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Altered Fine Structure of Amylopectin Is Induced by Exogenous Gibberellin During Rice Grain Ripening

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ABSTRACT : When GA_4 was applied to heading stage, it was examined to understand the change of plant hormones and starch during grain filling and ripening. Exogenous gibberellin caused a dramatic decrease in endogenous ABA content. Endogenous GA_4 content in both superior and inferior part was more promoted in GA_4-treated rice grain than in the control. GA_1 content of an inferior part was not detected in the control and GA_4-treated rice otherwise GA_4 was detected in all grain parts. Ripened grain rate in GA_4-treated rice grain was lower than that of the control plant. Amylopectin from GA_4-treated grain contained more very short chains with degree of polymerization (DP) between 4 and 8 than amylopectin from the control plant. It suggests strongly that fine structure of rice endosperm may be changed by exogenously applied GA_4 in rice plants.

Keywords: gibberellin A_4, superior and inferior grain, starch, *Oryza sativa.*

Abbreviations: GA_4 - gibberellin A_4, ABA - abscisic acid

Starch accumulation during rice grain filling is a key process determining grain yield and quality in rice plant. Changes in starch accumulation and quality affects many environmental factors such as fertilizer, watering depth, air temperature, and plant hormones in rice plants. Phytohormones are also considered as one of the key regulators to seed development (Karssen, 1982). There are some results that auxins, gibberellins and abscisic acid are involved in regulating grain development (Hansen and Grossmann, 2000). A rice panicle is composed of a number of spikelets or caryopses. Based on their flowering date and locations within a panicle, the spikelets can be classified as superior and inferior (Zhu *et al.*, 1988; Iwasaki *et al.*, 1992; Umemoto *et al.*, 1994). Superior spikelets flower earlier and located at the top of primary branches, whereas inferior spikelets flower later and are located at the base of secondary branches. Superior spikelets usually exhibit a faster rate of increase in dry weight during development and higher grain weight than inferior spikelets (Sikder and Gupta, 1976; Kato, 1989; Yang *et al.*, 2003). The physiological effect of exogenous gibberellic acid (GA_3) on rice plants is to break dwarfism and stimulate the stem elongation of genetically dwarf genotypes leading to potentially increasing grain yield (Singh *et al.*, 2003). Until now, however, there are no reports that the growth responses of rice plants to bioactive gibberellin such as gibberellin A_4 (GA_4) that are might be operated and may have a physiological function. The purposes of this study was to investigate the exogenous effects of gibberellin A_4 on the changes of endogenous plant hormones such as gibberellin and abscisic acid, and starch content in both the superior and inferior grains of rice during grain ripening

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MATERIALS AND METHODS

A japonica rice (*Oryza sativa* L. cv. Hwayoung) was grown in seed bed and forty-five-day-old seedlings was transplanted on June 15 into the plastic pot (17 cm height, 16 cm diameter, and 3.42 m^3 volume) which was filled with 4.5 kg sandy loam soil and with a hill per pot and three seedlings per hill. Two g of nitrogen as urea, 0.2 g of phosphorus as superphosphate, and 0.3g of potassium as KCl were mixed into the soil in each pot before transplanting. At mid-tillering, panicle initiation, and heading, 0.6, 1.2, and 1g of urea were top dressed into each pot, respectively. The rice plants were watered daily and the pots were kept at 1 to 2 cm water level for growing period. Starting at 9-d post anthesis (DPA), 200 $\mu g\ g^{-1}$ of gibberellin A_4 was sprayed at the rate of 50 cm^3 per pot on the leaves and panicles daily for 7 d. The rice plants sprayed with the same volume of distilled water were taken as the control. Panicles from each treatment were sampled for two times on 8 Sept and 26 Oct after GA application, respectively.

For endogenous gibberellins and ABA analysis, twenty panicles in one pot were harvested and divided into two groups. Half of them were analyzed for GA analysis. Another half-sampled panicles were used for ABA analysis. Ten panicles in another pot were also harvested for starch

analysis. Each measurement had five replicates.

Starch in rice panicles was measured from modification of the literature (Wang *et al.*, 1999). Rice endosperms were placed in 20 mM HEPES-NaOH buffer (pH 7.4) and heated at 90°C for 10 min. The heated sample was homogenized on ice with fine quartz sand, and then centrifuged at 18,000 *g* for 10 min at 4°C. The precipitated pellet was suspended in distilled water, and the suspension was heated at 90°C for 1h. The gelatinized starch was digested with amyloglucosidase at 50°C for 1h, and resultant glucose was determined according to the methods (Bermeyer and Bernt, 1974).

ABA was quantified following method of Browning and Wignall (1987) and Kamboj *et al.* (1999). Dry sample (0.5 g) was extracted with 30 cm^3 of 95 % isopropanol, 5 % glacial acetic acid, and 100 ng of [(±)-3,5,5,7,7,7-d6]-ABA standard. The extracts were dried and methylated by adding diazomethane and monitoring the responses ions, which were at m/e (mass versus charge) 162 and 190 for Me-ABA and 166 and 194 for Me-[$^2H_6$]-ABA (obtained from Dr. Suzanne R. Abrams, *Plant Biotechnology Institute, National Research Council of Canada, Canada*), respectively.

For analyzing gibberellins, the extraction and HPLC of GA-metabolites followed the reference (Lee *et al.*, 1998). Lyophilized tissues were ground to a fine powder in a mortar and extracted with 80% methanol (MeOH). After methanolic extraction, gibberellins were purified using a series combination with preparatory column chromatography, solvent partitioning, and *reverse-phase HPLC* (Foster and Morgan, 1995). Each deuterated internal standards were added. Tritiated (1500 Bq each of [1,2-$^3H_2$] GA_1 and [1,2-$^3H_2$] GA_4) standards were also added to the combined extract to monitor recovery through the purification procedure. Gibberellins were quantified using GC-MS selected ion monitoring by calculating the area ratio of endogenous GA to the deuterated GA, and the contribution from the deuterated standard to the non-deuterated GA was corrected (Beall *et al.*, 1991).

RESULTS AND DISCUSSION

Recently it was found that endogenous ABA content in the superior and inferior grains was altered by exogenously applied gibberellin compared to the control during rice grain filling. When GA_4 was applied to heading stage, ABA content was more increased in inferior grain than in superior part. Otherwise, ABA content of superior grain in the control was increased slightly compared to inferior grain. Exogenous gibberellin caused a dramatic decrease in endogenous ABA content. In the changes of bioactive gibberellins (GA_1 and GA_4), GA_1 content did not show remarkable change in the superior and inferior grain of the control plant, however, its content was dramatically twice decreased in inferior grain compared to the superior part when gibberellin was applied. In addition, endogenous GA_4 content in both superior and inferior part was more promoted in GA_4-treated rice grain than in the control. It was first found that total gibberellin content was increased in superior grain like the control plant although ABA content was increased in inferior grain during rice grain filling (Table 1). ABA content in GA_4-treated rice grain during grain ripening was similar to each

Table 1. Effect of exogenously applied gibberellin (GA_4) on endogenous ABA, gibberellin and ripened grain rate during rice grain filling.

Treatment	Panicle part	ABA [ng g^{-1} (d.m.)]	Gibberellin [ng g^{-1} (d.m.)]		
			GA_1	GA_4	Total
Control	Superior	34.3±1.2	7.8±1.0	4.5±1.4	28.5±2.7
	Inferior	32.6±2.2	8.1±0.3	3.9±0.8	27.3±1.9
GA_4 200 μg g^{-1}	Superior	24.6±0.9	8.2±1.7	9.6±0.6	33.8±2.1
	Inferior	27.1±0.4	4.5±1.1	5.7±0.5	29.6±1.1

Values in parenthesis indicate the data checked at harvesting stage. Means±SE, n = 5.
Total gibberellins are both the early C-13 hydroxylation and non C-13 hydroxylation pathway including active GA_1 and GA_4.

Table 2. Effect of exogenously applied gibberellin (GA_4) on endogenous ABA, gibberellin and ripened grain rate during rice grain ripening.

Treatment	Panicle part	ABA [ng g^{-1} (d.m.)]	Gibberellin [ng g^{-1} (d.m.)]		
			GA_1	GA_4	Total
Control	Superior	4.3±0.4	0.3±0.0	0.2±0.1	3.9±0.1
	Inferior	2.8±0.5	n.d.	0.1±0.0	4.2±0.3
GA_4 200 μg g^{-1}	Superior	3.8±0.3	0.5±0.2	0.3±0.1	4.9±0.2
	Inferior	3.9±0.0	n.d.	0.4±0.1	3.5±0.8

Values in parenthesis indicate the data checked at harvesting stage. Means±SE, n = 5. n.d.: not detected. Total gibberellins are both the early C-13 hydroxylation and non C-13 hydroxylation pathway including active GA_1 and GA_4.

grain part, otherwise ABA content showed different level in the control. It is interesting that GA_1 content of an inferior part was not detected in the control and GA_4-treated rice otherwise GA_4 was detected in all grain parts (Table 2).

When GA_4 was applied to rice plant, grain weight was dramatically decreased compared to the control plant, grain

Table 3. Effect of exogenously applied gibberellin (GA_4) on changes of grain weight and ripened grain rate at the rice harvesting stages.

Treatment	Panicle part	Grain weight (mg grain^{-1})	Ripened grain (%)
Control	Superior	24.2±1.2*	88.7
	Inferior	23.6±1.1	86.9
GA_4 200 μg g^{-1}	Superior	22.3±0.9*	82.2*
	Inferior	23.5±1.0	84.3*

Values in parenthesis indicate the data checked at harvesting stage. Means±SE, n = 25. Values significantly different at $P<0.05$.

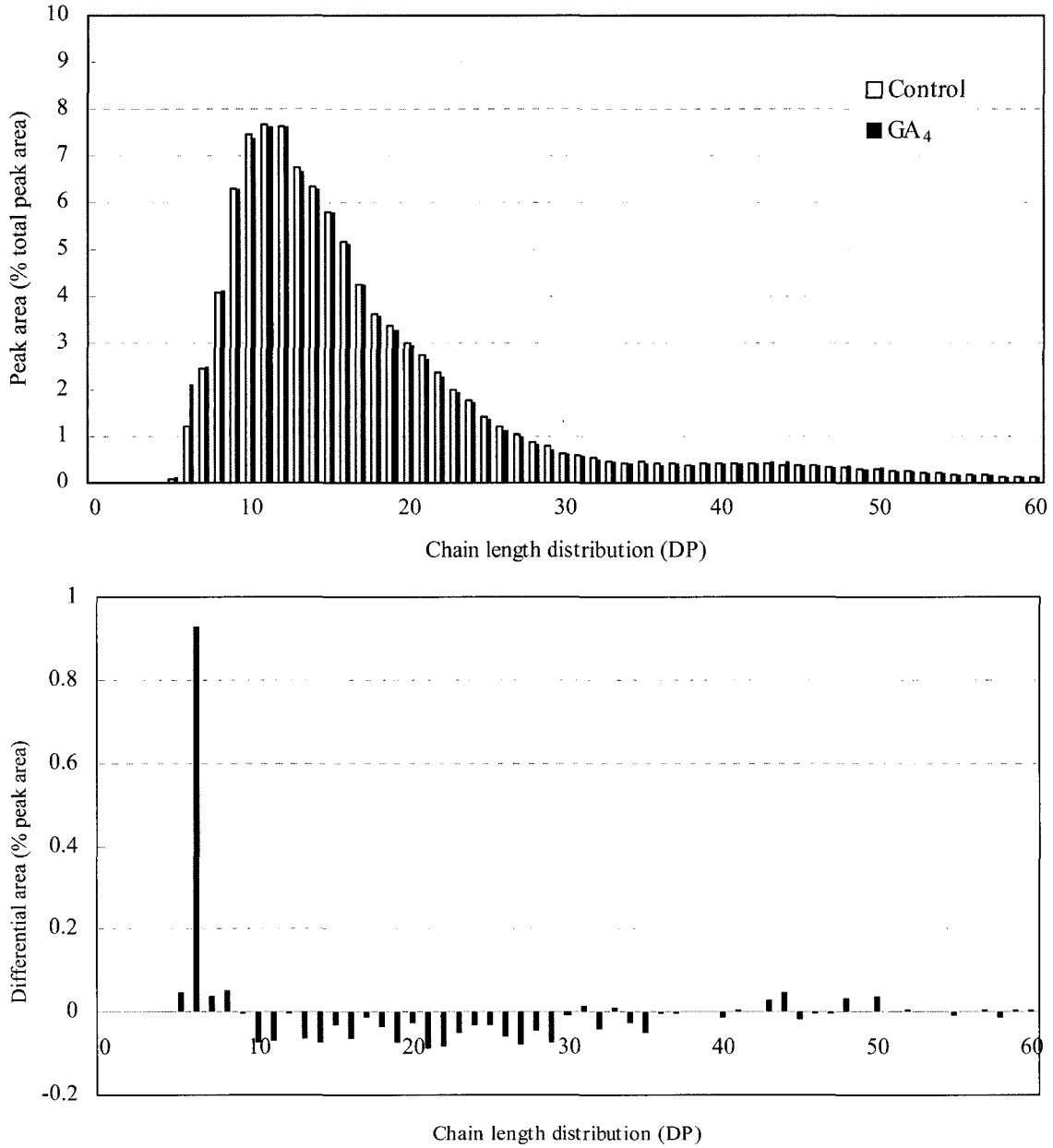

Fig. 1. Effects of GA_4 on chain length distribution of α-polyglucans in rice endosperm of Goamibyeo. (up) Chain length distribution and (down) Differences in chain length distribution of total α-polyglucans in the control and GA_4-treated rice endosperm.

weight of inferior part was more increased than that of superior. Ripened grain rate in GA_4-treated rice grain was lower than that of the control plant. It is also interesting that ripened grain rate was increased in the inferior part compared to the superior part (Table 3). It is generally recognized that gibberelllin (GA_3) promotes phloem uploading of metabolite in leaves to sink source, and accumulates starch into the leaves (Singh, 2003; Matsukura *et al.*, 1998). In spite of same group of gibberellic acid and GA_4, it shows different physiological roles in the rice plants. It can be explained possibly that two different gibberellin biosynthetic pathway is operated and the early C-13 hydroxylation route leading to GA_1 and GA_3 (gibberellic acid is weakly produced to conversion of GA_{20} and GA_5) and non C-13 hydroxylation pathway leading GA_4 may considers to have different action in rice plants. We also determined the chain length distribution of á-polyglucans in rice endosperm as affected by gibberellin and the chain profiles of the control plant and GA_4-treated rice plants (Fig. 1). Amylopectin from GA_4-treated grain contained more very short chains with degree of polymerization (DP) between 4 and 8 than amylopectin from the control plant. Otherwise relative long chains with degree of polymerization DP = 20 to 30 were depleted. It suggests strongly that fine structure of rice endosperm may be changed by exogenously applied GA_4 in rice plants. To clarify the change of fine structure, it needs further study on the thermal properties as affected by GA_4 in rice endosperms.

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