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CO_2 및 온도 상승 시 벼의 수량, 질소 이용 효율 및 질소 흡수 반응

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Yield, Nitrogen Use Efficiency and N Uptake Response of Paddy Rice Under Elevated CO_2 & Temperature

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ABSTRACT

Due to the acceleration of climate change or global warming, it is important to predict rice productivity in the future and investigate physiological changes in rice plants. The research aimed to explore how rice adapts to climate change by examining the response of nitrogen absorption and nitrogen use efficiency in rice under elevated levels of carbon dioxide and temperature, utilizing the SPAR system for analysis. The temperature increased by +4.7 °C in comparison to the period from 2001 to 2010, while the carbon dioxide concentration was held steady at 800 ppm, aligning with South Korea's late 21st-century RCP8.5 scenario. Nitrogen was applied as fertilizer at rates of 0, 9, and 18 kg $10a^{-1}$, respectively. Under conditions of climate change, there was an 81% increase in the number of panicles compared to the present situation. However, grain weight decreased by 38% as a result of reduction in the grain filling rate. BNUE, indicative of the nitrogen use efficiency in plant biomass, exhibited a high value under climate change conditions. However, both NUEg and ANUE, associated with grain production, experienced a notable and significant decrease. In comparison to the current conditions, nitrogen uptake in leaves and stems increased by 100% and 151%, respectively. However, there was a 25% decrease in nitrogen uptake in the panicle. Likewise, the nitrogen content and NDFF (Nitrogen Derived from Fertilizer) in the sink organs, namely leaves and roots, were elevated in comparison to current levels. Therefore, it is imperative to ensure resources by mitigating the decrease in ripening rates under climate change conditions. Moreover, there seems to be a requirement for follow-up research to enhance the flow of photosynthetic products under climate change conditions.

***Key words*: SPAR, Elevated CO_2, Elevated temperature, NUE, NDFF**

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I. Introduction

According to the 'South Korea Detailed Climate Change Outlook Report' based on the IPCC 6th Report (AR6), the late 21st-century annual mean temperature in South Korea is projected to increase by 2.3 to 6.3 ℃ compared to the current period (2000-2019), accelerating on greenhouse gas emissions levels. Additionally, the amount of precipitation is anticipated to rise by 4-16% (Kim *et al.*, 2022). South Korea's average temperature has notably surpassed the global average, experiencing a 1.5 ℃ increase, more than twice the global average temperature rising of 0.74 ℃ (Kwon, 2005). The particularly pronounced increase in winter temperatures is expected to significantly impact the agricultural ecosystem by altering the natural system, leading to shorter winters and longer springs and summers.

According to the Korea Meteorological Administration, in comparison to the past 30 years (1912-1940), the recent onset dates of spring and summer have shown a tendency to occur 17 and 11 days earlier, while the start dates of fall and winter have tended to be 9 and 5 days later. As a result, summer has extended by 20 days, and winter has shortened by 22 days. Consequently, it is anticipated that summer will increase by 64 days in the 2090s, reaching 109 days in the 2020s and 173 days in the 2090s (KMA, 2023). Regarding current rice cultivation, the rising temperatures have led to a shift of transplanting days approximately 5 days earlier than in 1990. It is expected that future temperature increases will pose a significant threat, potentially causing severe damage in ripening stage due to high-temperature.

The primary meteorological factors significantly influencing crop growth are temperature and CO_2 levels. Elevated temperatures tend to stimulate plant vegetative growth. However, research findings indicate that a shortened plant season, increased infertility rates due to high temperatures, and reduced ripening rates can lead to decreased yields (Ziska *et al.*, 1997). Conversely, an increase in CO_2 concentration has positive effects on plants. When CO_2 levels reach 660 ppm, rice yields are reported to increase by an average of 20%, the number of panicles increases by 12%, and the number of grains increases by 15%, attributed to enhanced photosynthesis (Kim *et al.*, 2003; Wang *et al.*, 2015). Studies have demonstrated that under elevated CO_2 conditions, nitrogen accumulation in plants influences biomass and contributes to increased rice yields alongside carbon accumulation. However, there are reports indicating a decrease in nitrogen concentration in plants and an increase in nitrogen use efficiency (Kim *et al.*, 2011; Wang *et al.*, 2012; Wang *et al.*, 2015).

It is known that nitrogen serves as a limiting factor for plant growth in an elevated CO_2 environment (Wang *et al.*, 2015). Crops acquire ammonium nitrogen and nitrate nitrogen from the soil, and in this process, variability increases due to the impact of elevated CO_2 and temperature on the dissolved nitrogen form in the soil (Pendall *et al.*, 2004). Consequently, it is crucial to assess plant absorption and utilization under diverse climate change conditions and nitrogen levels to deduce the optimal fertilization quantity that can enhance productivity. Currently, there is a gap in research regarding the analysis of physiological changes in rice productivity and nitrogen fertilization under the altered climate conditions expected in the late 21st century in South Korea.

This study examined changes in nitrogen use efficiency and absorption of rice under climate change conditions, and examined whether there was an effect on biomass and grain production. The climate of the late 21st century was simulated based on the RCP8.5 scenario which is elevated CO_2 and temperature using SPAR system, and nitrogen amounts were applied differently accordingly. The trend of nitrogen absorption during the ripening period was analyzed by site, and in particular, the movement of nitrogen applied at panicle initiation was analyzed using the 15N-urea isotope. In addition, the effect of nitrogen on yield in the future climate was analyzed through analysis of nitrogen use efficiency and yield components, and the nitrogen utilization of rice was

Table 1. CO_2 concentration and Mean air temperature in SPAR system, (ECET) elevated CO_2 concentration and elevated temperature during rice growing season, (ACAT) ambient CO_2 concentration and ambient temperature during growing seasons

Climate	CO_2 (ppm)	Mean Air Temperature (℃)											
		June		July			August			September			October
		Mid	Later	Earlier	Mid	Later	Earlier	Mid	Later	Earlier	Mid	Later	Earlier
ACAT	400	22.9	24.1	25.4	26.1	27.0	27.6	26.5	25.3	24.1	22.8	20.2	17.9
ECET	800	27.6	28.8	30.1	30.8	31.7	32.3	31.2	30.0	28.8	27.5	24.9	22.6

* ACAT was set to the average temperature at each time in Jeonju, from 2001 to 2010, and ECET was set to CO_2 & temperature based on the RCP8.5 scenario for the Jeonju reported by the Korea Meteorological Administration.

evaluated in the future climate.

II. Materials and Methods

2.1. Weather and Fertilization design using SPAR System

The study, conducted in 2021, utilized the SPAR (Soil-Plant-Atmosphere-Research) facility operated by the National Institute of Crop Science in Wanju-gun, Jeollabuk-do. SPAR is a specialized facility designed to precisely assess crop growth by dynamically adjusting CO_2 concentration and temperature on an hourly basis while utilizing natural light conditions (Allen *et al.*, 2020; Sang *et al.*, 2020). Real-time analysis and confirmation of the required CO_2 concentration settings were carried out using the LI-820 (Li-COR, USA). The LI-820 measures CO_2 concentration at 1-second intervals to maintain constant levels, and it is linked to the supply solenoid control system for feedback. The SPAR facility's exterior is constructed of plexiglass material, enabling the utilization of natural solar radiation, and it boasts a light transmittance rate exceeding 94%.

Temperature data for Jeonju-si from 2001 to 2010 were acquired from the Korea Meteorological Administration's historical records, averaged at 10-day intervals. These values served as the baseline for the control group, with a set CO_2 concentration of 400 ppm. For the RCP8.5 scenario, it is anticipated that Jeonju-si will experience an average temperature increase of 4.7 °C between 2071 and 2100 compared to the period from 2001 to 2010. To simulate climate change conditions, the temperature of the control group was adjusted upward by 4.7 °C for each time period, and the CO_2 concentration was set at 800 ppm (Table 1). The specific values for temperature and CO_2 were determined based on the detailed map of South Korea available on the climate information portal operated by the Korea Meteorological Administration (KMA, 2023). To ensure accuracy, the temperature error was maintained within a maximum of 0.2 ℃, and the CO_2 concentration was kept within a maximum error of 10%.

The fertilization parameters were established at three levels: N0 (no fertilizer: 0 kg $10a^{-1}$), N9 (standard fertilizer: 9 kg $10a^{-1}$), and N18 (twice the standard amount: 18 $10a^{-1}$). At the panicle initiation stage, ^{15}N labeled urea was applied, with concentrations tailored to each specific treatment.

2.2. Rice cultivation and Nitrogen fertilization

The rice variety selected for the study was *Oryza Sativa* L. cv. Shindongjin, a variety currently popularly cultivated in Korea. Transplanting was conducted on June 10th, precisely 20 days after sowing, with a planting density of 30 × 14.5 cm and a total area of 1.6 m². Nitrogen fertilization was implemented at rates of 0, 9, and 18 kg 10^{-1} for each treatment. Throughout the rice growth season, urea fertilizer was applied at 4.5 kg 10^{-1} at the basal, 1.8 kg 10^{-1} at the mid-tillering stage, and 4.8 kg 10^{-1} at the panicle initiation stage.

Given that nitrogen fertilization at the panicle initiation stage has the most significant impact on grain production compared to fertilization at other stages, ^{15}N labeled urea (Urea-$^{15}N_2$, SIGMA- ALDRICH, co, USA) was used to determine concentration and uptake contents. P_2O_5-K_2O were fertilized at the standard rate of 4.5-6 kg 10a^{-1} (Table 2).

Table 2. Climate (temperature and CO_2) and Nitrogen fertilizer contents for each condition in SPAR system, (ECET) elevated CO_2 concentration and elevated temperature, (ACAT) ambient CO_2 concentration and ambient temperature

Code	ACAT			ECET		
Temperature (℃)	Ambient			Ambient + 4.7℃		
CO_2 (ppm)	400			800		
N (kg 10a^{-1})	0	9	18	0	9	18

2.3. Analysis

2.3.1. Reserch on rice growth

For the rice yield survey, over 10 hills were harvested 40 days after heading. Various parameters were measured, including rice yields, the number of panicles per hill, the number of spikelets per panicle, and the ripened grain rate. To conduct a thorough analysis, the leaves, stems, and roots were carefully separated, dried, and their weights were measured and calculated. This approach aimed to provide a detailed assessment of the different components contributing to rice yield and growth, allowing for a comprehensive understanding of the plant's response under the experimental conditions.

2.3.2. Nitrogen uptake contents and utilization Efficiency (NUE)

For each treatment, plant samples were collected in triplicate at specific stages: heading days, 10 days after heading, 20 days after heading, and maturity. The collected samples were then segregated into leaves, stems, panicles, and roots. Subsequently, they were subjected to a drying process for three days in a dryer set at 70 °C, followed by grinding. The total nitrogen concentrations were determined using an elemental analyzer (Primacs SNC-100, Skalar, Netherlands). Nitrogen uptake content was calculated by multiplying the dry matter weight of each plant part by its respective nitrogen concentration. The calculation of nitrogen use efficiency (NUE) was analyzed using the formula described below (Cassman *et al*., 1998; Craswell and Godwin, 1984; Mahajan *et al*., 2010; Wang *et al*., 2020).

2.3.3. The Ratio of Nitrogen derived from fertilizer at panicle initiation (NDFF)

To determine NDFF values, samples were separated into spikes, stems, and leaves at maturity. The ^{15}N labeled urea was analyzed using a Stable Isotope Ratio Mass Spectrometer System (Elemental Analyzer, Isoprime, UK). Using the calculation formula provided below, the nitrogen derived from fertilized

$$\text{Biomass N use efficiency (BNUE)} = \frac{\text{Biomass dry weight at treat plot} - \text{Biomass dry weight at treat N0}}{\text{N uptake at treat Nx} - \text{N uptake at N0}} \qquad \text{(eq. 1)}$$

$$\text{Physiological N use efficiency (PNUE)} = \frac{\text{Grain yield at treat plot} - \text{Grain yield at treat N0}}{\text{plant N uptake at Nx} - \text{N uptake at N0}} \qquad \text{(eq. 2)}$$

$$\text{Agronomic N use efficiency (ANUE)} = \frac{\text{Grain yield at treat plot} - \text{Grain yield at treat N0}}{\text{Quantity of N applied}} \qquad \text{(eq. 3)}$$

$$\text{Recovery efficiency (RE)} = \frac{\text{Total N uptake at treat plot} - \text{Total N uptake at N0}}{\text{Quantity of N applied}} \qquad \text{(eq. 4)}$$

$$\text{Nitrogen use efficiency of grain (NUEg)} = \frac{\text{Grain yield}}{\text{Quantity of N applied}} \qquad \text{(eq. 5)}$$

$$\text{NDFF (\%)} = (\text{Plant 15N atom \%} - 0.3663)\ /\ (\text{Fertilizer 15N atom \%} - 0.3663) \times 100 \qquad \text{(eq. 6)}$$

$$\text{The amount of 15N labeled N (g hill}^{-1}) = \text{NDFF}\ /\ 100 \times \text{Total N in plant (g hill}^{-1}) \qquad \text{(eq. 7)}$$

$$\text{RE} - \text{15N (\%)} = \text{The amount of 15N labeled N}\ /\ \text{the amount of N applied} \times 100 \qquad \text{(eq. 8)}$$

(NDFF) ratio, the uptake amount of fertilized 15N, and the Recovery Efficiency of 15N-labeled fertilizer (RE-15N) were computed (Lee and Ryu, 1994; Lenka *et al*., 2019). In the formula, 0.3663 represents the percentage of 15N atoms naturally occurring in nature.

2.4. Statistical analysis

The experimental data of this study was repeated three times per treatment, and ANOVA was performed with climate conditions and nitrogen concentration as factors, and Tukey's HSD was performed as a post hoc test ($P < 0.05$). Statistical analysis was performed using R studio (ver. 4.0.2).

III. Results

3.1. Rice yield and biomass change under elevated CO_2 and Temperature

In the climate change condition (referred to as ECET) with the N9 treatment, the grain weight was 25.2 g hill^{-1}, indicating a twofold reduction compared to the current climate condition (referred to as ACAT). Conversely, there was a significant increase in grain weight under the N18 treatment, where nitrogen fertilization was higher (Table 3). While there was no statistical difference in the number of spikelets per panicle under ECET, the number of spikelets per unit area increased by 75%. However, due to a substantial reduction in the ripening rate, approximately 29-33% lower in ECET compared to the control, the overall grain weight was reduced by about 30%.

In ECET, the dry weight of leaves and stems exhibited a significant increase, and this trend continued with higher fertilization amounts. In comparison to N0, N9 showed a two-fold increase, and N18 demonstrated a three-fold increase, indicating a notable enhancement in vegetative growth organs (Table 3). Contrary to the ACAT, the harvest index experienced a 47% decrease in ECET, attributed to the rise in vegetative growth organs and the reduction in grain weight. The leaf-stem-panicle ratio per hill was 14-25-61 in ACAT-N9, reflecting the current climate standard plot. However, in ECET-N9, representing the future climate standard plot, this ratio shifted to 17-51-33. The increase in the proportion of stems was significant, and while the proportion of leaves increased slightly, the proportion of panicles within each individual decreased (Fig. 1. (a)).

Under N9 conditions, the spikelet rate, which was 33% in ACAT, experienced a significant decrease of

Table 3. Agricultural characteristics of rice under elevated CO_2 and temperature and nitrogen levels

Climate	Nitrogen (kg 10^{-1})	Dry Matter Weight (g hill^{-1})		Rough Rice Weight (g hill^{-1})	The No. of panicle (EA hill^{-1})	The No. of spikelet (EA Panicle^{-1})	Ripened grain rate (%)	HI
		Leaf	Stem					
ACAT	N0	5.4 ± 1.11 bz	10.1 ± 1.60 bz	23.0 ± 2.07 az	8 ± 0.8 bz	102 ± 17.3 z	98 ± 0.5 ay	60 ± 1.6 ax
	N9	12.2 ± 1.21 by	21.2 ± 2.27 by	51.9 ± 4.24 ay	13 ± 1.8 by	157 ± 21.2 y	95 ± 1.8 az	62 ± 0.3 ay
	N18	16.1 ± 2.36 bx	34.5 ± 5.80 bx	53.0 ± 7.09 ax	17 ± 2.6 bx	133 ± 24.8 y	97 ± 0.6 ay	54 ± 1.9 az
ECET	N0	7.3 ± 0.98 az	23.8 ± 3.64 az	17.8 ± 1.60 bz	16 ± 1.8 az	110 ± 16.5 z	31 ± 5.8 by	35 ± 1.2 bx
	N9	13.7 ± 3.19 ay	41.2 ± 9.08 ay	25.2 ± 2.45 by	20 ± 4.3 ay	154 ± 26.4 y	33 ± 13.4 bz	30 ± 1.2 by
	N18	21.7 ± 3.76 ax	59.1 ± 7.07 ax	35.3 ± 2.35 bx	28 ± 4.9ax	142 ± 22.9 y	28 ± 9.6 by	29 ± 0.5 bz
P-value	*Climate*	*	***	***	***	NS	***	***
	N*itrogen*	***	***	***	***	***	***	***
	I*nteraction*	NS	NS	***	NS	NS	*	***

* Different letters indicate significant differences between mean values at $P<0.05$ level using the HSD test after two-way ANOVA. Climate: a, b Nitrogen: x, y, z

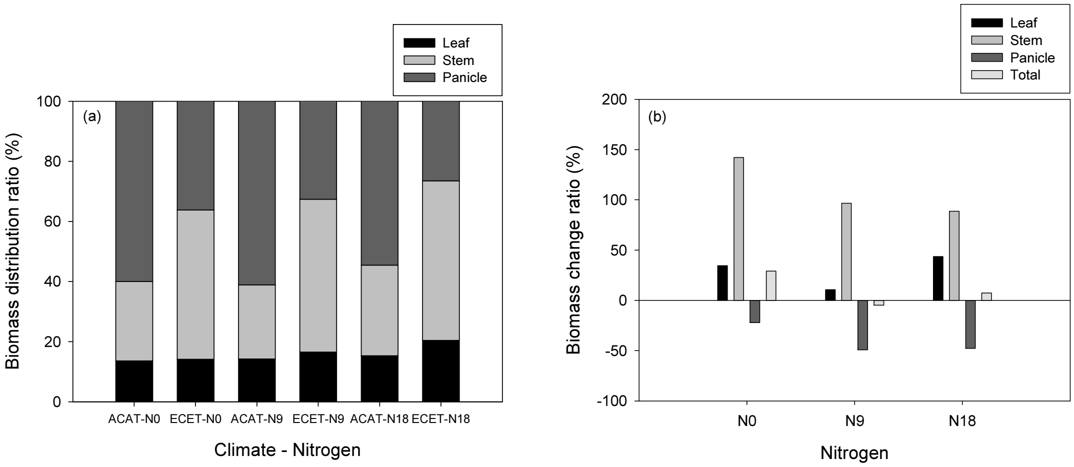

Fig. 1. Changes in biomass distribution by parts of rice in climate change compared to current climate (a) biomass distribution of leaves, stems, and panicles under climate change and nitrogen levels, (b) Biomass change ratio under Elevated CO_2 and elevated temperature (ECET) compared to current climate (ACAT).

49% to 61% in ECET. In comparison, under N0 and N18 conditions, the spikelet rate decreased by 22% and 48%, respectively, due to the impact of climate change (Fig. 1. (b)). Conversely, when N9 was applied, both leaves and stems within a hill increased by 11% and 97%, respectively, in ECET. This trend persisted even with variations in nitrogen levels.

3.2. N uptake concentration change of each part after heading stage

When comparing nitrogen uptake after the harvesting stage, the nitrogen uptake content per hill was 0.6 g $hill^{-1}$ in ECET, while it was 0.65 g $hill^{-1}$ in ACAT, indicating a 7% decrease during ECET. However, there was no significant difference between treatments (Fig. 2. (a)). Despite this, there was a highly significant difference in the uptake rates by

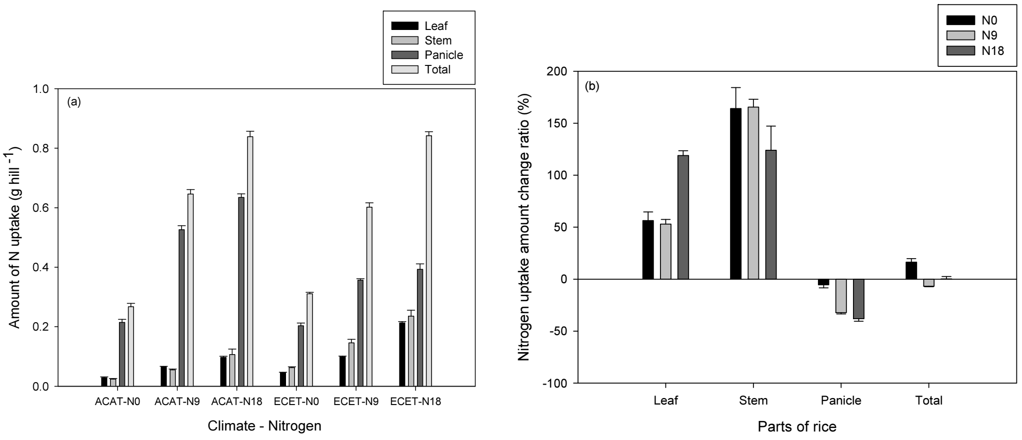

Fig. 2. Comparison of nitrogen by parts of rice in climate change (a)Amount of Nitrogen uptake by parts of rice (b) Nitrogen uptake amount changes of rice under Elevated CO_2 and elevated temperature (ECET) compared to the current climate (ACAT).

different plant parts. In leaves, N9 increased by 53% in ECET compared to ACAT, and in stems, ECET increased by 166%. However, in panicles, there was a decrease of 32%, and the trends were similar at other nitrogen concentrations (Fig. 2. (b)).

In the case of ECET, the nitrogen uptake trend varied depending on the amount of nitrogen application compared to ACAT. The trend of nitrogen uptake in ECET and ACAT varied based on the nitrogen fertilization levels. In ECET, the nitrogen uptake rate of leaves at N18 increased by 119%, while the change in the nitrogen uptake amount at N0 was 56%, representing a difference of about two times. Conversely, the nitrogen uptake rate in stems increased by 162% at N0 compared to ACAT in ECET, but it showed a decreasing trend, unlike the leaves, at 121% at N18. In panicles, similar to stems, there was a decrease in ECET compared to ACAT. The nitrogen uptake amounts at N18 showed a decrease of -38%, compared to -5% at N0.

Post-heading stage, there was a substantial difference in nitrogen content in leaves depending on climate change and nitrogen levels, but this difference gradually decreased. In contrast, the difference in panicles increased significantly (Fig. 3). There was no significant difference in nitrogen uptake by the stem. Under the same fertilization conditions, nitrogen uptake in leaves and stems was higher in ECET than in ACAT, whereas in the panicles, nitrogen uptake in ECET actually decreased. However, as the amount of fertilization increased, the nitrogen content consistently increased.

3.3. Nitrogen use efficiency (NUE)

The BNUE (Biomass Nitrogen Use Efficiency), which signifies the increase in biomass yield per incremental increase in plant nitrogen, significantly increased to 118 g g^{-1} for ECET compared to 109 g g^{-1} for ACAT. In contrast, it exhibited a decreasing trend in ACAT. This suggests that under ECET, there is an enhanced efficiency in utilizing nitrogen for biomass production compared to the current (ACAT).

The PNUE (Plant Nitrogen Use Efficiency), representing the incremental grain yield increase per incremental increase in plant nitrogen, was significantly reduced to an average of 32 g g^{-1} in ECET compared to an average of 68 g g^{-1} in ACAT. Similar patterns were observed for NUEg (Nitrogen Use Efficiency for grain production) and ANUE (Agronomic Nitrogen Use Efficiency), which were also significantly lower in ECET. Both NUEg, indicating grain production in relation to the amount of nitrogen applied, and ANUE, indicating incremental increase in grain yield due to applied nitrogen, were notably lower in ECET (Table 4). Additionally, the RE (Recovery Efficiency), reflecting the incremental increase in plant nitrogen

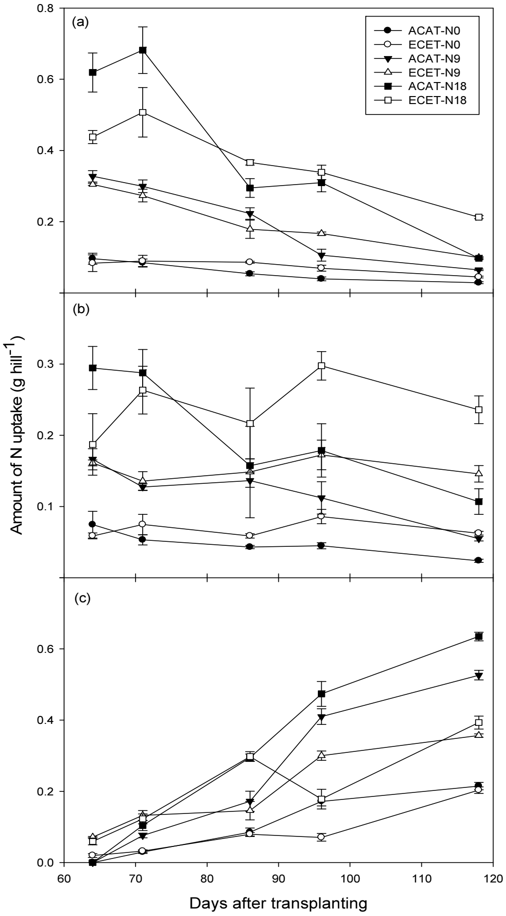

Fig. 3. The nitrogen uptake amount changes after heading stage, when cultivated in climate change (a) leaf, (b) stem, (c)panicle due to climate change and fertilization level.

Table 4. Effect of variable nitrogen levels on nitrogen use efficiency of rice. (BNUE) Biomass N- use efficiency, (PNUE) Physiological nitrogen use efficiency, (ANUE) Agronomic nitrogen use efficiency, (RE) Recovery efficiency, (NUEg) Nitrogen use efficiency of grain

Climate	Nitrogen (kg 10^{-1})	BNUE (g g^{-1})	PNUE (g g^{-1})	ANUE (g g^{-1})	NUEg	RE (g g^{-1})
ACAT	N9	112.41±4.75 b	78.52±4.11 a	72.74±1.85 ay	129.87±0.92 ay	0.93±0.04 ay
	N18	106.59±2.03 b	57.86±1.32 a	39.17±2.02 az	67.73±1.72 az	0.71±0.01 az
ECET	N9	113.46±9.00 a	26.24±0.43 b	17.76±1.23 by	59.77±0.75 by	0.72±0.03 by
	N18	124.21±3.24 a	38.51±5.93 b	23.80±3.86 bz	44.81±3.56 bz	0.66±0.22 bz
P-value	*Climate*	*	***	***	***	**
	N*itrogen*	0.54	0.14	***	***	***
	I*nteraction*	0.06	***	***	***	*

* Different letters indicate significant differences between mean values at $P<0.05$ level using the HSD test after two-way ANOVA. Climate: a, b Nitrogen: y, z

due to applied nitrogen, also decreased under ECET.

There was no significant difference in BNUE depending on the level of nitrogen fertilization. However, NUEg and ANUE significantly increased in N18 compared to N9. Similarly, RE also significantly increased in N18 compared to N9. It's worth noting that RE and NUEg were significantly reduced in N18 with high fertilization amounts. Unlike NUEg, ANUE showed a difference in ECET, with N9 decreasing compared to N18 at 17.23 g g^{-1}.

Table 5. Nitrogen derived from fertilizer (NDFF), the amount of ^{15}N labeled urea in plant and recovery efficiency of ^{15}N labeled fertilizer (RE-15N) when cultivated climate change & different ^{15}N top dressing at panicle initiation stage

Climate	Nitrogen (kg 10^{-1})	Grain	Leaf	Stem	Root	Total
NDFF (%)						
ACAT	N9	20.9 ± 0.52 az	17.3 ± 1.90 z	13.9 ± 0.72 z	7.4 ± 1.38 bz	14.9 ± 0.62 z
	N18	26.8 ± 0.60 ay	22.4 ± 2.27 y	20.6 ± 2.06 y	11.9 ± 1.36 by	20.4 ± 1.38 y
ECET	N9	16.8 ± 0.42 bz	17.6 ± 0.57 z	15.2 ± 2.21 z	12.2 ± 0.85 az	15.5 ± 0.42 z
	N18	19.1 ± 0.33 by	23.9 ± 1.58 y	21.9 ± 0.57 y	17.6 ± 1.00 ay	20.6 ± 0.63 y
The Amount of 15N labeled N in plant (g $hill^{-1}$)						
ACAT	N9	8.7 ± 0.30 az	1.3 ± 0.03 bz	1.0 ± 0.56 bz	0.11 ± 0.004 bz	11.2 ± 0.24 az
	N18	15.4 ± 1.96 ay	2.5 ± 0.25 by	2.5 ± 0.24 by	0.23 ± 0.012 by	20.6 ± 1.54 ay
ECET	N9	2.4 ± 0.07 bz	2.0 ± 0.12 az	2.9 ± 0.48 az	0.46 ± 0.005 az	7.7 ± 0.62 bz
	N18	4.7 ± 0.15 by	6.0 ± 0.18 ay	6.1 ± 0.57 ay	1.18 ± 0.024 ay	17.9 ± 0.53 by
RE-15N (%)						
ACAT	N9	91.9 ± 3.16 a	13.6 ± 0.27 bz	11.0 ± 0.59 b	1.2 ± 0.04 bz	29.4 ± 0.24 a
	N18	81.0 ± 10.35 a	13.4 ± 1.31 by	13.2 ± 1.27 b	1.2 ± 0.06 by	27.2 ± 1.54 a
ECET	N9	25.5 ± 0.71 b	20.8 ± 1.32 az	30.1 ± 5.08 a	4.8 ± 0.05 az	20.3 ± 0.62 b
	N18	24.7 ± 0.79 b	31.2 ± 0.92 ay	32.2 ± 3.02 a	6.2 ± 0.13 ay	23.6 ± 0.53 b

* Different letters indicate significant differences between mean values at $P<0.05$ level using the HSD test after two-way ANOVA. Climate: a, b Nitrogen: y, z

Table 6. Results of two-way ANOVA (*p*-values) on different parameters of rice when cultivated climate change & different ^{15}N top dressing at panicle initiation stage

Factor	Grain	Leaf	Stem	Root	Total
NDFF					
Climate	***	0.47	0.30	***	0.59
Nitrogen	***	**	***	***	***
Interaction	***	0.60	0.97	0.64	0.79
The Amount of 15N labeled fertilizer in plant					
Climate	***	***	***	***	**
Nitrogen	***	***	***	***	***
Interaction	*	***	*	***	0.58
RE					
Climate	***	***	***	***	***
Nitrogen	0.61	***	0.13	***	0.05
Interaction	0.47	***	0.79	***	*

3.4. NDFF analysis using 15N-urea

The ^{15}N labeled urea was surface-applied before the panicle initiation stage, and ^{15}N-uptake results were subjected to a significance test (Table 5, Table 6). NDFF varied depending on climatic conditions, with ECET being statistically significantly lower in grains but higher in roots. Specifically, NDFF was statistically significantly lower in ECET than ACAT in grains, but conversely, in roots, it was higher in ECET than ACAT. Leaves and stems showed no differences depending on climate change conditions.

The RE-15N, representing the amount of ^{15}N-labeled nitrogen uptake contents absorbed by the plant from the applied ^{15}N-urea, was more than 3 times lower for grains in ECET than in ACAT. Additionally, RE-15N in roots was about 2 times lower in ECET compared to ACAT. However, in leaves and stems, the RE-15N was significantly higher in ECET, approximately two times higher. The RE-15N of the entire hill appeared to be reduced by about 7% compared to ACAT.

NDFF and 15N-labeled nitrogen uptake contents were significantly higher in N18 compared to N9. While there was no statistical difference in RE-15N for grains and stems, the RE-15N was significantly higher for leaves and roots.

The proportion of ^{15}N labeled nitrogen uptake contents within an individual did not differ significantly between nitrogen concentrations. However, there was a substantial difference depending on climate change. In the control (ACAT), the grain-leaf-stem-root ratio was 76-12-11-1, which had a relatively higher proportion of grains compared to other parts. On the other hand, under ECET, the ratio shifted to 29-29-36-6, indicating a higher abundance of vegetative growth organs (Fig. 4). In

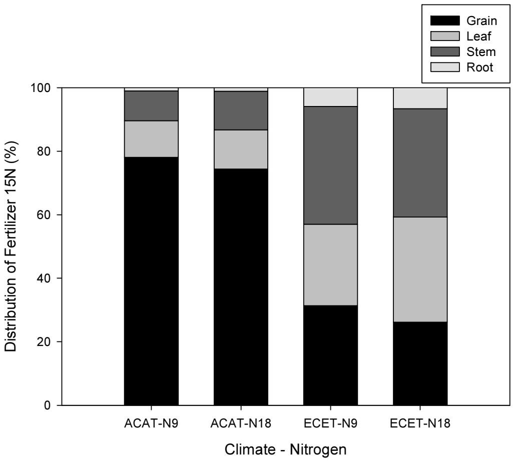

Fig. 4. Balance of ^{15}N labeled fertilizer use in rice when cultivated climate change & different ^{15}N top dressing at panicle initiation stage.

grains, the nitrogen content decreased by about 62% due to climate change, but increased by 146% in leaves, 231% in stems, and 484% in roots (Fig. 4).

IV. Discussion

4.1. Changes in seed production and dry matter

In Elevated CO_2 and elevated temperature (ECET), the number of spikelets increased significantly, with no difference in the number of spikelets per panicle (see Table 3). Consequently, the number of spikelets per unit area increased, but the ripening rate decreased, leading to a decline in the total number of grains. Previous studies have reported decreases of 10% or 30-40% depending on the rice variety, but the decrease observed in this study appears to be greater, possibly due to the higher CO_2 and temperature changes compared to other studies (Kim *et al*., 2011; Wang *et al*., 2015).

The reduction in rough rice weight under ECET seems to be attributed to an increase in infertility rate and a decrease in ripening rate due to the rise in temperature. Among the various warming factors affecting crops, temperature appears to have the most significant negative impact, surpassing the positive effects of the CO_2 fertilizer mentioned earlier (Lee *et al*., 2012). The decrease in fertility and ripening rate due to high temperature has been consistently revealed in numerous studies, suggesting challenges in the flow of photosynthetic products to the sink (Lee *et al*., 2012; Lee *et al*., 2015).

Contrary to the decrease in grain production, stem and leaf dry weight significantly increased under climate change conditions, leading to a 42-52% decrease in Harvest Index (HI). This decrease in HI was attributed to an increase in the rate of change between plant organs (Fig. 1). In ACAT-N9, the ratio of leaf-stem-panicle of a hill was 14-25-61, but in ECET-N9, the ratio of stems increased to 17-51-33, and the ratio of leaves increased significantly. Increased CO_2 stimulates photosynthetic production in leaves, which doubles as temperatures rise (Wang *et al*., 2020). Although an increase in CO_2 typically reduces the nitrogen content of leaves and has a negative effect on Rubisco content related to nitrogen oxide production, there are reports that these effects are alleviated when CO_2 and temperature rise together (Wang *et al*., 2020).

4.2. Differences in nitrogen uptake and NUE by each part

Under ECET compared to ACAT, nitrogen uptake differed by plant parts, with a 100% increase in leaves and a 151% increase in stems, but a 25% decrease in panicles (Fig. 2). This trend aligns with the differences in biomass increase shown in Fig. 1. While there are reports that an increase in CO_2 tends to decrease leaf nitrogen concentration, this effect seems to be counteracted by a simultaneous increase in temperature. When the same nitrogen concentration is applied, the nitrogen content per plant remains high under ECET (Fig. 3). Conversely, stems exhibited a similar trend to leaves, but nitrogen concentration in panicles decreased further under ECET.

BNUE, which represents the incremental biomass yield increase per incremental increase in plant nitrogen, reflected the increase in vegetative growth organs due to enhanced photosynthesis under ECET, and it was higher compared to ACAT (Table 4). However, PNUE, ANUE, and NUEg, indicating grain production and the rate of grain production increase in rice relative to nitrogen fertilization, were lower in ECET compared to ACAT. As suggested in many studies, the cause of this is a decrease in grain yield due to a reduction in the grain ripening rate caused by high temperature (Kim *et al*., 2011; Lee *et al*., 2012; Sang *et al*., 2018). Additionally, it can be observed that the ripening period was shortened due to high temperatures, leading not only to reduced production but also inhibited movement of nitrogen compounds and photosynthetic products (Lal *et al*., 2022).

In relation to this, RE, which represents the incremental increase in plant nitrogen due to applied nitrogen in Table 4, shows the opposite trend to

BNUE. The decrease in nitrogen, despite the increase in biomass, indicates a reduction in nitrogen concentration accordingly. This can be confirmed in Table 5, where NDFF applied at panicle initiation decreased in grains in ECET. This phenomenon is known as the “dilution effect,” and it has been reported that nitrogen concentration in rice decreases due to an increase in dry matter under high CO_2 (Kim *et al.*, 2011).

In a comparison within the current climate (ACAT), the NUE in N9 is twice as high as in N18. Conversely, PNUE and ANUE are lower in N9 than in N18 in ECET. In the absence of environmental stress, unnecessary nitrogen absorption is limited, and excessive nitrogen fertilization is suppressed. However, under elevated CO_2 and temperature, the demand for nitrogen use increases as carbon source absorption increases (Yang *et al.*, 2007).

4.3. NDFF analysis at panicle initiation stage

Rice is typically fertilized at transplanting, tillering stage, and panicle initiation stage, with nitrogen application at the panicle initiation stage having the most significant impact on grain yield production (Nam *et al.*, 2013). In this study, ^{15}N-urea was applied during this period. There are findings suggesting that both high temperature and high CO_2, when elevated together, lead to a significant increase in the biomass of the plant’s vegetative growth organs, and nitrogen uptake also appears to be similar compared to when temperature and CO_2 are treated separately (Nam *et al.*, 2013). The results of this study also demonstrated a significant increase in ^{15}N-labeled nitrogen uptake contents in all vegetative growth organs under ECET.

However, under ECET, nitrogen content in the grains decreased by 44% compared to ACAT, indicating that the ^{15}N-labeled nitrogen in the leaves was not effectively transferred to the grains (Table 5). Additionally, the ratio of ^{15}N-labeled nitrogen in the plant (in the order of grains, leaves, stems, and roots) was 20-29-36-6 under ECET, contrasting with 76-12-11-1 under ACAT. This suggests a significant reduction in movement into grains (Fig. 4). Nitrogen primarily flows from the source to the sink through the sieve tube in the form of amide. In the case of rice, approximately 80% of the nitrogen in the panicle is transmitted through the sieve tube in the form of glutamine and asparagine from roots and leaves (Tabuchi *et al.*, 2007). These compounds are then delivered to the sink and become a nitrogen source for various biosynthetic reactions through glutamic acid synthase.

Furthermore, nitrogen absorbed from the roots is transported in the form of compounds such as amino acids and proteins. In the leaves, it combines with photosynthetic products to produce starch. Starch, in turn, is converted into a sugar form that is easily transportable and moves into the grains. This movement is enhanced under high CO_2 conditions. However, it is known that at high temperatures, movement through the sieve tube is hindered, resulting in a reduction in the size and quality of rice grains. Therefore, if rice is cultivated under elevated CO_2 and temperature using current varieties, additional nitrogen fertilization is likely to be necessary. In the long term, research efforts will be needed to counteract the inhibition of the movement of nitrogen compounds and carbohydrates in plants caused by high temperatures.

적 요

기후변화가 심화됨에 따라 작물에 미치는 영향을 평가하고 개선 방안을 도출하는 것은 필수적이다. 본 연구에서는 고온, 고이산화탄소 조건에서 벼의 질소 흡수 반응 및 질소이용효율 등을 분석하여 기후변화에 따른 벼의 적응 대책을 검토하고자 수행하였다.

21C 후반 RCP8.5 시나리오에 근거하여 온도는 2001~2010년 대비 +4.7 ℃ 상승, CO_2는 800 ppm을 기후변화 조건으로 하였으며, 질소를 0, 9, 18 kg 10a^{-1} 수준으로 각각 시비하였다. 그리고 벼 낟알의 질소 흡수를 보기 위해 수비 시비시 안정동위원소^{15}N-urea를 표층 시비하였다.

기후변화 조건에서는 현재 기후 대비 잎, 줄기의 바이오매스량은 증가하나 등숙률 감소로 정조중이 38 %

감소하여 수확지수도 47% 감소하였다. 기후변화로 인해 잎과 줄기에서 질소흡수량은 현재기후 대비 각각 87%, 139% 증가하였으며, 반대로 곡실의 질소함량은 31% 감소하는 경향을 보였다. 기후변화 조건에서 ANUE, NUEg는 표준시비 시 각각 76%, 54% 유의하게 감소하였으며, 수비 질소의 흡수량과 회수율(RE)도 이와 동일한 경향을 보였으며 질소시비를 증가하였을 경우에도 동일한 경향을 보였다. 임실 및 등숙률 저하로 sink/source 균형이 무너져 질소 화합물 및 광합성 산물의 이동이 저하되어 질소함량이 영양생장기관에 머물러있고 곡실로의 전류가 되지 않으므로 향후 이를 극복할 수 있는 고온 적응 품종 육성과 이앙시기 조절 등이 선행되어야 한다.

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