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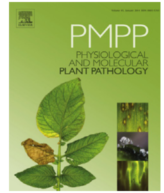

The role of phytohormones in relation to bakanae disease development and symptoms expression

Shireen A. Jahan Quazi [a, b, *], Sariah Meon [c], Hawa Jaafar [d], Zainal Abidin B.M. Ahmad [c]

[a] Institute of Tropical Agriculture, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia
[b] Plant Pathology Division, Bangladesh Rice Research Institute, Gazipur 1700, Bangladesh
[c] Department of Plant Protection, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia
[d] Department of Crop Science, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia

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ABSTRACT

Fusarium proliferatum, the causal agent of bakanae disease was found to be up-regulated gibberellic acids (GAs) in bakanae diseased rice plants and thereby contributing to the different types of symptoms observed associated with the disease. The up- and down regulation of GAs, IAA and ABA were studied in susceptible (MR 211) and resistant (BR3) varieties artificially inoculated with *F. proliferatum* at different times after inoculation. The increase in GAs including fungiproduced GA3 and IAA were higher in inoculated MR 211 rice plants (GAs = 26%, IAA = 40.39%) as compared to resistant BR3 (GAs = 19%, IAA = 4.27%), 7 days after inoculation (disease score 1, S-1) in whole plant sample analysis. The increase were higher in both roots and leaves but not in the stem, thus the main symptom observed as stunting of the susceptible plants at disease score 1. However, 14 days after inoculation (disease score 3, S-3), both phytohormones were observed to increase in whole plant sample analysis as well as in all plant parts sampled. This increasing effect was found associated with elongation of internodes and chlorosis of leaves in susceptible plants of MR 211. As the symptoms progressed to disease score 5 (S-5, after 21 days of inoculation) susceptible plants of MR 211 were started to collapse and lodged due to over elongation, and finally dead followed by a decrease in GAs and IAA but an increase in ABA. In resistant variety BR3, marginal up regulation of GAs and IAA were observed only at 21 days after inoculation in stems with no typical symptom of bakanae disease.

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Introduction

Phytohormones are plant hormones, produced inside the plant that help plant cells to stimulate or inhibit specific enzyme systems and in the regulating of plant physiological activities including metabolism, growth and development. Apart this, plant pathogens may also produce the same phytohormones in infected plants. In addition, it was observed that pathogen can alter metabolic activity by producing different phytohormones by themselves in host plant cells [24]. Singh et al. (1997) [22] reported that plant pathogen interaction associated with disease susceptibility or resistance reaction and was related with alterations in the levels of phytohormones in plants. A relationship between production of different phytohormones, quantity and plant resistance has already been established by Ref. [20].

A number of phytohormones have been identified and found responsible for plant growth development as well as for disease resistance or susceptibility. Among the identified and reported phytohormones gibberellic acids (GAs), indole acetic acid (IAA) and abscisic acid (ABA) are considered as major and important considering their role in developmental process and their involvement in disease resistance mechanism in plants [11,13,16].

Among the three important phytohormones, only the role of increased GA_3 by the causal fungal pathogen has been reported for abnormal increase of plant height especially internodes in bakanae diseased rice plants [8,23,29]. However, the interrelationship between other major phytohormones and increased GA_3 produced by the pathogen in inoculated plants of susceptible variety had not been identified. Moreover, the reasons behind expression of other symptoms of bakanae such as

* Corresponding author. Plant Pathology Division, Bangladesh Rice Research Institute, Gazipur 1701, Bangladesh. Tel.: +8801855873259.
E-mail address: shireenbrri@yahoo.com (S.A.J. Quazi).

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stunting in plant height, chlorosis, leaf and stem browning, crown rot and root necrosis have not been reported yet. Considering the synergistic relationship between GA_3 and IAA, and antagonistic relationship between GA_3 and ABA [6,28], it was postulated that GA_3, IAA and ABA phytohormones might be involved in different bakanae symptoms expression. As endogenous GA_3 levels were found to be correlated with tallness (hypertrophy) in bakanae diseased plants [17], therefore, the justification of the current study was that the up-regulation level of GA_3 might also be responsible for the up- and down-regulation of IAA and ABA and thereby associated with other symptoms expression. Moreover, in relation to bakanae disease development, IAA and ABA that might have influences on disease susceptibility/resistance and have not been identified yet.

Apart this, *Fusarium proliferatum* is a member of *Fusarium (F.) fujikuroi* species complex and had been found associated with bakanae disease as this pathogen was isolated from bakanae diseased plant samples [27,29]. Although, *Fusarium fujikuroi,* has been identified and reported as a virulent pathogen of bakanae disease [1,29] but, recently *F. proliferatum* also has been identified and reported as a bakanae causing pathogen through morphological and molecular identification [19]. Moreover [12], reported that phylogenetically *F. fujikuroi* and *F. proliferatum* are closely related fungus and bakanae disease caused by *F. proliferatum* produced different types of bakanae symptoms through pathogenicity test similar as produced by *F. fujikuroi* [19]. Therefore, the aims of this research were 1) to find out the relationship between up- and down regulation of GAs (including GA_3), IAA and ABA in infected MR 211 (susceptible) and in infected BR3 (resistant) variety with bakanae causing pathogen *F. proliferatum* and 2) to determine the role of these three phytohormones in bakanae symptoms expression.

Materials and methods

Inoculation method and plant growth management

Seeds of susceptible variety, MR 211 (collected from Malaysian Agricultural Research and Development Institute, Malaysia) and resistant variety BR3 (collected from Bangladesh Rice Research Institute, Bangladesh) to bakanae disease were used in this study. Before conducting this study, MR 211 and BR3 were identified as susceptible and resistant to bakanae disease, respectively, through varietal screening test. The varietal screening test was evaluated using disease severity index (DSI) where, DSI in the range of <0.2, between 0.2 and 1.0, and >1.0 designated as resistant, moderately resistant and susceptible (data not presented here). The seeds were inoculated with *F. proliferatum* according to [19] in varietal screening test method. In brief, the seeds were surface sterilized with 70% ethanol, washed with sterilized distilled water and then soaked overnight in sterilized distilled water. Then, the water was drained out and the seeds were further soaked in spore suspension (10^6 conidia⁻mL) of *F. proliferatum* isolate for 48 h. The seeds were then planted in sterilized soil (40% sand, 30% clay, 30% peat) in trays (2 kg soil per tray) measuring $38 \times 28 \times 11$ cm and arranged in a completely randomized design with 3 replications (60 seeds per replication). For the control treatment, pre-soaked seeds with water were soaked further in sterile distilled water for 48 h before sowing. All trays were placed in a glasshouse with day and night temperatures of 30–35 °C and 23–30 °C respectively, and watered once daily with a hand sprinkler. Fertilizer comprising of N: P: K (15:15:15) were applied at 4 g per tray, twice at 15 day intervals.

Fungal mycelium harvest

Three mycelial plugs (5 mm) from 5 day old single conidium culture of virulent *F. proliferatum* isolate was inoculated aseptically into 100 mL of potato dextrose broth and incubated for 7 days at 28 °C. The mycelial mat grown on the medium was filtered through a double layer of sterile muslin cloth, washed with sterile distilled water, dried on filter paper and stored at −20 °C until further analysis.

Sampling procedure

Disease severity scale from 1 to 5 for bakanae disease assessment following the method of by Refs. [29] and [1] with some modifications was used and illustrated in Table 1.

Disease symptoms expression in disease score 2 quickly turned into disease score 3, and similar observation was also found in disease score 4 that turned rapidly to disease score 5. Disease scores 2 and 4 were mainly the initial symptoms expression of disease score 3 and 5, respectively. The most prominent and typical symptoms associated with bakanae disease were observed in disease score 1, 3 and 5. Therefore, only three disease score levels {score 1 (S-1) = plant stunted with chlorotic leaves (7 days after inoculation), score 3 (S-3) = abnormal elongated internodes with chlorotic or brownish leaves (14 days after inoculation), and score 5 (S-5) = leaf and stem browning with elongated internodes, fungal masses produced on the infected plant or dead plant (21 days after inoculation)} were chosen for sampling of phytohormones analysis. During each sampling time plants were collected randomly from each of the disease score level (disease score 1, 3 and 5) along with control (healthy) plants, immersed in liquid nitrogen immediately and then stored at −20 °C until analysis. Analysis was carried out on whole plant samples (WP) and from individual plant parts viz. leaves, stems and roots.

Extraction, detection and quantification of phytohormones

Chemicals and reagents used

Methanol (HPLC grade), phosphate buffer components: [monobasic sodium phosphate monohydrate (Sigma–Aldrich, >99.0%), and dibasic sodium phosphate dihydrate (Sigma–Aldrich, >99.0%)], ethyl acetate (Sigma–Aldrich, HPLC grade), hydrochloric acid (Sigma–Aldrich, 36.5–38.0%), diethyl ether (Sigma–Aldrich, HPLC grade), acetic acid (Sigma–Aldrich, >99.0%), sodium sulfate (Sigma–Aldrich, HPLC grade), and deionized ultra pure water (MQ) were used.

Chromatographic procedure

The HPLC (Model: Agillent, 1100 series) machine was equipped with a degasser (DEGASSER, G1322A), quaternary pump (Quat Pump, G1311A), an auto injector (ALS, G 1313A) and detector (DAD, G1315B). The column used was a Phenomenex C18 Torrance CA (150 × 4.6 mm id) stainless steel analytical column with 5 μm particle size. The column temperature was maintained constant at 30 °C in the column oven (COLCOM, G 1216A).

Standard solution and standard curve preparation: The standard solution of the individual phytohormone (GA_3, IAA and ABA) was dissolved in HPLC grade methanol (Sigma–Aldrich), prepared in a series of dilutions and chromatographed separately to determine the retention time for each phytohormone [15]. These solutions were filtered using Whatman cellulose membrane filter (0.2 μm, Germany) before injection. The signal of the compounds was monitored at 208, 265 and 280 nm for identification of better peak resolution of each phytohormone. The retention times of the solutions were determined based as three different determinations.

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