J.Food Pharm.Sci. 1 (2013) 14-17

Journal of Food and Pharmaceutical Sciences

Available online at jfoodphamsci.com

Research Article

Changes in Gibberellic Acid (GA_3) Content in *Oryza sativa* Due to Paclobutrazol Treatment

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ARTICLE INFO

Received 07/01/2012
Received in revised form 17/01/2012
Accepted 27/02/2012
Available online 01/03/2012

ABSTRACT

The objective of this study was to determine the level of plant hormone gibberellic acid (GA_3) in paddy due to the treatment of Paclobutrazol (PBZ) treatment using high performance liquid chromatography (HPLC) with UV-vis detection at 208 nm. The separation was achieved using reversed column Crestpak C18 (150 mm x 4.6 mm i.d; 5 µm) at 30 ± 1° C using mobile phase of acetonitrile-water (30:70%; v/v), pH 6.80. The treatment of PBZ with different concentration of 100, 200, 400, and 600 mg/L reduced the concentration of GA_3 in paddy. The level of GA_3 in paddy treated with 100 mg/L of PBZ did not show significant difference from untreated one. However, the level of GA_3 in paddy treated with other concentrations (200, 400, and 600 mg/L) of PBZ was significantly different ($P < 0.05$) from untreated paddy.

Keywords: analysis, gibberellic acid, paclobutrazol, HPLC

1. Introduction

Currently, in order to succeed in agriculture activities, careful planning based on the understanding of local climate, soil characteristics and controlled growth using pesticides, fertilizers and growth chemical promoters must be adhered. One of the main internal factors controlling the growth and development of plant is plant hormones (Kelen *et al.*, 2004).

Gibberellic acid or chemically named as 2,4a,7-trihydroxy-1-methyl-8-methylenegib-3-ene-1,10-dicarboxylic acid 1,4a-lactone and other related compounds are often exploited in rice plantation (Santos *et al.*, 2004). GA_3 can be taken into account as vegetal growth promoter commonly used in the crop. Chemically, this compound is a carboxylic acid (Figure 1) produced by submerged fermentation or solid substrate cultures of the fungus *Gibberella fujikuroi* (Berrios *et al.*, 2010).

Several analytical methods have used for determination of GA_3 and other phytohormones, most of them are based on chromatographic techniques, namely:

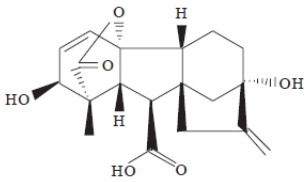

Fig 1. The chemical structure of gibberellic acid (GA_3)

thin layer chromatography-densitometric (Yayas *et al.*, 1997), high performance liquid chromatographic using mass spectrometric (LC-MS) detection (Hou *et al.*, 2008), LC-MS using solid phase extraction as sample preparation technique (Ma *et al.*, 2008), UV-vis detection (Kelen *et al.*, 2004; Wu and Hu, 2009; Tansupo *et al.*, 2010) (most of the developed methods were exploited UV-Vis detection; therefore, in this study we use HPLC in combination with UV-vis detector for quantitative analysis of GA_3).

In addition, paclobutrazol (PBZ) is a triazole derivative and has been reported to inhibit GA

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biosynthesis in plant by inhibiting kaurene oxidase, a Cyt P-450 oxidase, thus, blocking the oxidation of kaurene to kaurenoic acid (Rademacher, 2000) resulting in retardation of plant height. Various researchers have reported on the positive effect of PBZ in controlling internodes elongation (Wahyuni, 2002; Yim *et al*., 1997; Yoshinaga *et al*., 2005). The objective of the present study was to determine the levels of GA_3 due to the treatment of PBZ in paddy.

2. Materials and Methods

2.1 Materials

Pro analytical grade of reagents and solvents were used, unless otherwise indicated. Water, with a conductivity lower than 0.05 µS/cm, and acetonitrile purchased from E. Merck (Darmstat, Germany) were of HPLC grade. Hydrochloric acid and phosphoric acid used for pH adjustment as well as potassium dihydrogen phosphate, potassium hydrogenphosphate, methanol, ethyl acetate, and sodium sulfate used for extraction of GA_3 were all of analytical grade obtained from E. Merck. The standard of GA_3 was obtained from Sigma (St. Louis, USA).

2.2 Experimental design

A field trial was carried out from August 2007 to December 2007. Two locations having environmental difference which allowed the chemical testing in broader scope were used, namely at Perak and Kedah, Malaysia. A plot of 0.5 hectare was divided into 20 subplots of 5 m x 5 m and the experiment was done in five replicates using a complete randomized block design. The variety of paddy used was MR219 with a sowing rate of 150 kg/ha using the broadcast method. The field was flooded when the paddy plants were about two weeks old and water in the field was maintained at about 15-20 cm deep. Subplots were separated by 2 meter distance among them. PBZ with a concentration of 0, 100, 200, 400 and 600mg/L was applied at panicle initiation stage (55-57 days after showing). The treatment was applied with a knap-sack sprayer using a spray volume equivalent to 350 l/ha at a pressure of 300 kPa.

2.3 Determination of Gibberellic acid (GA_3)

2.3.1 Sample preparation

The levels of GAs was determined using high-performance liquid chromatography (HPLC) using UV-Vis detector according to Durley *et al.*, 1982 and Wurst *et al*., 1984, with slight modification. The sample was taken two weeks after application of PBZ. Approximately 5 g of fresh weight of third internode and freeze-dry tissue was accurately weighed with analytical balance having sensitivity of 0.1 mg. Samples were placed into Beaker filled with 30 mL methanol 70% (v/v) and then kept at 4°C until overnight. The extract was filtered through a Whatman filter and the methanol was evaporated under vacuum. The aqueous phase was adjusted to pH 8.5 with 0.1 M phosphate buffer and then partitioned with ethyl acetate 3 times. After removal of the ethyl acetate phase, the pH of the aqueous phase was adjusted to 2.5 with 1 N HCl. The solution was partitioned with diethyl ether 3 times, and then passed through andydrous sodium sulfate. After that, the diethyl ether phase was evaporated under vacuum and the dry residue containing hormones of GA_3 was dissolved in 2.0 mL of absolute methanol and stored in vial at 4°C.

2.3.2 Chromatographic condition

The chromatographic analysis was performed using Shimadzu Model LC equipped with reversed phase column Crestpak C18 (150mm x 4.6 mm i.d; 5 µm) maintained at constant temperature of 30 ± 1° C. The mobile phase used was acetonitrile-water (30:70%; v/v), pH adjusted to 6.80 with dilute HCl delivered isocratically with flow rate of 1.0 mL/min. An injection volume of 10 µL was used for each analysis. The standard solution of the individual acid was prepared in the mobile phase and chromatographic separately to determine the retention time for acid. The signal of the compound was monitored at 208 nm for GA_3, respectively. The standard solution of GA_3 was made in the mobile phase and chromatographed separately to identify retention time of GA_3.

2.3.3 Statistical analysis

Data of GA_3 due to the treatment of PBZ were analyzed using one-way analysis of variance procedure (ANOVA) in Syntax. Treatment means were compared by LSD test.

3. Results and discussion

Due to its versatility, rapidity, simplicity and ease in optimization, HPLC using UV-Vis spectroscopy detection is the most common analytical technique used for quantification of GA_3 and other plant hormones such as abscisic acid and indole-3-acetic acid (Kelen *et al.*, 2004). In this study, an absorbance at 208 nm was selected for such determination. Figure 2 showed the HPLC chromatogram of GA_3 in paddy samples. The peak of GA_3 was well separated from others.

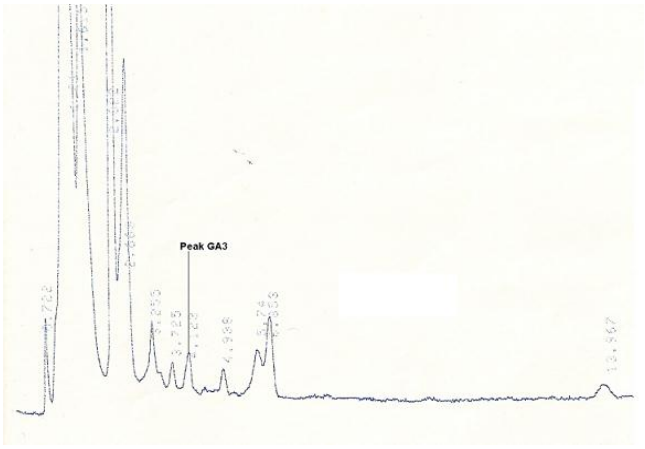

Fig 2. The chromatogram of GA_3 in paddy sample using the optimized condition.

Figure 3 exhibited calibration curve for the determination of GA_3. There was a linear relationship between concentration (x) and peak area as response (y) with the coefficient of determination (R^2) of 0.9984. This curve was further used to predict the levels of GA_3 using interpolation technique.

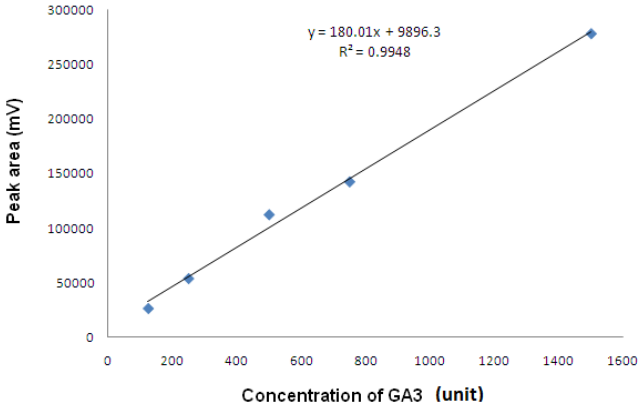

Fig 3. The calibration curve for the relationship between concentration of GA_3 and peak area as response

The analytical figure of merits revealed that the recovery percentage to access the analytical accuracy was in the range of 93. 12 – 105.25 %. According to Horwitz, the acceptable value of recovery for ppm level was in the range of 80 – 110 %. Furthermore, the method precision was evaluated using inter-day precision giving relative standard deviation (RSD) of 6.15 – 12.5 %. The maximum RSD values allowed using Horwitz method is 15 % with the analyte level of 1 ppm (Gonzalez and Herrador, 2007). The sensitivity of the used HPLC method, expressed with limit of detection was relatively low, i.e. 1.97 ppm.

Table 1 exhibited the levels of GA_3 calculated as µg GA3/100g fresh weight due to the treatment of PBZ. The level of GA_3 in the untreated paddy was 0.069 ± 0.003, which is not statistically different from that treated with PBZ at 100 mg/L. However, the level of GA_3 in paddy treated with other concentrations was significantly different ($P < 0.05$). There is a decrease of GA_3 due to the treatment of PBZ.

Table 1. The concentration of GA_3 due to the treatment of PBZ

Paclobutrazol (PBZ)Treatment (mg/L)	GA_3 concentration (µg/100g FW)
D0 (control)	0.069 ± 0.003^a
D1 (100)	0.065 ± 0.004^a
D2 (200)	0.047 ± 0.003^b
D3 (400)	0.016 ± 0.002^c
D4 (600)	0.009 ± 0.001^c

The result described support the notion that the growth rate of rice plant and internode is determined by a growth-promoting plant hormone GA_3. We showed that the level of endogeneous GA_3 in rice plant is reduced by PBZ treatment (Table 1). There is strong evidence that GA_3 is catalyzed via an indirect pathway from involve cytochrome P450 whereby oxidized ent-kaurene to ent-kaurenoic acid (Rademacher, 2000). PBZ is an inhibitor of biosynthesis ent-kaurene to ent-kaurenoid acid (Yamaguchi, 2008), its effect on GA_3 levels and growth in rice plant can be explained in terms of reduced GA_3 formation. The levels of GA_3 with PBZ treatment in several concentrations decreased following increased in PBZ concentration, but optimum concentration of PBZ is 400 mg/L since 600 mg/L was insignificantly different as compared to 400 mg/L.

4. Conclusion

The treatment of PBZ with different concentration of 100, 200, 400, and 600 mg/L can reduce the concentration of GA_3 in paddy. The level of GA_3 in paddy treated with 100 mg/L of PBZ did not show significant difference from untreated one. Meanwhile, the level of GA_3 in paddy treated with PBZ at concentrations of 200, 400, and 600 mg/L was statistically different ($P < 0.05$) from untreated one. However, there was a significant decline in GA_3 concentration with the increase in concentration of PBZ up to 400 mg/L. The use of PBZ at 600 mg/L was not advantageous compared to 400 mg/L. Therefore, the optimum concentration of PBZ in rice field is 400 mg/L, to reduce internode length and hence increase lodging resistance.

Acknowledgement

The authors thank to Ministry of Science, Technology and Innovation (MOSTI) Malaysia for the grant No. 05 - 01 – 04 – SF0164 awarded to Assoc. Prof. Dr. Uma Rani Sinniah.

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