Songklanakarin J. Sci. Technol.
43 (2), 344-351, Mar. - Apr. 2021

http://www.sjst.psu.ac.th

Original Article

Effect of product containing spores of *Bacillus cereus* strain RS87 on growth and yield of Thai rice cultivar RD49 under water stress conditions

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Received: 10 January 2019; Revised: 16 September 2019; Accepted: 21 January 2020

Abstract

The objectives of this study were (1) to explore the potential of the rhizo-product *Bacillus cereus* strain RS87 [hereafter, "product"] in combination with different fertilizer regimes for enhancing rice tolerance to water stress while still maintaining good yield components, and (2) to examine the antioxidant defense system during water stress conditions. Results showed that rice treated with the product with at least 75% recommended fertilizer rate (RFR) tolerated water stress well and had mean total filled grain weight equivalent to rice growing naturally and receiving RFR alone. Meanwhile, water-stressed rice treated with RFR alone had mean total filled grain weight about 30% less than non-stressed rice with the same amount of fertilizer. In addition, two enzymatic components, SOD and PO activities, were significantly enhanced in rice treated with the product before and after water stress conditions compared to rice receiving RFR alone.

Keywords: rhizo-product strain RS87, water stress, rice yield components, antioxidative defense system, rice cultivar RD49

1. Introduction

Climate change, or global warming, has several negative impacts including rising temperature and sea water level, unexpected strong weather, and shifting precipitation patterns which may result in water stress in some agricultural areas (Dai, 2011). Water stress in plants occurs when the demand for water exceeds available water during a certain period. Plants experience this stress when the water supply to their roots becomes limited or when the transpiration rate intensifies. The impact of water stress can affect plant growth and productivity (Jaleel *et al.*, 2009; Lesk, Rowhani, & Ramankutty, 2016). Plant physiological and biological changes that occur under water stress include a reduction of photosynthesis due to a decrease in chlorophyll content of leaves (Chernyad'ev, 2005), changes in plant morphology, anatomy and cytology, i.e., leaf size shrinkage, thickening of the leaf cell wall, induction of early senescence (Iannucci, Rascio, Russo, Di Fonzo, & Martiniello, 2000), and the formation of reactive oxygen species (ROS) causing cellular damage (Nayyar & Gupta 2006).

Plant growth-promoting rhizobacteria (PGPR) have been reported to enhance plant growth and crop yield, and also to alleviate plant drought stress through several mechanisms (Arzanesh, Alikhani, Khavazi, Rahimian, & Miransari, 2011; Arshad, Sharoona, & Mahmood, 2008;

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Cassan *et al.*, 2009; Creus, Sueldo, & Barassi, 2004; Mayak, Tirosh, & Glick, 2004; Sandhya, Ali, Grover, Reddy, & Venkateswarlu 2009). One of these mechanisms is PGPR involvement in the alteration of the antioxidant defense system. Both non-enzymatic components such as glutathione and ascorbic acid and enzymatic components such as superoxide dismutase (SOD, EC 1.15.1.1), catalase (CAT, EC 1.11.1.6) and peroxidase (PO, EC 1.11.1.7) (Gusain, Singh, & Sharma, 2015; Heidari & Golpayegani, 2011; Naseem & Bano, 2014; Sandhya, Ali, Grover, Reddy, & Venkateswarlu, 2010) are thought to be involved.

Bacillus cereus strain RS87 enhances plant growth and promotes yield in several plants, including rice (Jetiyanon & Plianbangchang, 2012; Jetiyanon, Wittaya-areekul, & Plianbangchang, 2008). A biofertilizer, formulated as a pellet and containing spores of *Bacillus cereus* strain RS87 named rhizo-product strain RS87 (product), developed by our research team has demonstrated early rice seedling growth promotion with potential for partial fertilizer replacement under greenhouse conditions (Jetiyanon Wittaya-areekul, Plianbangchang, & Lohitnavy 2017). This product has also been preliminarily tested for improving drought resistance in Thai rice cultivar RD49 under greenhouse conditions (Jetiyanon personal communication). Results show that plants treated with the product recovered from drought conditions after receiving water for seven days, while non-bacterized plants did not recover and later died (Figure 1). This indicates that the product RS87 may somehow play a crucial role in plant induction of systemic tolerance to water stress.

The objectives of this study are (1) to explore the potential of the product for enhancing rice tolerance to water stress while still maintaining good yield components, and (2) to examine the enzymatic components involved with the antioxidant defense system during water stress condition.

Figure 1. Drought resistance of Thai rice cultivar RD49 treated with rhizo-product strain RS87 (three pots from the right) compared with non-bacterized plants (three pots from the left). The plant age was 60 days after transplanting

2. Methodology

2.1 Rhizo-product of *Bacillus cereus* strain RS87

The tested product was formulated as compaction granules roughly 2-3 cm in diameter. The active ingredient in the tested product was spores of *B. cereus* strain RS87 prepared with the procedure described by Jetiyanon *et al.* (2008). The pellets also contain clay powder, corn starch, and zeolite powder as inactive ingredients. There were approximately 10^9 spores of strain RS87 per gram of tested product.

2.2 Sources of seed rice and clay soils

Photoperiod insensitive Thai rice (*Oryza sativa* L.) cultivar RD49 used in this study was purchased from a rice seed retail store in Phitsanulok province, Thailand. Clay soils used in the greenhouse experiment were collected from growers' rice paddy fields adjacent to Naresuan University. Approximately 7.5 kilogram of natural clay soils were transferred to each plastic pot (12 inches in diameter), and water was added to saturate the soil before planting.

2.3 Pot experiment

The experimental design was a randomized complete block with five treatments, including control treatments, applied with recommended fertilizer rate (RFR) with and without water stress condition and rice treated with rhizo-product strain RS87 using three different fertilizer regimes (RFR, 75% RFR, and 50% RFR) with water stress condition. Each treatment was replicated five times. A 12-inch-diameter plastic pot represented an experimental unit. Each pot contained five seedlings. All rice plants were grown to maturity in each pot. The experiment was conducted twice. Two types of commercial fertilizer, complex (16-20-0) and nitrogen (46-0-0), were applied to rice plants depending on their growth stage. The RFR of complex was applied at a rate of 30 kg N, 16.4 kg P, ha^{-1} at 30 days after transplanting, and the RFR of fertilizer nitrogen was applied at a rate of 57.5 kg N ha^{-1} at 60 days after transplanting (maximum tillering stage). In the rhizo-product treatments, 0.5 gram of product was applied concurrently with (1) 100% RFR, (2) 75% RFR, and (3) 50% RFR.

The first experiment was conducted between February and June 2017. The average temperature in the greenhouse was 33 °C during the day and 26 °C at night. The relative humidity was approximately 80–85%. The second experiment was performed between July and October 2017. The average temperature in the greenhouse was 34 °C during the day and 27 °C at night. The relative humidity was approximately 80–85%.

Rice seeds were surface sterilized with 3% NaOCl for 20 min and rinsed three times with sterilized ddH_2O. Three grams of rice seeds were soaked in a suspension of the product for 24 hrs. The suspension was prepared by combining three grams of the tested product with 30 mL of sterilized ddH_2O in a 100 ml sterilized beaker. The tested product was dispersed evenly using a magnetic bar on a stirrer. Seeds soaked in a sterilized ddH_2O, only, served as a non-bacterized control. Tested product suspensions and ddH_2O were discarded and seeds were further incubated at 30 °C while covered with foil to prevent dehydration for seven days before transferring to clay soils in the plastic tray for another fourteen days. Then, seedlings of each treatment were transplanted into clay soils in a plastic pot (5 plants/pot) under greenhouse conditions. Pots were flooded starting from the day of transplanting, and the water was maintained above the soil line in each pot. Seven days later, the second split of

fertilizer was applied and plant height and leaf chlorophyll concentration were measured. A non-destructive measurement of leaf chlorophyll concentrations was determined using the Soil and Plant Analyzer Development (SPAD) meter (model SPAD 502 Plus Chlorophyll Meter, Konica Minolta INC., Japan). The SPAD values are reported to be generally proportional to the amount of chlorophyll present in the leaf (Ling, Huang, & Jarvis, 2011). Studies of Wakiyama (2016) also confirm the nonlinear model of the relationship between the SPAD value and rice leaf blade chlorophyll content with high coefficients of determination.

One week later, water stress was initiated in all rice treatments (except control treatment without water stress condition) by stopping watering until the soil reached the critical water stress period of approximately 20-24% of clay soil moisture (source: https://nrcca.cals.cornell.edu/soil/CA2/CA0212.1-3.php). A soil moisture meter (M0750 model, EXTECH instrument, USA) was used to monitor critical water stress. Once plants reached the critical water stress period, pots were flooded again until rice approached the harvesting period.

Two further investigations of the plant antioxidant defense system were conducted. In the first experiment, the 6th true leaf from treatments of each replication was randomly collected at four different times: (1) the day before water stress condition, (2) at the critical water stress period, (3) 24 hr after plants received water, and (4) 48 hr after plants received water. For the second experiment, the 6th true leaf from treatments of each replication was randomly collected at: (1) the day before water stress condition, (2) 48 hr after water stress condition, (3) 96 hr after water stress condition, (4) at the critical water stress period, and (5) 24 hr after plants received water. All leaf samples were immediately transferred to the ultralow freezer (-80 °C) for further biochemical analysis.

During the harvesting period, water was drained from each plastic pot. The soil was allowed to dry gradually while the whole rice panicles approached maturity. Rice panicles from each cultivar were harvested. Rice yield components, including number of panicles per pot, panicle length, number of filled grains per pot, grain yield, and percentage of unfilled grains per panicle of each treatment were recorded. All data were analyzed by analysis of variance (ANOVA), and the treatment means were separated using Fisher's protected least significant difference (LSD) test at $P \leq 0.05$ using SAS software (Statistical Analysis System Institute [SAS]).

2.4 Estimation of antioxidative enzymes

Superoxide dismutase (SOD) and peroxidase (PO) enzymes were investigated since they have been reported to ameliorate plant cell damage caused by biotic and abiotic stress conditions (Alscher, Erturk, & Heath, 2002; De Gara, de Pinto, & Tommasi, 2003; Jetiyanon & Plianbangchang, 2013; Zhang & Kirham, 1994).

Leaf samples (0.4 g) kept in the ultralow freezer from each replication were pooled, flash-frozen in liquid N_2, and crushed into a fine powder with a sterile mortar and pestle. They were homogenized in 3.6 ml of a pre-chilled 0.1 M Tris–HCl buffer, pH 7.0 containing 1% polyvinyl-polypyrrolidone (Sigma–Aldrich, Inc., MO, USA). The homogenate was centrifuged at 10,000*g* in a benchtop refrigerated centrifuge (VEROCITY 14R, Dynamica (Asia) Limited, Kowloon, Hong Kong) for 10 min at 4 °C. The supernatant for the enzymatic activity assay was transferred to a 1.5 ml vial and stored at 20 °C. A colorimetric assay for enzyme activity was performed with a Double Beam UV/Vis Spectrophotometer (OPTIZEN 3220UV, Mecasys Co., Ltd. Daejeon, South Korea). The reaction rates were linear and proportional to the enzyme or protein concentration added. The standard Bradford assay (Bradford, 1976) was employed to test the protein concentration for plant extracts in each sample.

2.4.1 Superoxide dismutase activity (SOD)

All extracts were tested for SOD activity using the riboflavin/methionine system (Beauchamp & Fridovich, 1971). The 1 ml reaction mixture in 3 ml tube contained 50 mM potassium phosphate buffer (pH 7.8), 0.1 mM EDTA, 13 mM methionine, 75 μM nitroblue tetrazolium, 2 μM riboflavin, and the enzyme extract. Riboflavin was added last. The tubes were stirred and the reaction was initiated by placing the tubes under two 18 W fluorescent lamps. After 10 min, the reaction was terminated by switching off the light. Non-illuminated tubes served as blanks. The tubes were stirred, and the reaction mixture was then poured into 1.5 ml cuvettes. Total SOD activity was assayed spectrophoto metrically at 560 nm. One unit of SOD activity was defined as the amount that inhibits nitroblue tetrazolium photoreduction by 50% under the assay conditions. All chemicals are purchased from Sigma–Aldrich, Inc.

2.4.2 Peroxidase activity (PO)

All extracts were tested for peroxidase activity using guaiacol as the hydrogen donor. Procedures were modified from Hammerschmidt, Nuckles, and Kuc´ (1982). The 1 ml reaction mixture in 1.5 ml cuvette contained 0.25% (v/v) guaiacol in 0.01 M sodium phosphate buffer (pH 6.0), enzyme extract, and 0.1 M H_2O_2. Enzyme extract was added last to initiate the reaction. The changes in absorbance at 470 nm were recorded at 30 s intervals for 3 min. The enzyme activity was expressed as changes in the absorbance min^{-1} mg protein^{-1}. All chemicals were purchased from Sigma–Aldrich, Inc.

3. Results

3.1 Plant height and SPAD value before water stress

Seven days after the second fertilizer application, the rice treatments in both experiments mostly had similar plant height, ranging from 60.5-62.8 cm and 75.5-77.6 cm in the first and the second experiments, respectively. However, the height of plants in the first experiment treated with the product and RFR were significantly greater than that of plants treated with the product and 50% RFR (Table 1). At fourteen days after the second fertilizer application (before starting water stress condition on the following day), the SPAD values of the rice leaf in all treatments of both experiments were similar, ranging from 38.58-39.50 in the first experiment and 43.76-44.95 in the second experiment (Table 1). There was no statistically significant difference among the treatments.

Table 1. Plant height and SPAD value of rice treated with rhizo-product strain RS87 using three different fertilizer regimes before water stress conditions.

Treatment[p]	Mean plant height (cm)		Mean SPAD value	
	1st experiment	2nd experiment	1st experiment	2nd experiment
100% fertilizer without water stress	61.71ab	77.57a	39.28a	44.23a
100% fertilizer with water stress	61.70ab	77.54a	39.55a	44.91a
Rhizo-product RS87+100% fertilizer with water stress	62.78a[q]	77.06a	39.70a	43.89a
Rhizo-product RS87+75% fertilizer with water stress	61.47ab	76.00a	38.58a	44.95a
Rhizo-product RS87+50% fertilizer with water stress	60.51b	75.51a	39.26a	43.76a
$LSD_{0.05}$	2.23	4.32	1.37	1.37

[p]The experimental design was a randomized complete block. Each experiment contained 5 replications (5 plants/rep.) per treatment. The first fertilization (16-20-0) was applied when rice plants were 30 days old and the second fertilization (46-0-0) was applied when plants were 60 days old. Rhizo-product strain RS87 was concurrently applied with three different fertilizer regimes. Plant height and SPAD value were measured 7 days after the second fertilization. [q]Values followed by a different letter(s) within a column are significantly different at $P \leq 0.05$, according to Fishers's protected least significant difference (LSD) test.

3.2 Rice yield components

In treatments under water stress condition, rice treated with the product combined with different fertilizer regimes in both independent experiments mostly resulted in better total filled grain weight and numbers of filled grains compared to rice treated with RFR alone. The percentage of unfilled grain per panicle in rice treated with RFR alone under water stress condition was the highest. Panicle length and number of panicles per pot were similar among all treatments, except for the number of panicles per pot in the second experiment in which rice treated with the product combined with 50% RFR was significantly lower than for rice treated with the rhizo-product strain RS87 combined with 75% RFR and rice treated with RFR alone without water stress conditions. Rice treated with the rhizo-product strain RS87 and with different fertilizer regimes under water stress conditions, showed similar yield components to rice treated with RFR alone without water stress conditions. (Table 2). Rice under water stress conditions showed total filled grain weight decrease of about 30% in both experiments compared to rice without water stress condition (Table 2).

3.3 Antioxidant defense system in rice before and after water stress condition

3.3.1 Superoxide dismutase activity (SOD)

For the first experiment, before water stress conditions, total SOD activity in rice treated with the product using three different fertilizer regimes (RFR, 75% RFR, 50% RFR) was generally higher than in rice treated with RFR alone. Only rice treated with the product using RFR and 75% RFR had significantly higher total SOD activity (about 30% higher) than rice receiving RFR alone. At the critical water stress period, there was a significant incremental increase in total SOD activity in rice treated with the product in combined with different fertilizer regimes compared to rice treated with RFR alone. Rice treated with the product and RFR had the greatest total SOD activity (≈107 mg^{-1} protein), which was significantly higher than the rest of the treatments. The total SOD activity in both rice treatments of product using 75% RFR (≈87 mg^{-1} protein) and 50% RFR (≈82.1 mg^{-1} protein) was significantly greater than the total SOD activity in rice treated with RFR alone (≈70 mg^{-1} protein). Nevertheless, the total SOD activity in all rice treated with rhizo-product strain RS87 using different fertilizer regimes (ranging from 80-82 mg^{-1} protein) was still significantly higher than rice receiving RFR alone (≈72 mg^{-1} protein) one day after re-establishing watering. The significant difference in the total SOD activity among all rice treatments under water stress conditions disappeared the next day (Table 3).

Generally, all rice treated with rhizo-product strain RS87 applied with different fertilizer regimes showed higher SOD activity than rice treated with RFR alone. Similar to the first experiment, the second experiment showed that the total SOD activity in rice treated with rhizo-product strain RS87 in combined with RFR exhibited the most SOD activity before and after water stress conditions. Before water stress conditions, only rice treated with rhizo-product strain RS87 combined with RFR showed significantly greater SOD activity (81 mg^{-1} protein) compared to rice treated with RFR alone (70 mg^{-1} protein). The increased SOD activity occurred in all rice treatments two days after water stress conditions. Again, the rice treated with rhizo-product strain RS87 combined with RFR only, showed significantly higher SOD activity (131 mg^{-1} protein) compared to rice treated with RFR alone (87 mg^{-1} protein). Both rice treatments of rhizo-product strain RS87 combined with RFR and 75% RFR had significantly higher SOD activity compared to rice treated with RFR alone through four days after water stress condition, at the critical water stress condition, and one day after receiving water. Natural SOD activity in rice applied with RFR alone without water stress conditions ranged from 61-74 mg^{-1} protein, throughout the sampling times (Table 4).

In general, some amount of total SOD activity was detected in rice treated with RFR alone without water stress condition, ranging from 61-74 mg^{-1} protein, in the first experiment, and from 62-75 mg^{-1} protein in the second experiment throughout the sampling times (Tables 3 and 4).

3.3.2 Peroxidase activity (PO)

Before water stress conditions, in the first experiment, rice treated with the product supplemented with recommended fertilizer rate (RFR) showed the greatest total

Table 2. Effectiveness of rhizo-product strain RS87 with different fertilizer regimes on rice yield components after water stress conditions in two independent experiments.

Treatment	Mean total filled grains weight (gram)		Mean number of filled grains per pot		Mean percentage of unfilled grains per panicle		Mean panicle length (cm.)		Mean number of panicles per pot	
	1st exp.	2nd exp.	1st exp.	2nd exp.	1st exp.	2nd exp.	1st exp.	2nd exp.	1st exp.	2nd exp.
100% fertilizer alone without water stress	78.8a*	6.58a	50.299a	277.75a	57.62a	33.79a	22.20a	23.15ab	10.50a	6.75a
100% fertilizer alone with water stress	6.14b	4.58a	237.75a	205.50a	61.93a	45.50a	23.00a	22.67b	8.75a	5.75ab
Rhizo-product RS87+100% fertilizer with water stress	7.46ab	6.97a	257.75a	275.75a	56.80a	32.23a	22.07a	24.40a	9.75a	5.75ab
Rhizo-product RS87+75% fertilizer with water stress	6.95ab	7.03a	248.75a	304.00a	56.03a	33.56a	22.25a	23.99ab	9.25a	6.50a
Rhizo-product RS87+50% fertilizer with water stress	6.90ab	5.42a	247.75a	248.75a	52.55a	36.84a	21.67a	23.15ab	9.50a	5.00b
$LSD_{0.05}$	1.99	2.61	75.09	99.32	11.80	20.39	2.71	1.38	3.08	1.18

*Values followed by a different letter(s) within a column are significantly different at $P \leq 0.05$, according to Fishers's protected least significant difference (LSD) test.

Table 3. Effect of rhizo-product strain RS87 applied with various fertilizer regimes on both total SOD and PO activity of Thai rice cultivar RD49 before and after water stress conditions in the first experiment conducting during February-June 2017.

Treatment[e]	Antioxidant defense enzyme activity (mg^{-1} protein)							
	Day before water stress		At critical water stress period		24 hours after receiving water		48 hours after receiving water	
	SOD	PO	SOD	PO	SOD	PO	SOD	PO
100% fertilizer alone without water stress	68.43b	471.35d	75.59cd	434.24b	62.19c	345.50c	71.92b	415.17ab
100% fertilizer alone with water stress	64.41b	419.71d	70.15d	454.22b	72.43b	379.50b	84.73ab	447.50a
Rhizo-product RS87+100% fertilizer with water stress	83.57a[f]	855.26a	106.82a	1,052.20a	82.30a	466.00a	95.31a	416.32ab
Rhizo-product RS87+75% fertilizer with water stress	83.28a	691.85b	87.37b	752.11a	81.20a	447.89a	84.46ab	400.00ab
Rhizo-product RS87+50% fertilizer with water stress	71.66b	593.50c	82.06bc	463.50b	80.26a	403.81b	89.98a	372.78b
$LSD_{0.05}$	8.92	75.96	11.02	139.35	2.76	39.63	13.46	48.34

[e]The experiment was a randomized complete block design. Each treatment consisted of 5 replications. Each replication was a plastic pot containing 5 rice plants. The first fertilization (16-20-0) was applied when rice plants were 30 days old and the second fertilization (46-0-0) was applied when plants were 60 days old. Rhizo-product strain RS87 was concurrently applied with three different fertilizer regimes. [f]Values followed by a different letter(s) within a column are significantly different at $P \leq 0.05$, according to Fishers's protected least significant difference (LSD) test.

PO activity. All rice treated with the product in combination with different fertilizer regimes generally yielded significantly higher total PO activity, ranging from 1.4 to 2 times, when compared to rice treated with RFR alone. At the critical water stress period, there was little increase in the total PO activity between rice treated with the product in combination with 50% RFR and rice treated with RFR alone. In contrast, the total PO activity in rice treated with the product in combination with RFR and 75% RFR was significantly greater compared to the treatments previously mentioned above. One day after resumption of watering the total PO activity of all treatments declined to enzyme activity levels lower than before water stress conditions. However, the enzyme activity in rice treated with the product in combination with RFR (466 mg^{-1} protein) and 75% RFR (447 mg^{-1} protein) was still significantly higher (about 22% and 17%, respectively) compared to rice treated with RFR alone. The following day, the total PO activity in rice treated with the product in combination with different fertilizer regimes still decreased, while PO activity in rice treated with RFR alone increased but only significantly for rice treated with the product in combination with 50% RFR (Table 3).

In the second experiment, the trend of higher PO activity was similar in rice treated with the product in combination with different fertilizer regimes when compared with rice treated with RFR alone. Rice treated with the product in combination with RFR and 75% RFR showed significantly higher PO activity when compared with rice treated with RFR alone on both the day before water stress conditions and two days after water stress conditions. Four

days after water stress conditions, only rice treated with the product in combination with RFR resulted in significantly higher PO activity when compared to rice treated with RFR alone. At the critical water stress condition, all rice treated with the product in combination with different fertilizer regimes had significantly higher PO activity, ranging from 14-40%, when compared with rice treated with RFR alone. Even though the PO activity in all treatments declined at 24 hr after resumption of watering, rice treated with the product in combination with different fertilizer regimes still had significantly higher enzyme activity, ranging from 10-23%, when compared to rice treated with RFR alone (Table 4).

In both experiments throughout the sampling times, some amount of natural PO activity was measured in rice treated with RFR alone without water stress conditions, ranging from 345-471 mg^{-1} protein, in the first experiment and from 283-379 mg^{-1} protein in the second experiment (Tables 3 and 4).

4. Discussion and Conclusions

Before initiation of water stress, rice plants treated with rhizo-product strain RS87 (product) in combination with 75% RFR and 50% RFR had similar plant height and SPAD values compared to rice treated with RFR alone. These results suggest that partial growth promotion in rice treated with the product comes from the actions of strain RS87 such as IAA production (Jetiyanon *et al.*, 2008), phosphate solubilization and siderophore production (Jetiyanon & Plianbangchang, 2010), and nitrogen fixation (Jetiyanon, 2015) to compensate for the lesser amount of fertilizer that the plants received.

Rice is one of the most drought-susceptible cultivated crops, especially at the reproductive stage (Agarwal *et al.* 2016) leading to low crop productivity (Pantuwan, Fukai, Cooper, Rajatasereekul, & O'Toole, 2002). In this study, the critical water stress period in rice (about 7-10 days after termination of watering) occurred at the reproductive stage of rice plant cultivar RD49. After resuming growth to maturity, only rice treated with the product in combination with RFR and 75% RFR had similar yield components, especially total filled grain weight (the main component of grower concern after harvest), in both experiments compared to rice receiving RFR alone without water stress, while rice with water stress conditions and receiving RFR alone showed lower crop productivity in both experiments. This suggests that the rhizo-product strain RS87 somehow plays a role in alleviating water stress to enhance rice plant productivity.

Water deficiency in plants can cause production of reactive oxygen species (ROS) such as hydrogen peroxide, hydroxyl radicals, and superoxide radicals (Guo, Ou, Lu, & Zhong, 2006). The accumulation of this ROS may result in a decrease in the accumulation of above-ground vegetative biomass subsequently, lower crop yield (Vile *et al.* 2012). It has been shown that one of the mechanisms of drought tolerance in some crops such as green gram plants (Saravanakumar, Kavino, Raguchander, Subbian, & Samiyappan, 2011), maize (Sandhya *et al.*, 2010), and rice (Gusain, Singh, & Sharma, 2015) is induced tolerance by plant growth promoting rhizobacteria (PGPR) through the manipulation of antioxidant enzymes. Gusain and colleagues (2015) reported that their tested PGPR strains benefit rice plants by inducing higher amounts of defense related enzyme activities, including SOD and PO activities, for drought tolerance under water deficit conditions. The results from our study agree with these findings (Tables 3 and 4). In our experiments, the SOD and PO enzyme activities in rice cultivar RD49 when treated with the product in combination with RFR and 75% RFR show greater activity compared to the activity in rice treated with RFR alone before water stress. The activity of the two enzymes is enhanced after starting water stress condition and continues until the critical water stress period is reached. In contrast, the level of both enzyme

Table 4. Effect of rhizo-product strain RS87 applied with various fertilizer regimes on total SOD activity of Thai rice cultivar RD49 before and after water stress conditions in the second experiment conducting during July-October 2017.

Treatment[e]	Antioxidant defense enzyme activity (mg^{-1} protein)									
	Day before water stress		2 days after water stress		4 days after water stress		At critical water stress period		24 hours after receiving water	
	SOD	PO	SOD	PO	SOD	PO	SOD	PO	SOD	PO
100% fertilizer alone without water stress	69.44b[f]	378.89d	74.30c	283.34d	71.36c	287.75c	60.77c	302.86d	64.58c	330.98e
100% fertilizer alone with water stress	69.85b	408.20cd	87.54bc	550.20c	84.60c	654.50b	86.93b	505.42c	76.92bc	435.42d
Rhizo-product RS87+100% fertilizer with water stress	80.72a	625.54a	131.12a	751.54a	125.14a	867.73a	110.75a	708.64a	100.64a	537.12b
Rhizo-product RS87+75% fertilizer with water stress	75.70ab	469.32b	118.66ab	604.09b	117.54ab	734.50ab	109.83a	665.40a	100.37a	604.10a
Rhizo-product RS87+50% fertilizer with water stress	70.97ab	445.00bc	107.02ab	595.96bc	104.97b	632.17b	99.81ab	579.58b	87.91ab	479.60c
$LSD_{0.05}$	10.01	36.88	31.24	51.58	18.99	135.75	19.39	61.1	22.65	42.31

[e]The experiment was a randomized complete block design. Each treatment consisted of 5 replications. Each replication was a plastic pot containing 5 rice plants. The first fertilization (16-20-0) was applied when rice plants were 30 days old and the second fertilization (46-0-0) was applied when plants were 60 days old. Rhizo-product strain RS87 was concurrently applied with three different fertilizer regimes. [f]Values followed by a different letter(s) within a column are significantly different at $P \leq 0.05$, according to Fishers's protected least significant difference (LSD) test.

activities in rice treated with RFR alone slowly increased but was still significantly lower compared to rice treated with the product in combination with RFR and 75% RFR during water stress condition. This indicates that the product may stimulate rice before encountering water stress by producing higher activities of both SOD and PO. Once encountering water stress factors, both previously induced SOD and PO activities in rice by the strain RS87 in the product result in enhanced plant amelioration during stress. The enhanced SOD and PO activities have also been shown to occur in biotic stress caused by pathogen invasion in which plants induced by PGPR show increased levels of the enzyme activities before and after pathogen challenge when compared to the non-bacterized control treatment (Jetiyanon, 2007; Jetiyanon & Plianbang chang 2013).

In conclusion, rhizo-product strain RS87 in combination with 75% RFR can ameliorate the effects of water stress on rice plants by enhancing antioxidative enzymes (SOD and PO activities) during water stress condition, resulting in yield components equivalent to those of rice without water stress conditions. Further study is required to examine the practicality of this product under field conditions.

Acknowledgements

The authors are highly grateful for the Thailand Research Fund (grant no. RDG5920040) for financial support and also would like to thank Dr. W.P. Moss for his kind editorial assistance.

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