Effects of Plant Growth Promoting Rhizobacteria on Rice (*Oryza Sativa*) in Salinity Stress Condition

ผลของแบคทีเรียส่งเสริมการเจริญต่อข้าวในสภาวะที่มีความเครียดของเกลือ

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ABSTRACT

Salinity stress is a major yield loss problem in rice cultivation. Moreover it also plays a vital role in growth reduction. Important effective of plant growth promoting rhizobacteria is promoting plant growth through their mechanism. In addition PGPRs can promote plant growth under various abiotic stress conditions including salinity stress. PGPRs inoculated in KDML 105 rice variety, promoted rice growth under both normal and salt stress conditions. KDML105 rice variety inoculated with PGPR as R-HW-15 and R-HW-22, rice seedling was cultivated in salinity condition including 120 and 180mM NaCl salt concentration. Shoot height of rice seedling in inoculated treatment is higher than un-inoculated treatment. Moreover, R-HW-22 inoculated treatment can increase percentage of root length by 58.46% (cultivated at 180mM salt concentration). Hydroponic experiment, rice seedlings were cultivated with various concentration of NaCl for 15 days. R-NP-8 can survive in high salt concentration (180mM) or 19.15 ds/m. This results can be further develop and determine in pot and green house trials.

บทคัดย่อ

ความเค็มคือปัจจัยสำคัญอย่างหนึ่งที่ก่อให้เกิดความเสียหายในการเพาะปลูกข้าว นอกจากสาเหตุในการลดลงของผลผลิตแล้วยังส่งผลต่อการเจริญของพืชอีกด้วย แบคทีเรียส่งเสริมการเจริญนั้นมีประสิทธิภาพในการส่งเสริมการเจริญผ่านกลไกของแบคทีเรีย ยิ่งไปกว่านั้นยังสามารถส่งเสริมการเจริญในสภาวะแวดล้อมที่ไม่เหมาะสมต่อการเจริญของพืช โดยเฉพาะความเค็มในดิน ผลการทดลองในงานวิจัยครั้งนี้ชี้ให้เห็นว่าชุดทดลองที่มีแบคทีเรีย R-HW-15 และ R-HW-22 เจริญร่วมด้วยมีแนวโน้มส่งเสริมการเจริญของต้นกล้าข้าวได้ดีที่สุด และเมื่อเปรียบเทียบความสามารถในการส่งเสริมการเจริญของแบคทีเรียจากความยาวรากที่ความเข้มข้นเกลือเท่ากับ 0mM พบว่า ในชุดทดลองที่มีแบคทีเรีย R-HW-22 มีผลของการเจริญที่สูงกว่าชุดที่ไม่มีแบคทีเรียสูงถึง 58.46% นอกจากนี้เมื่อทาการทดลองเพาะปลูกข้าวในสารละลายHoagland solution แบบระบบHydroponic พบว่าชุดทดลอง R-NP-8 นั้นสามารถรอดชีวิตได้แม้มีความเข้มข้นเกลือสูงถึง 180mM หรือ ค่า EC เท่ากับ 19.15 ds/m จากผลการทดลองในห้องปฏิบัติการพบว่าชุดทดลองที่มีแบคทีเรียส่งเสริมการเจริญนั้นสามารถส่งเสริมการเจริญของต้นกล้าข้าวขาวดอกมะลิ 105 ได้ดีทั้งในสภาวะที่มีเกลือโซเดียมคลอไรด์ และไม่มีเกลือ

Keywords: Salinity, plant growth promoting rhizobacteria, Hydroponic
คำสำคัญ: ความเค็ม แบคทีเรียส่งเสริมการเจริญในพืช ไฮโดรพอนิกส์

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Introduction

Salinity soil is one of the most important factors which cause growth reduction and yield loss in crop plant. Moreover, high concentration of salt has toxicity to soil element deficiencies and nutrient uptake in plant. Saline soil was described by that having electrical conductivity (EC) more than 4dsm^{-1}. If EC is higher than 8dsm^{-1}, it will damage and becomes toxic to plant. Salinity stress induced several morphological symptoms on rice including tip burning, stunted growth, tillering reduction and yield loss. North eastern of Thailand has agriculture area estimate 17.12 billion ha^{-1}. But 2.848 billion ha^{-1} of agriculture area has saline stress (EC estimate 6-8 dsm^{-1}). Statistic value shows, saline stress can damage 50% of crop per year. Management of saline soil usually suggests including cover crop plantation or flood in paddy field before rice cultivar season. But it's not long term management. Nowadays, bio-agriculture is made practical use to resolve salinity soil in paddy field. Microorganism can promote plant growth through their mechanisms and activities as well known as plant growth promoting rhizobacteria (PGPR). Lugtenberg (2014) reported PGPR could against soil borne pathogen and also support adaptation of plant in abiotic environment. Moreover, 20 halo-tolerant free-living and nitrogen fixation bacterial strains, DL2, DL3 and DL6 isolation have optimum growth in various salt concentrations by 4% , 6 and 10% . The plant treated with bio-inoculums has shoot height and root length increase at 12.61 and 82.35%. The results can indicate halo-tolerant bacteria have effective to growth induction under saline soil condition (Shubhagi S. 2016). The previous studied would explain effective of PGPRs on rice growth promotion and alleviate salt stress in plant cultivation.

Objective of the study

The aim of this study was to assess the effectiveness of plant growth promoting rhizobacteria on rice growth under both normal and salinity stress conditions.

Materials and methods

Bacterial and rice seedling preparation

Bacteria isolates was selected from rice paddy field in Khon Kaen province, Thailand. The bacterial isolates were determined about growth promoting properties including nitrogen fixation, phosphate solubilization and chitinase enzyme production. They were cultivated in nutrient broth and a shaken incubation at 150 rpm, 30^{0}C for 18 hours.

For rice seedling preparation, KDML 105 rice grain (Ubonratchathani rice department, Thailand) was cleaned up with sterile ddH_2O and 95% of ethanol. After that rice grains were soaked in 0.2% of mercuric chloride for 30 minutes. Cleaned up grains with sterile ddH_2O twice time and cultivated in dark place and room temperature (25-30^{0}C) for 3 days before bacterial colonization. After seed germination, twenty healthy grains of KDML 105 were colonized with 10% (v/v) of PGPRs (R-HW-15, R-HW-22, R-NP-20, R-HW-25, R-HW-24 and R-NP27). And rice was cultivated again in the dark place and room temperature (25-30^{0}C).

Salt tolerance of rice at the germination stage

The rice seedling was separated to 5 treatments following salt concentration (NaCl) as 0, 60, 80, 120 and 180mM using 20 rice seedlings per group and cultivated in dark place at room temperature for 3 days. Growth of tested rice seedlings (i.e. shoot height, root length and dry weight percentage) were measured for salinity effect determination. Effect of salt concentration was described through growth tested.

Salt tolerance of rice seedling grown in hydroponic solution

The 7 days old rice seedlings were colonized with PGPR (CD-3, R-NP-7, R-NP-6, R-NP-15, R-NP-16, R-NP-19, R-NP-20, R-NP-21, R-NP-22, R-NP-23, R-NP-24, R-HW-22, R-HW-23, R-HW-24, R-HW-25, and R-HW-26). The rice seedling was separated to 4 treatments following NaCl concentration (0, 160, 180 and 240mM) and all treatments were grown in Hoagland solution. The tolerance was observed after 15 DAT (days after transplanting) and determined by seedling growth appearance.

Results

Salt tolerance

Affection of plant growth promoting rhizobacteria on KDML 105 rice variety, R-HW-15 and R-HW-22 could promote rice growth both germination and seedling periods. Even thought stress condition like high concentration of NaCl (180mM), colonized treatments still have growth rate better than un-colonized at 47% and 30%, respectively (Figure1). Moreover, shoot height of rice seedling in R-HW-15 were also higher than un-colonized treatment (Ctrl) at 8% and 16% (Figure2).

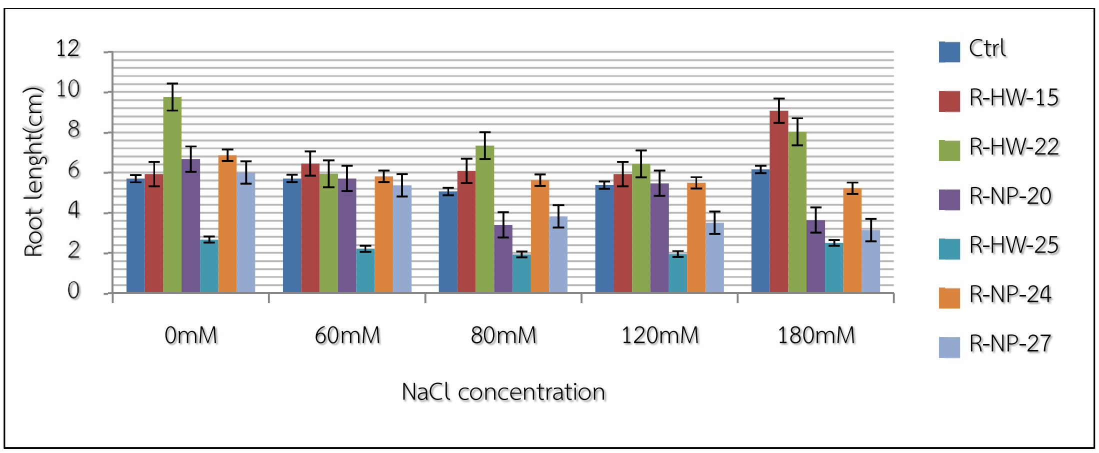


Figure 1 Root length (cm) of rice seedlings (6 days after transplanting) in various salt concentrations. (Ctrl is un-colonized treatment and all treatments was triplicate)

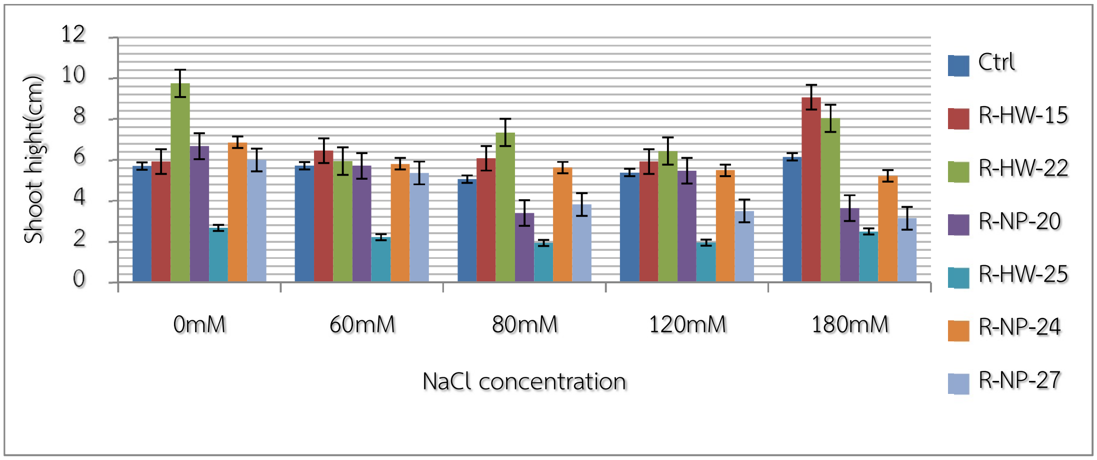


Figure 2 Shoot heights (cm) of rice seedlings (6 days after transplanting) in various salt concentrations. (Ctrl is un-colonized treatment and all treatments was triplicate)

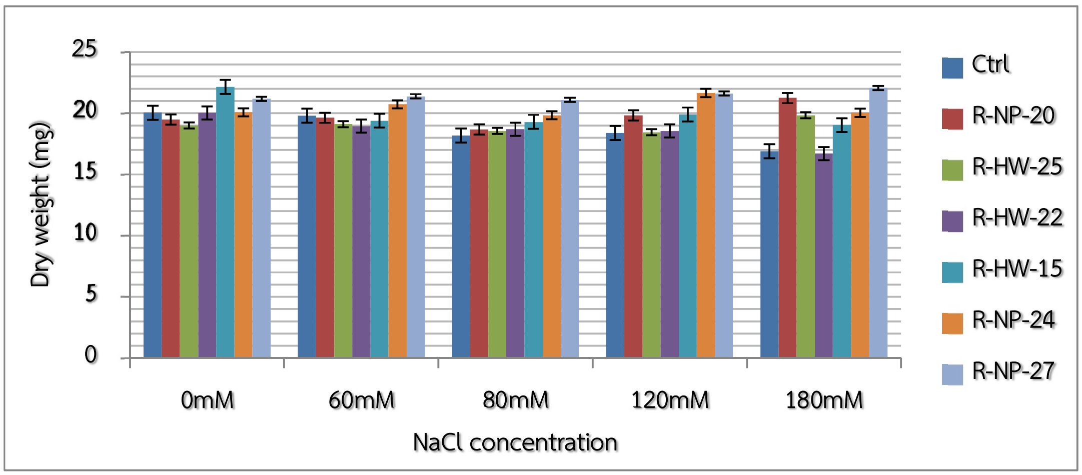


Figure 3 Dry weight (mg) of rice seedlings (6 days after transplanting) in various salt concentrations. (Ctrl is un-colonized treatment and all treatments was triplicate)

Salt tolerance in hydroponic experiment

The affect of PGPRs on salt tolerance tend to increasing growth in stress condition so researcher would expand level of salt concentration and period of plantation time. 15 days after transplanting in salt stress condition for R-NP-8 could survive in high level of NaCl. And all bacterial treatments have better resulting in growth than un-colonized condition (Table1).

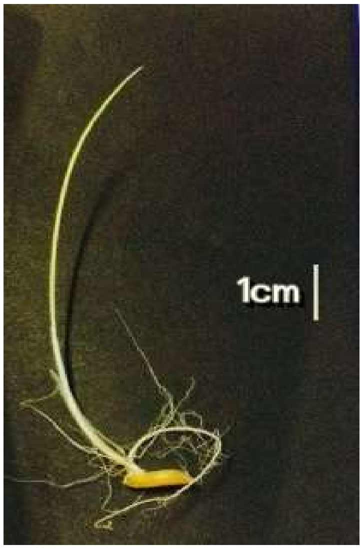
Table 1 Appearance of hydroponic plantation

Treatments	Concentration of NaCl (mM)			
	0 mM	**160 mM**	**180 mM**	**240 mM**
Un-inoculated	+	-	-	-
CD-3	++	++	++	+
R-NP-7	+++	++	++	+
R-NP-6	+++	+++	+++	+++
R-NP-15	+++	+++	++	++
R-NP-16	++	++	++	+
R-NP-19	+++	+++	+++	++
R-NP-20	++	++	++	+
R-NP-21	+++	+++	+++	+++
R-NP-22	++	+++	+++	++
R-NP-23	+++	++	++	+
R-NP-24	+++	++	++	+++
R-HW-22	+++	++	++	++
R-HW-23	+++	+++	+++	++
R-HW-24	++	+	+	+++
R-HW-25	+++	++	+	++
R-HW-26	+++	+++	+++	++

(+++ , rice could grow better other treatment and grow without abnormal appearance as dry tip)

(++ , rice could grow at good but fibrous root was dry, less and tip of shoot also dry)

(+ , rice has atrophic shoot and less fibrous)



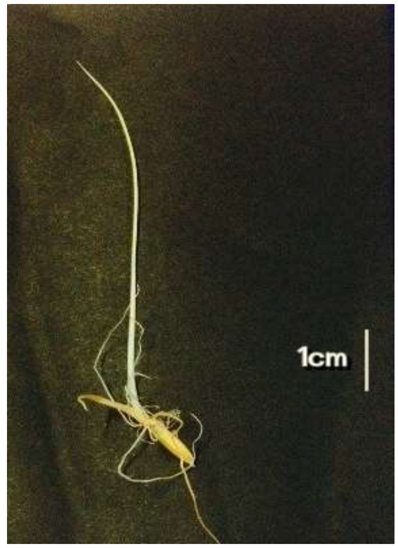


Figure 3 All pictures presence 14 days old rice seedling in R-NP-24 treatment. Right picture is seedling at 80mM, Rice seedling has many fibrous roots and still green which slightly different from left picture which seedling at 120mM. Although seedling was dried but it still has fibrous root.

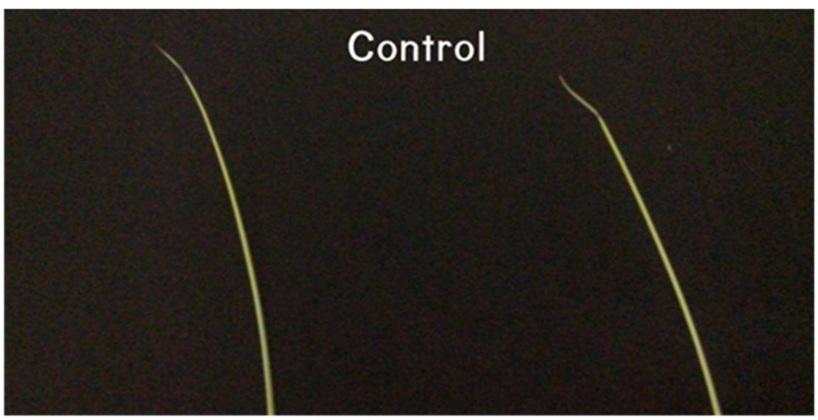


Figure 4 The picture presence tip of un-colonized rice seedling, it shows abnormal appearance, tip of rice shoot so dry and brownness.

Discussion

Salinity soil problem is major problem in north eastern of Thailand for rice cultivation which can cause by naturally and farmer practice. Normally plants can tolerance in salt stress condition estimate 4-6 dsm^{-1}. In previous study, Hashemi rice variety was cultivated in various water salinity concentrations as 2, 4, 6 and 8dsm^{-1}. The result shown that both NaCl and $CaSO_4$ had effect on rice growth, they reduce growth rate reach 47% and effect on yield lees more than 50% when comparison with no salinity water (Hassan et al. 2011). However, there were many studies shown that plant growth promoting rhizobacteria could promote growth and yield of wheat (*Triticum aestivum L.*) under salinity stress condition both pot and field experiment. The results shown *Planococcus rifietoensis* SAL-15 increase overall plant growth at 37% differ from un-colonized which reduce growth and yield up to 60% (Lubna et al. 2013). Habib(2016) also reported *Enterobacter* sp. UPMR18 had bio-resource effective and salt tolerance enhancing by antioxidant enzyme activities including ACC determination or Catalase. In addition, PGPRs as *Bacillus pumilus* and *Pseudomonas pseudoalcaligenes* also were able to colonize in GJ-17 rice variety under greenhouse condition. They increased germination at 16% , plant height at 31% and 27% of dry weight. Moreover they also reduced salt concentration as 71% of Na and 36% of Ca when comparing with non-colonized. The results of this study shown that after treated with PGPRs, the KDML 105 rice seedlings could survive in high level of NaCl stress condition reaching 180 mM or 13.8 dS/m. Besides, PGPR could promote rice growth under both normal and stress condition. R-HW-15 and R-HW-22 are on top of growth promotion and salt tolerance. It can conclude PGPRs were effective in enhancing salt tolerance of rice at germination and seedling stage. Further study is required to selected PGPRs isolation and study about their application on rice growth with other stress condition as rice blast disease.

Acknowledgment

This study thanks Microbiology, Faculty of Science, Khon Kaen University, Thailand.

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