

## An Enhanced Root System Developmental Responses to Drought by Inoculation of Rhizobacteria (*Streptomyces mutabilis*) Contributed to the Improvement of Growth in Rice

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**Drought limits rice production under upland conditions. This study quantified the effect of rhizobacteria inoculation on root system development during germination stage under laboratory conditions and in early vegetative stage in soil subjected to drought, as well as their contribution to soil water uptake and dry matter production using NSIC Rc192 variety. The source of inoculant was *Streptomyces mutabilis*, a bacterium capable of producing plant growth promoting compounds such as ACC deaminase, indole-3-acetic acid, and phosphatase. In the first experiment, pre-germination inoculation of seeds by *S. mutabilis* significantly increased the shoot and seminal root length as well as root hair lengths, relative to the uninoculated control. In the second experiment, inoculation of *S. mutabilis* generally had longer total root length under drought – regardless of the timing of inoculations – relative to the uninoculated control. Consequently, improved root system development contributed to the increase in soil water uptake under drought and thus, resulted in the increase in dry matter production. Among inoculation treatments, one-time inoculation of *S. mutabilis* either at pre-germination or pre-drought stress at 14 days after sowing (DAS) had significantly greater shoot dry matter production than three-time inoculation at pre-germination, thinning (3 DAS), and pre-drought stress (14 DAS). This study demonstrated the effectiveness of rhizobacteria (*S. mutabilis*) containing growth promoting compounds for enhancing drought dehydration avoidance root traits and improving the growth of rice plants under drought conditions.**

Key words: drought, dry matter production, rhizobacteria, root system development, upland rice, water use

### INTRODUCTION

Drought is one of the most serious abiotic stresses that limit crop production under rainfed conditions. There are about 100 M hectares of rice areas in the world and 89% of them are in Asia. Of the total rice area in Asia, 45% are

rainfed areas, of which 25% is never flooded and thus, classified as upland conditions (Serraj et al. 2009). The rainfed upland field has poor accumulation of water due to uneven upper toposequence, absence of bunds, and lower water-holding capacity of the soil (Bernier et al. 2008). Generally under upland conditions, it is thought that deep and thick roots are the key traits for adaptation in rice (O'Toole & Bland 1987) particularly roots below

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30 cm from the soil surface (Yoshida & Hasegawa 1982). This is highly influenced by both genotypic variations and intensity of drought stress (Price et al. 2002; Kamoshita et al. 2002; Kato et al. 2006, 2007; Uga et al. 2011), as well as cultural management practices such as the application of sufficient amount of nitrogen (N) (Tran et al. 2014) regardless of N forms (Tran et al. 2015).

Root growth can be regulated by ethylene levels (Sharp & LeNoble 2002; Pierik et al. 2007) and the biosynthesis of ethylene is regulated by biotic and abiotic stresses (Hardoim et al. 2008). In the biosynthetic pathway of ethylene, S-adenosylmethionine (S-AdoMet) is converted by 1-aminocyclopropane-1-carboxylate synthase (ACS) to 1-aminocyclopropane-1-carboxylate (ACC), the immediate precursor of ethylene. Under environmental cues such as drought, production of ACC oxidase (ACO) is abundant which catabolizes ACC to ethylene. However, to limit ethylene production under drought, ACC must be catabolized by enzyme other than ACO. For instance, ACC can be sequestered and degraded by ACC deaminase producing bacteria to supply N and energy, and reduce the deleterious effect of ethylene (Glick 2005). ACC exuded from seeds, roots, or leaves (Penrose et al. 2001) and may be taken up by the bacteria associated with these tissues, and subsequently cleaved by ACC deaminase (Penrose & Glick 2003). This consequently stimulates plant ACC efflux, decrease the root ACC concentration and ethylene evolution, and increase root growth (Glick et al. 1998; Mayak et al. 2004). Thus, microbial production of ACC deaminase such as rhizobacteria plays an important role in improving abiotic stress tolerance in plants (Glick 2005, 2012; Yaish et al. 2015).

Among plant growth promoting bacteria (PGPB), actinomycetes are the most economically and biotechnologically valuable prokaryotes. They produce half of the discovered bioactive secondary metabolites. More than 50 genera have been used in human, veterinary medicine, agriculture, and industry. One of the genera of actinomycetes is *Streptomyces*. Generally accounting for a large percentage of the soil microflora, *Streptomyces* is an effective colonizer of plant root systems and is able to survive unfavorable growth conditions like drought by forming spores. The production of growth-promoting compounds is part of the metabolism of various bacteria associated with plants, causing modifications in the morphology of roots, influencing nutrient and water absorption, and consequently promoting plant growth (Bashan & Holguin 1997).

Recently, an actinomycete was isolated from Binangonan soil in Rizal Province, Philippines with 98% probable identity as *Streptomyces mutabilis* based on 16S rDNA analysis (Cruz et al. 2015). *Streptomyces mutabilis*

produced plant growth-promoting compounds such as ACC deaminase, indole-3-acetic acid, and phosphatase, either of which was responsible for promoting rice growth under laboratory conditions (Cruz et al. 2014, 2015). In this study, the researchers hypothesized that the application of rhizobacteria such as *S. mutabilis* could promote root system development even under drought stress and contributes to the increase in water uptake and consequently, maintain greater dry matter production in rice. Thus, this study aimed to evaluate the effect of rhizobacteria (*S. mutabilis*) inoculation on root system development during germination stage at controlled conditions and in early vegetative stage in soil subjected to drought, and their contribution to water uptake and dry matter production.

## MATERIALS AND METHODS

### Time and Place of Study

Two experiments were conducted: one each under laboratory and greenhouse conditions during Jan-Feb 2016 at the Philippine Rice Research Institute - Central Experiment Station (PhilRice-CES), Muñoz, Nueva Ecija, Philippines (15°40' N, 120°53' E, 57.6 m above sea level).

### Source of Inoculant

An actinomycete identified as *Streptomyces mutabilis* (Cruz et al. 2015), which is capable of producing growth-promoting compounds (Cruz et al. 2014, 2015), was used as source of inoculant for both experiments. This bacterium was maintained on arginine-glycerol-salt (AGS) agar slants.

### Rice Genotype Used

A rice variety, NSIC Rc192 (Sahod Ulan 1) was used in both experiments. This variety is known for its good yield performance ranging at 3.7-5.5 t ha<sup>-1</sup> under drought-prone rainfed lowland ecosystem.

### Experiment 1. Effectiveness of *S. mutabilis* in Enhancing the Shoot and Growth of Rice Seedlings During Germination Stage

One hundred (100) g of the sterilized carrier was used to make *Streptomyces mutabilis* inoculant. One (1) mL of actively growing *S. mutabilis* isolate was inoculated in 22 mL AGS broth and was cultured for 3-4 days. Broth culture was inoculated into the 100 g sterilized soil-based carrier, which brought the soil moisture to approximately field capacity. Seeds of NSIC Rc192 were soaked in concentrated H<sub>2</sub>SO<sub>4</sub> for 30 s and washed with sterile distilled water seven times to

remove H<sub>2</sub>SO<sub>4</sub>. Surface sterilized seeds were pre-soaked in a 7-day old culture broth for 30 min. Germination tests were carried out in a petri dish lined with filter paper. Seminal root length of five randomly selected seedlings from each treatment were monitored daily and measured until 7 days after germination. Root hair formations were examined at 3 days after germination by cutting a 1-cm segment from the seminal root and observed under a system microscope (Olympus CX41, Olympus Corporation, Tokyo, Japan) at 400x magnification. Digitized images were taken from a camera attached to the microscope.

## Experiment 2. Shoot Growth, Root System Development and Water Use of Rice Under Drought Conditions as Affected by Different Inoculation Treatments of Rhizobacteria (*S. mutabilis*)

### Cultural Management and Imposition of Drought Stress

Three pre-germinated seeds of NSIC Rc192 were grown in each box (25 cm x 2 cm x 40 cm, L x W x H) filled with 2.8 kg soil (mixture of 1.7 kg garden and 1.1 kg sandy loam soils), following the method of Kano-Nakata and colleagues (2012). The soil in each box was pre-mixed with 730 mg ammonium sulfate (21-0-0), 460 mg solophos (0-20-0), and 160 mg muriate of potash (0-0-60).

The soil in each box was initially saturated with water (24% soil moisture content, SMC) and maintained at the same level SMC from the day of sowing up to 14 days after sowing (DAS). Thereafter, watering was withheld and the soil was allowed to dry down to 10% SMC and maintained to that level of SMC until 40 DAS. Boxes were weighed daily using a digital balance to record the wet mass of the soil. The soil moisture content (% by mass) in each box was calculated as the ratio between water mass (difference between the wet mass of the soil excluding the box on a given day and the dry mass of the soil at 2.8k g) and the dry mass (2.8 kg) of soil. Once the target SMC during drought period was reached, watering was done to replace the amount of water lost and maintain the desired SMC. Drought treatments imposed through progressive soil drying were terminated at 40 DAS.

### Inoculation Treatments

One hundred (100) g of the sterilized carrier were used to make *Streptomyces mutabilis* inoculant. One (1) mL of actively growing *Streptomyces mutabilis* isolate was inoculated in 22 mL AGS broth and was grown for 3-4 days. Broth culture was inoculated into the 100 g sterilized soil-based carrier which brought the soil moisture to approximately field capacity. The inoculation treatments were as follows: 1) uninoculated

(control); 2) inoculated at pre-germination; 3) inoculated before the imposition of drought (pre-drought, 14 DAS) and; 4) inoculated each at pre-germination, at thinning and at pre drought. At pre-germination, surface sterilized seeds were pre-soaked in a 7-day old inoculant suspension for 30 min. During thinning and pre-drought stress, inoculation was done by watering the inoculant suspension directly to the soil with plants.

Sampling was done at 40 DAS after 14 days of well-watered, and then 26 days of progressive drought stress conditions. Three plants (1 plant = 1 replication) were collected from each treatment.

### Shoot and Root Growth Measurements

The shoots were cut and oven dried at 70°C for 48 h before weighing. The whole root system was sampled using a pinboard following the methods of Kono and co-workers (1987). The extracted root systems embedded in plastic sheets were temporarily stored in alcohol for further measurements. Prior to measurements, alcohol was removed and root systems were stained in 0.25% Coomassie Brilliant Blue R 250 aqueous solution for at least 24 h. This staining procedure was indispensable for taking high-resolution digital scans of the entire root system, including the fine lateral roots (LR). After staining, the root samples were washed gently with running water to remove excess stains.

The total number of nodal roots (NR) was manually counted. The length of each NR was measured using a meter stick. After manual measurements, the root system of each sample was scanned at 600 dpi (EPSONv700 Perfection) and analyzed for total root length (TRL) using WinRhizo v. 2007d (Régent Instruments, Québec, Canada). A pixel threshold value of 175 was set for the root length analysis. After analysis, the roots were oven-dried at 70°C for 48 h prior to weighing of the root dry weight (RDW). The lateral root length (LRL) was computed as the difference between the TRL and total nodal root length (TNRL). The mean nodal root length (mean NRL) was computed as the ratio between TNRL and NRN.

### Statistical Analysis

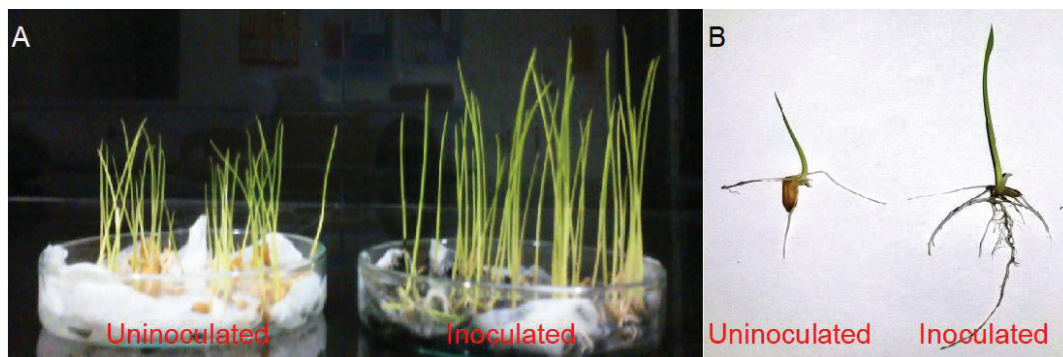
The experiment 1 was laid out in CRD while the experiment 2 was laid out in RCBD, both with three replications. In experiment 2, the ANOVA and calculation of means were done for all traits measured using IRRISTAT program (version 4.1). Means were compared using Tukey's HSD at 5% level of significance.

## RESULTS AND DISCUSSION

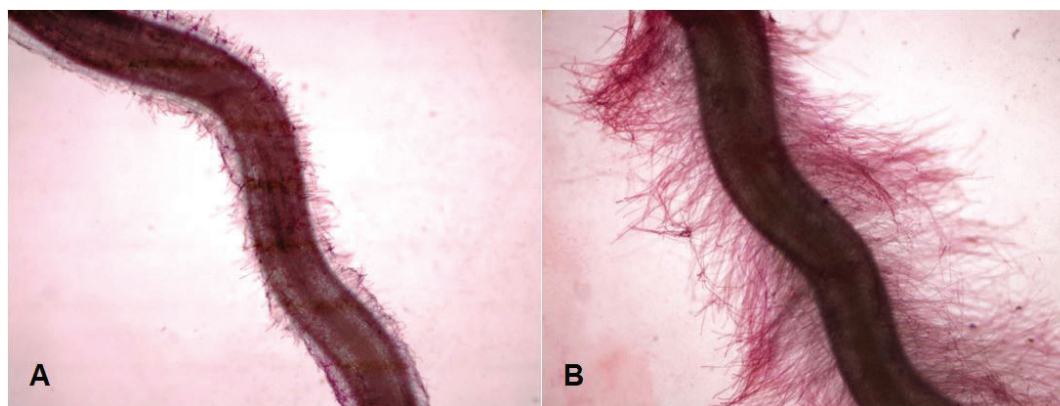
### Shoot and Root Growth of Rice Seedlings During Germination as Affected by Inoculation of Rhizobacteria (*S. mutabilis*) During Seed Soaking

The effect of *S. mutabilis* inoculation on the seminal root elongation and root hair production of NSIC Rc192 are presented in Figures 1 and 2, respectively. Visually, inoculation of seeds with *S. mutabilis* significantly promoted the shoot and root growth of rice seedlings (Fig. 1A and B), compared to the uninoculated seeds at 5 days after germination. Shoot length increased by 28% in *S. mutabilis* inoculated seeds over uninoculated control at 5 days after inoculation (Fig. 1A). Similarly, inoculation with *S. mutabilis* increased seminal root length of NSIC Rc192 by 35% relative to uninoculated control (Fig. 1B). Furthermore, root hair length was also promoted due to *S. mutabilis* at 3 days after germination relative to uninoculated control (Fig. 2). The daily seminal root elongation from seed germination to 7 days after germination is presented in Figure 3. The difference in seminal root length between inoculated and uninoculated seedlings became apparent at 4 days after germination. The maximum seminal root length in inoculated seedlings

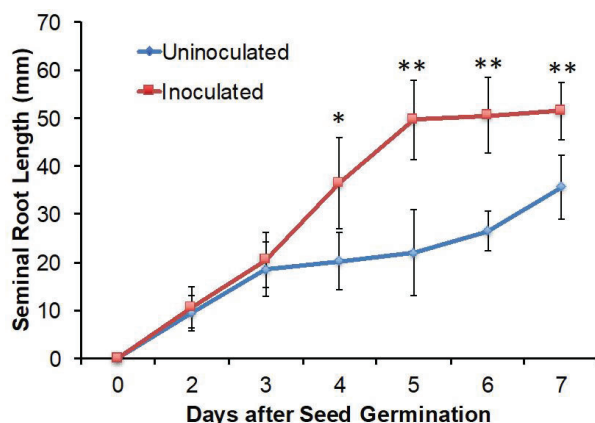
peaked at 5 days until 7 days after germination at 50 mm length. The seminal root length in uninoculated seedlings also continued to increase but at a lower rate and reached to about 35 mm only at 7 days after germination. Overall, the seminal root length was 145% greater in *S. mutabilis* inoculated rice seeds than in uninoculated ones at 7 days after germination. The above results suggest that inoculation with *S. mutabilis* promoted the shoot and root growth of rice seedlings during germination stage. In this study, there was no imposed abiotic stress during germination stage of seedlings. Thus, the promotion of root and shoot growth with the inoculation of *S. mutabilis* was most probably attributed to the effect of its growth-promoting compound such as indole-3-acetic acid (auxin) and phosphatase (Cruz et al. 2015). Auxin through its morphogen, IAA, has been documented to promote stem elongation in many plants (Gallavotti 2013) and increase root hair formation (Patten & Glick 2002), while high phosphatase in roots promoted root hair elongation in *Arabidopsis thaliana* (Smith et al. 1994). Harikrishnan and co-authors (2014) also showed an enhanced growth of rice seedlings when inoculated with *Streptomyces* sp. VSMGT1014. They also showed that seed germination, root length, shoot length, fresh and dry weight significantly increased both culture and culture filtrate compared to control.



**Figure 1.** Effect of rhizobacteria (*S. mutabilis*) inoculation on the shoot and root growth of NSIC Rc192 rice seedlings.



**Figure 2.** Effect of rhizobacteria (*S. mutabilis*) inoculation on root hair length of NSIC Rc192 rice seedlings at 3 days after inoculation: A) uninoculated, B) inoculated (magnification: 400x).



**Figure 3.** Daily seminal root length of NSIC Rc192 rice seedlings inoculated with rhizobacteria (*S. mutabilis*). Values are means of three replicates  $\pm$  standard errors. \* and \*\* indicate significant differences between inoculation treatments at 5 and 1% Tukeys HSD, respectively.

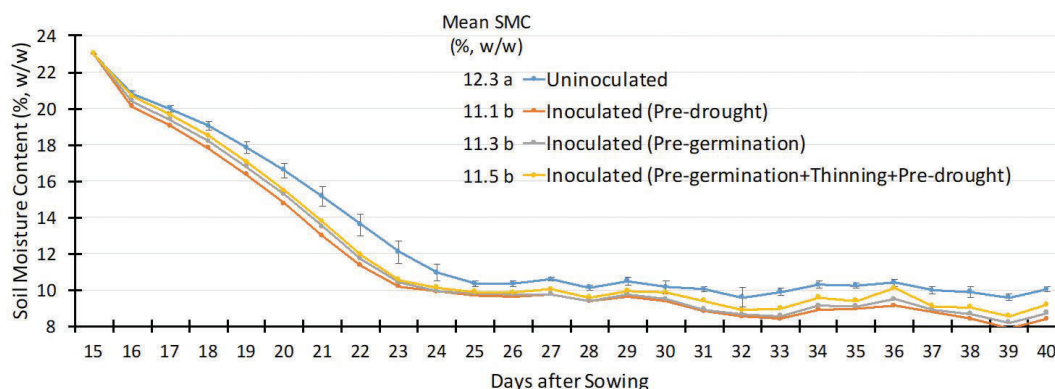
#### Soil Moisture Dynamics in Different Inoculation Treatments of Rhizobacteria (*S. mutabilis*) During Progressive Drought Stress

The rate of soil moisture decline during progressive drought stress in inoculated and uninoculated treatments is presented in Figure 4. Soil moisture content was generally decreased earlier in *S. mutabilis* inoculated seedling regardless of time of application, relative to the uninoculated seedlings. In fact, the soil moisture in inoculated treatments reached 10% SMC 6 days earlier than the uninoculated treatment. In general, inoculated treatments lower SMC (0.8-1.2%) during progressive drought than that of uninoculated treatment. Kato and co-authors (2006) used SMC to assess the soil water uptake rate of evaluated rice genotypes; the lower the SMC, the higher the soil water uptake ability and vice versa. Thus,

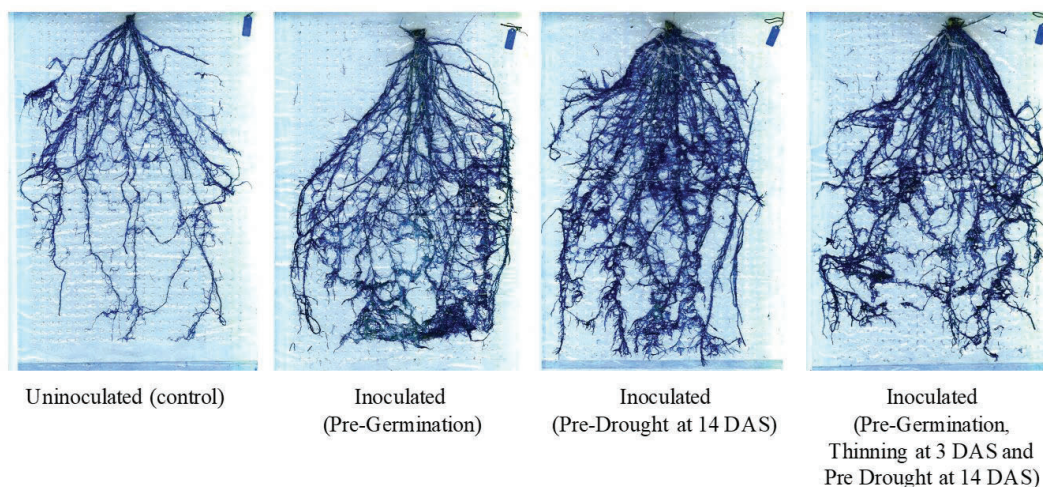
the result suggests that plants inoculated with rhizobacteria (*S. mutabilis*) had greater ability to take up water from the drying soil, which was directly related to the size of their root system architecture as will be discussed in the succeeding section.

#### Root System Development, Water Use, and Dry Matter Production of NSIC Rc192 Under Drought Conditions with Different Inoculation Treatments of Rhizobacteria (*S. mutabilis*)

Root system development of NSIC Rc192 with different rhizobacteria (*S. mutabilis*) inoculation treatments is shown in Figure 5. Visually, the root system development in inoculated treatments had generally larger and more extensive root (many lateral roots) systems than in the uninoculated control. The quantitative analysis of each root trait component is presented in Table 1. Roots system development based on TRL were significantly longer in treatments with one-time inoculation either at pre-germination or at pre-drought stress (14 DAS) than in uninoculated control (Table 1). On the other hand, plants with three-time inoculations at pre-germination, thinning (3 DAS), and pre-drought stress (14 DAS) had an intermediate total root length, which was not significantly different from either uninoculated control or inoculated once either at pre-germination or pre-drought stress (14 DAS). The number of nodal roots per plant was not significantly different among inoculation treatments, while the total lateral root length had the same trend with that of the total root length (Table 1). Furthermore, the root dry weight, total nodal root length, and the mean nodal root length were generally and significantly higher in inoculated treatments regardless of the timing of application, compared with the uninoculated control. Among the root trait components, the total lateral root



**Figure 4.** Soil moisture dynamics during progressive drought stress at 15-40 days after sowing as affected by different inoculation treatments of rhizobacteria (*S. mutabilis*). Values are means of three replicates  $\pm$  standard errors. Values inside the graph are the mean soil moisture content corresponding to each inoculation treatment from the start of progressive soil drying (16 DAS) up to the termination of the experiment (40 DAS). Means followed by the same letters are not significantly different at 5% Tukey's HSD.



**Figure 5.** Effect of different inoculation treatments of rhizobacteria (*S. mutabilis*) on the root system development of NSIC Rc192 grown under progressive drought stress.

**Table 1.** Effect of the different inoculation treatments of rhizobacteria (*S. mutabilis*) on root growth and development of NSIC Rc192 grown under progressive drought stress.

Inoculation Treatments	Total Root Length (cm plant <sup>-1</sup> )	Nodal Roots (no. plant <sup>-1</sup> )	Root Dry Weight (g plant <sup>-1</sup> )	Total Nodal Root Length (cm plant <sup>-1</sup> )	Total Lateral Root Length (cm plant <sup>-1</sup> )	Mean Nodal Root Length (cm plant <sup>-1</sup> )
Uninoculated	4585.4 b	43.0 a	0.27 b	685.5 b	3899.9 b	16.1 b
Inoculated (Pre-germination)	11735.2 a	54.7 a	0.57 a	1062.9 a	10672.3 a	19.4 a
Inoculated (Pre-drought)	12670.9 a	65.3 a	0.59 a	1265.6 a	11405.3 a	20.1 a
Inoculated (Pre-germination + Thinning + Pre-drought)	9271.1 ab	61.5 a	0.47 a	1240.1 a	8031.1 ab	20.1 a

In a column, means followed by the same letters are not significantly different at 5% Tukey's HSD.

length had the greatest influence on the changes on root system development ( $r^2=0.99$ , significant at  $p=0.01$ ) (data not shown). Since lateral roots mainly comprised the whole root system in terms of number and length (Yamauchi et al. 1996), their response to inoculation of rhizobacteria (*S. mutabilis*) directly dictates whole root system development.

In the present study, shoot dry matter production was significantly increased by the inoculation of rhizobacteria regardless of the timing of applications, relative to the uninoculated control (Table 2). Among inoculated treatments, three-time inoculation at pre-germination, thinning, and pre-drought stress had significantly lower shoot dry matter production than either of the one-time inoculation treatment (Table 2). The leaf area in inoculated treatments, particularly those which was inoculated once either at pre-germination or at pre-drought stress, were significantly higher than in uninoculated control (Table 2). In contrast, the leaf area in inoculated treatment applied three times at pre-germination, thinning, and pre-drought stress had intermediate value between the uninoculated control and one-time inoculation treatments (Table 2). Other shoot traits such as plant height and number of

tillers per plant were not significantly different among inoculation treatments. Furthermore, total water use after drought stress imposition was generally and significantly higher regardless of inoculation treatments, compared to uninoculated treatment (Table 2).

Upland rice areas had very low yield because of drought stress occurrence all throughout the growing season. Root elongation and lateral root production are some of the key root traits for adaptation and maintenance of higher dry matter production and yield. And thus, continued root growth and development is a must. In the present study, the rhizobacteria (*S. mutabilis*) used in the inoculation treatments contained growth promoting compounds such ACC deaminase, indole-3-acetic acid, and phosphatase, which effectively promoted rice growth under laboratory conditions (Cruz et al. 2014, 2015). Thus, the improvement of NSIC Rc192 root system under drought conditions was attributed to the integrated effects of growth promoting compounds produced by *S. mutabilis*. The enzyme directly responsible for the ethylene biosynthesis during drought stress is 1-aminocyclopropane-1-acid carboxylic oxidase (ACO or EFE - ethylene forming enzyme), which converts ACC into this plant hormone (Hegg & Que 1997).

**Table 2.** Effect of the different inoculation treatments of rhizobacteria (*S. mutabilis*) on shoot growth and development of NSIC Rc192 grown under progressive drought stress.

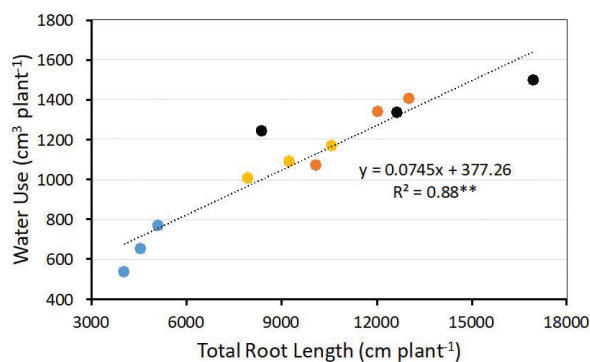
Inoculation Treatments	Shoot Dry Weight (g plant <sup>-1</sup> )	Leaf Area (cm <sup>2</sup> plant <sup>-1</sup> )	Plant Height (cm)	Tillers (no. plant <sup>-1</sup> )	Water Use (g plant <sup>-1</sup> )
Uninoculated	1.3 c	205.6 b	78.5 a	1.5 a	651.7 b
Inoculated (Pre-germination)	2.4 a	360.6 a	80.5 a	3.0 a	1267.7 a
Inoculated (Pre-drought)	2.3 a	350.4 a	76.8 a	3.0 a	1355.3 a
Inoculated (Pre-germination + Thinning + Pre-drought)	1.8 b	279.3 ab	77.8 a	2.5 a	1085.0 a

In a column, means followed by the same letters are not significantly different at 5% Tukey's HSD.

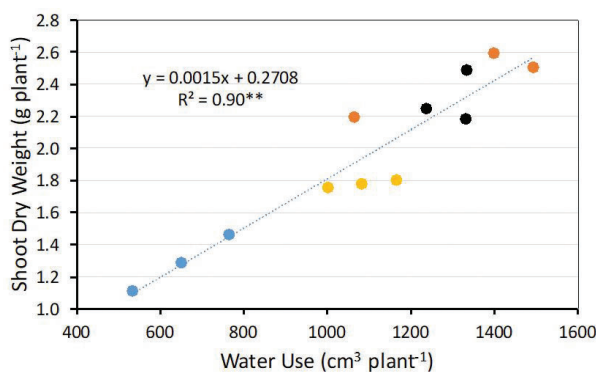
However, in the presence of ACC deaminase bacteria such as the *S. mutabilis* used in this study, ACC is converted to ammonia and  $\alpha$ -ketobutyrate ( $\alpha$ -KB) instead of ethylene (Glick 1998) and thus, allowing the rice roots to continue higher rate of elongation even under progressive drought stress (Table 1). The extensive branching of lateral roots along the nodal roots in inoculated treatments suggests the growth promotive effect of phosphatase enzyme (Smith et al. 1994). Taken together, these promotive effects have facilitated higher rate of nodal root elongation into the soil profile while simultaneously and extensively promoting lateral root production, which increased soil water extraction and water use (Suralta et al. 2010). Consequently, this contributed to the increase in shoot dry matter production (Bañoc et al. 2000; Siopongco et al. 2006; Suralta et al. 2010; Kano et al. 2011; Kano-Nakata et al. 2011). Also, the action of IAA from the rhizobacteria may also further enhance the dry matter production under drought stress via higher rate of stem elongation. Mohite (2013) showed that rhizosphere soil isolates significantly increased the plant height of wheat seedlings along with increase in chlorophyll content relative to the control. In response to the presence of tryptophan and other small molecules in the plant root exudates, the rhizobacteria synthesize and secrete the phytohormone indole-3-acetic acid (IAA), some of which is taken up by the plant and stimulate plant cell proliferation and/or plant cell elongation (Gallavotti 2013; Glick 2014).

In the present study, there was a positive and significant relationship between the size of root system developed under drought as influenced by inoculation treatments and the extent of water use (Figure 6), indicating the functional ability of these roots for increased water uptake from the drying soil. Consequently, greater water use directly influenced the amount of dry matter produced under drought stress (Figure 7).

It is interesting to note that there may be a potential tradeoff of rhizobacterial inoculations in treatment with repeated inoculations. A three-time inoculation of rhizobacteria (*S. mutabilis*) tended to have lower



**Figure 6.** The relationship between total root length and water use at the end of drought stress at 40 DAS. Values are means of 3 replications. Inoculation treatments of rhizobacteria (*S. mutabilis*): blue (uninoculated); black (inoculated at pre-germination); orange (inoculated at pre-drought, 14 DAS); and yellow (inoculated each at pre-germination, thinning at 3 DAS and at pre-drought at 14 DAS). Values are means of three replications. \*\*, significant at  $p=0.01$ .



**Figure 7.** The relationship between total root length and water use at the end of drought stress at 40 DAS. Values are means of 3 replications. Inoculation treatments of rhizobacteria (*S. mutabilis*): blue (uninoculated); black (inoculated at pre-germination); orange (inoculated at pre-drought, 14 DAS); and yellow (inoculated each at pre-germination, thinning at 3 DAS and at pre-drought at 14 DAS). Values are means of three replications. \*\*, significant at  $p=0.01$ .

efficiency in increasing shoot dry matter production than to those inoculated only once either at pre-germination or at pre-drought stress (14 DAS), relative to uninoculated control (Table 2). This may be partially attributed to the potential competition of soil nutrients between the roots and the inoculated rhizobacteria as the population of this microorganism may increase with number of repeated inoculations. Kuzyakov and Xu (2013) showed that shortly after nitrogen (N) mobilization from soil organic matter and litter, microorganisms take up most N especially in soil environments with low N concentrations. This may have deprived the roots of available soil nutrients during drought stress. This phenomenon, if proven, may have implications on the optimum amount of inoculations, timing, as well as the corresponding nutrient management under upland conditions to avoid critical competition between the roots and the inoculated rhizobacteria.

## CONCLUSION AND RECOMMENDATIONS

This study showed that the rhizobacterial inoculation improves the root elongation rates and root hair formation and shoot growth during rice germination under favorable conditions. Furthermore, one-time inoculation of rhizobacteria (*S. mutabilis*) during pre-germination of seeds or during pre-drought treatment of rice seedlings at 14 DAS significantly improved the root system size of rice under drought stressed soil conditions. This consequently maximized greater soil water uptake during progressive drought and thus, contributed to the maintenance of greater dry matter production. The result demonstrates the effectiveness of rhizobacteria (*S. mutabilis*) containing growth-promoting compounds for enhancing drought dehydration avoidance and improving the growth of rice under drought conditions. Field trials are needed to carefully validate these findings and potentially translate into the production environment.

On the other hand, repeated inoculations of *S. mutabilis* have lower efficiency in improving root growth and functions for contributing to shoot dry matter production under drought compared to one-time inoculation. The mechanism is currently subject for further investigations.

## ACKNOWLEDGMENT

The authors acknowledge research funding by the Crop Biotechnology Center (CBC) and Applied Biology Center for the Rice Environment (ABCRE) of PhilRice.

## STATEMENT ON CO-AUTHORS' CONTRIBUTIONS

MYTB, JCTB, LMC, VHL, JUP, AJOS, and KMDL contributed equally to this work.

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