Korean J. Crop Sci. 49(2): 73~81(2004)

Response of Soybean to Elevated CO_2 Concentrations and Temperatures at Two Levels of Nitrogen Application

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ABSTRACT: Effects of ambient and elevated CO_2 and high temperature, and their interactions with zero and applied nitrogen supply (NN - no nitrogen and AN - applied nitrogen) were studied on soybean (*Glycine max* L.) in 2001. In this experiment, elevated CO_2 (650 μmol· mol^{-1}) and temperature (+ 5°C) increased total dry mass at final harvest by 125% and 119% and seed weight per plant by 57% and 105% for NN and AN plants, respectively. Although the influence of temperature and temperature × CO_2 were not significant, the influences of CO_2 concentration and temperature × CO_2 concentration were significant on total dry weight and seed weight, respectively. In particular, seed weight per plant was increased, while weight per one hundred seed weight was decreased with elevated CO_2 and temperature. The N supply increased biomass and seed weight per soybean plants. The results of this study suggest that the long-term adaptation of soybean growth at an elevated CO_2 concentration and high temperature might potentially result in a increase in dry matter production and yield.

***Keywords*:** soybean, CO_2, temperature, nitrogen, dry matter, yield

The mean global surface temperature is expected to increase about 2 ~ 6°C. In addition, it is predicted that the atmospheric CO_2 concentration may reach two times CO_2 concentration within the 21th century (Burroughs, 2001). The atmospheric CO_2 concentration and temperature could influence on plant growth and crop production (Morison & Lawlor, 1999). Many scientists have suggested that the response of C_3 plants to elevated CO_2 concentration and temperature is significant for future agricultural and biomass production. In particular, determinate plants like cereal crops sometimes exhibit a predicted greater stimulation of production by increased CO_2 and temperature (Fritschi *et al.*, 1999; Rawson, 1995; Wheeler *et al.*, 1994).

A strong positive relationship for biomass accumulation by natural increase or decrease in temperature and stimulation by CO_2 enrichment in four species, including carrot and radish, has been reported (Mitchell *et al.*, 1993; Sionit *et al.*, 1990; Kimball, 1983). The effect of elevated CO_2 concentration on plant growth is primarily due to changes in the composition and dry mass per unit area of leaf. The factors influencing these changes are found to be primarily temperature-dependent.

Beans grown in elevated CO_2 (700 μmol · mol^{-1}) have shown an increase photosynthesis, plant growth, and beans and cereal crops yield (Wu & Wang, 2000; Sims *et al.*, 1999; Lal *et al.*, 1998). By the report of Manderscheid *et al.* (1997), elevated CO_2 concentration did not affect magnesium concentration of the plant cell, but decreased potassium and sulphur concentrations, and also the CO_2 effect on calcium and phosphorus concentration was various depending on harvest date. The previous studies (Heagle *et al.*, 1993; Overdieck, 1993) reported a significant decrease of potassium, but not of magnesium, phosphorus, and calcium under CO_2 enrichment. And Ludewig *et al.* (1998) reported that increased concentrations of CO_2 stimulate photosynthesis, and many species accumulate nonstructural carbohydrates in the leaf under these conditions, and moreover the leaves of plants grown at elevated CO_2 contains nitrogen concentration lower than those at ambient CO_2. By Rogers *et al.* (1996), leaf N concentration per unit leaf dry weight in plants grown at elevated CO_2 (550 and 900 μmol · mol^{-1}) was up to 33% which was lower than those of ambient CO_2. However, these conditions were brought about the lowered activity of Rubisco, the primary carboxylating enzyme in C_3 plants, and the inhibition of photosynthesis due to the feed hack inhibition of gene expression by accumulations of sucrose and starch.

Many scientists only reported increase in agricultural productivity in cotton, white clover and an apple tree under CO_2 enrichment and increased temperature (Ro *et al.*, 2001; Manderscheid *et al.*, 1997; Rogers *et al.*, 1996). However, there is a little information about effect of elevated CO_2 and temperature with two levels of nitrogen applications on soybean during the growth period.

The objectives of this study were to determine the effects of increased CO_2 (650 μmol · mol^{-1}) and temperature (+ 5°C) on the growth and yield of soybean at two levels of nitrogen fertilization.

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MATERIALS AND METHODS

CO_2 and the temperature-controlled closed environment facility for plant growth

The closed environment plant growth facility consisted of 8 experimental units arranged in two rows. Each unit was comprised of a soil compartment (3 × 3 × 3 m), a transparent canopy enclosure (4 × 3 × 6 m), and a utility space for the temperature control unit located on the north side. A weather station measured air temperature, relative humidity, and solar radiation using sensors connected to a data logger (21X, Campbell Scientific, USA). An open architecture, distributed control system (DCS), POREX 6800 (POSCON Institute), developed by POSCON of POHANG Steel Korea was used to control the environment in the facility and to collect the data automatically from the sensors. The POREX 6800 DCS consists of two UNIX-based workstations (SPARCstation 20, Sun Microsystems, USA) that provide user-friendly interfaces for process and engineering operations. It also has a process control station that performs various real-time processing functions for field input/output points and scheduled transfers of real-time data to a central database. Each unit independently controls the atmospheric CO_2 and air temperature in growth facility.

The mixed gas with compressed CO_2 and flow fresh air, depending on the preset CO_2 concentration of the bulk air in the enclosure, was brought into the enclosure using an air blower. The purity of the CO_2 gas was regularly inspected, and chilled or heated water was supplied to the fan coil unit to control the inside temperature of enclosure. Conditioned air passed through the plant canopy with sufficient flux enough to cause slight leaf flutter. An atmospheric CO_2 concentration of ± 0.5°C of an ambient regime was independently controlled in each unit, and reference values for the ambient regime were taken in real-time from a weather station. The solar radiation, air temperatures, and relative humidity inside the enclosure were measured and multiplexed to a 21X data logger. The atmospheric CO_2 concentrations in the enclosure were measured using infrared CO_2 analyzers (ZRH, Fuji Electric, Japan), thus controlling the CO_2 level. Elevated temperature was indicated by a 5°C step above the ambient regime. The data of daily average day time temperatures during the growth of soybeans in the growth facility are shown in Fig. 1.

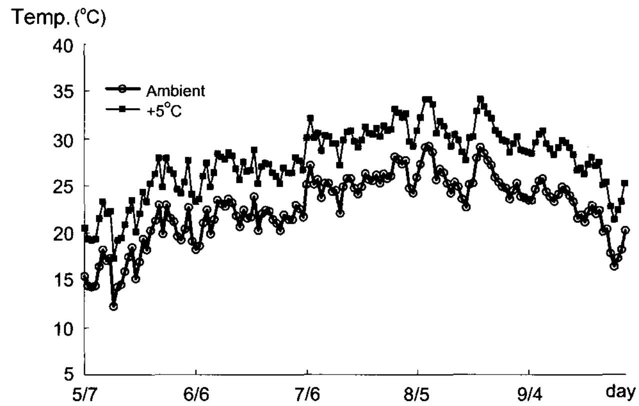

Fig. 1. Average daytime temperatures during the grown period of soybeans in the growth facility.

Plant materials and growth conditions

Soybean seeds (Sinpaldalkong #2) were sown on 7 May 2001 into plastic pots (27 cm in diameter and 30 cm in height) filled with a sandy loam soil. Three seeds were sown in each pot and the nitrogen fertilization was carried out with two levels of concentrations: 40 $kg{\cdot}ha^{-1}$ (nitrogen supply per 10,000m^2) or 0 $kg{\cdot}ha^{-1}$ (no nitrogen supply). Other major nutrient components were applied with same level, with potassium at 60 $kg{\cdot}ha^{-1}$ and phosphate at 70 $kg{\cdot}ha^{-1}$. The pots were placed in the closed environment plant growth facility of the National Horticultural Research Institute in Korea. Plants were exposed to one of two CO_2 concentrations: ambient (360 $\mu mol\ CO_2{\cdot}mol^{-1}$) or elevated (650 $\mu mol\ CO_2{\cdot}mol^{-1}$). This was combined with two temperature regimes: ambient, and elevated temperature throughout the growing season. The chamber received solar radiation with natural day length. In chamber, place of the pots were weekly rotated to remove a chamber effect.

Growth measurements and analysis

The soil was sampled and analyzed in a soil and plant chemical testing laboratory at the National Horticultural Research Institute in Korea. Plants were harvested six times from 20 days after sowing at the intervals of 20 days during the growing season. The harvested plants were separated into leaf, stem, root, nodule, and pod. Total shoot dry weight, seed dry weight per plant, seed number per plant, and average seed dry weight in each pot were determined. Dry weights of all components were obtained following oven drying at 80°C and 72 hours. Leaf area was determined with an electronic planimeter (LI-3100, Li-Cor, NE, USA) and the nitrogen concentrations of leaf and seed were measured using the Kjeldhal method. The soil properties, based on the results of the analysis, are shown in Table 1.

Statistical analysis

Data were evaluated using General Linear Model (GLM)

Table 1. Chemical properties of the pot soil in the experiment.

pH	EC	T-N	OM	P_2O_5	Ex. cations			
					K	Ca	Mg	Na
(1:5)	$dS{\cdot}m^{-1}$	$mg{\cdot}Kg^{-1}$	%	$mg{\cdot}Kg^{-1}$	$cmol^{+}{\cdot}Kg^{-1}$			
6.5	0.6	610.0	1.0	274.9	0.4	4.5	1.6	0.1

procedures and treatment means were compared using Tukey's multiple comparison tests. A two-way analysis of variance (ANOVA) was used to test the main treatment effects (CO_2 and temperature) and the interaction effects of CO_2 × temperature with two levels of nitrogen applications (SAS Institute Inc., 1998).

RESULTS AND DISCUSSION

Plant growth response

At the beginning of the season, the changes of total dry matter showed no significant positive relationship with CO_2 and temperature before 60 days (Fig. 2). However, at later growth stages, highly significant effects on total dry matter was observed, where elevated CO_2 increased total dry matter by 78.9% (with applied nitrogen 91.8%) and higher temperature (+ 5°C) increased total dry mass by 22.9% (with applied nitrogen 12.7%). Since the accumulated biomass represents the sum of whole season photosynthesis and respiration, and it can be considered to be an integrated response of soybean to the elevated CO_2 and high temperature (+ 5°C) treatment.

Emergence was occurred about 14 days after sowing, and data were collected from 20 days after sowing. The 80 days after sowing, pod development to ripening, were occurred in the fastest plant growth among treatments (Fig. 2).

Leaf weight increased rapidly from about 50 days after sowing. In the treatment of elevated CO_2 at both ambient and higher temperature, the total amount of leaves displayed sigmoidal pattern until seed filling stage. The treatment of ambient CO_2 at both ambient and higher temperature displayed a flattened S-type curves, which were slightly different between AN (applied nitrogen) and NN (no nitrogen) (Fig. 3). The stem weight showed in similar tendency at 50 days after sowing (Fig. 4). Dry weights of the leaf, stem, root and nodule were still increased at 100 days after sowing, while dry weight of the pod was increased at maturity. Generally, the increase of dry weight displayed S-type under LL, LH, HL, and HH with two kinds of N-applications, however nodule weight was increased almost straight linear curve at those conditions (Figs. 3, 4, 5, 6, and 7). Pod weight increased very rapidly from 80 days (ambient) and 100 days (CO_2) after sowing (Fig. 6). The changes in plant growth with AN (applied nitrogen) were very similar to NN (no nitrogen).

CO_2 treatment had highly significant effects on total dry weight ($P<0.001$), while temperature treatment and temperature × CO_2 interaction was not significant (Table 2). Total dry weight of aerial part increased with an increasing level of CO_2 and with nitrogen supply, except for nodule weight, which reduced with the N supply (Table 2). Apart from nodule weight, there was a significant effect of CO_2 concentration on leaf weight, stem weight, and root weight (Table 2).

In particular, leaf and stem weights showed the greatest sensitivity to elevated CO_2, increasing by an average of 145 % and 225% over ambient CO_2, respectively (Figs. 3 and 4).

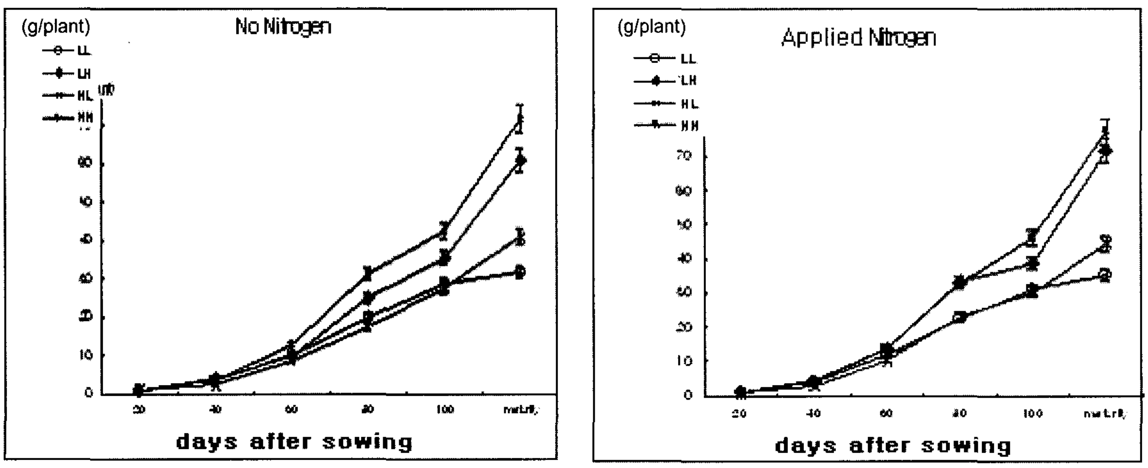

Fig. 2. Changes in plant total dry weight in soybeans grown with different CO_2 concentration and temperature (LL: ambient temperature and 360 μmol $CO_2{\cdot}mol^{-1}$, LH: ambient temperature and 650 μmol $CO_2{\cdot}mol^{-1}$, HL: + 5°C temperature and 360 μmol $CO_2{\cdot}mol^{-1}$, HH: + 5°C temperature and 650 μmol $CO_2{\cdot}mol^{-1}$).

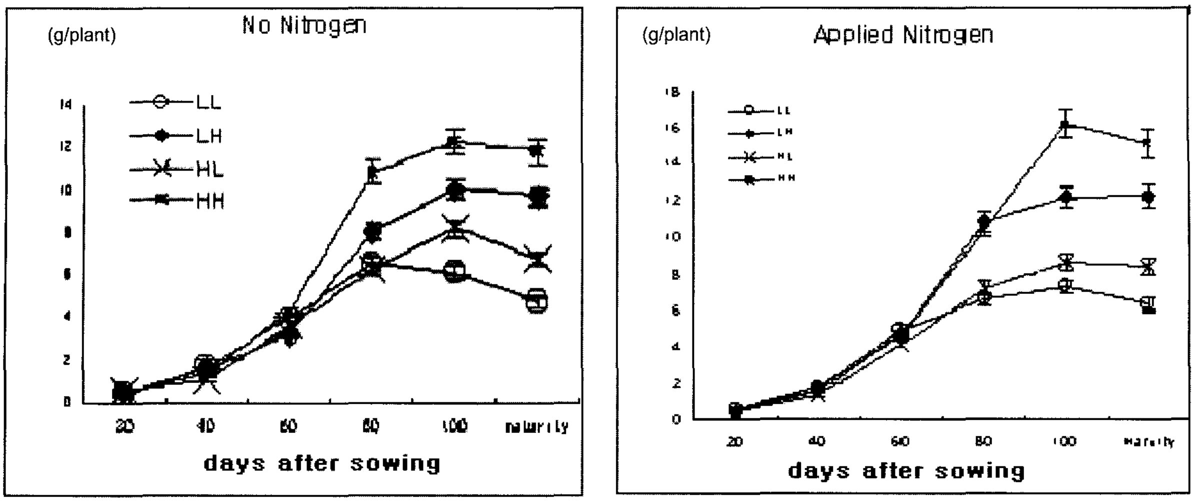

Fig. 3. Changes of leaf weight in soybeans grown in the closed environment plant growth facility. (LL: ambient temperature and 360 μmol $CO_2 \cdot mol^{-1}$, LH: ambient temperature and 650 μmol $CO_2 \cdot mol^{-1}$, HL: + 5°C temperature and 360 μmol $CO_2 \cdot mol^{-1}$, HH: + 5°C temperature and 650 μmol $CO_2 \cdot mol^{-1}$).

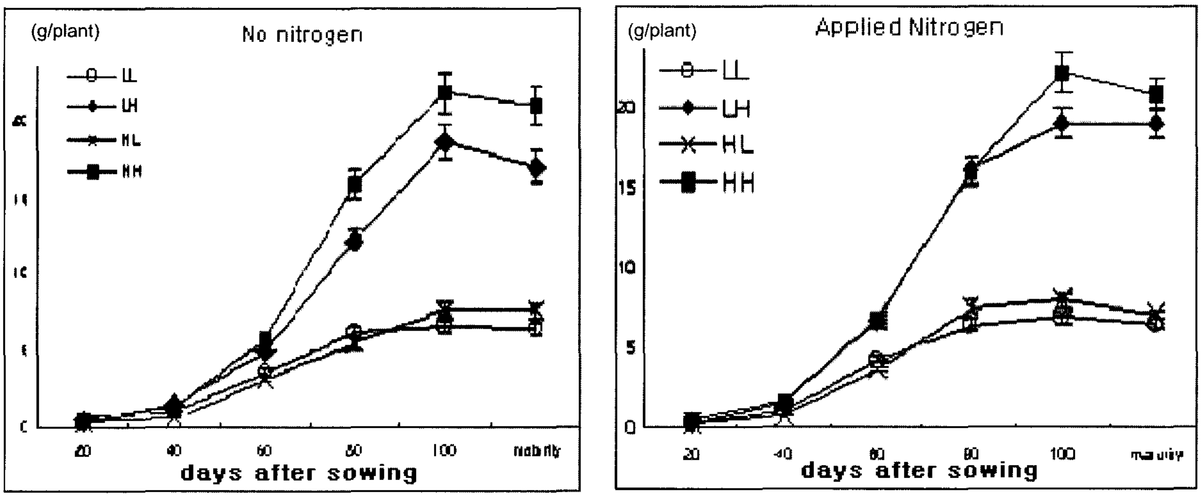

Fig. 4. Changes of stem weight in soybeans grown in the closed environment plant growth facility (LL: ambient temperature and 360 μmol $CO_2 \cdot mol^{-1}$, LH: ambient temperature and 650 μmol $CO_2 \cdot mol^{-1}$, HL: + 5°C temperature and 360 μmol $CO_2 mol^{-1}$, HH: + 5°C temperature and 650 μmol $CO_2 \cdot mol^{-1}$).

The increases in leaf area at high CO_2 were not as great as the increase in leaf and whole plant weight, which is a common response of C_3 plants to CO_2-enriched and high temperature conditions (Rogers *et al.*, 1996; Manderscheid *et al.*, 1997).

The experiment showed a significant increase in total dry weight, leaf area, plant height, and seed weight in response to elevated CO_2 and temperature conditions (Table 3). Subsequently, the seed weight per plant increased, while 100 seeds weight decreased with elevated CO_2 and temperature. In particular, the nitrogen supply increased biomass and seed weight per plant. The results suggest that the long-term adaptation of soybean growth to the elevated CO_2 concentration and high temperature may potentially increase growth and yield.

The positive yield response of soybean to CO_2 enrichment was closely associated with an increase in the number of seeds per plant. This is supported by earlier findings that an increase in yield with high CO_2 and temperature was largely resulted from an increase in grain number (Jennifer & Daniel, 1988).

In the present experiment, the seed numbers per pod were relatively stable, but the seed numbers per plant were affected by applied nitrogen. However, 100 seeds weight decreased with elevated CO_2 and temperature. A slight decrease in 100 seeds weight of plants in the LH-treatment,

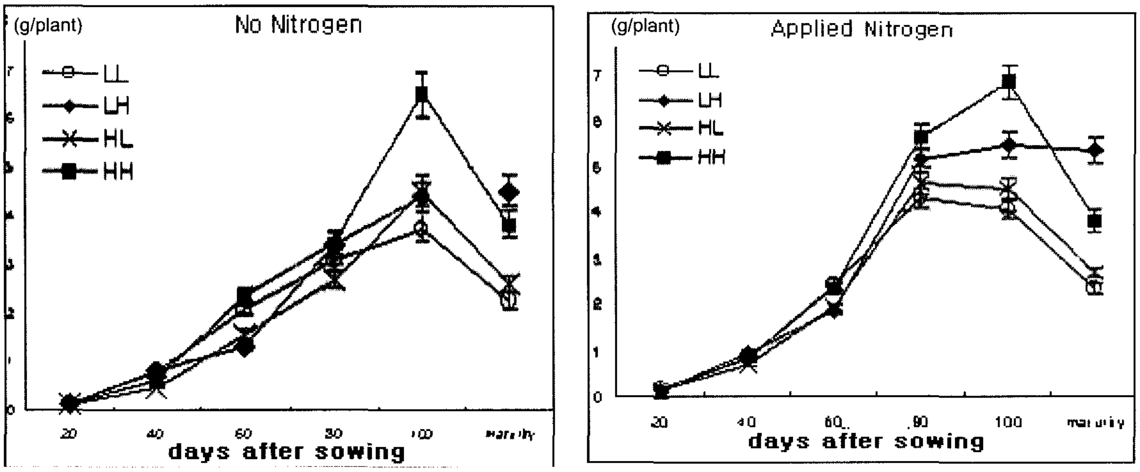

Fig. 5. Changes of root weight in soybeans grown in the closed environment plant growth facility (LL: ambient temperature and 360 μmol CO_2·mol^{-1}, LH: ambient temperature and 650 μmol CO_2·mol^{1}, HL: + 5°C temperature and 360 μmol CO_2·mol^{-1}, HH: + 5°C temperature and 650 μmol CO_2·mol^{-1}).

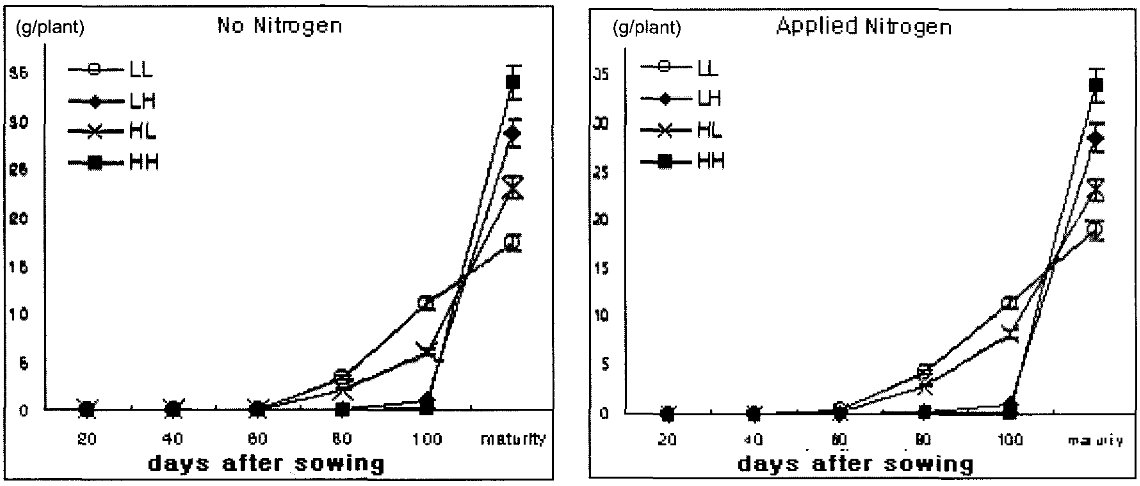

Fig. 6. Changes of pod weight in soybeans grown in the closed environment plant growth facility (LL: ambient temperature and 360 μmol CO_2·mol^{-1}, LH: ambient temperature and 650 μmol CO_2·mol^{-1}, HL: + 5°C temperature and 360 μmol CO_2·mol^{-1}, HH: + 5 temperature and 650 μmol CO_2·mol^{-1}).

elevated CO_2 and ambient temperature, was also observed. A higher number of seeds per plant were observed in HH and HL (ambient + 5°C and 650 μmol·mol^{-1}; ambient + 5°C and 360 μmol·mol^{-1}) compared to the control (LL: ambient and 360 μmol·mol^{-1}). Elevated CO_2 and increasing air temperature were also associated with an increase in final node number on main stem and branches (Jones *et al.*, 1984) (Table 3). Baker & Allen (1989) also reported an increase in the final node number on main stem for soybean resulting from CO_2 enrichment, as well as increases in final leaf number with increasing air temperature.

Generally, nodulation is needed to stimulate by a micro quantity nitrate at the early stage. However, this study showed that nodule development on the roots was much more prolific in NN was much more prolific than AN (Table 2). Baker & Allen (1989) found increasing soybean development rates with increasing temperature and CO_2 treatments. These results, and those reported by Baker & Allen (1989), indicated that across the temperature and CO_2 regimes studied thus far, temperature influences soybean developmental rates to a far greater degree than CO_2 concentration. However, this experiment showed a different result. Days to flowering appeared to be more strongly influenced by the CO_2 treatment than by air temperature; reproductive development began earlier in the ambient CO_2 (360 μmol·mol^{-1}) treatment than the elevated CO_2 (650 μmol·mol^{-1}) treatment.

In the current study, the vegetative response at flowering was not a good predictor of seed yield sensitivity to elevated CO_2 and temperature. In addition, partitioning among the

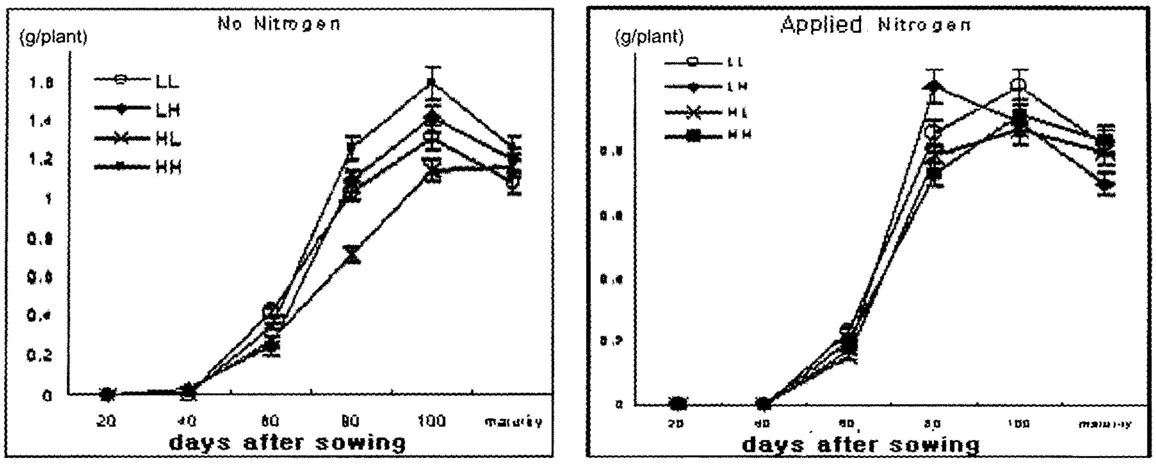

Fig. 7. Changes of nodule weight in soybeans grown in the closed environment plant growth facility (LL: ambient temperature and 360 μmol $CO_2 \cdot mol^{-1}$, LH: ambient temperature and 650 μmol $CO_2 \cdot mol^{-1}$, HL: + 5°C temperature and 360 μmol $CO_2 \cdot mol^{-1}$, HH: + 5°C temperature and 650 μmol $CO_2 \cdot mol^{-1}$).

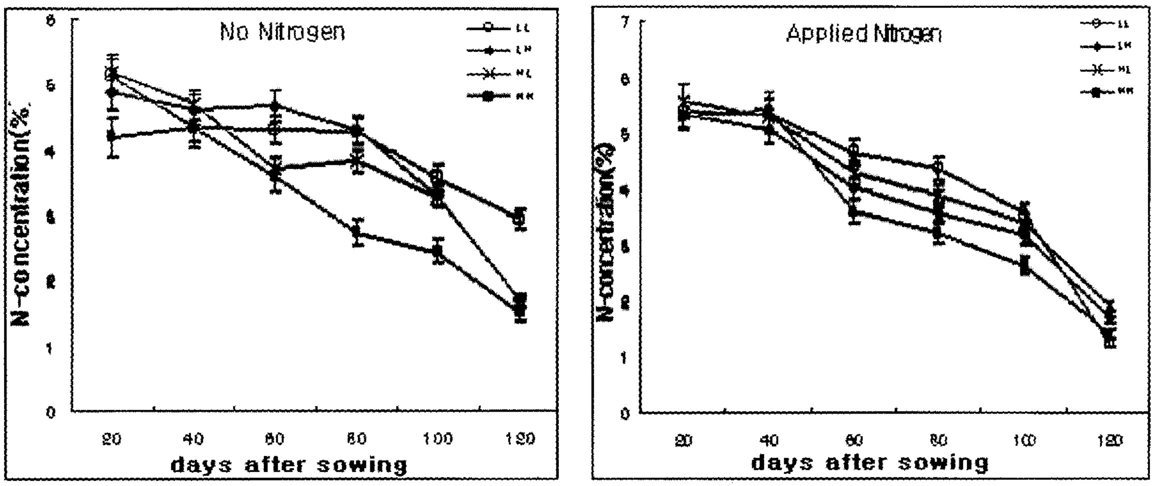

Fig 8. Changes in the N concentration in the leaf of soybeans grown in the closed environment plant growth facility (LL: ambient temperature and 360 μmol $CO_2 \cdot mol^{-1}$, LH: ambient temperature and 650 μmol $CO_2 \cdot mol^{-1}$, HL: + 5°C temperature and 360 μmol $CO_2 \cdot mol^{-1}$, HH: + 5°C temperature and 650 μmol $CO_2 \cdot mol^{-1}$).

vegetative organs, or changes in determinacy or plant architecture were also not correlated with the relative sensitivity of seed yield to elevated CO_2 (Zerihun & BassiriRad, 2000; Ludewig *et al.*, 1998; Manderscheid *et al.*, 1997).

Nutrient concentrations and other macronutrients in the seed and leaf

Nutrient concentrations of harvested soybean seed are summarized in Table 5. Elevated CO_2 and temperature did not affect nitrogen, phosphorus, and potassium concentration in the seed, but decreased the concentrations of calcium and magnesium under elevated CO_2, and it is increased in calcium and magnesium under + 5°C temperature (Table 5). Also there was no significant temperature × CO_2 interaction for all nutrients except nitrogen concentration under applied nitrogen treatment (Table 5). Approximately, change of the N concentration in the soybean leaf was expressed as a decreasing curve (Fig. 8), with a severely larger decrease at 80 days after sowing. Rogers et al. (1996) reported that the plants grown under no nitrogen treatment had higher nitrogen concentrations in leaf than those of applied nitrogen treatment under ambient temperature and CO_2, and then this fact was accorded with the result of this study. Also soybeans grown at elevated CO_2 had a lower leaf nitrogen concentration compared to those of ambient CO_2 (Fig. 8).

In general P, K, Ca, and Mg were decreased in soybean seeds under all conditions, liked to nitrogen concentration in

Table 2. Influence of atmospheric temperature and CO_2 concentration at two levels of nitrogen supply on leaf weight, stem weight, root weight, and nodule weight of 120-day-old soybean plants.

Temperature	CO_2 concentration (μmol·mol^{-1})	Leaf Weight (g)		Stem Weight (g)		Root Weight (g)		Nodule Weight (g)		Total dry Weight (g)	
		NN†	AN‡	NN	AN	NN	AN	NN	AN	NN	AN
Ambient	360	4.8	6.4	6.4	6.5	2.3	2.3	1.1	0.8	14.6	16.0
	650	9.7	12.3	17.0	19.0	4.5	5.3	1.2	0.7	33.0	37.3
Ambient + 5°C	360	6.8	8.4	7.1	7.6	2.6	2.7	1.2	1.1	17.7	19.8
	650	11.8	15.1	20.8	20.9	3.8	5.0	1.3	0.8	37.7	41.8
$LSD_{0.05}$		1.3	1.7	1.7	1.6	0.6	0.3	0.1	0.1	13.6	15.4
ANOVA											
Temperature		**	**	*	ns	ns	ns	ns	**	ns	ns
CO_2 concentration		***	***	***	***	***	***	ns	**	***	***
Temperaturex CO_2 concentration		ns	ns	ns	ns	ns	ns	ns	ns	ns	ns

†NN: no nitrogen (0 Kg·ha^{-1}), ‡AN: applied nitrogen (40 Kg·ha^{-1})
a ***P<0.001, ** P<0.01, *P<0.05, ns: not significant

Table 3. Influence of atmospheric temperature and CO_2 concentration at two levels of nitrogen supply on leaf area, number of branches, number of nodes, and plant height of 120-day-old soybean plants.

Temperature	CO_2 concentration (μmol·mol$^{-1)}$	Leaf area (cm^2)		Number of branch		Number of node		Plant height (cm)	
		NN†	AN‡	NN	AN	NN	AN	NN	AN
Ambient	360	1530	1702	4.3	3.7	8.8	9.3	37.2	37.1
	650	2470	2270	6.5	6.0	13.0	13.7	67.9	71.6
Ambient + 5°C	360	1946	2031	4.8	4.8	8.8	8.8	41.8	42.0
	650	2236	2617	6.3	7.2	12.3	13.0	55.8	55.2
$LSD_{0.05}$		433	513	1.8	1.2	1.2	1.1	9.2	7.7
ANOVA									
Temperature		ns	ns	ns	*	ns	ns	ns	ns
CO_2 concentration		*	*	*	***	***	***	***	***
Temperature × CO_2 concentration		ns	ns	ns	ns	ns	ns	ns	***

†NN: no nitrogen (0 Kg·ha^{-1}), ‡AN: applied nitrogen (40 Kg·ha^{-1})
a ***P<0.001, **P<0.01, *P<0.05, ns: not significant

Table 4. Influence of atmospheric temperature and CO_2 concentration at two levels of nitrogen supply on pod numbers, pod weight, number of seeds, seed weight, and 100 seeds weight of final harvest soybean plants.

Temperature	CO_2 concentration (μmol·mol^{-1})	number of pods/ plant		Pod Weight/ plant (g)		Number of seeds/ plant		Seed weight/ plant(g)		100 seeds weight (g)	
		NN†	AN‡	NN	AN	NN	AN	NN	AN	NN	AN
Ambient	360	33.2	33.3	17.3	19.0	68	74	10.1	11.6	14.9	15.6
	650	54.8	73.0	28.7	34.2	105	131	20.5	24.2	18.4	19.4
Ambient + 5°C	360	40.7	52.0	23.2	24.8	78	95	14.3	15.6	16.4	18.2
	650	72.8	98.7	34.0	35.3	124	176	15.9	23.8	13.5	12.8
$LSD_{0.05}$		12.6	15.1	1.1	1.4	9.6	10.7	0.4	0.5	1.1	1.3
ANOVA											
Temperature		**	ns	***	***	**	***	ns	***	**	**
CO_2 concentration		***	**	***	***	***	***	***	***	ns	ns
Temperature×CO_2 concentration		ns	ns	ns	**	ns	*	***	***	***	***

†NN: no nitrogen (0 Kg·ha^{-1}), ‡AN: applied nitrogen (40 Kg·ha^{-1})
a: ***P<0.001, **P<0.01, *P<0.05, ns: not significant

Table 5. Influence of atmospheric temperature and CO_2 concentration at two levels of nitrogen supply on seed nutrient concentrations.

Temperature	CO_2 concentration ($\mu mol \cdot mol^{-1}$)	N		P		K		Ca		Mg	
		NN†	AN‡	NN	AN	NN	AN	NN	AN	NN	AN
		$g \cdot kg^{-1}$									
Ambient	360	54	57	14	15	29	28	5.1	5.5	3.7	3.6
	650	57	60	14	13	29	25	3.4	3.1	2.4	3.1
Ambient +5°C	360	53	66	15	12	29	28	5.4	5.4	3.6	4.4
	650	52	53	15	17	27	27	4.3	4.3	2.2	3.4
$LSD_{0.05}$		6.1	8.4	0.7	0.6	1.1	1.3	0.3	0.2	0.2	0.2
ANOVA											
Temperature		ns	ns	ns	ns	ns	ns	**	**	**	**
CO_2 concentration		ns	ns	ns	ns	ns	ns	**	**	**	**
Temperature × CO_2 concentration		ns	*	ns	ns	ns	ns	ns	ns	ns	ns

†NN: no nitrogen (0 $Kg \cdot ha^{-1}$), ‡AN: applied nitrogen (40 $Kg \cdot ha^{-1}$)
a: ***P<0.001, **P<0.01, *P<0.05, ns: not significant

parts of the leaf at both no and applied nitrogen treatments, but only nitrogen was increased in soybean seeds under ambient temperature and elevated CO_2, respectively. Among the nutrients, Ca and Mg were highly correlated with temperature or CO_2 concentration and N-fertilizer application level. Also nutrients including Ca and K in seeds were not correlated with nitrogen application or not, while nitrogen concentrations in seeds were increased by nitrogen application, and showed a higher increase at elevated temperature and ambient CO_2, especially (Table 5). These results indicated that the fertilization practices should be modified with changing temperature and CO_2 concentrations.

ACKNOWLEDGEMENT

This paper was supported by Konkuk University in 2004.

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