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Effect of Nitrogen and Silicon Nutrition on Bioactive Gibberellin and Growth of Rice under Field Conditions

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Abstract

Gibberellins are growth hormones that play a pivotal role in the growth and development of plants. Present investigations were carried to check the effect of nitrogen (N) and silicon (Si) on bioactive GA_1 and its immediate precursor GA_{20} at different growth stages of two rice cultivars with different maturity traits. It was observed that the endogenous bioactive GA_1 level gradually increased during vegetative stage and anthesis stage of both Junghwabyeo (early flowering cultivar) and Daesanbyeo (late flowering cultivar). However, the GA_1 and GA_{20} content start decreasing during the seed filling stage in both rice cultivars, which indicated a possible relationship of bioactive GA_1 and floral development. Our results also confirmed that early 13-hydroxylation pathway was operated at all developmental stages of rice plant. Variation in the levels of the endogenous gibberellins in rice shoots were measured by GC-MS-SIM using $^2H_2$-labeled gibberellins as internal standards. Combined application of N and Si enhanced growth parameters and reduced lodging index of both rice cultivars. It was thus concluded that the level of physiologically active GA_1 increased during vegetative and early reproductive stage, but starts declining at seed filling stage.

Key words: Rice cultivars, bioactive GAs, nitrogen, silicon, growth and development, lodging, GC-MS-SIM

Introduction

Plant hormones play roles in the growth and differentiation of cells or tissues by regulation of hormone-responsive genes. They may be either growth inhibitors or promoters depending on the site of action and concentration of the substance. Gibberellins are a group of naturally occurring plant hormones that affect cell enlargement and division which leads to internode elongation in stems. They affect many developmental processes such as seed and plant dormancy, germination, seed stalk and fruit development, particularly interacting with temperature and light. Physiological and biochemical studies have shown that the GA hormones are involved in controlling height and mutants deficient in production or sensitivity to these compounds have altered stature (Potts *et al.* 1985; Talon *et al.* 1990).

Development of an annual crop from emergence to maturity can be divided into three major physiological phases i.e. from emergence to flower initiation (vegetative development); from floral initiation to anthesis (reproductive development); and from anthesis to physiological maturity (seed filling) (Ritchie 1991). Photoperiod is a major factor affecting growth development in many species. Principally it determines time to floral initiation, and hence anthesis date. There are many reports that photoperiod affects floral development subsequent to floral initiation in rice (Coolhaas and Wormer 1953), Caryopteris (Piringer *et al.* 1963; Evans 1969), wheat (Slafer and Rawson 1994), and

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barley (Kernich *et al.* 1996).

However, little is known about the role of plant hormones during different developmental stages of plant, and how plant growth hormones regulate plant developmental stages. Current study was conducted to analyze the amounts of physiologically active GA_1 in rice shoots at vegetative and reproductive growth stages and to evaluate the role of N and Si in growth and lodging trait of early flowering and late flowering rice cultivars.

Materials and Methods

Plant materials, growth conditions and samplings

This experiment was conducted in the experimental field of National Yeongnam Agricultural Experiment Station, Milyang, South Korea. Seeds of two rice ecotypes, Junghwabyeo (early flowering i.e. 70 DAS) and Daesanbyeo (late flowering i.e. 90 DAS) were sown in nursery boxes and seedlings were then transplanted to paddy field.

The experiment consisted of three treatments viz. control (110 kg/ha (N)), 180 kg/ha (N) and 180 kg/ha (N) + 2000 kg/ha (SiO_2) with 3 replications per treatment. The experimental plot size for each replication was 20 m^2.

Extraction and quantification of endogenous gibberellins

Rice shoot samples of different developmental stages i.e. 58 days after sowing (DAS), 79 DAS, 96 DAS, 103 DAS, and 110 DAS were collected from the paddy field. Rice samples were immediately frozen in liquid N_2 and stored at -70°C till GA analysis. Lee *et al.* (1998) procedure was followed for extraction and quantification of endogenous GAs.

High-performance liquid chromatography (HPLC)

The GAs were chromatographed on a 3.9 x 300 mm μ BondaPak C_{18} column (Waters Corp. Milford, MA, USA) and eluted at 1.5 ml min^{-1} with the following gradient: 0 to 5 min, isocratic 28% MeOH in 1% aqueous acetic acid; 5 to 35 min, linear gradient from 28 to 86% MeOH; 35 to 36 min, 86 to 100% MeOH; 36 to 40 min, isocratic 100% MeOH. Up to fifty fractions of 1.5 ml each were collected. Small aliquots (15μl) from each fraction were taken, and radioactivity was measured with liquid scintillation spectrometry (Beckman, LS 1801) to determine accurate retention times of each GA based upon the elution of ^{3}H-GA standards. The fractions were dried on a Savant Speedvac and combined according to the retention times of ^{3}H-GA standards and previously determined retention times of the labeled (deuterated) GA standards.

GC-MS-Selected ion monitoring

Each dried GA fraction was re-dissolved in 100% methanol, transferred to a 1 ml reaction vial and dried under N_2 at 40°C. The sample was solubilised in 35μl of methanol, and the GA methyl ester was prepared with ethereal diazomethane. The sample was dried under N_2, re-dissolved in methanol and methylated one more time. The sample was solubilised in 35μl pyridine, and silylated for 30 min at 65°C with the same amount of N, O-Bis (trimethylsilyl)-trifluoroacetamide (BSTFA) with 1% TMCS (Pierce Chemical Co.). The sample was then reduced to dryness with N_2 and solubilised in anhydrous dichloromethane. One μl of each sample was injected on-column on a 30 m, 0.25 mm (i.d.), 0.25 μm film thickness HP-1 capillary column (J & W Scientific Co, Folsom, CA, USA). The GC (Hewlett Packard 6890) oven temperature was programmed for a 1 min hold at 60°C, then to rise at 15°C min^{-1} to 200°C followed by 5°C min^{-1} to 285°C. Helium carrier gas was maintained at a head pressure of 30 kPa. The GC was directly interfaced to a Mass Selective Detector (5973) with an interface and source temperature of 280°C, an ionizing voltage of 70 eV and a dwell time of 100 ms.

Quantification of endogenous GAs

Collection and analysis of the GC-MS data was accomplished with a GC-MS (6890 Chemstation). Three major ions of the supplemented [$^2H_2$]GA internal standards (obtained from Prof. Lewis N. Mander, Australian National University, Canberra, Australia) and the endogenous GA were monitored simultaneously (Table 1).

Table 1. GC-MS analysis of HPLC fractions from acidic ethyl acetate fractions of rice shoots (pre-flowering).

HPLC fraction	GAs	KRI[a]	Source	m/z (%, relative intensity of base peak)[b]
12~14	GA_1	2674	Sample	506(100) 448(20) 313(17)
		2674	Standard	508(100) 450(19) 315(14)
24~26	GA_{20}	2485	Sample	418(100) 375(45) 403(14)
		2485	Standard	420(100) 377(45) 405(13)

[a]KRI, Kovats retention indices. [b]Identified as methyl ester trimethylsilyl ether derivatives by comparison with reference spectra and KRI data (Gaskin and MacMillan 1991)

Field environmental conditions

The environmental conditions were recorded on sampling times in order to get better understanding of the field conditions (Table 2).

Table 2. General agronomic conditions during the course of experiment (2003)

Sampling time	Min. day temp. (°C)	Max. day temp. (°C)	Rainfall (mm)	Day wind velocity (m/sec)	Av. day humidity (%)	Day irradiancy (hr)	Av. day clouds (1/10)
7th July (58 DAS)	15.3	27.6	-	1.3	67.0	9.9	5.3
28th July (79 DAS)	23.5	29.0	15.5	4.0	76.8	2.9	8.6
14th August (96 DAS)	18.6	30.9	-	1.2	69.8	8.5	3.9
21th August (103 DAS)	22.5	30.1	10.0	1.0	82.0	2.9	9.4
28th August (110 DAS)	19.6	29.9	-	0.8	76.1	6.3	6.9

Growth and yield parameters

Stem height, panicle length, 3rd Internode length, ear weight (Fresh), plant shoot weight (Fresh) and stem lodging index were measured. 50 plants per treatment were randomly selected for calculating these parameters. The stem breaking strength was measured with Digital Force Gauge (Model: WW-59845-04). The standard breaking position of stem is 10 cm from the ground level and the time of calculating stem breaking strength is 20 days after flowering in rice (Kim *et al.* 1995). The lodging index was calculated by applying formula; Lodging Index= Culm length x Fresh weight/breaking strength of N_3.

Results

Changes in GA_1 and GA_{20} levels of two rice cultivars

The endogenous GA_1 and its immediate precursor GA_{20} contents of two rice cultivars, Junghwabyeo and Daesanbyeo were measured at different developmental stages. N fertilization of 110 kg/ha and 180 kg/ha, the bioactive GA_1 contents of Junghwabyeo, increased during vegetative stage till anthesis but start decreasing after 28th July (79 DAS) while in Daesanbyeo, the GA_1 contents gradually increased till 21st August (103 DAS) but declined later on (Fig. 1 & Fig. 2).

Silicon application increased the average GA_1 and GA_{20} level in both cultivars though the pattern of changes in GA levels was not affected (Fig. 3).

Growth parameters of two rice cultivars

Different growth attributes of two rice cultivars were signifi-

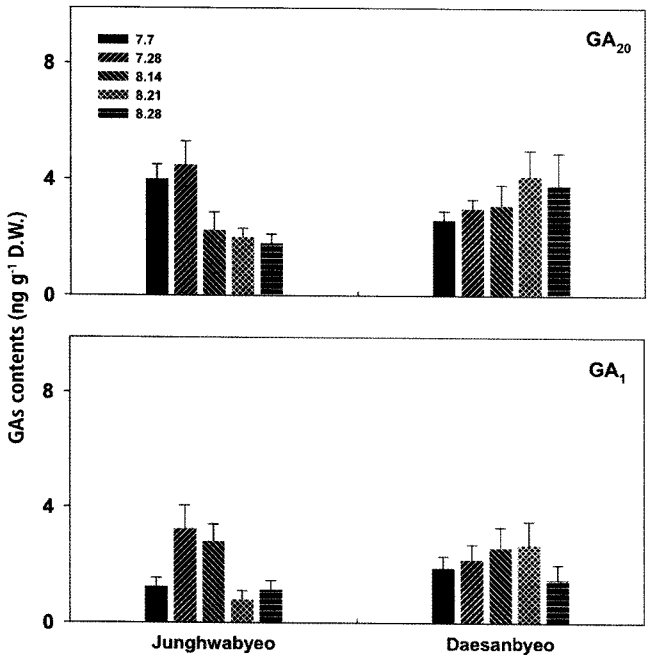

Fig. 1. Variation in GA_1 and GA_{20} contents of two rice cultivars at different growth stages under 110 $kgha^{-1}$ N nutrition

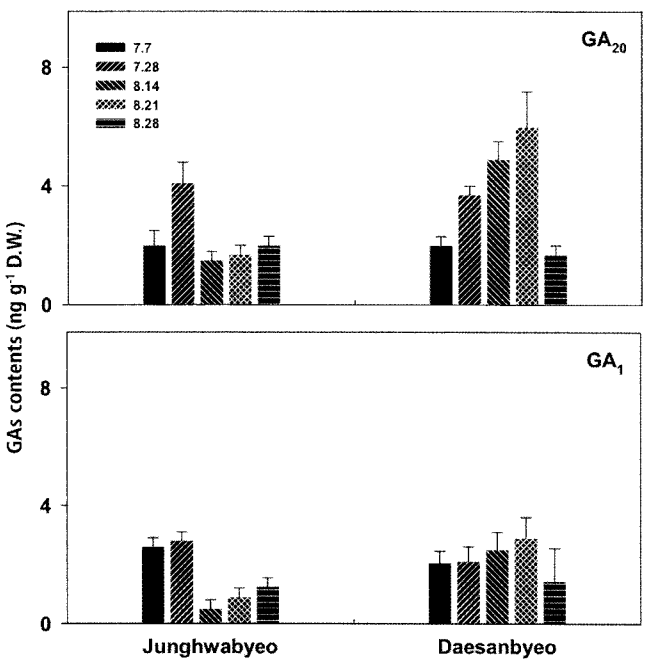

Fig. 2. Variation in GA_1 and GA_{20} contents of two rice cultivars at different growth stages under 180 $kgha^{-1}$ N nutrition

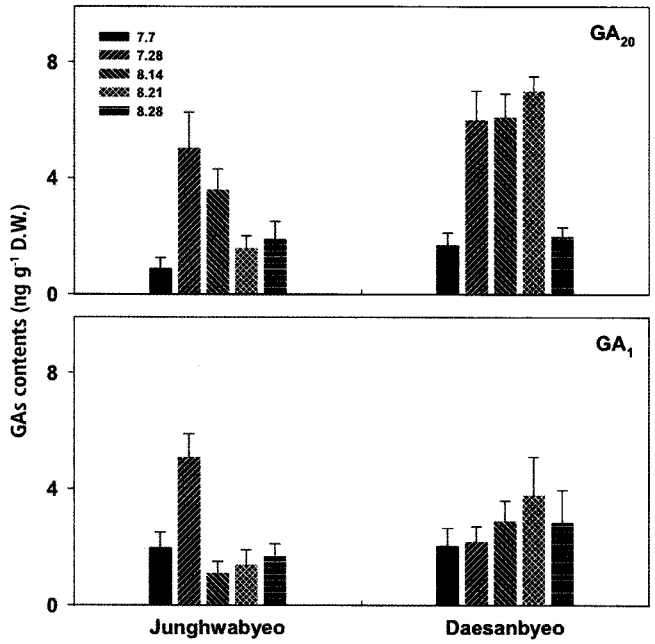

Fig. 3. Variation in GA_1 and GA_{20} contents of two rice cultivars at different growth stages under 180 $kgha^{-1}$ N + 2000 $kgha^{-1}$ SiO_2 nutrition

cantly affected by increased N level but little improved by the addition of SiO_2. The stem length and 3rd internode length significantly increased with higher N nutrition, while panicle length, fresh ear weight and fresh shoots weight was not significantly affected. In both rice cultivars, pronounced increase in lodging index was recorded for sole N applied treatments, though combined application of N and Si reduced lodging index (Table 3).

Discussion

Nitrogen and silicon play a vital role in plant growth although little attention has been paid to the importance of Si due to its abundance in lithosphere. These nutrients also regulate the endogenous gibberellins content of plants but little is known about the mechanism through which they affect the activity and level of phytohormones in plants. Our present study focused on the effect of N and Si on physiologically active GA_1 in rice revealed that GA_1 and its precursor GA_{20} level enhanced with N and Si application but their content greatly varied with the sampling time. The levels of GA_1 during late seed filling stage of both rice cultivars were lower than vegetative and early reproductive growth stages. In Junghwabyeo, an early-flowering cultivar, GA_1 level increased during vegetative stage and was maximum at early grain filling stage i.e. at 96 DAS, while highest at 110 DAS in Daesanbyeo (late-flowering cultivar). It suggests that at later growth stages, the gibberellins biosynthesis rate slowed down, coinciding with normal growth patterns in plants. The GA_1 and GA_{20} content gradually start declining after heading, a little recovered at maturing stage in early-flowering cultivar (Junghwabyeo) and late-flowering cultivar (Daesanbyeo), which was linked with different maturity durations of two rice ecotypes. The increase in GA content during flowering time may indicate a possible relationship of gibberellins and flower initiation in rice, as gibberellins has been suggested as intermediate messengers for the transition to flowering induced by environmental or endogenous signals (Parcy 2005). Several findings have suggested that an increase in gibberellin biosynthesis contributes to the promotion of flowering by long photoperiods in different species. On one hand, gibberellins accumulate when plants were induced to flower by transferring them to inductive conditions (*Arabidopsis*, Xu *et al.* 1997; *Spinacea oleracea*, Zeevaart et al. 1993; *Lolium temulentum*, King and Evans 2003). However, these results cannot be extrapolated to all plant species as gibberellins do not seem to be a major flowering signal in *Sinapis alba* (Corbesier *et al.* 2004). Furthermore, in *Fuchsia* (King *et al.* 2000) and grapevine (Boss and Thomas

Table 3. Growth components of two rice cultivars as affected by N and Si nutrition

Rice cultivar	Fertilizer (kg/ha)	Stem length (cm)	3rd internode length (cm)	Panicle length (cm)	Fresh ear weight (g)	Fresh weight (g/shoot)	Lodging index
			(N3)				
Junghwabyeo	110 (N)	73.78±1.2	12.44±0.4	19.40±0.6	3.44±0.2	9.24± 1.1	118.27±14.2
	180 (N)	78.77±0.8	15.32±0.2	19.92±0.4	3.40±0.5	10.17±1.6	138.07±12.1
	180 (N)+ 2000 (SiO_2)	79.24±1.5	15.97±0.5	19.88±0.8	2.90±0.4	10.89±1.3	105.74±11.4
Daesanbyeo	110 (N)	75.67±1.1	11.26±0.3	19.20±0.3	3.30±0.2	8.17± 1.5	96.65±8.7
	180 (N)	78.60±1.7	12.54±0.7	20.45±0.7	3.24±0.2	8.76± 1.3	100.76±16.4
	180 (N)+ 2000 (SiO_2)	79.45±1.9	12.75±0.6	19.44±0.5	2.68±0.3	8.91± 1.7	88.00±10.9

2002), gibberellins actually inhibit flowering. Our results also confirmed that in both rice cultivars, early 13-hydroxylation pathway was operated in shoots at all developmental stages. Similar observations were also documented by Hwang *et al.* (2007) and Kobayashi *et al.* (1994).

The growth parameters of two rice cultivars were increased by N and Si application, suggesting that both of these elements are important sources of rice nutrition. Si application has decreased lodging index of both rice cultivars, showing that under Si nutrition, the rice plants were more resistant to lodging. This might be due to possible rigidity of cell walls, imparted by Si nutrition. Yosuke *et al.* (2000) reported that silicon application increased lodging resistance through increased breaking strength and increased pushing tolerance. Similarly, Jang *et al.* (2007) observed that Si application enhanced plant height and culm length but high doses of Si above a critical level may be less effective in maintaining higher growth rates.

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