RESEARCH ARTICLE
J. Crop Sci. Biotech. 2008 (December) 11 (4) : 223 ~ 226

Growth and Gibberellins Level of Two Rice Cultivars as Influenced by Different Nitrogen Containing Compounds

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<Received September 16, 2008 / Accepted September 30, 2008>

Abstract

Seedlings of two rice cultivars i.e. cv. Daesanbyeo and cv. Dongjinbyeo were analyzed for growth and endogenous gibberellins (GAs) in response to nitrogen nutrition applied in the forms of KNO_3, $(NH_4)_2SO_4$ and NH_4NO_3. All the growth parameters showed an increase in N applied treatments and their magnitudes of increase were different depending on different nitrogen fertilizer forms. The endogenous GAs contents were increased with N application but differentially affected by various N-forms in both rice cultivars. In cv. Daesanbyeo, maximum amount of bioactive GA_1 was recorded for $(NH_4)_2SO_4$, while maximum amount of bioactive GA_1 in cv. Dongjinbyeo was observed in NH_4NO_3 applied treatments. In both rice cultivars, KNO_3 applied rice plants contained least GA_1 contents. Also, GA_{19} was the most abundant GA found in rice seedlings whereas GA_4 was absent at seedling stage. Our study indicated that different rice cultivars showed different responses for the same fertilizer depending upon the response potential of each cultivar and the pertinent physiological responses to changes of endogenous GAs in rice cultivars, which were comparatively lesser in magnitude.

Key words: GC-MS-SIM, Gibberellins analysis, Nitrogen forms, Plant growth, Rice cultivars

Introduction

The phytohormones play a vital role in the growth and development of plants. They act at micro molar or even lower concentration to regulate physiological and developmental processes, such as seed germination, leaf expansion, stem elongation, flowering, and seed formation. These structurally diverse compounds include auxins, cytokinins (CK), abscisic acid (ABA), Gibberellins (GA), ethylene, polyamines, jasmonates, salicylic acid and brassinosteriods (Davies 1995). Gibberellins (GAs) are a large family of tetracyclic and diterpenoid compounds and function as endogenous plant growth regulators. Through phenotypic analyses of mutants with reduced GA production, it has been revealed that bioactive gibberellins play an essential role in many aspects of plant growth and development, such as stem elongation, flower and fruit development and seed germination (Ross et al. 1997). The first committed step of GA biosynthesis is the formation of ent-kaurene from geranylgeranyl pyrophosphate, with copalyl pyrophosphate as an intermediate. This reaction is catalyzed by the enzymes ent-copalyl diphosphate synthase and *ent*-kaurene synthase, which have been cloned from various plant species (Sun and Kamiya 1997). *ent*-Kaurene is metabolized to GAs by membrane-associated monooxygenases and soluble, 2-oxoglutarate-dependent dioxygenases (Graebe 1987). Quantitative analysis using combined gas chromatography-mass spectrometry (GC-MS) and bioassays with dwarf plants have revealed that GAs are mainly present in actively growing and elongating tissues, such as shoot apices, young leaves and flowers (Kobayashi et al. 1988; Potts et al. 1982). This clearly suggests that GAs are primarily synthesized at the site of their actions. In contrast, there is some evidence for the presence of GAs in xylem and phloem exudates, indicating a long-distance transport of GAs through these tissues (Hoad 1995).

The application of chemical fertilizers has resulted in an increased production of rice thus helped to alleviate hunger and poverty across the globe. Nitrogen (N), phosphorus (P) and potassium (K) are the three most important macro nutrients required for plant growth and development and present in the soil along with other micronutrients. In agricultural lands, these nutrients are frequently added for sustainable crop yield, as

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excessive utilization of NPK by the crops results in their depletion. Nitrogen is mostly provided to different crops in the form of urea [$(NH_2)_2CO$], ammonium nitrate (NH_4NO_3), potassium nitrate (KNO_3) and ammonium sulfate [$(NH_4)_2SO_4$]. Although higher plants have the capacity to utilize organic N (Na¨sholm et al. 1998), the major sources for N acquisition by roots are considered to be nitrate (NO3-) and ammonium (NH_4+) (Haynes and Goh 1978). Plants vary substantially in their relative adaptations to these two sources of N (Kronzucker et al. 1997). Although NH_4+ should be the preferred N source due to less metabolic energy requirements than NO_3- (Bloom et al. 1992), only few species actually perform well when NH_4+ is provided as the only N source.

$(NH_4)_2SO_4$, NH_4NO_3 and KNO_3 are three important available sources of N for plant nutrition. However, we have limited information on the comparative effects of these N containing compounds on the growth and development of rice. Furthermore, the status of GA in relation to nitrogen application has never been investigated in rice, although considerable attention has been focused on the role of gibberellins in controlling shoot elongation (Graebe 1987). Therefore, in this study, we investigated the effect of $(NH_4)_2SO_4$, NH_4NO_3 and KNO_3 on the growth and endogenous GA content in two rice cultivars.

Materials and Methods

General procedures

The complete randomized block design (CRBD) was used for this experiment with each treatment consisted of 6 replications, comprising 24 plants in each replication.

Seeds of rice cultivars viz. Daesanbyeo and Dongjinbyeo were procured from Yeong-Nam Agricultural Research Institute, Milyang, Korea. Rice (*Oryza sativa* L.) seeds were surface-sterilized in 5% NaOCl for 10 min, rinsed with deionized water, left to imbibe in aerated deionized water and incubated in nursing beds for 5 days. The germinated seeds were transplanted into plastic pots (22 × 15 × 7 cm) filled with paddy soil. The pots were supplied with phosphorus and potassium at the rates of 95 kg/ha and 144 kg/ha, respectively. The $(NH_4)_2SO_4$, KNO_3 and NH_4NO_3 fertilizers were applied at the rate of 150 kg/ha at 5th day of transplantation. The plants were grown in a controlled environment chamber with a 16 hr-30°C day and 8 hr-20°C night regimen and light intensity of 1000 μmol $m^{-2}s^{-1}$.

Extraction and quantification of endogenous GAs

The plants were harvested 17 days after sowing (DAS) and the shoots were immediately frozen in liquid nitrogen and stored at -80°C. When all the required materials for GA analysis had been collected, the samples were lyophilized for 24 h. The extraction and quantification of endogenous gibberellins were followed as described by Lee et al. (1998). GAs were quantified using [17, 17-$^2H_2$]-GAs (20 ng each) as internal standards (obtained from Prof. L.N. Mander, Australian National University, Canberra, Australia). The three prominent ions were analyzed by GC-MS-SIM (6890N network GC system and 5973 network mass selective detector; Agilent Technologies, Palo Alto, CA, USA) with dwell times of 100 ms. The endogenous GAs contents were calculated from the peak area ratios respectively. Retention time was determined by the hydrocarbon standards to calculate the KRI (Kovats Retention Indices) value (Kovàts 1958).

Growth parameters

The plant height, plant dry weight, shoots height and shoots dry weight were measured on the 7th day of N application to the plants. Dry weights were measured after drying samples at 70 °C for 48 h in an oven (Bohm 1979).

Soil analysis

A representative random soil sample was taken from air dried, grinded, homogenized and screened (2 mm sieve) paddy soil and analyzed for physicochemical properties (RDA, 1988). Nitrogen was analyzed through modified Kjeldahl method (Paul and Berry 1921). The paddy soil used in the experiment was of silt-loam texture. The physicochemical property of paddy soil used in the experiment was as follows

Statistical analysis

The data were subjected to Duncan's multiple range test (DMRT) (SAS 9.1; SAS Institute, Cary, NC, USA). And, the standard deviation was calculated using Sigma plot 2001 software (Jandel Scientific, San Rafael, CA, USA).

Results

Effect of different N-forms on plant growth

The growth parameters were differentially affected by different N fertilizer forms. In Daesanbyeo cultivar, the maximum plant height (29.9±2.0cm) was recorded for treatments supplied with $(NH_4)_2SO_4$, while in Dongjinbyeo, it was 32.4±0.6cm at NH_4NO_3 application. The KNO_3 application produced the least growths as compared with other N fertilizer forms. The plant dry

Table 4. Effects of different N-forms on plant height, plant dry weight (DW), culm length and culm dry weight (DW) in two rice cultivars (recorded at 17 DAS)

Rice cultivar	N- forms	Fertilizer level (kg/ha)	Plant height (Shoot+Root) (cm)	Plant DW (g)†	Culm length (cm)	Culm DW (g)†
Daesanbyeo	Control	0	$25.3^{d*}\pm1.6$	$6.56^{a}\pm0.1$	$10.1^{b}\pm0.2$	$1.08^{c}\pm0.1$
	KNO_3	150	$27.1^{c}\pm1.2$	$7.4^{a}\pm0.9$	$11.8^{ab}\pm0.5$	$1.39^{abc}\pm0.2$
	$(NH_4)_2SO_4$	150	$29.9^{b}\pm2.0$	$8.30^{a}\pm0.5$	$13.2^{ab}\pm1.0$	$1.87^{a}\pm0.3$
	NH_4NO_3	150	$28.7^{b}\pm1.4$	$7.89^{a}\pm0.7$	$12.5^{ab}\pm0.5$	$1.69^{ab}\pm0.3$
Dongjinbyeo	Control	0	$26.9^{c}\pm1.9$	$6.85^{a}\pm0.2$	$10.4^{ab}\pm0.4$	$1.13^{c}\pm0.1$
	KNO_3	150	$29.7^{b}\pm1.7$	$7.64^{a}\pm0.6$	$11.5^{ab}\pm1.0$	$1.27^{bc}\pm0.1$
	$(NH_4)_2SO_4$	150	$31.6^{a}\pm1.6$	$7.80^{a}\pm0.4$	$12.8^{ab}\pm1.0$	$1.68^{ab}\pm0.2$
	NH_4NO_3	150	$32.4^{a}\pm0.6$	$8.31^{a}\pm0.8$	$13.7^{a}\pm0.9$	$1.86^{a}\pm0.3$

In a column, means followed by the same letter are not significantly different at $P < 0.05$ according to Duncan's multiple range tests. † Total dry weight of 15 hills weight (DW) was also affected by different N fertilizer forms and heaviest mean dry weight were shown by NH_4NO_3 application in both rice cultivars followed by $(NH_4)_2SO_4$, while KNO_3 treated plant was observed at least dry weight compared with control treatments (Table 1).

The maximum mean culm length (13.2±1.0 cm) and culm DW (1.87±0.3) were observed in $(NH_4)_2SO_4$ treatments for cv. Daesanbyeo while cv. Dongjinbyeo showed the maximum mean culm length of 13.7±0.9 cm and culm DW (1.86±0.3) in treatment wiith NH_4NO_3. The least culm height and culm DW were recorded for KNO_3 applied treatments. On average, the maximum mean growth parameters were observed for NH_4NO_3 application followed by $(NH_4)_2SO_4$ and KNO_3 in both rice cultivars (Table 1).

Effect of different N-forms on GAs content

The endogenous GA contents were significantly enhanced by different N fertilizer forms in both rice cultivars. In Daesanbyeo, endogenous GA_1, GA_{20}, GA_{19}, GA_{53} and GA_{12} contents were maximum in treatments where N was applied in the form of $(NH_4)_2SO_4$ (Fig. 1). But, GA_1, GA_{20}, GA_{19}, GA_{153} and GA_{12} amounts in Dongjinbyeo were higher in plants treated with NH_4NO_3. The endogenous concentration of bioactive GA_1 and its immediate precursor GA_{20} were highest with $(NH_4)_2SO_4$ in Daesanbyeo (9.88 ng/g and 7.18 ng/g respectively), while highest GA_1 and GA_{20} contents (12.65 ng/g and 10.47 ng/g, respectively) were recorded for NH_4NO_3 treated Dongjinbyeo. GA_{19} was found to be the most abundant endogenous GA in rice (Fig. 1).

Discussion

On global basis, more than 70% of the rice is produced in the highly managed irrigated systems in the lowlands of Asia (IRRI 1998). Different chemical fertilizers are used for producing maximum rice yield on per hectare basis and nitrogen is considered to be one of the most important macronutrients. The supply of nitrogen fertilizer during vegetative growth poses the most critical limitation to the realization of yield potential in the field (Cassman et al. 1998; Kropff et al. 1993; Sheehy et al. 1998).

Our results showed that growth parameters and endogenous gibberellins were differentially affected by KNO_3, $(NH_4)_2SO_4$ and NH_4NO_3. The endogenous GA contents were significantly higher in the treatments where N fertilizer source was NH_4+ rather than NO_3-. Similarly, the plant height and dry weight, culm length and dry weight were also higher in ammonium based N fertilizer than nitrate based. In rice, the application of NH_4+ is also preferred to NO_3- as N source because NH_4+ metabolism requires less energy than that of NO_3-. Similarly, the previously studies reported that in contrast to most agricultural soils, where nitrate (NO_3-) is the predominant N source, hypoxic conditions in the paddy environment largely preclude the microbial formation of NO_3- through nitrification (Arth et al. 1998; Kronzucker et al. 1998; Wang et al. 1993). Thus, NH_4+ is the main form of N available to rice in the paddy field. It is, therefore, that NH_4+ as compared to NO_3- has received exclusive attention and extensively used in rice.

GA_{19}, a precursor of GA_{20}, was the most abundant, while GA_{12} was found to be least in all treatments. The amount of GA_{19} was about 20 to 30 folds more than GA_{20}, a precursor of bioactive GA_1 in both rice cultivars, coinciding with the results of Appleford and Lenton (1991). The levels of bioactive GA_1 content of cv. Daesanbyeo, and cv. Donjinbyeo were different in response to plant acquisition of three N forms. The differential bioactive GA_1 content in two rice cultivars suggests that GAs biosynthesis rates were different not also within different rice cultivars but also by different N forms.

The N application enhanced different growth parameters and endogenous GAs contents in all rice cultivars were tested as compared to control. These suggested that plant growth and development in rice depended on nitrogen metabolism and GAs. The results also demonstrated that different rice cultivars

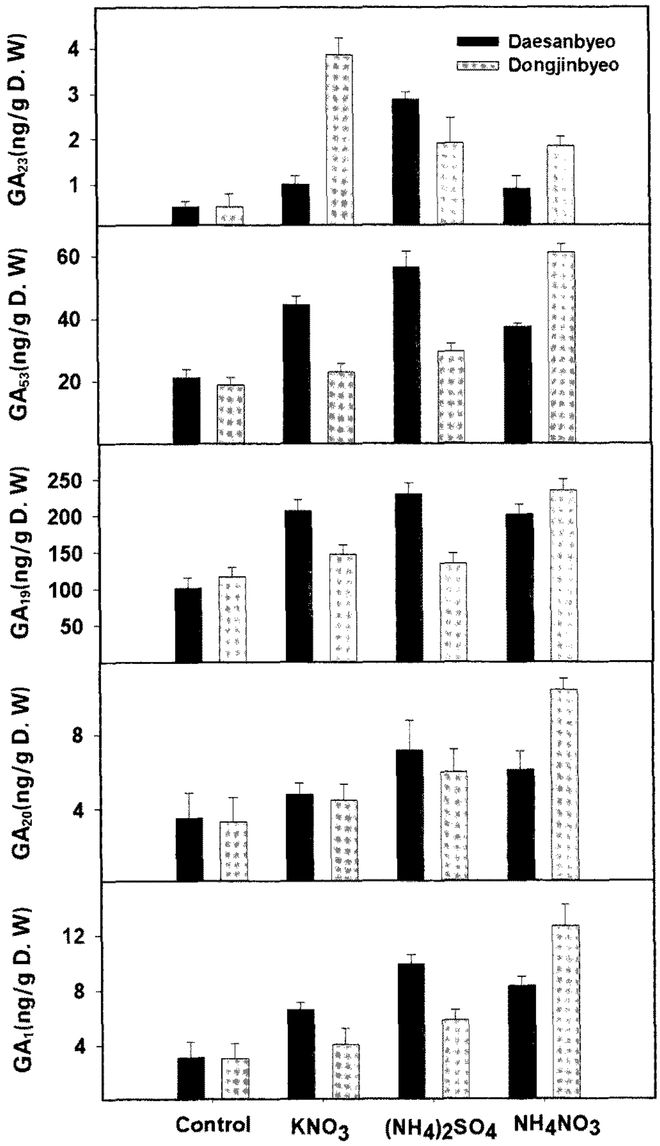

Fig. 1. Endogenous GA contents in rice cv. Daesanbyeo and Dongjinbyeo treated with different N-forms of N fertilizers. Plants for GA assessment were sampled at 17 DAS. Error bars show standard deviation.

responded differently to the application of different nitrogen forms. Current study confirms previous reports on the role of N forms on growth of different plant species (Grindal et al. 1998; Ingram et al. 1986; MacKenzie-Hose et al. 1998; Ross et al. 1989).

Acknowledgements

This research was supported by the Korea Research Foundation Grant (KRF-2000-005-G00002).

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