

Induction of Disease and Drought Resistance in Rice by *Pseudomonas fluorescens* SP007s

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ABSTRACT

A plant growth promoting rhizobacterium *Pseudomonas fluorescens* SP007s suppressed the incidence of various diseases of several economic crops over 12-field trials. In this work, strain SP007s developed in a new formulation could increase resistance to not only diseases but also drought stress in rice plant. Application of the new SP007s-bioproduct (ISR-P/K) as seed treatment, broadcasting, and foliar spray significantly performed the best results in reduction of six-important diseases (bacterial leaf blight, blast, brown spot, narrow brown spot, sheath blight; and dirty panicle caused by *Xanthomonas oryzae* pv. *oryzae*, *Pyricularia grisea*, *Helminthosporium oryzae*, *Cercospora oryzae*, *Rhizoctonia solani*; and complex pathogens including *C. oryzae*, *Curvularia lunata*, *H. oryzae*, *Fusarium semitectum*, *Alternaria padwickii*, and *Sarocladium oryzae* respectively) and increased yield with 52.1%. This enhanced effectiveness positively correlated with defense enzyme accumulation in rice leaves compared to conventional routine practice under field conditions at Suphanburi province ($P=0.05$). In a natural case of 2-week water deficit during investigation at 90-day-old plants, activity of protective enzymes including β -1,3-glucanase, guaiacol peroxidase (GPX), peroxidase (POX), phenylalanine ammonia lyase (PAL), and superoxide dismutase (SOD) detected by $3.3 \mu\text{g}^{-1}$ glucose min^{-1} protein, $0.83 \text{ min}^{-1} \text{ mg}^{-1}$ protein, $1.4 \text{ min}^{-1} \text{ mg}^{-1}$ protein, $9 \text{ nmol tran-cinnamic acid min}^{-1} \text{ mg}^{-1}$ protein, and 9 unit mg^{-1} protein respectively, was sharply increased in drought-stress rice. The content of these enzymes increased according to disease severity was also observed at same water deficit period. These results indicate that *P. fluorescens* SP007s in ISR-P/K product protects rice plant from disease and drought stress with expressing related-enzyme accumulation.

Key words: Biocontrol, Bioformulation, Induced systemic resistance, Protective enzymes, Reduced chemical application

INTRODUCTION

Thailand produces rice (*Oryza sativa* L.) as an economical crop and a main food throughout history of the country. Improved rice yield with various technology inputs including crop protection is therefore, an urgent attention. Plant stress by biotic (disease) and abiotic (drought) causes are one of the most damage to rice production. Among them, leaf; and panicle diseases caused by multiple bacteria and fungi; and a complex parasitic-fungi are now assumed epidemic proportions and becoming an important problem, whereas rice growth under global warming crisis has been also limited the production (Prathuangwong et al., 2010). Upon these stress conditions, excess energy may result, which is potentially harmful to plant physiology affecting disease expression. Plants have numerous defenses against both pathogen attack and unfavorable effect which are based on constitutive barriers and induced resistance upon contact with invaders (Van Loon et al., 1998). This type of resistance is referred to acquired or induced resistance (SAR or ISR). Plants

may fail to protect themselves if heavy attack or severe disease is occurred. To increase resistance upon plant defenses, plant growth promoting rhizobacteria (PGPR) including *Pseudomonas fluorescens* SP007s using as a biological control or protective agent can develop a form of protection of induced systemic resistance in multiple economic crops (Chuaboon and Prathuangwong, 2009; Prathuangwong and Chuaboon, 2010; Prathuangwong et al., 2009). ISR has been reported as one of the mechanism by which PGPR reduce plant diseases, functioning through the manipulation of the physical and biochemical properties of host plants (Van Loon et al., 1998). The metabolic response characteristic of systemic defense mechanisms such as induction of enzymatic activities related to increased plant resistance had been widely reported (Hemsanit et al., 2010; Prathuangwong and Buensanteai, 2007). Another phenomenon involved SAR or ISR called priming (Figure 4) is metabolic responses triggered by PGPR that will be only appreciated upon pathogen challenge or abiotic stress and consist in a more rapid and stronger defense expression (Buensanteai et al., 2008, 2009).

Several studies have shown that SP007s is the most effective PGPR in controlling a range of infectious diseases, but not yet for abiotic disease such as drought stress (Chuaboon and Prathuangwong, 2009; Prathuangwong and Chuaboon, 2010; Prathuangwong et al., 2009). These works also demonstrated that SP007s in a stable formulation were more effective in disease control than its fresh cells (Prathuangwong et al., 2007; Preecha and Prathuangwong, 2009). The objective of this study was then to investigate in field experiment if the application of different SP007s-based formulations enhanced the efficacy of biocontrol treatment and was able to induce resistance in rice plant against both infectious diseases and drought stress that correlated with increased defense-related enzyme accumulation.

MATERIALS AND METHODS

Culture conditions and formulation of *P. fluorescens* SP007s

Strain SP007s isolated from cauliflower rhizosphere and kept at -80°C was recovered on King's medium B and tested *in vitro* on nutrient glucose agar for efficacy in growth inhibition of *Xanthomonas oryzae* pv. *oryzae* (Xoo), the causal agent of bacterial leaf blight of rice (Prathuangwong et al., 2007). After pathogen inhibition was identified, the strain was proceeded for formulated preparation. Cells of SP007s resuspended in sterile distilled water with original 1×10^{13} cfum l^{-1} were formulated in 2-different products including talc-and kaolin-based formulations. The talc-based formulation of the marked *P. fluorescens* SP007s strain using patented technology developed by S. Prathuangwong (2009 patent submission: "ISR-P", number 0901001791) and the kaolin base developed in this study (ISR-P/K) were investigated. Both bioproducts (ISR-P and ISR-P/K) were developed into 2-formulation types, powder; and granule for seed and foliar treatment; and broadcast respectively. These bioproducts were sealed in an aluminium foil bag and kept at room temperature. Survival of SP007s in different formulations was determined during storage period and before use (Preecha and Prathuangwong, 2009).

Plant cultivation and field experiment

Seeds of rice cv. Phitsanulok 60 were soaked in sterile water over night and incubated (shaded-dried) another day to initiate sprout seeds at room temperature. Bacterization of these sprout seeds with each bioproduct (ISR-P and ISR-P/K) in powder formulation at 1 gkg^{-1} seed (1×10^6 cfum l^{-1} SP007s of 6-month-old bioproduct) added with 0.001% Tention T7 was conducted before seeding into the field by $125 \text{ kg seed broadcast ha}^{-1}$ with no transplantation.

Field experiment with treatments of each SP007s bioproduct (ISR-P and ISR-P/K) in T1 to T3 and a conventional standard practice (synthetic chemical application including chemical fertilizer, fungicides and insecticides) in T4 severed as control check was conducted in randomized complete block design with three replications under farmer's field at Suphan Buri. Standard plot size of $400 \times 400 \text{ m}$ at the spacing around $4 \times 5 \text{ cm}$ was maintained for all 4 treatments (Figure 1). Level of field

irrigation and other cultivated-routine practices during the growing season were done according to conventional standard protocols for all treatments.

The talc- and kaolin-based powder products of SP007s of 6-month-old were thoroughly mixed in water (20gL^{-1} with 1×10^7 cfum l^{-1}) and sprayed weekly; begun from 14-throughout 63-day-old plants (6-foliar sprays), except at 21, 42, and 70-day-old the broadcasted application (bottom fertility). Each ISR-P/K (in a granular formulation in T3) and ISR-P bioproduct (in a powder and granule formulation in T1 and T2) at the rate 1:1 granule urea fertility $[(\text{NH}_2)_2\text{CO}]$ of total 94 kg ha^{-1} (47 bioproduct : 47 urea kg ha^{-1}) were added to soil by 3-broadcasts applied twice weekly, begun at 21-day-old plants. The conventional treatment was applied with recommended dose of urea fertilizer (94 kg ha^{-1} of 46% N at same 3 times mentioned above), fungicides (propiconazole + difenoconazole applied 4 times during 14-until 70-day-old plants), and insecticides (cypermethrin + dinotefuran and chlorpyrifos at 30, 37, and 45-day-old plants).

During the growing season, the irrigation system at the experimental location was naturally broken for 2-week and continued-water deficit equivalent to drained rice plot begun at 90-day-old plants. Leaf tissues of drought condition were rapidly frozen in liquid nitrogen and stored at -80°C until analysis for protective enzyme activity.

Enzyme activity bioassay of ISR in the field

Assay of defense-related enzymes was performed weekly in treated plants that were naturally infectious and noninfectious (drought) diseases under field conditions. A total of these replicates consisting of random plants each were used. Leaves from these plants in each replicate were pooled to obtain 1 g fresh weight. After frozen in liquid nitrogen, leaves were powdered with mortar and pestle that homogenized with 2 ml of extraction buffer (0.1 M Tris-HCl buffer, pH 7, 0.1 M KCl, 1 mM phenylmethanesulfonyl fluoride, 10 mL^{-1} Triton-X100, 30 gL^{-1} polyvinylpyrrolidone K30) at 4°C . The homogenate was centrifuged for 10 min at 12,000 rpm. The supernatant was used as crude enzymes (total proteins) extract and kept on ice until the enzyme activity assays. Total protein concentration was determined in the plant extract by the Bradford method (Bradford, 1976) according to the manufacture's instruction (Sigma, MO) with minor modification (Buensanteai et al., 2009).

Enzyme extracted in 0.1M buffer was used for the estimation of β -1, 3-glucanase, peroxidase (POX), phenylalanine ammonia-lyase (PAL), and guaiacol peroxidase (GPX) following the method described by Pan et al. (1991), Hammersmidt et al. (1984), Prathuangwong and Buensanteai (2007), Upadhyaya et al. (1985), and Chatnaparat et al. (2009) with less modification (Buensanteai et al., 2009) respectively. Total superoxide dismutase (SOD) was also measured using the method described by Dhindsa et al. (1981) with minor modification (Hemsanit et al., 2010).

Data analysis

All disease incidence in the entire plots of naturally infested field were assessed weekly begun at 14-day after planting (DAP) and continued until 2 weeks before harvest at 110 DAP. Disease rating was performed as disease incidence using W-random sampling as described by Delp et al. (1986), then calculated the area under disease progress curve (AUDPC) according to the procedures described by Campbell and Madden (1990).

Plant growth promotion assay by PGPR, *P. fluorescens* SP007s was also determined. Plants were harvested weekly after planting begun 15-throughout 70-day-old plants; and root length, plant height, and plant weight (fresh and dry) were measured. Plant growth parameter index was determined using the equation of plant growth index = (root length + plant height + plant fresh weight + plant dry weight) / 4. Grain yield was recorded at the harvesting time (110 DAP). All data recorded were subjected to analysis of variance (ANOVA) followed by determining difference between treatment means by the Duncan's multiple range test.

RESULTS AND DISCUSSION

Rice growth promotion assay

Application of *P. fluorescens* SP007s in T3 increased field growth parameter of rice plants that was observed as equivalent as chemical treatment in an average throughout 70 DAP (Figure 1, Table 1). But the increase in total yield of rice plants due to all 3-bioproduction applications (T1 to T3) was significant higher compared to the plants from T4-chemical treatment (Table 1). The PGPR strain SP007s tested in this study had previously exhibited a growth promotion effect on multiple plant species (Chuaboon and Prathuangwong, 2009; Hemsanit et al., 2010; Prathuangwong and Buensanteai, 2007; Prathuangwong and Chuaboon, 2010; Prathuangwong et al., 2010). Its positive effect on rice growth and yield was shown again in the experiment. The significance increased in plant growth index suggests that growth promotion is mediated through auxin production, given that SP007s strain may be able to release auxin-like compounds to culture media (Buensanteai et al., 2008; Prathuangwong, 2009). The ISR-P/K formulation applied with seed and foliar treatment in a powder foliar spray and granular broadcast (T3) achieved the greatest increase in plant growth promotion of rice. All date evaluation except at 56 DAP that the T2 (ISR-P applied with powder spray and granule broadcast) showed higher promotion of plant growth. However, the ISR-P application in T1 (only powder formulation for spray and broadcast) gave lower efficacy in plant growth promotion than ISR-P in T2 (Figure 1). Formulation is a key to bioproduct success that its effectiveness for disease control and plant growth enhancement depends on several factors including the development of a carrier for bacterial antagonist SP007s and the method of application (Prathuangwong, 2009). The carrier could improve the bacterial activity and stability, shelf-life, and also protect the bacteria against environmental extreme in storage period, and an initial source of nutrient after application. Differences between ISR-P (talc-based) and ISR-P/K (kaolin-based) formulation including homogenous base of talc and kaolin and source of iron compounds in the formulate component were performed in this study (data not shown). A component and portion of the carrier may affect a pH nature of these bioformulations resulting biocontrol efficacy of PGPR strain SP007s. We conclude that SP007s compounds (such as auxin and various secondary metabolites) in ISR-P/K are better diffusible and its production involved is independent of the pathogen presence, compared to a previous ISR-P formulation. Also, higher levels of iron diffuse in ISR-P/K adversely affected the efficacy of SP007s to compete with other microorganisms (Prathuangwong et al., 2010). However, efficacy of SP007s in ISR-P bioproduct alone; in a form of powder and granule, and application method between spray and broadcast, was not consistent in plant growth promotion and disease reduction (Figure 2). The granule broadcast directly delivered SP007s to soil base and roots as the bottom fertility, providing a good result in crop production against diseases. The increase in aerial plant growth (Figure 1) and yield (Table 1) was found, suggesting that the positive effect of SP007s on rice was due to different mechanisms including a direct growth promotion. In this study, rice plants applied with PGPR strain SP007s bioproducts (T1 to T3) could promote higher plant growth and total yield than plants treated with urea fertilizer in T4, demonstrating that *P. fluorescens* SP007s also increased the mineral uptake by plants and may fix nitrogen in the free-living state and plant colonization (Fallik et al., 1991). The specific ability of strain SP007s as a nitrogen-fixer is needed for further study on an organization of the genes involved in this strain. The result obtained also reveals that SP007s bioproduct application provides the reduction of fungicide and insecticide sprays while increases qualitative yields of rice at the same time.

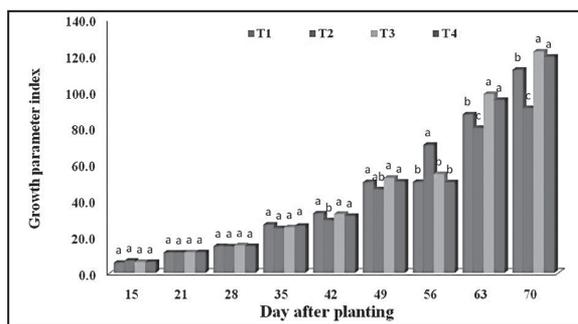


Figure 1. Growth parameter index calculated from root length and plant height, and fresh and dry weight of rice plants treated seeds, 3-weekly broadcasts, and 6-weekly sprays with PGPR strain *Pseudomonas fluorescens* SP007s by different formulations (T1 to T3) and chemical-treated control (T4). Values are the mean of three replicates. Different letters indicate the existence of significant differences according to DMRT ($P=0.05$). T1=ISR-P powder formulation, T2=ISR-P powder and granule formulation, T3=new formulation (ISR-P/K) with powder and granule type, and T4=conventional chemical standard.

Disease reduction and obtainable yield

The 4 treatments tested among naturally rice pathogens were significantly differed in their effect on AUDPC (Figure 2) and disease reduction and total yield (Table 1). Figure 2 showed that the SP007s bioformulations (T1 to T3) significantly reduced AUDPC for 4 out of 6 diseases including blast (caused by *Pyricularia grisea*), brown spot (*Helminthosporium oryzae*), narrow brown spot (*Cercospora oryzae*), and sheath blight (*Rhizoctonia solani*) compared to chemical control treatment in T4. The other 2 diseases, bacterial leaf blight (*Xanthomonas axonopodis* pv. *oryzae*) and dirty panicle (a complex pathogens including *C. oryzae*, *Curvularia lunata*, *H. oryzae*, *Fusarium semitectum*, *Alternaria padwickii*, and *Sarocladium oryzae*) were achieved a level of control by these SP007s bioproducts similar to chemical treatment in T4. According to the AUDPC values for bioproducts, the SP007s treatment in T3 gave a significant reduction in severity of bacterial leaf blight and blast compared with SP007s treatment in T1 and T2; whereas better control in brown spot and narrow brown spot; and sheath blight was found in T1; and T2 respectively, probably due to SP007s expressed diverse mechanisms in different products against these diseases (Table 1). Brown spot occurred with the high degree of severity that the disease at the beginning of assessment (14 DAP) was observed except sheath blight. Once infection was established by this disease, the chemical treatment (T4) showed the highest severity of brown spot. Disease peaks of all natural infections except dirty panicle were highest in all treatments at week 10th evaluation (70 DAP) then, reduced to the lesser extent and were not observed at week 12th and 13th. However, over the 2-last weeks assessed, the drained rice plots have been occurred for 2 weeks continuously at weeks 12th and 13th resulting in the severity peak of dirty panicle infection present at week 13th. Among natural infections, dirty panicle typically occurs in adult plants (Prathuangwong et al., 2010) that may explain why the infection was not found at the beginning and more severe in the last month of the field trial for all treatments tested. The AUDPC values for treatments however, indicated that T1 to T4 resulted in considerable disease control after occurrence of infection, their severity remained stable over time. Treatment T3 protected the rice plants satisfaction against all diseases, confirming the benefit of a new ISR-P/K formulation and application techniques. Treatments T1 and T2 also provided efficient for disease control but to a lesser extent than T3. Treatment T4 of solely fungicide application reduced AUDPC in a same manner as efficient as SP007s bioproduct. The results provided a satisfied reduction in severity of the diseases were therefore, due to *P. fluorescens* SP007s, even without the use of fungicides. The use of SP007s has been reported for the control of different fungal and bacterial diseases on various crops (Chuaboon and Prathuangwong, 2009; Prathuangwong and Chuaboon, 2010; Prathuangwong and Buensanteai, 2007; Prathuangwong

et al., 2010), but a few studies have been carried out on rice diseases, specially the dirty panicle disease. In this study, *P. fluorescens* SP007s bioformulation consistently reduced dirty panicle better satisfaction than the fungicide treatment (Table 1).

The positive effects were recorded during the course of experiment on plant growth and yield enhancement (Figure 1, Table 1). *P. fluorescens* SP007s treatments (T1 to T3) increased rice yield significantly, compared to chemical treatment T4 (Table 1). Treatments with SP007s-ISR-P/K (T3), a new formulation developed in this study and a previous ISR-P bioformulation (T2) resulted in 52.1% higher average yield than the chemical treatment in T4.

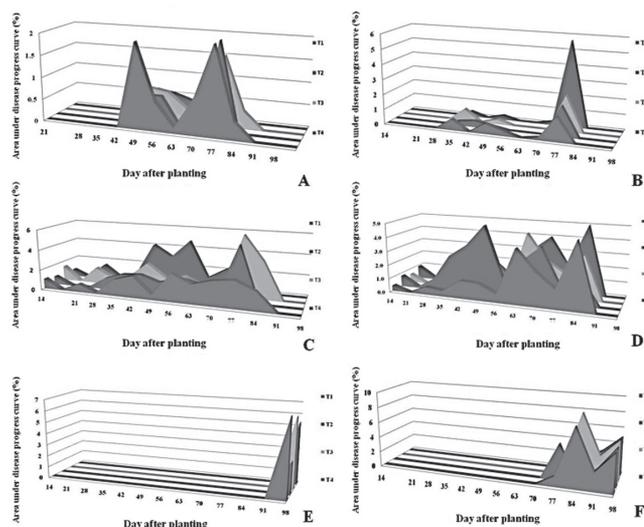


Figure 2. Disease progress curves for bacterial and fungal infections in rice plants treated with *Pseudomonas fluorescens* SP007s bioproducts (T1 to T3) and chemicals (T4) over 98 days. Diseases caused by A, *Xanthomonas oryzae* pv. *oryzae* (caused bacterial leaf blight); B, *Pyricularia grisea* (blast); C, *Helminthosporium oryzae* (brown spot); D, *Cercospora oryzae* (narrow brown spot); E, *Rhizoctonia solani* (sheath blight); and F, a complex pathogens including *C. oryzae*, *Curvularia lunata*, *H. oryzae*, *Fusarium semitectum*, *Alternaria padwickii*, and *Sarocladium oryzae* (dirty panicle). Details of T1 to T4 treatments are as same as marked in Figure 1.

Surprisingly, the final yield obtained from these 2-SP007s treatments (T2 and T3) significantly differed from the same SP007s-ISR-P formulation in T1 that treated by only powder formulation for both spray and broadcast. This may be due to the suitable form of SP007s bioproduct between powder-and granule-broadcast that reached the peak as bottom fertility. Since *P. fluorescens* SP007s have been known as a plant growth promoter resulting yield increase that reported in various crops (Chuaboon and Prathuangwong, 2009; Prathuangwong and Chuaboon, 2010; Prathuangwong and Buensanteai, 2007; Prathuangwong et al., 2009; Prathuangwong et al., 2010) similar to Wei et al. (1991), the direct growth promotion with increased hormones and mineral up take by PGPR can improve the efficacy in disease control and then achieve a significant increase in yield (Prathuangwong and Chuaboon, 2010; Van Loon et al., 1998; Wei et al., 1991). Moreover, the potential of *P. fluorescens* SP007s in ISR-P formulation applied by granule broadcast may maximize in mineral uptake from soil compared to same SP007s ISR-P applied as powder broadcast. Therefore, higher growth promotion and yield enhancement, and the disease reduction obtained from ISR-P granule broadcast provides better results than ISR-P powder broadcast. The inadequate data of uniformity between the two forms of SP007s bioproduct (powder and granule), application method, SP007s population in the environment, and mode of control will affect the differences of disease severity,

plant growth, and yield causing the use of SP007s bioproduct may not always be predictable. Further studies should be undertaken to solve these problems for achievement use of SP007s bioformulation produced at manufacture level.

Table 1. Efficacy of *Pseudomonas fluorescens* SP007s bioformulation on reduced diseases and increased plant growth promotion and yield of rice plants under field experiment¹.

Treatment ²	Disease reduction (%) ³						Plant growth index	Yield (Ton ha ⁻¹)
	BA	BL	BS	SB	NB	DP		
T1	20.0b	15.0b	22.9a	0c	17.6a	2.9b	43.5b	6.4b
T2	41.1a	43.8a	9.8b	66.7a	0c4	40.0a	41.6c	7.3a
T3	50.0a	56.3a	12.6b	16.7b	5.8b	31.4a	46.5a	7.3a
T4	0c	0c	0c	0c	0c	0c	45.0a	4.8c
CV	30.9	36.2	10.6	1.2	3.2	28.0	4.8	18.2

¹Means followed by a same letter in a column are not significantly different according to Duncan’s multiple range test ($P=0.05$).

²Details of treatment are same as marked in Figure 1.

³Disease reduction by T1 to T3 is compared with T4. BA= bacterial leaf blight, BL=blast, BS= brown spot, SB= sheath blight, NB= narrow brown spot, and DP=dirty panicle.

⁴0=control efficacy is equivalent to T4.

Induced protection enzymes in association with disease resistance by SP007s

Plant has numerous defenses against pathogens (biotic stress) and unfavorable effects (abiotic stress) which can be systemically activated upon exposure of plants to these stresses known as SAR or ISR response (Figure 4). Activation of plant resistance can be induced by both abiotic and biotic agents. Abiotic inducers such as plant regulators including salicylic acid (SA), jasmonic acid (JA), and ethylene (ET) and various elicitors obtained from pathogens, PGPR, or from plants themselves (elicitors from plant-pathogen interaction), have been reported (Van Loon et al., 1998). The biotic inducers include PGPR and pathogens that each rhizobacterium will activate different defense mechanisms within ISR response, resulting in differential disease reduction of various cause agents (Wei et al., 1991). The mechanism involved may be physical or chemical barriers arising from increased activity of enzymes associated with induction of systemic resistance (Prathuangwong and Buensanteai, 2007). The defense-related enzymes were determined in PGPR *P. fluorescens* SP007s-treated rice plants with natural pathogen challenge under field conditions (Figure 4). Figure 3 showed that all SP007s bioformulations in T1 to T3 induced a greater amount of defense enzymes in the SP007s-treated plants than the rice plants treated by chemical control in T4 at every week assay. Total POX levels were significantly induced in weeks 2, 3, 6, and 10; and reached a maximum in 4, 8, and 11th (Figure 3A). Results from PAL activity in Figure 3B in plants treated with SP007s had higher PAL induction in weeks 4, 5, 6, 9, 10, 12, and 14 and reached peak levels in 8 and 13 compared to 3, 7, 9, and 11th. A maximum peak induction of β -1,3-glucanase activity was observed in weeks 8 and 13th (Figure 3C), whereas GPX enzyme was higher increase in 4 weeks (in 5, 7, 10, and 12th) and reached peak level in 13th (Figure 3D). The SOD activity was however, sharply induced almost week assays and reached highest accumulation in week 11th (Figure 3E). Among SP007s bioformulation-treated plants, a new ISR-P/K formulation developed in this study (T3) performed a highest induction of all defense-related enzymes accumulated in rice plants (Figure 3). These increased enzymes activities assayed in rice leaves that indicated the resistance induction (Van Loon et al., 1998) were directly correlated with enhanced plant growth (Figure 1), giving protection against diseases (Figure 2), and resulting increased yield (Table 1). Increased accumulation of these protective enzymes to the peak levels in weeks 11 and 13th was also recorded in agreement with the 2-week water deficit with the total average detection of 3.3 μg^{-1} glucose min^{-1} protein, 9 unit

mg⁻¹ protein ; and 9 nmol tran-cinnamic acid min⁻¹ mg⁻¹ protein, 0.83, and 1.4 min⁻¹ mg⁻¹ protein for β -1,3-glucanase, SOD; and PAL, GPX, and POX respectively (Figure 2 and 3). These results demonstrate the linking ability of PGPR strain SP007s to decrease plant stress with activation of defensive enzyme activities involved in systemic resistance induction. PGPR mediated SAR or ISR may elicit different pathways simultaneously conferring additive responses (Figure 4) that are more effective than single-elicited pathways (Van Loon et al., 1998).

Biochemical activation of SAR or ISR that correlated with systemic accumulation of pathogenesis-related proteins (PR) such as β -1,3-glucanase is host-coded proteins with direct action against pathogen glucan resulting its growth inhibition (Buensanteai et al., 2009; Prathuangwong and Buensanteai, 2007). PAL is an enzyme related to phenylpropanoid metabolism that known to be a key point synthetic pathway of flavonoid phytoalexins which are antimicrobial compounds. POX is a key in the lignin biosynthesis and its role on formation of cell wall barriers has been reported in ISR response (Hemmerschmidt et al., 1984; Prathuangwong and Buensanteai, 2007). GPX and SOD are the member of the enzymatic antioxidant defense system that catalyze H₂O₂ and reactive oxygen species (ROS) in plants. These free radicals cause injuries upon oxidative stress resulting down regulation of physiological function of the plants (Chatnaparat et al., 2009; Hemsanit et al., 2010). These protective enzymes and antioxidants have important roles in protection of plants from oxidative damage and *P. fluorescens* SP007s showed efficiently more resistant rice plants to both disease and drought-mediated oxidative stress (Figure 4).

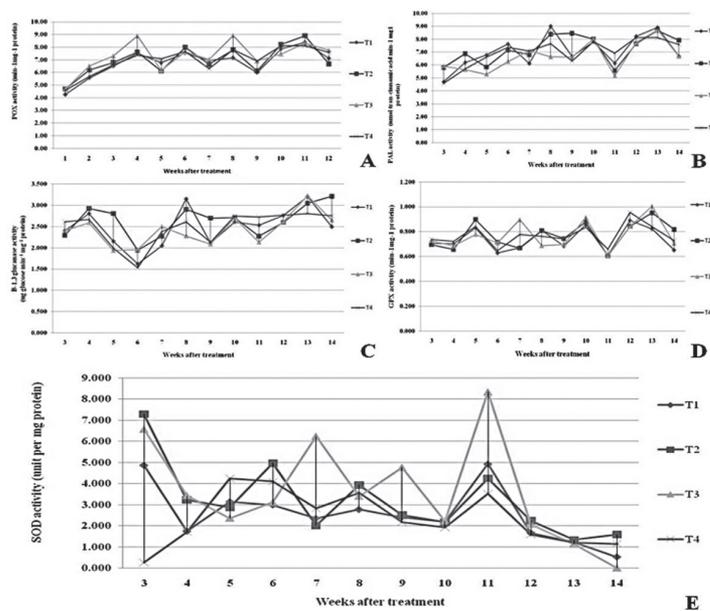


Figure 3. The defense-related enzyme activities induced by *Pseudomonas fluorescens* SP007s bioformulations (T1 to T3) and chemical treatment (T4) determined in rice-treated plants under field conditions. Protective enzymes include (A) POX, (B) PAL, (C) β -1,3-glucanase, (D) GPX, and (E) SOD. Details of treatments T1 to T4 are same as marked in Figure 1.

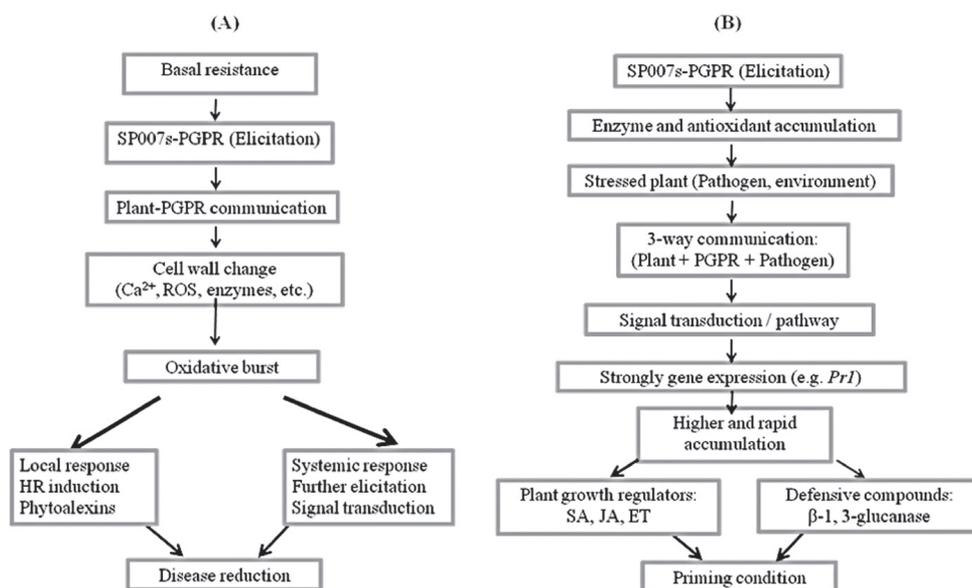


Figure 4. Model for plant resistance induction against disease and water stress. Defense compounds may less increase after SP007s treatment (A) and rapid accumulation when plants infected with pathogen (B). Induction by PGPR led to only one between SA-or- JA- dependent defense pathway; and / or JA / ET pathway.

CONCLUSION

The study revealed the application of PGPR *P. fluorescens* SP007s treatment under field conditions, is a beneficial management of infectious disease and drought protection of rice plants. Treatments of SP007s bioformulations induced plant resistance with increased defense-related enzymes against biotic and abiotic stress as well as enhanced plant growth promotion resulting in significant increase in biomass and yield achievement, when compared to the chemical routine treatment. The results also indicated that the efficacy of *P. fluorescens* SP007s treatment could be increased by use of ISR-P/K, a new bioformulation; and the granule is a better form than powder for broadcast application. Further research on development of a bioproduct is needed to minimize synthetic chemicals for pest management in rice plants.

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