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Growth and Yield Comparison of Rice Plants Treated with Encapsulated *Trichoderma asperellum* (UPM 40) in Response to Drought Stress

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ABSTRACT

During low rainfall periods, rice plants often face drought stress, which would significantly affect rice yield. One of the methods to mitigate the problem is incorporating rice plants with fungi such as *Trichoderma*. This study evaluated the effects of encapsulated *Trichoderma asperellum* (UPM 40) on the growth and yield of rice plants planted in saturated and flooded soil conditions in response to drought stress. A randomized complete block factorial design was implemented with four replications and two factors. The first factor was encapsulated *T. asperellum* (UPM 40) concentration of 0 and 5 g. The second factor was the soil condition: saturated and flooded soil. The drought stress was imposed by halting watering during early anthesis for 14 days and resumed afterward. One of the significant interaction effects detected was on the relative water content of rice plants planted in flooded soil conditions and treated with *T. asperellum* (UPM 40), where the value was 78.51%, higher than the control of 72.09%, which showed the ability of the fungus to help rice plants alleviate detrimental effects of drought stress and delay the onset of adverse effects of drought stress. Thus, it contributed to the crop's simultaneous improvement in rice yield compared to untreated plants in saturated soil. Applying 5 g encapsulated *T. asperellum* (UPM 40) to the rice plants would perform best in flooded soil conditions during drought stress.

Keywords: Ceptometer, chlorophyll, photosynthesis, radiation, weight

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INTRODUCTION

Many regions struggle against drought in producing food crops (Isendahl & Schmidt, 2006). Drought is a global crisis that is severely affecting grain production and quality. On top of that, the inevitably increasing world population and global climate change are making it a lot worse (Hongbo et al., 2005). As a field crop, rice (*Oryza sativa* L.) is defenseless against water stress (Tao et al., 2006). Factors such as unpredictable, insufficient, and uneven rainfall patterns during the vegetative phase, reproduction phase, and ripening stage lead to drought, thus decreasing an estimated 50% of global rice production (Crosson, 1995). Along with the complexity of the drought itself, the way the plant responds to it is also complex. The plant will adopt numerous mechanisms to survive drought (Trethowan et al., 2002). Among them are increased plant water uptake, reduced water loss by enhanced diffusive resistance, and increased water uptake through prolific and deep root systems (Farooq et al., 2009).

There are known methods to enhance crop yield in response to water stress caused by drought, such as applying periodical water stress and potassium fertilization (Zain et al., 2014), application of exogenous hydrogen peroxide and salicylic acid (Sohag et al., 2020) and through genetic improvement (Sahebi et al., 2018). The outcome is encouraging but is limited by time and requires additional resources. A deeper understanding of plant drought tolerance's physiological and molecular basis is required to refine or possibly develop new and improved methods (Trethowan et al., 2002).

Evidently, *Trichoderma* is applied in many kinds of cereal, such as *Triticum aestivum* L., *Vigna radiata* (L.) R. *Wilczek* and *Sorghum bicolor* shows an improved drought response by plants (Kaur & Kumar, 2020; Shukla et al., 2014; Sugiharto et al., 2020). Favorable activities ascribed to the *Trichoderma*-plant interactions include induced disease resistance, plant growth promotion, and tolerance to abiotic stresses (Harman et al., 2004). The mechanisms triggered by *Trichoderma* in the adjustment of drought response are drought avoidance through morphological adaptations, drought tolerance through physiological and biochemical adaptations, and enhanced drought recovery (Malinowski & Belesky, 2000). Yield performance is dictated by the root size and the surrounding architecture, especially when water availability is limited. In cacao, root colonization by *Trichoderma* enhances the growth of roots and the entire plant, increasing plant productivity and yield of reproductive organs (Bae et al., 2009). Therefore, this study evaluated the effects of encapsulated *Trichoderma asperellum* (UPM 40) on the growth and yield of rice plants planted in saturated and flooded soil conditions in response to drought.

MATERIALS AND METHODS

The experiment was conducted in a glasshouse at Field 10, Rice Research Site, Faculty of Agriculture, Universiti Putra Malaysia (UPM), Serdang (3˚02'N, 101˚42'E, 31 m a. s. l). The soil for this

experiment was obtained from a rice field at Kuala Selangor. The soil type was silty clay with the classification Beriah Series, Inceptisol order, and subgroup Typic Endoaquept (Tan et al., 2017).

Unsterilized rice field soil from Kuala Selangor was utilized in this experiment to mimic real situations. The soil was packed into sacks before being transported to the glasshouse in Field 10, UPM. A quick soil test was done before planting to determine the soil pH and contents of total nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), and magnesium (Mg). The soil air-drying process was done by laying the soil on a plastic canvas inside the glasshouse for 14 days. The dried soil was then crushed into small particles and sieved. Fifteen kg of sieved soil was filled into plastic pails at size 37.5 cm × 31.5 cm. In total, there were 192 plastic pails. Those pails were then filled with water until reaching a similar level to the soil surface and stirred to obtain a muddy-soil texture. Approximately 34.5 g of dolomite (Perlis Dolomite Industries, Malaysia) was mixed thoroughly into the soil. The soil mixture-filled pails were left for 14 days before transplanting.

Preparation of *T. asperellum* (UPM 40) conidial suspension and encapsulation were done according to the methods described by Adzmi et al. (2012). In preparation for *T. asperellum* (UPM 40) suspension, for every 250 ml of encapsulated (UPM 40) liquid mixture, 20 plates of freshly matured *T. asperellum* (UPM 40) were needed. Each plate was filled with 10 ml of sterile distilled water. The surface of potato dextrose agar (PDA) media (Fisher Scientific, USA) in each plate was scraped lightly to harvest conidial suspension, which was needed for making *T. asperellum* (UPM 40) capsules. The conidial suspension was then poured into a centrifuge tube. Each tube contained 40 ml of conidial suspension from 4 plates of fresh matured *T. asperellum* (UPM 40). These filled tubes were centrifuged at 6,026 x g for 10 min. The supernatant was discarded, and only pellets were kept for the next stage. Ten ml of sterile distilled water was filled into the pellet-contained test tubes and shaken until no pellet stuck on their walls. An amount of 2.5 g montmorillonite clay (MMT, Sigma-Aldrich, USA) was dissolved into 250 ml sterile distilled water for 24 hr at room temperature. After 24 hr, 12.5 g of corn starch (R&M Chemicals, Malaysia) was added into the MMT solution and stirred for 1 hr. Following that step, 7.5 g of alginate (Fisher Scientific, USA) was added into the mixture and left to dissolve for 3 hr. After dissolving for 3 hr had completed, 7.5 ml of 30% glycerol (Acros Organics, USA) was added to the mixture. The conidial suspension was also added to the mixture. A 5-ml syringe without a needle was used to make the *T. asperellum* beads by pressing out the mixture through it and dropped into 0.5 M calcium chloride ($CaCl_2$) (Systerm Chemicals, Malaysia) precooled sterile aqueous solution under mild agitation. The beads were collected by sieving and washed with sterile distilled water. After that, they were left to air dry for 24 hr at 30 ± 2ºC. The viability of conidia in the beads was maintained in the 2.69×10^4 to 1.56×10^3 cfu/g range.

Rice seeds of the MR219 variety were obtained from Federal Land Consolidation and Rehabilitation Authority (FELCRA) Berhad Seed Center in Seberang Perak, Perak, Malaysia. The selection of rice seeds weighing approximately 0.02 g/seed was conducted to obtain the physical uniformity of the seeds used for the study. The seeds were then surface sterilized by Miché and Balandreau's method (2001). It was followed by soaking off the seeds in sterile distilled water for 24 hr and arranging the seeds on moist filter papers placed inside Petri dishes. The rice seeds were then left for 24 hr for germination.

Then the germinated rice seeds were transferred into a seedling tray and left to grow for 14 days to ensure uniform growth. On day 14, the rice seedlings were transferred into soil containing plastic pails. Each pail was planted with 3 rice seedlings. The pails were watered daily to field capacity for 7 days and flooded to 5 and 1 cm depth according to the treatments. Watering was halted at early anthesis for 14 days and as an implementation of drought stress. After 14 days, watering was resumed according to treatments until two weeks before harvesting.

The experimental design was a two-factorial randomized complete block design (RCBD) with 4 replications. The first factor was 0 and 5 g encapsulation of *T. asperellum* (UPM 40). The second factor implied drought stress in the two different soil conditions: saturated and flooded during the early anthesis stage. A 1 cm of standing water above the surface determined the saturated soil condition. As for flooded conditions, the plastic pails of soil were filled with water until there was 5 cm standing water above the soil surface.

The measured variables for the experiment: the total intercepted photosynthetically active radiation, radiation use efficiency (RUE), relative water content, total chlorophyll content, relative water content, maximum root area, root length, and root volume were recorded as physiological attributes. At the same time, the weight of 1,000 grains, harvest index, total yield per culm, spikelet weight per panicle, percentage of filled grains per panicle, and number of filled grains per panicle were recorded as yield attributes of the conducted experiment.

Physiological Attributes

Light Interception. Above and below photosynthetically active radiation (PAR) was recorded weekly using AccuPAR Ceptometer (Decagon Devices, USA) between 1200 and 1300 hr. The readings were taken three times for each replication, with an average reading recorded. The fraction of PAR was calculated using the following formula (Gallagher & Biscoe, 1978).

$$\mathbf{Fi} = \mathbf{1} - \frac{I_o}{I_t} + \times \mathbf{100}$$

where Fi is the fractional amount of radiation interception (%), *Io* is the measured incident PAR on the surface of the ground, and I_t is the radiant flux density on top of the canopy. The value of *Io* was measured at the vertical height level.

The total incident PAR was taken from daily incident solar radiation recorded by Serdang Meteorological Station. Fifty percent (50%) of the incident solar radiation received was taken as PAR (Monteith, 1972). The amount of intercepted PAR by the crop (Sa) was calculated by the following formula (Szeicz, 1974):

$$Sa = Fi \times Si$$

where Sa is the amount of intercepted PAR, Si is the total amount of incident PAR, and Fi is the fractional amount of radiation interception.

Radiation Use Efficiency (RUE)

The radiation use efficiency (RUE) was calculated as the slope of the linear relationship between accumulated crop biomass and accumulated intercepted PAR. The regression line was forced through the origin based on the assumption that no dry matter was produced when accumulated intercepted PAR was zero (Monteith, 1977).

Chlorophyll Content

The fully expanded leaf, normally found on the second or third leaf from the top of a rice plant, was taken on the 14th day after drought stress implied. The leaf samples were placed into paper bags and brought to the Physiology Laboratory, Department of Crop Science, UPM, for further analysis.

The fully expanded leaf of the main tiller of the rice plant was homogenized with 80% acetone. The absorption of the extracts at wavelengths of 663 and 645 nm was measured with a spectrophotometer. Chlorophyll *a*, chlorophyll *b*, and total chlorophyll content were determined in mg/g FW according to the formula given below (Arnon, 1949):

$$\text{Chlorophyll } a = 12.72A663 - 2.59A645$$
$$\text{Chlorophyll } b = 22.9A645 - 4.67A663$$
$$\text{Total chlorophyll} = 20.31A645 + 8.05A663$$

where A663 is the spectrophotometer reading at wavelength 663 nm, and A645 is the spectrophotometer reading at wavelength 645.

Data for relative chlorophyll content was taken by using portable chlorophyll meter (MINOLTA™ SPAD-502) (Konica Minolta, Japan) on top fully expanded leaf. The leaf chlorophyll content was taken by scanning five different points on the leaf and the data taken was averaged using the portable chlorophyll meter.

Relative Water Content (RWC)

Fully expanded rice leaves on the main tiller of the rice plants were snipped and kept inside paper bags. The RWC of leaf samples was measured at the Physiology Laboratory, Department of Crop Science, UPM.

The rice leaf tissues were weighed immediately to get the fresh weight (FW). The leaf tissues were rehydrated in water for 24 hr until they attained full turgidity, surface-dried, and reweighed to get the turgid weight (TW). Finally, the tissues were oven dried at 80°C for 48 hr (until constant weight) to obtain the dry weight (DW). The RWC was calculated using the equation below (Bhushan et al., 2007).

$$RWC = FW - DW / TW - DW \times 100 \quad (20)$$

where RWC = Relative water content (%), FW = Fresh weight, DW = Dry weight, TW = Turgid weight.

Photosynthesis

The rate of photosynthesis was recorded before and after drought treatment was imposed on the rice plants. Data were taken on fully expanded leaves on the main tiller of the rice plants. Each data was taken five times, and the average value was recorded. The data of photosynthesis rate were examined using an LI-6400XT Portable Photosynthesis System (LI-COR Inc., USA) between 0900 and 1000 hr.

Root Attributes

The roots of the rice plants were harvested after the final harvest. The roots were separated from the upper part of the crop. The roots were then cleansed thoroughly until no soil or other debris was on top.

Since the roots were condensed, they were divided into sections depending on the root size to enable them to be analyzed properly. Each part of the roots was floated in acrylic trays and arranged to reduce overlapping. The roots were placed on a transparent acrylic tray and filled with distilled water until submerged. The container was placed on the surface of the Epson Perfection V700 Photo scanner (Epson Malaysia Sdn. Bhd., Malaysia). The roots were analyzed using WinRhizo software (Regent Instruments Inc., Canada) for their length, surface area, and volume from the scanned image.

Yield Attributes

Weight of Grains. A thousand grains were counted from collected grains from all treatments and weighed on a digital balance.

Harvest Index. Harvest index (HI) was calculated as a ratio of crop yield to total above-ground crop biomass (Donald & Hamblin, 1976):

$$\mathbf{HI = GYBY \times 100}$$

where HI = Harvest index (%), GY = Grain yield, and BY = Dry biomass yield.

During the final harvest, the grains were harvested by separating the panicles from the rice plants. The panicles were kept inside paper bags and labeled for their treatment and replication. The rice plants without panicles were also harvested and kept inside paper bags with their respective labels. Those samples were weighed for fresh weight. The samples were kept inside an oven at 70°C for 48 hr and until constant weight was achieved. The dried grains and tillers were weighed.

Number of Filled Grains. Filled and unfilled grains were detached from the panicles and soaked in a 20% sodium chloride solution ($CaCl_2$) (Systerm Chemicals, Malaysia). The floated and sunk grains were separated and counted. The sunk grains were recorded as filled grains, and the floated grains were categorized as unfilled.

Percentage of Filled Grains. The percentage of filled grains was obtained by dividing the number of filled grains by

the total number of grains per panicle and multiplying by one hundred.

Statistical Analysis

The data were analyzed using the software SAS® 9.4 (SAS Institute, USA). Following the analysis of variance (ANOVA), differences among means were separated using the least significant difference (Fisher LSD) at $p \leq 0.05$.

RESULTS

Interaction between encapsulated *T. asperellum* (UPM 40) application and soil conditions was insignificant for total PAR (Table 1). No significant difference in total PAR between encapsulated *T. asperellum* (UPM 40) application and soil conditions was recorded. However, a significant interaction of RUE of rice plants treated with encapsulated *T. asperellum* (UPM 40) and different soil conditions was observed (Table 1) and presented in Figure 1. The relative water content of rice plants treated with encapsulated *T. asperellum* (UPM 40) in different soil conditions showed no significant interaction between the two factors. The relative water content of rice plants treated with encapsulated *T. asperellum* (UPM 40) was 78.51%, significantly higher than that of rice plants planted without encapsulated *T. asperellum* (UPM 40) at 72.09%. Rice plants planted in saturated soil conditions had significantly lower relative water content than those planted in flooded water conditions at 70.60 and 78.21%, respectively. Net photosynthetic rate of rice plants treated with encapsulated *T. asperellum* (UPM 40) was higher ($p < 0.05$) than the non-treated rice plants at 11.38 and 8.18 μmol CO_2/m^2 s. As for rice plants planted in different soil conditions, rice plants planted in flooded soil conditions recorded a higher net photosynthetic rate than in saturated conditions.

Table 1

Total photosynthetically active radiation (PAR), radiation use efficiency (RUE), and relative water content (RWC) of rice plants treated with encapsulated Trichoderma asperellum *(UPM 40) and planted in different*

Treatment	Total PAR (g/MJ)	RUE (g/MJ)	RWC (%)	Net photosynthetic rate ($\mu molCO_2 \cdot m^{-2} \cdot s^{-1}$)
Rate of encapsulated *T. asperellum* (UPM 40) (g) (T)				
0	$371.72^{a} \pm 8.47$	$0.09^{b} \pm 0.01$	$72.09^{b} \pm 1.90$	$8.18^{b} \pm 0.79$
5	$386.98^{a} \pm 4.04$	$0.38^{a} \pm 0.14$	$78.51^{a} \pm 2.07$	$11.38^{a} \pm 0.85$
Significance level	ns	***	**	**
Soil condition (S)				
Saturated	$372.08^{a} \pm 8.52$	$0.08^{b} \pm 0.01$	$70.60^{b} \pm 2.13$	$8.32^{b} \pm 0.95$
Flooded	$386.62^{a} \pm 4.21$	$0.39^{a} \pm 0.14$	$78.21^{a} \pm 2.07$	$11.25^{a} \pm 0.79$

Table 1 *(Continue)*

Treatment	Total PAR (g/MJ)	RUE (g/MJ)	RWC (%)	Net photosynthetic rate ($\mu molCO_2 \cdot m^{-2} \cdot s^{-1}$)
Significance level	ns	***	**	**
Interaction				
T × S	ns	***	ns	ns

Note. Means within column followed by the same letter are not significantly different by least significant difference, $p \geq 0.05$; * = Significant at $p \leq 0.05$; ** = Significant at $p \leq 0.01$; *** = Significant at $p \leq 0.001$; ns = Not significant

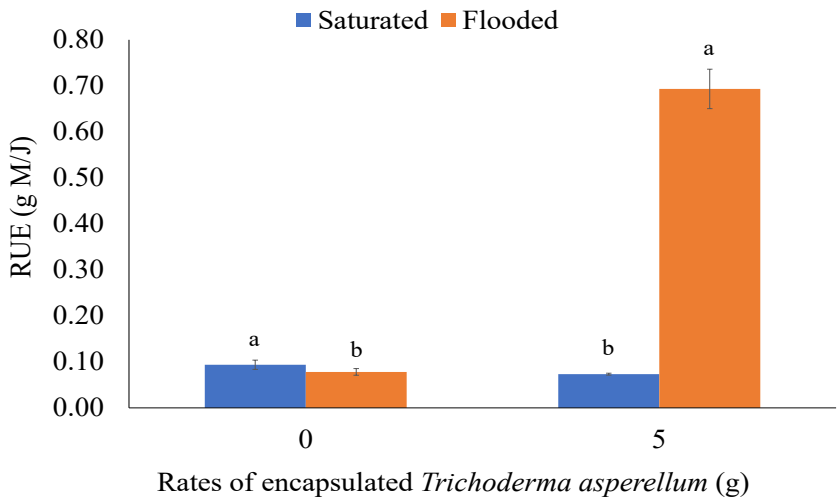

Figure 1. The RUE of rice plants in response to the application of *Trichoderma asperellum* (UPM 40) under saturated and flooded soil conditions

Note. Radiation use efficiency (RUE) followed by different letters indicate significance at the 0.001 probability level

No significant interaction was observed from Table 2 of the two factors on rice plants' total chlorophyll, and relative chlorophyll contents inoculated with *T. asperellum* (UPM 40) in different soil conditions. The total chlorophyll content of rice plants treated with 5 g encapsulated *T. asperellum* (UPM 40) was 6.98 mg/g, significantly higher than rice plants with 0 g treatment with 6.00 mg/g. It agreed with the result for the relative chlorophyll content of both treatments, with values of 45.45 and 41.20%, respectively. The total chlorophyll content of the rice plants planted in saturated soil conditions was 6.20 mg/g, significantly lower than those planted in flooded soil at 6.78 mg/g. The relative chlorophyll content of rice plants planted in flooded soil conditions also was significantly higher than that of saturated soil conditions, with values of 45.10 and 41.55, respectively.

Table 2

Chlorophyll a*, chlorophyll* b*, total chlorophyll, and relative chlorophyll content of rice plants treated with encapsulated* Trichoderma asperellum *(UPM 40) and planted in different soil conditions*

Treatment	Chlorophyll *a*	Chlorophyll *b*	Total chlorophyll mg/g	Relative chlorophyll content
Rate of encapsulated *T. asperellum* (UPM 40) (g) (T)				
0	$3.46^{b} \pm 0.23$	$2.54^{a} \pm 0.20$	$6.00^{b} \pm 0.21$	$41.20^{b} \pm 1.65$
5	$4.30^{a} \pm 0.11$	$2.68^{a} \pm 0.13$	$6.98^{a} \pm 0.18$	$45.45^{a} \pm 1.32$
Significance level	**	ns	**	*
Soil condition (S)				
Saturated	$3.89^{a} \pm 0.22$	$2.31^{b} \pm 0.11$	$6.20^{b} \pm 0.25$	$41.55^{b} \pm 1.64$
Flooded	$3.87^{a} \pm 0.30$	$2.91^{a} \pm 0.08$	$6.78^{a} \pm 0.27$	$45.10^{a} \pm 1.32$
Significance level	ns	**	***	
Interaction				
T × S	ns	ns	ns	ns

Note. Means within column followed by the same letter are not significantly different by least significant difference, $p \geq 0.05$; * = Significant at $p \leq 0.05$; ** = Significant at $p \leq 0.01$; *** = Significant at $p \leq 0.001$; ns = Not significant

Table 3 shows no significant interaction between the application of encapsulated *T. asperellum* (UPM 40) and soil conditions on the root area and root volume of cultivated rice plants. The root area of the rice plants was significantly affected by the application of encapsulated *T. asperellum* (UPM 40). Five (5) g applications of encapsulated *T. asperellum* (UPM 40) showed a higher root area produced by the rice plants at 2,281.80 cm^2 than the root area of rice plants planted without encapsulated *T. asperellum* (UPM 40) at 1,847.70 cm^2. Root area planted in flooded soil conditions was significantly higher than in saturated soil conditions, which were 2,402.50 and 1,726.90 cm^2, respectively. The root length of rice plants treated with and without encapsulated *T. asperellum* (UPM 40) significantly interacted with soil conditions (Figure 2). The root volume of rice plants treated with different rates of encapsulated *T. asperellum* (UPM 40) showed no significant interaction with different soil conditions. However, applying the encapsulated *T. asperellum* (UPM 40) on the rice plants caused the root volume to be significantly higher than the non-treated rice plants, with values of 298.90 and 202.28 cm^2, respectively. As for rice plants planted in different soil conditions, the root volume planted in saturated soil conditions was significantly lower than in flooded soil conditions. The values of the root volumes were 220.37 and 280.81 cm^2, respectively.

Table 3

Maximum root area, root length, and root volume produced by rice plants treated with encapsulated Trichoderma asperellum *(UPM 40) and planted in different soil conditions*

Treatment	Maximum root area (cm^2)	Root length (cm)	Root volume (cm^3)
Rate of encapsulated *T. asperellum* (UPM 40) (g) (T)			
0	$1847.70^b \pm 150.63$	$1366.70^b \pm 256.37$	$202.28^b \pm 17.54$
5	$2281.80^a \pm 193.56$	$3329.20^a \pm 117.70$	$298.90^a \pm 19.36$
Significance level	***	***	***
Soil condition (S)			
Saturated	$1726.90^b \pm 144.26$	$2018.40^b \pm 550.46$	$220.37^b \pm 24.10$
Flooded	$2402.50^a \pm 112.02$	$2677.50^a \pm 343.88$	$280.81^a \pm 25.89$
Significance level	**	**	**
Interaction			
T × S	ns	*	ns

Note. Means within column followed by the same letter are not significantly different by least significant difference, $p \geq 0.05$; * = Significant at $p \leq 0.05$; ** = Significant at $p \leq 0.01$; *** = Significant at $p \leq 0.001$; ns = Not significant

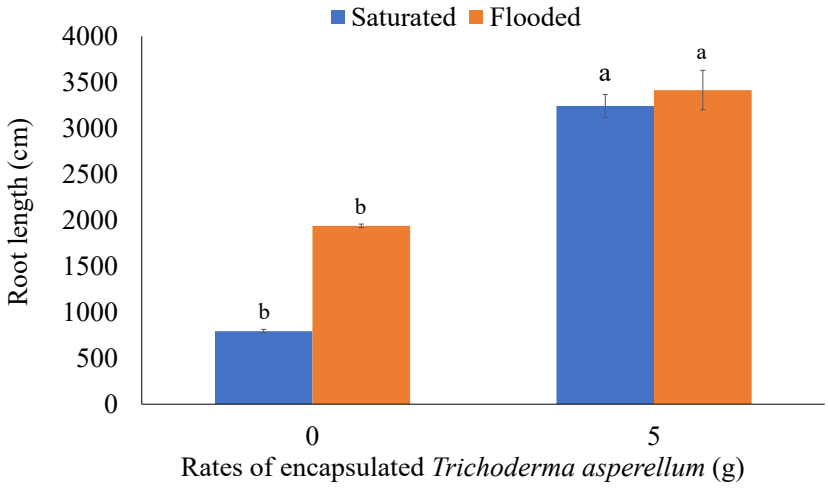

Figure 2. The root length of rice plants in response to the application of *Trichoderma asperellum* (UPM 40) under saturated and flooded soil conditions

Note. Root length followed by different letters indicate significance at the 0.05 probability level

Results in Table 4 show that the weight of 1,000 grains of rice plants treated with different rates of encapsulated *T. asperellum* (UPM 40) had no significant interaction with soil conditions. The 1,000 grains weight produced by the 5 g encapsulated

T. asperellum (UPM 40) treatment was significantly higher than the 0 g treatment, with 21.07 and 19.49 g, respectively. There was also a significant difference for 1,000-grain weight produced by rice plants planted in saturated and flooded soil conditions. The 1,000-grain weight planted in flooded soil was 21.75 g, higher than in saturated condition, 19.28 g. No significant interaction was found between rates of encapsulated *T. asperellum* (UPM 40) and soil conditions on the harvest index of rice plants. Rice plants treated with 5 g encapsulated *T. asperellum* (UPM 40) at 52.57% was significantly higher than 0 g treatment with 41.51%.

As for different soil conditions, the harvest index of rice plants planted in flooded soil conditions was significantly higher than in saturated soil conditions, with 52.00 and 42.08%, respectively. The total yield per culm of rice plants was independent of the effects of different rates of encapsulated *T. asperellum* (UPM 40) and soil conditions and showed that total yield was produced from rice plants treated with 5 g encapsulated *T. asperellum* (UPM 40) with 153.27 g, as compared to 0 g treatment encapsulated *T. asperellum* (UPM 40) with 147.78 g. The yield produced by rice plants planted in flooded soil conditions was significantly higher at 115.07 g than those planted in saturated soil conditions with 107.83 g.

Table 4

Weight of 1000 grains, harvest index, and total yield of rice plants per culm treated with different rates of encapsulated Trichoderma asperellum *(UPM 40) and planted in different soil conditions*

Treatment	Weight of 1000 grains (g)	Harvest index (%)	Total yield per culm (g)
Rate of encapsulated *T. asperellum* (UPM 40) (g) (T)			
0	19.49^{b}±0.41	41.51^{b}±1.96	147.78^{b}±1.58
5	21.07^{a}±0.49	52.57^{a}±2.91	153.27^{a}±0.80
Significance level	***	***	*
Soil condition (S)			
Saturated	19.28^{b}±0.32	42.08^{b}±1.93	107.83^{b}±2.22
Flooded	21.75^{a}±0.40	52.00^{a}±3.31	115.07^{a}±2.01
Significance level	***	***	**
Interaction			
T × S	ns	ns	ns

Note. Means within column followed by the same letter are not significantly different by least significant difference, $p \geq 0.05$; * = Significant at $p \leq 0.05$; ** = Significant at $p \leq 0.01$; *** = Significant at $p \leq 0.001$; ns = Not significant

There was significant interaction found between rates of encapsulated *T. asperellum* (UPM 40) and soil conditions of spikelet weight per panicle (Figure 3). Table 5 reveals no significant interaction between encapsulated *T. asperellum* (UPM 40) application and soil conditions for the percentage of filled grains per panicle. Rice plants treated with 5 g encapsulated *T. asperellum* (UPM 40) had a higher percentage of filled grains per panicle, which was 50.67%. Non-treated rice plants had a lower percentage of filled grains, 41.00%. Rice plants planted in flooded soil produced a significantly higher percentage of filled grains per panicle at 58.49% than those planted in saturated soil at 43.18%. The number of filled grains per panicle of the rice plants showed that the application of encapsulated *T. asperellum* (UPM 40) had significant interaction with soil conditions (Figure 4).

Table 5

Spikelet weight per panicle, percentage of filled grains per panicle, and number of filled grains per panicle of rice plants treated with encapsulated Trichoderma asperellum *(UPM 40) and planted in different soil conditions*

Treatment	Spikelet weight per panicle (g)	Percentage of filled grains per panicle (%)	Number of filled grains per panicle
Rate of encapsulated *T. asperellum* (UPM 40) (g) (T)			
0	$44.65^{b} \pm 2.65$	$41.00^{b} \pm 1.52$	$64.00^{b} \pm 3.80$
5	$60.98^{a} \pm 4.18$	$50.67^{a} \pm 1.31$	$83.50^{a} \pm 1.93$
Significance level	***	***	***
Water condition (S)			
Saturated	$45.53^{b} \pm 2.82$	$43.18^{b} \pm 2.39$	$67.67^{b} \pm 5.42$
Flooded	$60.10^{a} \pm 4.89$	$58.49^{a} \pm 2.05$	$79.83^{a} \pm 3.47$
Significance level	***	***	***
Interaction			
T × S	*	ns	*

Note. Means within column followed by the same letter are not significantly different by least significant difference, $p \geq 0.05$; * = Significant at $p \leq 0.05$; ** = Significant at $p \leq 0.01$; *** = Significant at $p \leq 0.001$; ns = Not significant

DISCUSSION

Relative water content (Table 1) shows the amount of solute available in the leaves of rice plants treated with encapsulated *T. asperellum* (UPM 40), which was less than rice plants planted without the fungal capsules after facing drought stress. According to Bhushan et al. (2007), relative

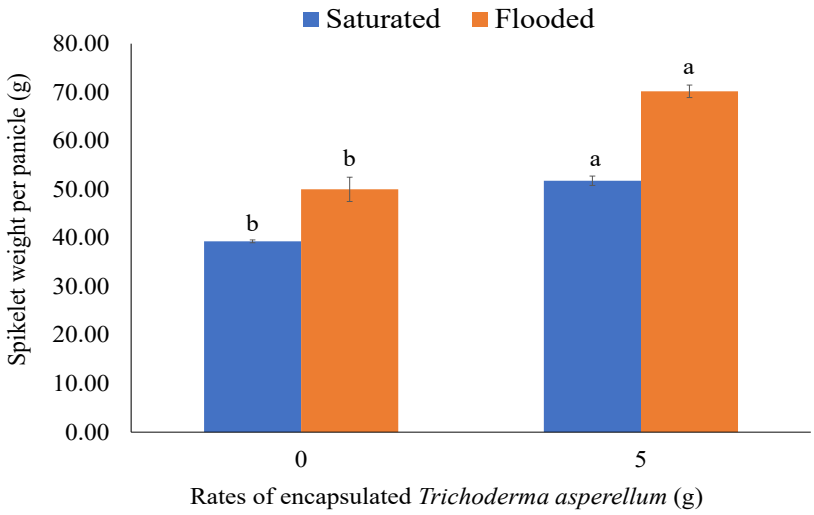

Figure 3. The spikelet weight per panicle of rice plants in response to the application of encapsulated *Trichoderma asperellum* (UPM 40) under saturated and flooded soil conditions

Note. Spikelet weight per panicle followed by different letters indicate significance at the 0.05 probability level

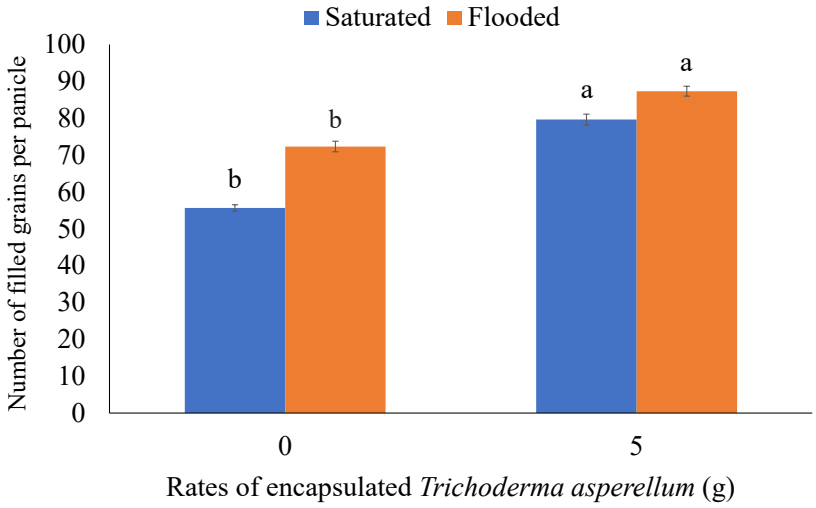

Figure 4. The number of filled grains per panicle of rice plants in response to the application of *Trichoderma asperellum* (UPM 40) under saturated and flooded soil conditions

Note. Number of filled grains per panicle followed by different letters indicate significance at the 0.05 probability level

water content is a term used to describe compatible solute accumulation like proline and an increase in cell membrane permeability causing ions and electrolyte leakage. It shows that the presence of *T. asperellum* (UPM 40) on the roots of the inoculated rice plants triggered the plants to accumulate the needed solutes or release

ions and electrolytes or both. Better RWC also led to a better net photosynthetic rate of the inoculated rice plants. However, since the RWC of non-inoculated rice plants was lower than the inoculated plants, the net photosynthetic rate was also lower, proving the higher drought stress level faced compared to the inoculated plants. Jayaweera et al. (2016) stated that lower performance of the net photosynthetic rate also influenced crop dehydration tolerance, including osmotic adjustment and cell membrane stability. Sanders and Ardnt (2012) reported that one of the plant's mechanisms to fight against drought stress is by accumulating osmotic proteins in the cells. An increase physiological processes in rice plants, such as net photosynthetic rate and water use efficiency, was a way *Trichoderma* spp. contributes to improving rice yield (Doni et al., 2014).

In addition, higher total chlorophyll content and relative chlorophyll content of the *T. asperellum* (UPM 40) inoculated rice plants in Table 2 also contributed to better net photosynthetic rate than the non-inoculated rice plants when exposed to drought stress. Rice seedlings were enhanced to become drought tolerant by *T. hamatum* 219-b by promoting greenness and chlorophyll content (Bae et al., 2009). Shukla et al. (2012) also reported the effect of drought stress on relative greenness (soil plant analysis development [SPAD] value), which was delayed from three to nine days. *Trichoderma harzianum* T 22 intensified leaf greenness through chlorophyll measurement, which provided further energy and carbon source for the development of maize plants (Harman, 2000). Another important evidence that *T. asperellum* (UPM 40) helped to enhance the growth of rice plants during drought was the maximum root area and root volume (Table 3) of the inoculated rice plants, which were found to be higher than the non-inoculated plants.

A study by Shukla et al. (2012) also found that during water cycle alteration, the presence of *Trichoderma* isolates increased the root and shoot length of the plants. The formation of more roots caused more water to be extracted into the soil during drought resulting in less stress held by the rice plants, bringing a higher net photosynthetic rate. Photosynthesis is an important process in cells to produce food to be stored in a sink for plants which for rice plants is the grains. An optimum photosynthesis rate may result in a satisfying yield. In this experiment, applying encapsulated *T. asperellum* (UPM 40) to rice plants resulted in a better net photosynthetic rate, believed to contribute to higher yield. In Tables 4 and 5, the weight of 1,000 grains, harvest index, total yield per culm, spikelet weight per panicle, percentage of filled grains per panicle, and number of filled grains per panicle showed the presence of *T. asperellum* (UPM 40) had significantly caused the yield attributes to be higher than the non-inoculated plants. Improved plant performance living under different biotic and abiotic stress was shown when treated with *Trichoderma* fungus (Mastouri, 2010).

Li et al. (2012) and Makino (2011) agreed that improving rice's physiological characteristics is needed to achieve high yields. A study by Charoenrak and Chamswarng (2016) also found that applying *Trichoderma* spp. to rice seedlings improved 1,000-grain weight by up to 1.65% compared to the untreated control. Similar findings were recorded by Chamswarng and Kumchang (2012), where wettable pellet formulation of *T. asperellum* isolate 01-52 caused the yield of rice vars. Pathum Thani 80 and Pin Kaset to increase. Khadka and Upkhoff (2019) also recorded that incorporating *Trichoderma* on rice seedlings under the System of Rice Intensification (SRI) produced a 26% yield increment compared to non-treated samples. These reports support the current research findings, where the involvement of encapsulated *T. asperellum* (UPM 40) could increase yield.

The yield attributes of rice plants planted in different soil conditions were also significantly different from each other (Tables 4 and 5). As in flooded soil conditions, more standing water caused less drought stress in the rice plants. Less water in saturated soil conditions caused a long drought stress period experienced by the rice plants. These events resulted in physiological and yield differences in both soil conditions. The situation caused less leaf senescence to occur, which led to the total biomass of the rice plants planted in flooded soil conditions than in saturated soil conditions. Chaves et al. (2003) explained that plants develop drought-coping mechanisms to complete their life cycle to avoid dehydration and combat drought stress. This circumstance was also described by Jamieson et al. (1995), where leaf senescence is the main cause of decreased radiation intercepted while causing biomass production to decrease. Bat-Oyun et al. (2011) reported that water stress caused the senescence of plants and brought a reduction in canopy photosynthetic capacity; such a situation also occurred in this experiment, where rice plants planted in saturated soil conditions had a lower net photosynthetic rate compared to flooded soil conditions.

Significant interactions existed between the *T. asperellum* treatment and soil conditions in RUE, root length, number of filled grains per panicle, and spikelet weight per panicle. The interactions were visible, where the listed attributes were higher when the rice plants were treated with encapsulated *T. asperellum* (UPM 40) and planted in flooded soil conditions than the non-inoculated rice plants planted in saturated soil conditions. However, RUE for inoculated rice plants planted in saturated soil conditions was lower than the non-inoculated rice plants. The rice plants focus on lengthening their roots to help increase the ability to look and absorb available water in the soil and to decrease drought stress held by the plants. As for roots increment rate of rice plants planted in flooded soil conditions from non-inoculated rice plants to inoculated rice plants was lower than in saturated soil conditions. *Trichoderma* root colonization promotes plant growth (Adams et al., 2007; Lynch, 2004). Spikelet weight per panicle of rice plants planted in

flooded soil conditions had a higher rate of increase from no application of encapsulated *T. asperellum* (UPM 40) to the presence of encapsulated *T. asperellum* (UPM 40) than in saturated condition (Figure 3).

The presence of the fungus encouraged rice plants planted in flooded soil conditions to improve their spikelet weight when faced with drought threats. As for the number of filled grains per panicle rate of increase from the non-inoculated *T. asperellum* (UPM 40) to the inoculated rice plants was higher when planted in saturated soil conditions than flooded soil conditions (Figure 4). The fungus facilitated the rice plants in producing a higher number of filled grains while combating drought stress. Producing more filled grains while having non-favorable conditions, which for this experiment was drought, was a way for the plants to ensure their legacy continues. A study by Asseng and van Herwaarden (2003) and Plaut et al. (2004) also stated that although drought stress caused an increase in the remobilization of assimilates to the grains, early senescence and reduction in grain filling also occurred.

CONCLUSION

During the drought season, the planting of rice plants in flooded soil conditions combined with the application of encapsulated *T. asperellum* (UPM 40) at 5 g was found to result in increased growth of the roots and yield of rice plants as indicated by higher spikelet weight per panicle and number of filled grains per panicle. On the contrary, rice plants planted in saturated soil conditions without inoculation of the fungus did not perform as well.

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