weight (*d*DNF/*d*TOPDW) calculated for the first three estimates before stage R5 for each cultivar grown in 1989 and 1991.

Year Cultivar No.	Date of R5	NDW/TOPDW AUG. 4	AUG. 14	AUG. 26	DNF/NDW* EMG** -AUG. 4	AUG. 4 -14	AUG. 14 -26	*d*DNF/*d*TOPDW (r^2)
		$g\ g^{-1}$			mg $day^{-1}g^{-1}$			mg $day^{-1}g^{-1}$
1989								
ENREI	AUG. 16	—	—	—	—	—	—	1.78 (0.89)
TAMAHOMARE	AUG. 26	—	—	—	—	—	—	1.58 (0.89)
MIZUKUGURI	AUG. 25	—	—	—	—	—	—	1.44 (0.96)
AKAZAYA	AUG. 23	—	—	—	—	—	—	1.68 (0.85)
T201	AUG. 15	—	—	—	—	—	—	— —
1991								
1 HAROSOY	AUG. 15	.0492	.0440	.0239	20.0	74.2	49.9	1.29 (0.47)
2 MANADARIN	AUG. 15	.0569	.0441	.0151	21.8	50.1	68.2	1.59 (0.92)
3 NORIN 1	AUG. 20	.0591	.0396	.0324	24.0	48.2	45.1	1.53 (0.88)
4 FUSANARI	AUG. 26	.0564	.0434	.0270	20.5	32.4	68.2	2.23 (0.93)
5 TOSAN-155	AUG. 23	.0501	.0364	.0387	24.9	39.5	47.0	1.84 (0.97)
6 FUKUSENNARI	AUG. 25	.0594	.0340	.0272	28.1	44.5	56.4	1.60 (0.94)
7 TACHINAGAHA	AUG. 22	.0556	.0471	.0307	28.4	37.0	53.4	1.84 (0.99)
8 TOSAN 61	AUG. 25	.0554	.0498	.0325	25.9	46.4	45.3	1.67 (0.82)
9 TOSAN Q310	AUG. 24	.0536	.0460	.0435	26.5	44.0	40.8	1.77 (0.97)
10 ENREI	AUG. 22	.0486	.0401	.0415	27.9	41.8	45.9	1.88 (0.99)
11 MIZUKUGURI	SEPT. 1	.0659	.0366	.0295	21.9	49.4	46.8	1.38 (0.89)
12 AKAZAYA	AUG. 30	.0538	.0492	.0303	27.6	38.0	44.2	1.54 (0.80)
13 SHIIN 4	SEPT. 1	.0529	.0453	.0315	28.1	37.4	54.3	1.94 (0.99)
14 KAKUSHIN 1	SEPT. 1	.0621	.0491	.0330	22.6	31.1	44.1	1.68 (0.98)
15 TAMAHOMARE	SEPT. 2	.0433	.0417	.0339	27.7	39.8	40.5	1.40 (0.97)
T201	AUG. 24	—	—	—	—	—	—	— —

* : The averaged value for the two dates between which DNF was estimated. ** : Emergence.

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difference in DNF among cultivars indicated by standard deviation (vertical lines in the figure) became greater later in the season. In view of the time course of DNF in 1991, the third estimate in both years seemed to represent the peak of nitrogen fixation activity, which is at roughly stage R5 (Table 1). DNF values at the third interval ranged from 0.3 to 0.5 g $day^{-1}m^{-2}$ among cultivars, and is a range similar to the observation of Hoshi and Kuwabara[3] for soybean crops on highly productive field conditions in Hokkaido.

In Fig. 2, DNF calculated for all cultivars and periods is plotted together against the top biomass. The top biomass is the average of values on two dates between which DNF was calculated.

Before R5, DNF of all cultivars showed a close linear relationship with top biomass in both years ($r^2=0.91$ and 0.92 for 1989 and 1991, respectively). This linearity is consistent with a report by Denison et al.[1] who showed a significant positive correlation between shoot dry weight and acetylene reduction activity for field-grown soybean plants. The slopes of the regression lines (*d*DNF/*d*TOPDW mg day^{-1} g^{-1}) calculated for each year in this experiment agreed well with each other, at 1.62 and 1.69 mg $day^{-1}g^{-1}$ in 1989 and 1991, respectively.

After R5, DNF no longer increased with top biomass, as shown with the last DNF estimate in 1991 (Fig. 2B). Some cultivars had significantly decreased their nitrogen fixation activity after R5. Since the developmental stage when the measurements occurred differed among cultivars (Table 2), DNF for the 3rd and 4th estimation periods in 1991 for each cultivar were re-plotted again against the days before and/or after R5 (Fig. 3).

Although some cultivars (cultivar number 4, 8, 11 and 13) had substantial decreases in DNF at roughly stage R5, most cultivars maintained or only slightly decreased DNF after R5. And the two earliest cultivars, Harosoy and Mandarin had substantial declines in DNF after 15 days following stage R5. The results for these two cultivars may have been a consequence of ontogenetic progress causing increased competition for assimilate during rapid pod fill[6,13].

Based on the above observations, the seasonal pattern of nitrogen fixation can be generally described as follows: DNF increases with a constant coefficient related to top growth approximately until or shortly before R5; it remains fairly stable during the initial stage of seed growth; and then a rapid decline follows, possibly at mid-grain filling. Such a pattern agrees well with previously reported observations[6,13].

3. Nodule growth and its activity

The nitrogen fixation activity can be divided into two components; nodule mass and nodule specific activity. Since DNF is found to be in close relation with top biomass, nodule mass was represented as the ratio to top dry weight (NDW/TOPDW g g^{-1}) in Table 2. While NDW increased through the three measurements before R5 in 1991 (data are not shown), the ratio NDW/TOPDW decreased during vegetative growth for all the cultivars, on average from 0.055 g g^{-1} on 4 Aug. to 0.031 g g^{-1} on 26 Aug., DNF per unit nodule weight (DNF/NDW) for each estimation period was calculated by dividing DNF with averaged value of NDW for the two dates for which DNF was estimated. DNF/NDW increased in most cultivars from approximately 25 mg day^{-1} for the first estimte (emergence to 4 Aug.) to 50 mg $day^{-1}g^{-1}$ for the last estimate (14 Aug. to 26 Aug.) Therefore, the increase of DNF/NDW coincided with the decrease in NDW/TOPDW resulting in a fairly constant ratio of DNF to top biomass during the vegetative growth period.

4. Variability of nitrogen fixation among cultivars

The close correlation in both years between top biomass and DNF before R5 when all cultivars are combined (Fig. 2) indicates that the primary difference among cultivars in the total amount of nitrogen fixed throughout the vegetative growth can be attributable to duration and rate of top biomass accumulation. Such a result helps to explain why Patterson and LaRue[9] found little variability in nitrogen fixation within maturity groups. Nevertheless, calculation of *d*DNF/*d*TOPDW for individual cultivars before R5 showed a substantial difference among cultivars (Table 2, linear regression coefficient between top biomass and DNF for each cultivar). The values of *d*DNF/*d*TOPDW in 1991 ranged from 1.29 mg day^{-1} g^{-1} ($r^2=0.47$) for Harosoy to 2.23 mg $day^{-1}g^{-1}$ ($r^2=0.93$) for Fusanari. Therefore, the differ-

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ence in dDNF/dTOPDW also can be a significant factor which causes the variance of nitrogen fixation among cultivars, though this is not so critical as the involvement of top growth. The reason for the cultivar difference in dDNF/dTOPDW is unknown in this study. Importantly, there was a good consistency in dDNF/dTOPDW between the two years for the four cultivars studied in both years. This consistency indicates there may be genetic control of dDNF/dTOPDW and that this trait may be altered by breeding selection.

Another cultivar difference in DNF was detected after R5, too (Fig. 3). Four cultivars Fusanari, Fukusennari, Mizukuguri and Shin-4 had rapid decline in DNF just after R5. Considering the possible improvement of soybean yield by enhancing nitrogen fixation during pod filling[4], this cultivar difference also is important. The four cultivars with rapid declines in DNF after R5 are old cultivars, and three of them had relatively less NDW/TOPDW at near R5 on 26 Aug. than more modern cultivars such as Tosan-155, Tosan-Q310 and Enrei (Table 2). Thus, withdrawal of carbon supply from the top to grow nodule might have occurred earlier in those old four cultivars.

Discussion

As shown in table 1, the nitrate levels of the soils used in this study seemed favorably to be moderate with the uptake fraction of one third in total nitrogen at later season which was similar as those of the reports which compared the estimates by the difference method and ^{15}N dilution method[9,14]. Thus estimates for nitrogen fixation in this study can be considered to be reflecting well the seasonal pattern and cultivar difference of nitrogen fixation activity.

As a whole, DNF of soybean crop increased with a constant coefficient related to top biomass until roughly R5 and tended to remain stable at the initial pod filling, and thereafter a rapid decline in DNF follwed. This description of the seasonal nitrogen fixation, especially the linear relationship between top biomass and DNF during vegetative growth, is considered to be fairly common to soybean cultivars.

There seems to be cultivar difference in nitrogen fixation activity in three aspects. First, based on the linearity between top biomass and DNF when all cultivars are combined, the primary difference among cultivars in nitrogen fixation during vegetative growth can be associated to top dry matter accumulation. Secondly, dDNF/dTOPDW of individual cultivars also is considered to cause some variance in nitrogen fixation during vegetative growth. And, following R5 too, genetic difference seems to exist in the earliness of decline in DNF after R5.

However, the latter two aspects are relatively minor differences. Considering undefined possibility that there might be some cultivar difference in the pattern of nitrogen absortion, further accurate estimation would be needed to quantify the difference in dDNF/dTOPDW and DNF after R5 among individual cultivars. At least, this study showed the evident existence of variability in nitrogen fixation activity among soybean cultivars in those two aspects.

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