Effect of CO$_2$ Concentration, Temperature and N Fertilization on Biomass Production of Soybean Genotypes Differing in N Fixation Capacity

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Abstract: We tested the hypothesis that elevated CO$_2$ concentration [CO$_2$]-induced enhancement of biomass production of soybean is greater in a genotype that has a higher nitrogen (N) fixation capacity. Furthermore, we analyzed the interactive effects of N fertilization, temperature and [CO$_2$] on biomass production. Three genetically related genotypes: Enrei (normally-nodulating genotype), Kanto 100 (supernodulating genotype), and En1282 (non-nodulating genotype) were grown in pots, with or without N fertilizer for two years (2004, 2005). They were then subjected to two different [CO$_2$] (ambient and elevated (ambient + 200 μmol mol$^{-1}$)) × two temperature regimes (low, high (low +4~5°C)). Top dry weight at maturity was the greatest in the elevated [CO$_2$] × high temperature regime, irrespective of genotype and N fertilization. The [CO$_2$] elevation generally enhanced N acquisition and dry matter production during the vegetative growth stage, and the enhancement was more pronounced in the nodulating genotypes (Enrei and Kanto 100) than in the non-nodulating genotype (En1282), indicating that N supply through N fixation contributes to elevated [CO$_2$]-induced biomass production in soybean. However, the relative responsiveness of biomass production to elevated [CO$_2$] was not necessarily higher in the supernodulating genotype than the normally-nodulating genotype. The N utilization efficiency to produce biomass was inferior in the supernodulating genotype than in the normally-nodulating and non-nodulating genotypes. These results did not fully verify the hypothesis that elevated [CO$_2$]-induced enhancement of biomass production of soybean is greater in a genotype with a higher N fixation capacity.

Key words: Biomass production, Elevated [CO$_2$], High temperature, N fixation, Photosynthesis, Soybean (Glycine max), Supernodulation.

Past population growth with the associated increase in human activities has created many environmental changes. Of the major changes, increasing atmospheric CO$_2$ concentration [CO$_2$] is a higher profile global issue because of its possible interactions with future climate changes, along with the effects of other greenhouse gases such as methane and nitrous oxide (Mearns, 2000). Historically, large increases in atmospheric [CO$_2$] have occurred during the past 200 years (Pritchard and Amthor, 2005). Although the future trend is uncertain, a continuous increase in [CO$_2$] is very likely for the 21st century (Cox et al., 2000; IPCC, 2007).

As a biophysical system dependent on climatic resources, crop production can be affected by changes in resources, including [CO$_2$]. Many previous studies have indicated that elevated [CO$_2$] generally increases the growth and yield of crops; often more markedly in species using the C$_3$ photosynthetic pathway than in plants using the C$_4$ pathway (Bowes, 1993; Rogers et al., 1997; Kimball et al., 2002; Pritchard and Amthor, 2005). Statistical summarization of numerous published data revealed that the increase in soybean shoot dry matter due to elevated [CO$_2$] (450–550 μmol mol$^{-1}$) was approximately 30%. However, the magnitude of the increase in dry matter production induced by elevated [CO$_2$] is inconsistent, and is probably dependent on the methodology of the experiments employed. For example, Tobert et al. (2004) found that elevated [CO$_2$] (twice ambient) increased total biomass production by ca. 40% compared to plants grown at ambient [CO$_2$], in a study using an open top chamber. In contrast, Morgan et al. (2005) reported in a free-air CO$_2$ enrichment (FACE) study that elevated [CO$_2$] (550 μmol mol$^{-1}$) increased above-ground net primary production by only 17–18%. They suggested that prior chamber studies may have overestimated the positive effect of elevated [CO$_2$]. Recently, Long et al. (2006) concluded, from a review of FACE studies, that FACE data show a much lower crop yield response to elevated [CO$_2$] than the data derived from enclosure studies, but this conclusion was argued by Tubiello et al. (2007).

It is well documented that growth in response...
to elevated \([\text{CO}_2]\) is often maximal in adequately fertilized plants (Rogers et al., 1999; Stitt and Krapp, 1999; Kim et al., 2005a, 2005b; Kobayashi et al., 2006). Furthermore, elevated \([\text{CO}_2]\)-induced enhancement in rice biomass production is defined by the amount of N fertilizer used (Nakagawa et al., 1994; Ziska et al., 1996). Proposed mechanisms to explain N limitation of biomass production at elevated \([\text{CO}_2]\) include N limitation accelerated by faster shoot growth (Farage et al., 1998; Stitt and Krapp, 1999), root volume restriction (Stitt and Krapp, 1999; Ainsworth et al., 2002; Long et al., 2004; Ainsworth and Long, 2005), enhancement of microbial N immobilization and/or N sequestration by plants (Diaz et al., 1993; Zak et al., 2000; Hungate et al., 2003; Luo et al., 2004). In contrast to non-leguminous crops, Allen et al. (1988) concluded that legume crops including soybean could supply an adequate amount of N through symbiotic N fixation to meet the requirement of the growth and grain yield. However, in terms of the N\(_2\) fixing capability of different genotypes, how soybean biomass production and seed yield would be maximized at elevated \([\text{CO}_2]\) remains unclear.

Soybean has three N sources: fertilizer N, soil N, and symbiotically fixed N. Of the three, the most common way to enhance N absorption by plants is to increase the use of N fertilizers (Yoshida, 1979; Watanabe et al., 1983). Given that a high input of N fertilizer increases the potential for groundwater contamination, soybean productivity should be improved by using biological N fixation. In this context, genetic improvement of symbiotic N fixation is another option for enhancement of N-acquisition. In the past two decades, several supernodulating soybean genotypes have been isolated, which form many more nodules than normally nodulating cultivars (Carroll et al., 1985a, 1985b; Day et al., 1986; Gremaud and Harper, 1989; Akao and Kouchi, 1992). However, most supernodulating lines bred so far have shown inferior growth and yield performance than their related normally nodulating ancestral cultivars. Hence, the supernodulating lines failed to produce practical benefits (Herrridge and Rose, 2000; Sinclair, 2004).

Recently, a new supernodulating cultivar “Kanto 100” became available, which was derived from high-yielding cultivar Enrei (Takahashi et al., 2003a). This genotype’s yield and agronomic performance are equal or superior to that of its ancestral cultivars under certain conditions (Takahashi et al., 2003b). However, its growth rate during the early growth stage tends to be inferior to its ancestral cultivar (Matsumani et al., 2004). Nevertheless, Kanto 100 is the first soybean cultivar ever bred that has the potential to be grown by farmers. It is characterized as having a superior ability to maintain high leaf N and photosynthesis, irrespective of the application rate and type of N fertilizer (Maekawa et al., 2003; Takahashi et al., 2005). This high photosynthetic capability of Kanto 100 depends on high leaf Rubisco and chlorophyll contents (Maekawa and Kokubun, 2005).

These lines of evidence raise a hypothesis: biomass production and yield of a soybean genotype with a superior capability for N fixation, such as Kanto 100, should be further enhanced under elevated \([\text{CO}_2]\). The first objective of this study was to verify the hypothesis that elevated \([\text{CO}_2]\)-induced enhancement of soybean biomass production, where N acquisition largely depends on symbiotic N fixation, is greater in a genotype with a higher N fixation capacity under elevated \([\text{CO}_2]\). The second objective was to identify the interactive effects of N fertilization and temperature with \([\text{CO}_2]\) on biomass production.

Materials and Methods

1. Plant materials

The supernodulating cultivar Kanto 100, its ancestral normally nodulating cultivar Enrei, and Enrei-derived non-nodulating genotype En1282, were used in this study. Kanto 100 is an improved genotype selected from the progeny of (\(\text{En-b0-1} \times \text{Enrei}\)×\(\text{En6500}\))×\(\text{Tamahomare}\) (Takahashi et al., 2003a; Yamamoto et al., 2004); En6500 is an EMS (ethyl-methyl-sulfonate)-induced mutant of Enrei (Akao and Kouchi, 1992). Four seeds per pot (16 cm diameter, 19 cm tall) were sown on 16 June 2004 and 7 June 2005 and were thinned to one plant per pot after emergence. Prior to sowing, fertilizer was applied at fixed rates: 0.3 g N, 1.0 g P\(_2\)O\(_5\), 1.0 g K\(_2\)O, and 5 g of slaked lime per pot. N was not applied to half the plants. The soil was a low-humic Andosol (Classification Committee of Cultivated Soils, 1996). The seeds were inoculated with a strain of \(\text{Bradyrhizobium japonicum}\) obtained from Tokachi-Nokoren (Obihiro, Japan).

The plants were grown, from sowing to maturity, in the temperature gradient chambers of National Agricultural Research Center for Tohoku Region, Morioka, Japan. Temperature gradients inside the chambers (24 m long) were created by pre-cooling the incoming outdoor air with an air-conditioner at the air inlet, and then warming the air with solar radiation or supplemental heat input from the air outlet of the chambers (Okada et al., 2000). A temperature difference as large as 7–8°C was maintained between the air inlet and outlet, by regulation of ventilating fans and an air heater. The plants used in the present study were placed on two different plots that provided either high (H) or low (L) temperature treatment during 2005, while the plants were grown only at the H temperature in 2004. The \([\text{CO}_2]\) inside the chambers was regulated at two levels: ambient (A) and elevated (E) \([\text{CO}_2]\) (ambient+200 \(\mu\)mol mol\(^{-1}\)). The treatments imposed in the experiments are summarized in Table 1. The \([\text{CO}_2]\) and temperature around the canopy of the plants were monitored every half hour, using...
sensors installed above the plots. Plants were irrigated twice a day with tap water, and pesticides were applied when necessary.

2. Measurement of growth and N content

In 2004, five plants were sampled from each plot at maturity (120 and 130 days after sowing (DAS) for En1282 and the other two genotypes, respectively), and top dry weights (excluding roots) were measured. In 2005, samples were taken at 35 and 69 DAS (69 DAS corresponded to R3, according to Fehr et al. (1971)). The period from 35 to 69 DAS corresponds to a stage of linear growth, and biomass production at maturity is largely dependent on the growth rate at this stage (Shibles et al., 1975; Kokubun, 1988). The samples were separated into various plant parts (leaves, stem + petioles + pods, root, nodules), oven dried at 80°C for three days, and then weighed. Prior to drying, leaf area was measured using a leaf area meter (Model AAM-9, Hayashi Denko Co. Ltd., Tokyo). Based on leaf areas, relative leaf growth rates (RLGR) were calculated from the following equation:

\[
(\log L_2 - \log L_1)/(t_2 - t_1),
\]

where \(L_1\) and \(L_2\) were leaf area at \(t_1\) and \(t_2\), respectively.

The dried samples in 2005 were ground in a mill and N content was analyzed by the Kjeldahl-Gunning procedure. Plants grown at the H temperature and fertilized with N were used for the measurements of leaf area and N content.

4. Measurement of photosynthetic rate

The apparent photosynthetic rate of the recently expanded terminal leaflet in five plants from each treatment was measured using a portable photosynthesis system (LI6400; Li-Cor Inc., NE, USA). Measurement was carried out at 1000 and 1200 h at 35 and 69 DAS using the plants that were fertilized with N and grown under the H temperature. The flow rate of air in the leaf chamber was controlled at 500 μmol s\(^{-1}\), and the [CO\(_2\)] supplied to the leaf chamber was maintained at concentrations similar to the respective growth environments (350 or 550 μmol mol\(^{-1}\) for ambient or elevated [CO\(_2\)] regime, respectively). Irradiance on the measured leaves (6 cm\(^2\)) was regulated at 1,500 μmol m\(^{-2}\) s\(^{-1}\) photosynthetic photon flux density (PPFD), and the chamber temperature was maintained at 25°C.

5. Statistical analysis

Analysis of variance for top dry weight at 35 DAS (only in 2005) and at maturity was performed to evaluate the effects of genotype, [CO\(_2\)], temperature, and N fertilization, and the effects of their interactions by using the JMP version 5.1 (SAS Institute Inc., NC, USA). Tukey’s means comparison test was applied to determine any significant differences between the means.

Results

1. [CO\(_2\)] and temperature

Table 2 shows monthly averages of daily mean [CO\(_2\)] and temperature inside the growth chambers for two years. Although [CO\(_2\)] fluctuated with a daily variation of 40–80 μmol mol\(^{-1}\), the elevated [CO\(_2\)] regime was approximately 200 μmol mol\(^{-1}\) higher than that of the ambient [CO\(_2\)] regime, as was intended. The mean [CO\(_2\)] of the ambient [CO\(_2\)] regime during the growth period (June–October) was 391 and 383 μmol mol\(^{-1}\) in 2004 and 2005, respectively, while that of the elevated [CO\(_2\)] regime was 595 and 584 μmol mol\(^{-1}\), respectively. The temperature difference between the ambient and elevated regimes was negligible.

The mean temperatures of the H and L temperature regime in 2005 were 24.9 and 20.5°C, respectively, with

| Year | [CO\(_2\)]   | Temperature | N fertilizer |
|------|-------------|-------------|-------------|
| 2004 | Ambient, Elevated | High       | Applied, Not applied |
| 2005 | Ambient, Elevated | High, Low  | Applied, Not applied |

For [CO\(_2\)] and temperature treatment, refer to Table 2. N applied: 0.3 g pot\(^{-1}\).
a difference of ca. 4.4°C. Differences between daily highs and lows were ca. 7–8°C, for both temperature regimes.

### 2. Growth and biomass production

Growth stages were significantly affected by temperature treatment, but little by [CO\(_2\)] and N fertilization irrespective of cultivar; H temperature regime, compared to L temperature regime, accelerated flowering time and maturity by 7–10 and 10–13 days, respectively, depending on genotype and year. Among genotypes, flowering time differed by less than 3 days, while maturity was about 10 days earlier in En1282 than the other genotypes.

Analysis of variance of the effect of treatments (genotype, [CO\(_2\)], temperature, N fertilization) on dry weight at maturity and at 35 DAS revealed that the effect of each treatment was significant (P<0.001), except that of N fertilization in 2005 (Table 3). The interactive effect of [CO\(_2\)] × genotype was significant in both years at maturity (P<0.001), but not at 35 DAS (P>0.05). The interaction between N fertilization × genotype was not significant at 35 DAS (P>0.05), while it was significant at maturity (P<0.001).

Fig. 1 shows top dry weight at maturity n 2004 and 2005, where the combined effects of [CO\(_2\)] elevation and N application were examined. The
positive effect of N application on top dry weight was obvious in En1282, whereas it was obscure in the two nodulating genotypes. Of all the plots, the greatest weight was obtained in Kanto 100, in the E [CO2] × H temperature × N application regime. The growth was more vigorous in 2004 than in 2005, probably reflecting more abundant solar radiation during the vegetative growth stage in 2004 (Table 2).

Fig. 2 shows total dry weights at 35 DAS and at maturity (144 DAS) of the plants with N fertilization in 2005, where the combined effects of [CO2] and temperature were examined. Irrespective of genotype and [CO2], enhancement of total dry weight induced by high temperature was generally substantial. However, differences in the weight between the H and L temperature regimes tended to be less at maturity than at 35 DAS, particularly in Enrei, where the difference was not significant (P > 0.05). Enhancement of total dry weight by [CO2] elevation tended to be greater at the H temperature regime irrespective of growth stage or genotype. At the L temperature regime, the enhancement was vague; the difference between ambient and elevated [CO2] was not significant (P > 0.05).

In 2005, the dry weights of various plant parts in the ambient and elevated [CO2] regimes were measured for the plants grown at H temperature with N application at 35 and 69 DAS, for each genotype (Table 4). At 35 DAS, dry weights of all plant parts were heavier in Enrei than in the other genotypes, irrespective of [CO2] At 35 DAS, an elevated [CO2]-induced increase in dry weight was observed in all plant parts (leaf, stem + petiole, root + nodules), irrespective of genotype. However, at 69 DAS, a similar increase was observed in the nodulating genotypes (Kanto 100 and Enrei). The increase was negligible or negative in En1282, except in the stem + petiole. Of all plant parts measured, the growth of stem + petiole was most markedly enhanced by [CO2] elevation, and enhancement was more pronounced in Kanto 100 than in the other genotypes.

3. Leaf area

Leaf area measured at 35 DAS was in the following order: Enrei > En1282 > Kanto 100, irrespective of [CO2] (Table 5). At 69 DAS this genotypic order was reversed in the elevated [CO2] regime; the leaf area was the greatest in Kanto 100, and was the least in En1282. This greater leaf area at 69 DAS in Kanto 100 was due to the higher RLGR during the mid-growth period than in the other genotypes. In the elevated [CO2] regime, RLGR was higher in Kanto 100 than in the other genotypes.

4. N concentration and N content of plant

The leaf N concentration was lower in the elevated [CO2] regime than in the ambient [CO2] regime.
However, no marked differences were observed in the N concentration in the other plant parts (Table 6). The genotypic difference in N concentration was obvious only in the leaves at 35 and 69 DAS and leaf N concentration was higher in Kanto 100 than in the other genotypes, irrespective of [CO₂]. The difference between Enrei and En1282 was negligible at 35 DAS. At 69 DAS, N concentrations in all plant parts except nodules were in the following order: Kanto 100 > Enrei > En1282.
A significant increase in N content of whole plants was observed in the elevated [CO₂] regime in Enrei at 35 DAS, and in Kanto 100 at 69 DAS (Table 6). The increase in total N uptake was primarily due to the increase in stem + petiole weight. In En1282 at 69 DAS, the N content was lower in the elevated [CO₂] regime, due to defoliation.

5. Nodulation and acetylene reduction activity (ARA)

At 42 DAS, ARA measured on a per plant basis tended to be increased by elevated [CO₂] in both cultivars (Fig. 3), although the differences between the ambient and elevated [CO₂] regimes were not statistically significant (P > 0.05). At 69 DAS, ARA tended to be lower in the elevated [CO₂] regimes in both cultivars, and the genotypic difference was not observed.

6. Photosynthetic rate

Generally, the apparent photosynthetic rate of the most recently expanded leaf tended to be higher in plants grown in the elevated [CO₂] than in the ambient [CO₂] regime, but the difference between the two [CO₂] regimes was not statistically significant.
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At 35 DAS, photosynthetic rates, irrespective of [CO$_2$], tended to be in the following order: Kanto 100 > Enrei > En1282. At 69 DAS, there was no obvious genotypic difference in the rates between Kanto 100 and Enrei, but the rate declined markedly in En1282.

**Discussion**

The positive effect of N application on top dry weight was obvious in the non-nodulating genotype En1282, irrespective of [CO$_2$] and temperature, but it was generally obscure in the nodulating genotypes (Fig. 1). The N-induced increase in dry weight did not exceed 22.0% in the nodulating genotypes. However, in the non-nodulating genotype En1282, it was as high as 91–203%. Thus, the N-fixing capability reduced the positive effects of N application on soybean biomass production.

The positive interactive effect of [CO$_2$] and N application on biomass production was observed in crops that lack N-fixing ability, such as rice (Kim et al., 2003a, 2003b), and wheat (Kimball et al., 2002).
In this study, the interactive effect in En1282 was significant only in the H temperature regime in 2005. Furthermore, the effect was not significant in the H temperature regime in 2004 or in the L temperature regime in 2005 (Fig. 1, P>0.05). This inconsistency seen in the non-nodulating genotype En1282 might be ascribed to a difference in temperature between the two years; it was warmer in 2005 than in 2004 (Table 2).

Generally, the enhancement of biomass production induced by elevated [CO2] and high temperature was more marked at 35 DAS, but enhancement was reduced with maturity (Fig. 2). An analysis of dry matter production during the linear growing period (from 35 to 69 DAS) revealed that the elevated [CO2]-induced increment of biomass during this period was most substantial in Kanto 100, less marked in Enrei, and negligible in En1282 (Table 4). This genotypic difference in biomass production during the linear growing period resulted in the differences in top dry weight at maturity.

Our hypothesis was that soybean biomass production under an elevated [CO2] regime is greater in a genotype with a higher capacity for N fixation. Irrespective of genotype, [CO2] elevation enhanced biomass at maturity except in En1282 under –N and L temperature in 2005 (Table 7). Particularly, the enhancement was more pronounced in the nodulating genotypes (Enrei and Kanto 100) than in the non-nodulating genotype (En1282), indicating that N supply through N fixation contributes to elevated [CO2]-induced biomass production in soybean. However, the relative responsiveness was not consistent between the two nodulating genotypes; the relative values were higher in Enrei under the –N and H temperature treatment in 2004 and 2005, while they were highest in Kanto 100 under the +N and H treatments (Table 7). Thus the responsiveness to elevated [CO2] did not consistently differ among the two nodulating genotypes. In this context, our hypothesis was not verified.

Elevation of [CO2] tended to increase photosynthetic rates irrespective of genotype, but it was not significant at the 0.05% probability level (Fig. 4). Kanto 100 exhibited a slightly higher photosynthetic rate than Enrei at 35 DAS, but the genotypic difference was negligible at 69 DAS. Previous studies indicated that the enhancement of soybean photosynthetic rates by elevated [CO2] is ranked the highest among the major crops (reviewed by Allen and Boote, 2000). Moreover, the acclimation of photosynthesis (i.e., loss of photosynthetic capacity) in response to elevated [CO2] is not likely to occur in soybean. However, long-term exposure to elevated [CO2], often lessens the enhancement of photosynthetic activity in many other species (Sasek et al., 1985; Tissue and Oechel, 1987). Rogers et al. (2004, 2006) found in a FACE study, that elevated [CO2] (ca. 550 μmol mol⁻¹) increased integrated CO2 assimilation by ca. 25%. In another FACE study by Bernacchi et al. (2005), elevated [CO2] increased light-saturated photosynthesis by 15–20%. Using a genotype that differed by a single gene and consequently altered the capacity of nodulation, Ainsworth et al. (2004) found that the non-nodulating genotype showed down-regulation of photosynthesis under elevated [CO2] (550 μmol mol⁻¹). The increased photosynthetic rates through [CO2] elevation in the present study were not as marked; they were 5.0, 9.5 and 22.8% at 35 DAS; 15.3, 8.6 and 13.2% at 69 DAS, in Enrei, Kanto 100 and En1282, respectively. The reason for the more modest increases in photosynthetic rates in our study compared with the previous studies remains unclear. It is possible that photosynthetic activity was adversely affected by water stress, which could be induced by high evaporative demand under high-temperature conditions, along with stomatal closure due to elevated [CO2] conditions (Pritchard and Amthor, 2005). Restriction of the rhizosphere in the pot-grown plants used in this study might have emphasized the stress compared to the FACE studies, because pot size affects soybean biomass production (Heagle et al., 1999; Booker et al., 2005).

The N-fixing activity estimated by RAD appeared to be enhanced by elevated [CO2] in both nodulating genotypes at 42 DAS, but the difference between the ambient and elevated [CO2] regimes was not significant at the 0.05% probability level (Fig. 3). This slight increase in N-[CO2]fixing activity was due to an increase in activity per nodule, and not to an increase in the number or weight of nodules per plant (data not shown). Since the photosynthetic rate tended

| Table 7. Enhancement of dry matter production by [CO2] elevation for three genotypes. Soybean plants were grown under two [CO2] (ambient (A) and elevated (E)) and temperature (high (H) and low (L)) regimes for two years (2004, 2005). |
|-----------------|-----------------|-----------------|-----------------|-----------------|
| Genotype       | 2004, H         | 2005, H         | 2005, L         |
|                | –N +N           | –N +N           | –N +N           |
| Enrei          | 17.3 (40)       | 11.0 (21)       | 8.8 (30)        | 2.1 (7)         | 0.1 (0)         | 2.9 (12)        |
| Kanto 100      | 11.2 (19)       | 25.6 (40)       | 7.6 (18)        | 7.0 (16)        | 8.9 (24)        | 5.5 (16)        |
| En1282         | 1.0 (8)         | 0.7 (3)         | 0.1 (3)         | 1.6 (15)        | –0.4 (0)        | 0.6 (8)         |

Values (g plant⁻¹) are the difference in top dry weight at maturity between the plants grown under E and A regime, and percent increase of E regime are shown in parentheses.
to be enhanced by [CO₂] enrichment (Fig. 4), the nodules might have received more photosynthate in elevated [CO₂], and thereby the N-fixing activity of nodulating genotypes might be more enhanced at elevated [CO₂] than at ambient [CO₂] at least during a vegetative growth stage (42 DAS). Although the plants grown at elevated [CO₂] had lower N concentrations than those grown at ambient [CO₂], irrespective of genotype (Table 6), which is consistent with the previous report (Rogers et al., 2006), N concentration of Kanto 100 remained markedly high even at elevated [CO₂]. Therefore, mutual enhancement of N fixation and photosynthesis during the vegetative growth stage appeared to be most pronounced in Kanto 100. However, this enhancement of ARA was not observed at the reproductive stage (69 DAS) in either genotype.

As described above, the enhancement of N acquisition induced by elevated [CO₂] and high temperature resulted in an increase in biomass production, irrespective of cultivar. However, the efficiency of acquired N utilized for biomass production could vary with the genotype. Fig. 5 shows the relationship of N content (ΔN) with increment of whole plant dry weight (ΔDW) (A), and nitrogen utilization efficiency (NUE) (B) during a period from 35 to 69 DAS for three genotypes. The plants of each genotype grown under ambient and elevated [CO₂] regimes were plotted on the same line, and the regression equations for each genotype were all significant (P<0.01). This indicated that [CO₂] elevation enhanced biomass production through a corresponding increase in N acquisition, but not necessarily through improved efficiency of dry matter production per increased N accumulation. A comparison of the regression lines of the relationship between ΔN and ΔDW revealed that steepness of the slope was in the following order: En1282 > Enrei > Kanto 100, indicating that N utilization efficiency (NUE) to produce dry matter is lower in the nodulating genotypes, particularly in the supernodulating genotype. This phenomenon appears to explain why the supernodulating genotype does not exhibit more marked responsiveness to elevated [CO₂] compared to the normally-nodulating genotype.

A possible reason why NUE of supernodulating genotype Kanto 100 was inferior to that of normally or non-nodulating genotypes might be the difference in energy costs between symbiotic N₂ fixation and nitrate uptake. Whole plant energy costs for symbiotic N₂ fixation were found to be greater than for nitrate metabolism (Ryle et al., 1979; Finke et al., 1982). For example, Finke et al. (1982) reported that 8.3 mol of C was respired per mol of N for N₂ fixation, while only 3.2 to 5.0 mol of C was respired per mol of N from nitrate assimilation, depending on the plant age. Thus, it is likely that a genotype with a higher capacity for N₂ fixation, such as Kanto 100, exhibits lower NUE than genotypes with a reduced capacity. Another possibility affecting the genotypic difference in NUE is the difference in N forms between N fixed symbiotically and that assimilated from nitrate. N forms of symbiotically fixed N₂ are predominantly ureides, whereas N forms assimilated from nitrate are predominantly amino acids (Harper, 1987). The different N forms could have different physiological functions and may affect the efficiency of dry matter production. Further studies are required to address these possibilities.

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* In Japanese with English abstract.

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