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Diurnal Variation in Endogenous Gibberellin Levels of Rice Shoots

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Abstract

Diurnal changes in levels of endogenous gibberellins (GAs) were investigated in three rice cultivars i.e. Sangjubyeo, Shingeumobyeo (photo-neutral) and Chucheongbyeo (photosensitive). The rice cultivars were grown under a 12-hr photoperiod and endogenous GA levels were assayed by gas chromatography-mass spectrometry (GC-MS-SIM) every 3 h for 24 h. The endogenous bioactive GA_1 and its immediate precursor GA_{20} contents were significantly different in both photosensitive and photo-neutral rice cultivars, though less pronounced differences were observed for endogenous GA_{12}, GA_{53}, GA_{19}, and GA_8 levels with in the three rice cultivars. The levels of bioactive GA_1 and its immediate precursor GA_{20} were significantly higher in Chucheongbyeo than in the other two cultivars. In Chucheongbyeo, the GA_1 contents increased significantly from 11.00 to 17.00 o'clock, thus indicating a correlation with light. In Shingeumobyeo, GA_1 contents slightly increased during morning hours, while a similar hike in GA_1 contents was observed for Sangjubyeo during evening hours. GA_{19} was found to be the most abundant GA form in the three rice cultivars. Our results suggested that GA production in rice depends upon the response potential of rice cultivars and that light positively correlated to GA production in photosensitive rice cultivar.

Key words: Rice cultivars, endogenous GAs, diurnal changes, photoperiod, GC-MS-SIM

Introduction

Gibberellins (GAs) are a large family of tetracyclic, diterpenoid compounds, some of which 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 effect of photoperiod in different developmental processes such as stem elongation, bolting, bud dormancy, flowering and tuber formation is immense. There are evidences that phytochrome-A and phytochrome-B play an important role in photoperiod perception in long day (LD) plant and short day (SD) plants, respectively (Jackson and Thomas, 1997). Light perception occurs in the leaves but the response depends on the signal being transported to other parts of the plant. A series of short-term developmental and morphological changes takes place immediately after transferring the plants from long day to short day condition. Some of these adaptive changes might be influenced by plant hormones in the complex regulatory process. For instance, induction of tuber formation in potato by short day is also partially mediated by the photoperiodic regulation of GA biosynthesis. Tuberization is inhibited by GA, the formation of which leaves is less when grown under a photoperiod of short days than under long days (García-Martínez *et al.*, 2002).

The physiological significance of the diurnal changes of GA content is still unclear for plants. There are only a few reports about changes of GA level related to circadian rhythms in plants, which suggested that circadian rhythms in plants are not identical. In present study, diurnal changes in GA levels of three rice cultivars i.e. Sangjubyeo, Shingeumobyeo (photo-neutral) and Chuchengbyeo (photo-sensitive) were observed.

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Materials and Methods

Seeds of three rice cultivars i.e. Sangjubyeo, Shingeumobyeo and Chuchengbyeo were procured from Yeong-Nam Agricultural Research Institute, Milyang Korea. Sangjubyeo and Shingeumobyeo are photo-neutral cultivars and are widely cultivated in Korea, especially in the South, while Chuchengbyeo is a typical photosensitive cultivar. Seeds were surface sterilized in 5% NaOCl for 1 min and then in methanol for 2 min, rinsed with deionized water, left to imbibe in aerated deionized water and incubated in nursing beds for 5 days. Germinated seeds were grown in pots (17.5 cm diameter × 19.5 cm depth) filled with a bed soil for paddy rice to prevent other soil effects. The paddy rice was silt-loam with following chemical composition: pH 6.1; 3.25 g/kg organic matter; 331 mg/kg available P_2O_5; 348 mg/kg available SiO_2 and 6.78, 20.28, 3.17 $cmol^+/\mu g$ of K, Ca and Mg respectively (RDA, 1988). Seedlings were watered with Hyponex and Hoagland solution everyday and with distilled water whenever needed. The Hoagland nutrient solution was slightly modified in order to fulfill the requirements of present experiment. The composition of nutrient solution was as follows: 0.625 mM K_2SO_4, 0.5 mM MgSO4 $7H_2O$, 0.25 mM KH_2PO_4, 0.5 mM $CaSO_4$ 1/2 H_2O, 1 mM KNO_3, 1 mM NH_4NO_3, 0.0095 mM Fe-EDTA, and other micro-nutrients in their original concentration.

The experiment was conducted under a complete randomized block design (CRBD) with each treatment (cultivar x sampling time) consisting of 3 replications.

Growth conditions

Plants were grown in a growth chambers under a mixture of cool-white fluorescent and halogen lamps, yielding a light intensity of 800 $\mu mol\ m^{-2}s^{-1}$ (400 to 700 nm) measured at the pot surface by a LI-COR Portable Spectroradiometer (LI-1800, L1-Cor Biosciences, Lincoln, NE, USA). Plants in growth chamber were grown under a 12 hr light regimen at 28°C and 12 hr dark regimen at 20°C Average relative humidity ranged from 70 to 80% ± 5% in all treatments.

Extraction and quantification of endogenous gibberellins

The plants shoots were harvested every 3 h for 24 h on 42nd day of sowing, immediately frozen in liquid nitrogen and stored at - 70°C. When all the required material for GA analysis had been collected, the samples were lyophilized. The extraction method used for extraction and quantification of endogenous gibberellins was based on the already established procedure of Lee *et al.* (1998).

High-performance liquid chromatography (HPLC)

The GAs were chromatographed on a 3.9 × 300 mm μ BondaPak C18 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 (15 μl) 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 35 μl 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 35 l 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]	m/z (%, relative intensity of base peak)[b]
6 ~ 8	GA_8	Sample	594(100) 448(25) 379(20)
		Standard	596(100) 450(24) 381(21)
12~14	GA_1	Sample	506(100) 448(20) 313(17)
		Standard	508(100) 450(19) 315(14)
24~26	GA_{20}	Sample	418(100) 375(45) 403(14)
		Standard	420(100) 377(45) 405(13)
29~31	GA_{19}	Sample	434(100) 374(59) 402(41)
		Standard	436(100) 376(57) 404(40)
35~37	GA_{53}	Sample	448(47) 251(30) 235(30)
		Standard	450(47) 253(28) 237(28)
41~43	GA_{12}	Sample	300(100) 240(31) 328(31)
		Standard	302(100) 242(32) 330(29)

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

Statistical analysis

The data was analyzed for standard deviation by using sigma plot software (2004).

Results

The levels of endogenous bioactive GA_1 and of its precursor GA_{20} were significantly different in two different rice ecotypes though less pronounced differences were recorded for GA_{12}, GA_{53}, GA_{19}, and GA_8 contents (see Fig 1 & 2). The level of GA_{12} was the lowest (0.47 ng g^{-1} DW in Sangjubyeo) in comparison with the content of the other GAs, regardless of the cultivar. The level of GA_{19} was always over 100 ng g^{-1} DW in all three rice cultivars clearly indicating that GA_{19} is the predominant endogenous GA in all rice cultivars. The level of GA_{20} was always higher in Chucheongbyeo (photoperiodic-sensitive) (9.64 ng g^{-1} DW) than in Sangjubyeo (6.64 ng g-1 DW) and Shingeumobyeo (6.50 ng g^{-1} DW) and increased as time progressed from 8 o'clock morning to 5 o'clock afternoon (see Fig. 2). The bioactive GA1 showed a maximum peak at 17:00 (9.69 ng g^{-1} DW), only in the photo sensitive cultivar (Chucheongbyeo). The diurnal changes observed for GA_8 contents in three rice cultivars were insignificant.

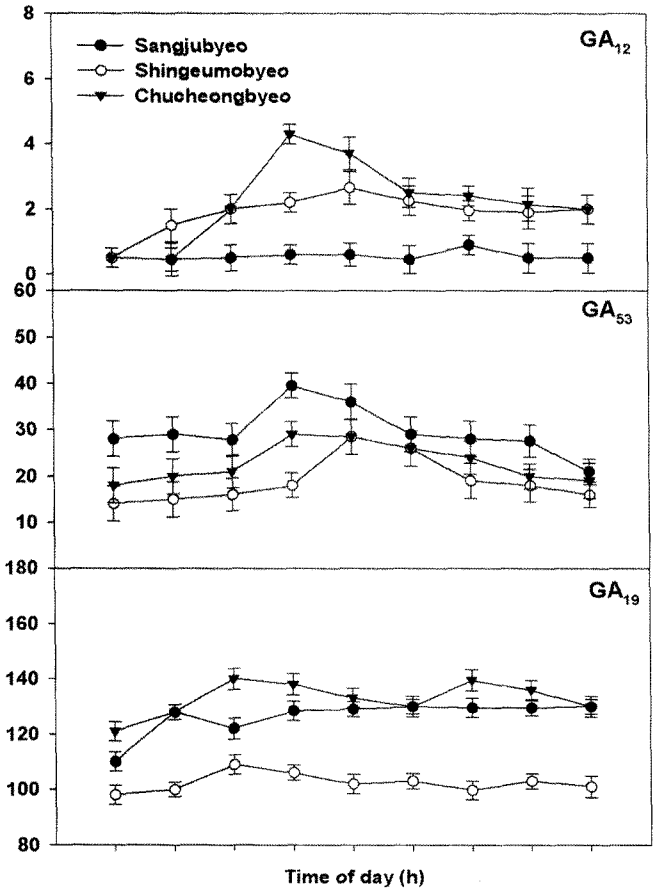

Fig. 1. Diurnal changes in GA_{12}, GA_{53}, and GA_{19} content of rice shoot (42 DAS), grown under 12 hr photoperiod. Bar at the bottom of graph indicated the dark period.

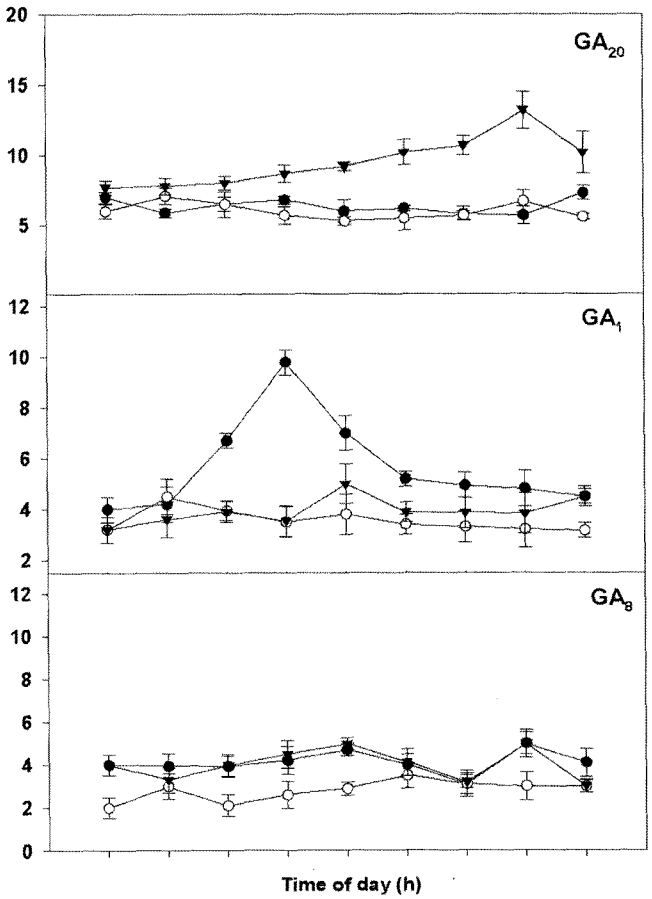

Fig. 2. Diurnal variations in GA_{20}, GA_1 and GA_8 content of rice shoot (42 DAS), grown under 12 hr photoperiod. Bar at the bottom of graph indicated the dark period.

The average content of bioactive GA_1 and of its precursor GA_{20} was, respectively, 1.5 and 1.7 times higher in the photoperiodic sensitive cultivar (Chucheongbyeo) than in the photo-neutral rice cultivars, regardless of analysis time (Fig. 3).

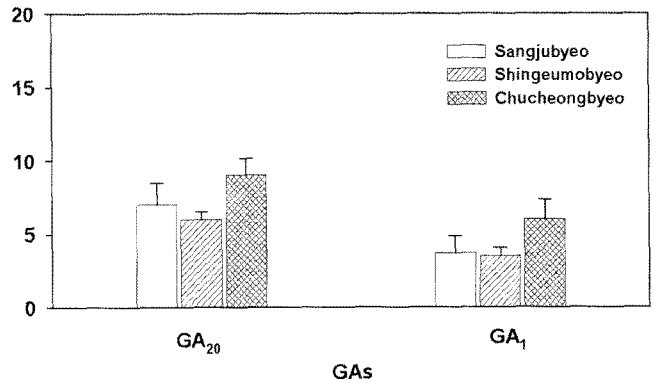

Fig. 2. Diurnal variations in GA_{20}, and GA_1 GA_8 content of rice shoot (42 DAS), grown under 12 hr photoperiod. Bar at the bottom of graph indicated the dark period.

Discussion

The photoperiod has pronounced effects on gibberellin production, as it regulates ent-kaurene synthesis, the first step in gibberellin biosynthetic pathway (Grennan, 2006). During present study an effort was made in order to evaluate the effect of similar light period on diurnal changes in endogenous GAs of rice cultivars with different response potentials for light. However, the actual differences in GAs levels in both types of rice cultivars at different times of day and night were insignificant. This suggests that the pulses or peaks are not physiologically important, although it is possible that the mass of tissue harvested and extracted, masked larger differences in GA levels in the apical meristems and young leaves.

In Chucheongbyeo, the GA_1 level increased sharply from 11.0 AM to 5.0 PM, demonstrating that bioactive GA_1 production in photosensitive rice cultivar was stimulated by light. However, Shingeumobyeo showed an increase in GA_1 levels during morning hours (8.0 AM to 11.0 AM), while Sangjubyeo recorded an almost equal increase in GA_1 contents during evening hours (5.0 PM to 8.0 PM). The level of bioactive GA_1 was not significantly different in the dark and exhibited almost similar biosynthesis patterns. Our results clearly indicated that the two ecotypes responded differently to the light period. Similarly, Foster and Morgan (1995) reported that in *Sorghum bicolor*, a SD plant, the GA_1 levels increased at lights-on, peaked in the afternoon, and decreased to a minimum in darkness. But in *Begonia*, no significant differences in GA content were found during the diurnal day and night cycle (Myster *et al.*, 1997).

It was thus concluded that the endogenous GAs content changes diurnally in plants, though the magnitudes of these variations are different in different species and even with in the same species. Our study also confirmed that early-13-hydroxylation pathway of GA biosynthesis was functional in rice. Similar observations were also reported by Kurogochi *et al.* (1979), Suzuki *et al.* (1981) and Kobayashi *et al.* (1988, 1993 & 1994). Variations in the endogenous GAs level of different rice ecotypes in response to light encourage us to investigate the relationship of light stimulus and GA metabolism in different rice ecotypes. However, to investigate such phenomenon, further research must be conducted.

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