Assessment of Probiotic Bacteria Effects on Rice Growth and Yield at Reduced Doses of Chemical Fertilizers in Pingtung, Southern Taiwan

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Abstract: ***Plant associated bacteria are promising alternatives to chemical fertilizers for plant growth and yield improvement in an eco-friendly manner. The objective of this study is to evaluate the effect of different amount of probiotic's solution (PS) and different recommended doses of chemical fertilizers (RDCF) doses on the growth and yields of rice in wet season. To achieve our goals, the experiment has been conducted at irrigated rice field at the National Pingtung University of Science and Technology (NPUST) in Taiwan. Five different treatments T1 (100% RDCF), T2 (75% RDCF + 25% PS), T3 (50% RDCF + 50% PS), T4 (25% RDCF + 75% PS) and T5 (100% PS) have been compared between each other's where T1 was considered as control treatment. Whole treatments have been subjected to alternate wetting and Drying Irrigation(AWD) and the System of Rice Intensification (SRI) practices along with rainfall. Data of growth parameters and yield components have been statistically analyzed and compared between treatments. Results shown that T3 has recorded best performances in plants height (111.62 cm), in chlorophyll content (36.82) and leaves area index (13.05) at maturity. Findings revealed also highest grain yield recorded in T1 (6.14 ton/ha) without any significant different with T3 (5.84 ton/ha). Results suggest that T3 could be applied in rice farming under similar soil characteristics and weather data to mitigate the huge use of chemical fertilizers for an eco-friendly agriculture.***

Keywords: Rice, AWD, SRI, chemical fertilizers, Probiotic.

1. Introduction

World's agricultural production systems face with tremendous challenges: demographic pressure, soil depletion, natural resources shortage, high-input and resource-intensive farming systems which are drastically coupled with the global climate change [1]. In such an environment of production, crops yield in general and in particular cereals crops productivity remains in constant constraints. According to reports the Food and Agriculture Organization of the United Nation (FAO), insistent challenges in recent researches will be focused on creating integrate approaches of eco-friendly crops production system which takes into account current risks factors in agriculture. Noticeably, it is known that more than 50% of world daily caloric intake is derived directly from cereal grain consumption [2] hence the importance to develop suitable and sustainable cropping systems of cereals' production. Among all those cereals, rice crop requires a need of reliable and efficient production system knowing its importance worldwide and its needs in inputs.

Rice is a staple food for more than half of the world's population, making it the most important cereal crop [3], [4]. Consequently, there will be a need of surplus in rice production of at least 800 million tons to feed the world's population by 2025 [5]. Over 90 percent of the world's rice production and consumption is located in Asia continent which accounted for 80% in the world's rice production [3], [6] in Africa, where populations live on rice. Face with demography and climatic concerns, rice cultivation system would require an ecofriendly production system. Effectively, it has been documented that in term of water consumption, rice consumes more than 50% of the total irrigation water in agriculture [7] under the conventional cropping system. The defy to save inputs such as water, seedlings and fertilizers in rice farming involve the implementation of innovated methods likewise Alternative wetting and drying (AWD) and the System of Rice Intensification (SRI) [8], [9], [10] which are well known in many countries including Taiwan as methods able to reduce inputs use while maintaining good yields and water productivity [11], [12], [13], [14]. Previous researches' results highlighted that the application of AWD along with SRI practices can provide a water saving up to 22.6% [15], 27 to 37% [16] or else between 31 to 37 % [7] and 55% to 74% [17] compared to continuous flooding (CF). In term of yields, equivalent yields of AWD (7.2t/ha) compared to CM (7.8t/ha) have been recorded [18] and sometimes even slight yields increase of 4%–6% have been obtained in AWD's practices compared to CF [5]. Other parameters like plant height (122cm vs. 130cm), number of effective tillers (310 vs. 338) and harvest index (43% vs. 44%) were not significantly different in AWD compare to CF [18]. Besides, high water productivity of 40% has been recorded while implementing 3 cm water depth weekly in rainy season in Southern Taiwan without compromising with rice growth and yields [11], [13], [19]. However, the optimization of fertilizers inputs in rice production is a required both for farmers and the environment [10], [4] and might be taken into account in current methods of farming. The improvement of these methods of rice cultivation are promising alternatives to conventional methods and might be promoted across the world both to respond to the demographic pressure, the climate changes, resources saving and environment protection.

In order to maintain rice productivity and meet with the world demand, high yielding varieties have been developed and popularized which involves an enormous use of fertilizers such as nitrogen (N), phosphorus (P) and pesticides [4]. From the point of view of these authors, 40 and 20 million metric tons of chemical N and P fertilizers respectively, will be required for food production by 2040. Subsequently, this alarming increase in synthetic chemical fertilizers would lead to degradation in soil, deterioration in air and water quality which is a threat to a sustainable environment. In addition, almost 75%–90% of applied chemical P fertilizers are rapidly immobilized by forming complex with Al^{3+} or Fe^{3+} in acidic soils or with Ca^{2+} in calcareous soils resulting in shortage of available P for plants nutrition [20], [4]. Therefore, enormous applications of chemical fertilizers to increase yield would be a waste both for plants and for producers. Alternatively, approaches to improve nutrients' use in agriculture under nutrients deficient in tropical soils are conceivable through micro-organisms inoculation [21].

Previous researches of microbial activity showed that plants would utilize diverse strategies to uptake adequate nutrients, including modifications to root morphology, carbon metabolism, membrane structure by passing the exudation of organic acids, protons and enzymes in association with mycorrhizal fungi and bacteria [22]. For instance, symbiotic associations between probiotic bacteria and plants have been reported by various researchers. Therefore, free-living plant-associated bacteria directly or indirectly exert beneficial effect on plant growth and development and are generally known as plants probiotic bacteria [20]. They are well-known to enhance plant growth and improve yield by increasing plant nutrient use efficiency through solubilization and mineralization of nutrient components particularly, mineral P [23], N-fixation and synthesis of phytohormones [4]. Thus, a significant decrease in the use of chemical fertilizers could be achieved with the application of probiotic bacteria as bio fertilizers which is an eco-friendly promising alternative that can be used to reduce chemical fertilizers inputs and costs [20], [4]. Reducing inputs in rice production can also be involved with the use of probiotic bacteria solutions. Following previous AWD investigations [11], [19] in rainy season in southern Taiwan, the current study aims to evaluate the effect of different probiotic doses on rice growth and yields under reduced doses of chemical fertilizers. The objective was to determine whether or not probiotic can be used to reduce fertilizers' use while maintaining equivalent grain yield.

2. Material and Methods

2.1 Study area description and experiment design

The experiment was conducted from August 14th to December 5th 2018, in the irrigation experimental field of the National Pingtung University of Science and Technology (NPUST) in Southern of Taiwan. The field is located at 71 m above sea level; at 22.39° (N) latitude and 34.95° (E) longitude. Previous analysis of the soil physical properties shows that the textural class of the soil in the experimental field was loamy soil (27% of sand and 24% of clay) [11], [13]. The wilting point was 15% volume, field capacity 30.5% volume; saturation 42.9% volume; bulk density 1.40g/cm3, matric potential 11.09 bar; and hydraulic conductivity at 57mm/h. The experimental field was a randomized complete block design (Figure 1) with three replications and five fertilizers and probiotic treatments composed of T1 (100% RDCF), T2 (75% RDCF + 25% PS), T3 (50% RDCF + 50% PS), T4 (25% RDCF + 75% PS) and T5 (100% PS). Each plot was 4 m long and 1.5 m wide with total area of 6 m2 and 0.3 m hardpan. The spacing between blocks was 1 m and 0.5 m between plots.

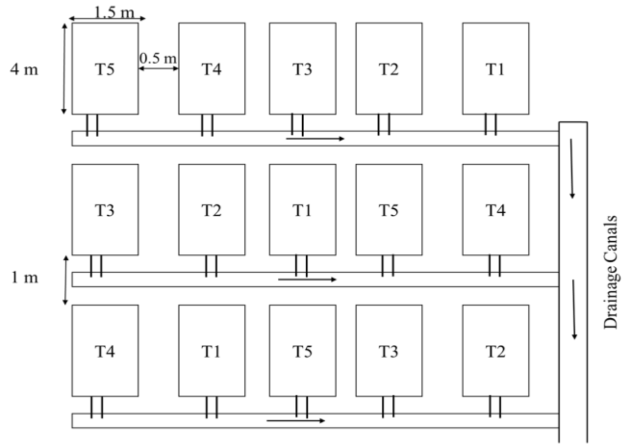

Figure 1: Layout of the randomized bloc

2.2 *Climatic* Condition

Meteorological parameters affect significantly the production most than any other factor. Some daily important climatological parameters (maximum and minimum temperatures, relative humidity, rainfall) factors of the experiment site were collected from NPUST meteorological station. Monthly average values of minimum and maximum temperature, relative humidity, and cumulative monthly rainfall are presented in Table 1.

Table 1: Climatic data during the cropping period

Months	Rainfall (mm)	Av. Temp (^{0}C)	Min. Temp (^{0}C)	Max. Temp (^{0}C)	Relative humidity
Aug.	41.6	28.48	23.70	32.58	87.61
Sep.	14.3	28.72	25.35	30.42	84.10
Oct.	5.56	28.27	25.58	30.12	82.12
Nov.	0.07	27.35	25.37	28.70	79.34
Dec.	0.00	26.00	23.35	28.22	76.77

Source: Meteorological station of NPUST

2.3 Land Preparation and transplanting

Two weeks before transplanting, soil has been labored in each plot handily. Paddling and leveling have been done a week before transplanting. Those operations have been necessary to ensure a proper growth of rice crops. The techniques applied in this experiment for rice growth were AWD and SRI methods These practices used young seedlings while the irrigation method was an alternated wetting and drying method along with rainfall. Two leaves age (20 days-old) obtained from the irrigation site nursery of Tainan certified as Kaohsiung 114 variety were manually transplanted on August 14th. Seedlings were transplanted, lined up with 25 cm hills spacing between rows and 25 cm between hills (75 hills per plot). Each hill received one seedling transplanted in a shallow ranged between 1–2 cm.

2.4 Irrigation and water management

Irrigation water depth of 5 cm was applied daily to each plot during the first 3 weeks (21days) when a rain did not occur to maintain and facilitate seedlings roots installation and development. Water treatment of 3 cm depth weekly was started 21 days after transplanting and the frequency of irrigation was initiated at seven (7) days accordingly to the irrigation interval commonly used by farmers. The use of previous approach [12], allowed to record the time necessary to apply the desired water in each plot following the equation below [11], [13], [19]:

$$t = (A \times d) / Q \qquad (1)$$

Where, t = Time required to irrigate fields (sec); A = Area of sub-plot (m^2); d = Depth of water applied according to the schedule (m); Q = Water flow Discharge (l /sec= 0.91 l/s).

The volume of water to be applied to reach the desired depths was obtained using the following equation (2) according to previous authors:

$$IR = A \times h \times 10^3 \qquad (2)$$

Where IR is the amount of irrigation water (liters) for a desired depth above the soil surface; A is the surface area of the plot (m2); and h is the desired water depth above the soil surface (m).

2.5 Chemical fertilizers and probiotic' treatments

The chemical fertilizers applied in this experiment consist of N-P2O5-K2O -MgO at the ratio of 15-15-15-4 and urea having respectively doses of 270 kg/ha and 150 kg/ha. These doses are recommended doses used by farmers in Taiwan [11], [24]. From these doses, the variation between our treatments have been set at reduce percentages of the total amount to define each treatment (Table 2). Rice growth cycle like development stage, tillering and panicle initiation are considered as sensitive stages and fertilizers applications have been planned and followed thoroughly according to these growing stages. At each stage, granules of chemical fertilizers (NPK) are weighed and applied according to the manufacturer recommendations. Consequently, each treatment has received three splits of NPK fertilizer during the rice growing cycle. In contrast, any NPK has been applied in the treatment named T5. Only Urea was applied in two (2) splits doses of 150 kg/ha (1/3 during development stage and 2/3 at panicle initiation) in this treatment in order to insure grains filling.

Table 2: NPK and Probiotic rate and application schedule

Treatment	Rate of probiotic applied (ml/plot)	Rate of NPK applied (g/plot)	Period of application (DAT: Days After Transplanting)
T1	0	162	• Development stage (7-20) • Tillering (21-40) • Panicle initiation (65-80)
T2	30	121.5	
T3	60	81	
T4	90	40.5	
T5	120	0	

The solution of probiotic as well has been applied according to the rice growing stages and treatments. A probiotic's solution containing five strains of bacteria (table 3) has been gotten at the laboratory of biotechnological of the National Pingtung University of Science and Technology (NPUST) for this purpose. Some of these bacteria have known by researchers in the literature review having the ability of promoting rice plants' growth [22], [25], [4]. In order to reduce the use of chemical fertilizers, the probiotic solutions have been applied at different rates to evaluate rice growth and yield. Their colony forming unit (cfu) has been estimated to be 107 cfu in agar plat under controlled conditions. Bacteria's cultures in the laboratory have been strictly done by the biotechnological laboratory and the methodology of bacteria cultures has not been taken into a count in this experiment. A recommended quantity to be applied has been therefore set by the laboratory to be 200 l/ha which is equivalent to 120 ml/ plot size of 6 m2 in this study. The probiotic's solution was applied in the required treatments 3 -5 days before the application of fertilizers in order to avoid undesirable effects within these two types of treatments. To allow bacteria to colonize the rhizosphere zone, the probiotic solution was thoroughly measured with a graduated cylinder and the liquid poured in the center of a given plot while irrigating at the same time.

Table 3: Bacteria strains isolated in the probiotic solution

Bacteria's strains
Bacillus aryabhattai
Stentrophomonas maltophilia
Sphingobium yanoikuyae
Shingomonas sp.
Burkholderia caribensis

2.6 Plant height estimation

For the plant height measurement, six (6) plants in a square meter were randomly chosen each time for the measurement of the height in each replication. Data were measured and recorded every two weeks (14 days). Plants height were measured from the base to the tip of the highest leaf and the mean were calculated. A ribbon was used for the measurement (figure 2). Measurements were done every two weeks starting from 30 DAT until the late season.

Figure 2: Plant height measurement

2.7 Leaf area index (LAI) measurement

According to previous works [26], the measurement of leaf area index (LAI) involves two techniques that are measuring the area of a leaf and selecting the correct leaf samples so that leaf area per plant can be adequately estimated. The previous authors suggested the selection of six hills per plot randomly. Therefore, hills were selected in the square meter placed in the center of the plot. For each selected hill, the length and width of the 3rd topmost leaf on the plant (or on the middle tiller) were measured. Length and width of the leaf were measured with a ruler. Leaf area was computed using the length-width method according to the following equations described previous authors investigations [26], [11], [14].

$$Leaf\,area\ (cm2) = L\ (cm) \times W\ (cm) \times K \quad (3)$$

Where, L = length of 3rd leaf from the top (cm), W is maximum width of the leaf (cm) and K is a correction factor of 0.75.

Leaf area index were calculated following the formula proposed by authors: LAI = average leaf size × number of leaves per shoot × number of shoots per hill (or plant) × number of hills (or plants) per unit of ground area. The LAI equation was derived as followed (4):

LAI= ((Sum of leaf area/hill of four hills (sq. cm))/ (Area of land covered by four hills (sq. cm) (4)

2.8 Plant chlorophyll content

The chlorophyll meter (model SPAD-502, MINOLTA, Japan) was used to determine leaf chlorophyll content (figure 3). From a square meter, six (6) hills per plot were selected randomly and three leaves from the uppermost fully expanded leaves were selected on each plant to measure the chlorophyll content. Readings were done on the 70% length starting from the top of the leaf. The average of these three observations were recorded as the leaf chlorophyll content of each plant. Chlorophyll content were measured every 14 days starting from 30 DAT until the maturity.

Figure 3: Plants chlorophyll measurement

2.9 Yield attributes and grain yield assessment

According to previous researchers [26], grain yield is related to characters like plant type, growth duration, and yield components. The yield attributes taken into account in this study were tillers number, grains' number per panicle, percentage of filled grains per panicle and 1,000 grain weight. In order to assess the yield components parameters, eight (8) hills within the meter square hills (25 hills) located in the center of the plot were chosen haphazardly and harvested. The unfilled grains (U) were separated from filled grains and the number of filled grains (F) and unfilled grains have been counted using a seed counter (Count-A-Pak model). Three samples of 1,000 grains were taken from the total grains production of each plot and these 1,000 grains weights were obtained from the filled grains weight (W) and the number of filled grains (F) according to equations 5 and 6 derived in previous works [26]. A total of 45 samples were considered for the 1,000 grains weight per plot and means per treatment have been determined.

Percentage of unfilled grains = (U/ (F+U)) X100 (5)
1000 grains weight = (W/ F) X100 (6)

In each replication, parameters were individually measured and the means of each treatment determined and reported. Grain yield was determined in a square meter area (1m² area) in each replication (Twenty-five 25 hills in total) as carried out by precedent [27]. The 25 plants from the meter square were harvested in the center of each plot (excluding the border rows) and threshed using a thresher and the yield per replication as well as yield per treatment were determined.

3. Statistical analysis of Data

Data were compiled and subjected to mean calculation using Statistical Analysis System (SAS) to evaluate the variance of treatment effects. The significance of the treatment effect was determined using F-test. When ANOVA indicated that there was a significant difference, multiple comparisons of means were performed using the Least Significant Difference method (LSD) at 0.05 probability level or Microsoft Excel wherever necessary.

4. Results and discussion

4.1 Comparison of plant height

Plant height has been evaluated by choosing six (6) plants in a plot during each stage of growth (n=6). Figure 4 displays the results relating to the height of rice plants under the different treatments. The maximum heights were recorded in T3 (50% RDCF + 50% PS). At every growth stage plants height evolution was significant in each treatment. The height was ranged between 53 cm at active tillering to 112 cm at maturity. In the whole season, plants growth was significantly equivalent with plants height recorded in [11], [19] investigations when compared with the irrigation water of 3 cm depth per week. This increase can be explained probably by the application of probiotic used which might has enhanced the nutrients uptake inducing hence plants growth. Some authors [11] and [24] have investigated also on the difference in water treatment in rainy season and have found a maximum height of 113.1cm at 90 DAT which was closed to our results. However, the fertilization doses were reduced and replaced with probiotic bacteria solution in our study. The similitude of growth results may be justified by the use of probiotic which positively has impacted plants growth and the higher rainfall recorded from the earliest season until the active tillering (August 14th to September 14th seen on the figure 16 above) (seen in table 1) has been also a great benefit to plant growth in the current experiment.

The analysis showed an increase in plant height from 57.37 cm in active tillering to 111.62 cm recorded in (T3) at maturity. This treatment recorded high height values over the cropping period compared to the other treatments. However, no significant difference was observed between treatment at each stage of growth. According to our results, T3 (50% RDCF + 50% PS) seems to perform well for the height growth parameter. Previously, some researchers [4] had found that 50% of fertilizers applied with 50% probiotic performed well in growth parameter which is in alignment with our results. These findings could be justified by the inputs (water, fertilizers and probiotic) management efficiency specifically the probiotic effects when looking at growth results in this investigation. Results of growth parameters confirm that bacteria are able to induce plants growth and are promising alternative to reduce the huge use of chemical fertilizer in agriculture mainly in rice production as asserted previous authors statements [8], [10], [20], [4], [28]. To abound in the same direction as previous researchers, ours results suggest that probiotic bacteria are promising alternatives to chemical fertilizers in rice cultivation and could enhance plants growth while promoting biodiversity.

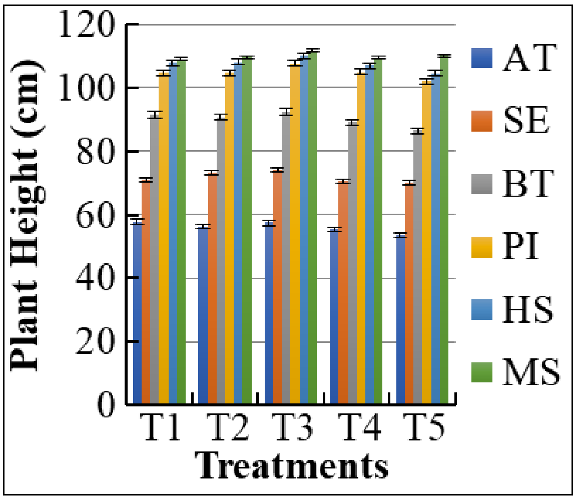

Figure 4: Evolution of plant height along the growing stages (n=6) (AT: active tillering; SE: stem elongation; BT: booting; PI: panicle initiation; HS: heading; MS: maturity)

4.2 Comparison of leaves chlorophyll content

Results of leaves chlorophyll content evolution every two weeks throughout the growing stages, from active tillering stage up to maturity are shown on the figure 5. Results recorded show a gradual increase along with the evolution of the crop age in each treatment until panicle initiation. However, this evolution of data displayed a decrease in leaves chlorophyll content from panicle initiation to maturation for almost all treatments. The decrease in chlorophyll content from the panicle initiation to maturity indicates that the chlorophyll is a key component in grains' formation and maturation. Our findings corroborate with previous investigations [13], [4], [29]. In T1 (100% RDCF), chlorophyll content increased from 37.25 at active tillering to 45.01 at panicle initiation. T3 recorded the highest chlorophyll content over other treatments from active tillering (38.97) up to maturity with a slight decrease of chlorophyll content (36.82) at maturity. Similar decrease in results have been got in previous SRI results under rainy season [11]. Moreover, in probiotic bacteria application, preceding researchers [20], [4] had revealed the efficiency of probiotic bacteria to enhance chlorophyll content in rice crops leaves. They had recorded high values in chlorophyll content (up to 47) in the development stage and a slight decrease in these values at maturity which match with our findings. Evidently, probiotic bacteria could play a key role in phosphorus solubilization and nitrogen fixation which are the main components of effective chlorophyll content in plants leaves. Soil characteristics might have also affected the performance of probiotic bacteria which probably have contributed to increase chlorophyll content.

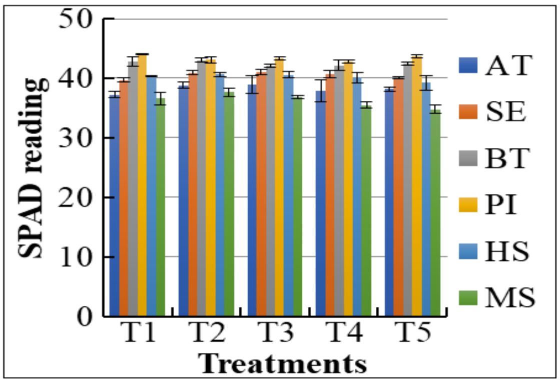

Figure 5: Leaves chlorophyll content at different stages of rice (n=6) (AT: active tillering; SE: stem elongation; BT: booting; PI: panicle initiation; HS: heading; MS: maturity).

4.3 Evolution of leaf area index (LAI) during the cropping season

LAI during the rice growth period was ranged from 1.77 in T3 at active tillering to 13.05 at maturity where its values were high compared to others treatments (figure 6). From development stage until the maturity, LAI increases with the plant age and in was convenience with growth parameters such as height and chlorophyll. From active tillering to the maturity, LAI averages were higher compared to the previous experiment carried out in previous investigations [11], [19], [14] under AWD and SRI in the same site. These high values may be due to SRI practices including plants spacing, water and nutrients management but also the probiotic application. Statistical analyses showed that there was not significant difference recorded between same stages at p value equal to 0.05 ($p > 0.05$), when treatments are compared to each other. However, the LAI evolution of T3 shows high values compared to other treatment suggesting that a balance use of fertilizers and probiotic should enhance plants growth when soil characteristics and weather data are similar likewise those displayed in the current study area. High values of LAI might be due both to the rainfall, the fertilizer management and probably the use of probiotic which might have positively affected crops LAI. The probiotic use might hence improve plants growth parameters and also reduce the application of chemical fertilizers for an ecofriendly agriculture as suggested precedent investigations [20], [4].

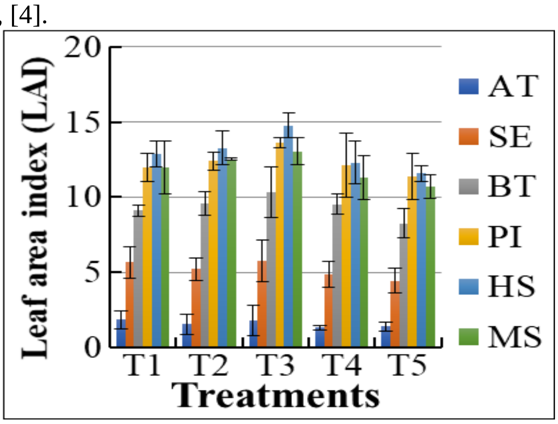

Figure 6: Trend of LAI content at different stages (n=6) (AT: active tillering; SE: stem elongation; BT: booting; PI: panicle initiation; HS: heading; MS: maturity).

4.4 Tillers number per hill

Tillers number is a determinant of yield given that every tiller could bear a panicle. Duncan grouping test carried out on the tillers' number varied in accordance with the treatments applied as indicated investigations [4], [17]. If some differences were noticed in terms of number of tillers, according to the statistical analysis, no significant difference was found between tillers number in the different fertilizers and probiotic's treatments. The minimum tillers number was 14 and was recorded in T2 (75% RDCF+25% PS). The maximum tillers number was found in T3 (50% RDCF+50% probiotic) with a value of 16.06 (figure 7). These findings were lower compared to those [5] recorded in some findings where an average between 20–30 tillers per hill has been found. However, tillers number per hill were coherent to the findings other authors [11], [17], [19] who founded an average of 16.08 to18.52 tillers per plant. The factor which could have affected the tillers number in the present trial might be the plant height which reached more than 100 cm. However, the slightly higher number of tillers in T3 suggest that efficient results could be reached in SRI by applying balance doses of fertilizers and probiotic bacteria. With wide spacing and low seedling density, water and nutrients management could create optimum conditions on microorganisms' activity. This study suggests that SRI practices including probiotic use could reduce inputs use in rice cultivation while maintaining reasonable tillering.

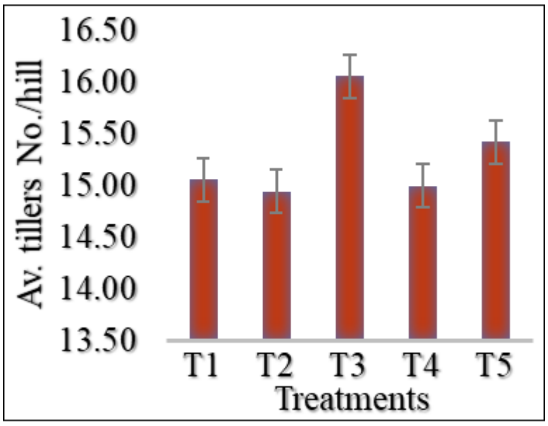

Figure 7: Tillering's response per treatments (n=8)

4.5 Yield components and grain yield of treatments

Grain yield performance is determined by different yield attributes like number of panicles per hill, total number of grains per panicle, filled grains percentage and 1000-grain weight at different percentages [26]. Following these authors recommendations, eight (8) hills in a square meter have been selected randomly in the center of each plot to assess panicles number per hill, grains number per panicle, filled grains percentage and 1000 grain weight while all plants (25) in the meter square have been harvested to assess grain yield per hectare. Results analyses are indicated in the table 4.

Table 4: Yield components and yields of treatments (n=8)

Treat	Pan. No./hill	No.	% of	1000 grain	Grain

		Grains/ pan	Filled grains	weight (g)	yield (ton/ha)
T1	$11.33^{ab} \pm 1.83$	130.67^{a}	90.33^{a}	28.67^{a}	6.14^{a}
T2	10.00^{b}	134.33^{a}	91.67^{a}	27.67^{ab}	5.89^{a}
T3	12.33^{a}	128.67^{a}	85.00^{b}	27.00^{ab}	5.84^{a}
T4	10.67^{ab}	138.67a	88.67^{ab}	28.33^{ab}	5.71
T5	10.67^{ab}	131.00^{a}	92.67^{a}	25.67^{b}	5.15^{a}
p	**	ns	**	**	ns

Notes: **: Means within columns not followed by the same letter are significantly different at $p < 0.05$ level by Duncan's Multi-range test; ns: Not significantly different.

In opposition with the other parameters, grain yield was influenced by the different fertilizers and probiotic treatments during the investigation carried out under SRI. Grain yield analysis recorded in the table 4 shows that the highest yield was realized in T1 (6.14 ton/ha). Results in grain yield decreased slightly within treatments when the fertilizes doses in a given treatment is decreased. However, no significant difference was revealed between treatments according to Duncan's Multi-range test of grain yield (ton/ha). The result in this case is opposed to the growth parameters results where T3 performed better than other treatments. This result may be due to the number of grains per panicle and the 1000 grains weight which are important parameters affecting the yield [26]. The significant difference between treatments of the number of panicles and of the percentage of filled grains has affected also the grain yield. Those parameters have been determinant on the grain yield but its effects were not evident to display a significant difference on the grain yield between treatments. The treatment T3 thereby could be an efficient treatment in term of growth parameters and grain yield. In addition to these results, incredible performance in growth parameters and grain yield have been achieved in T5 (5.17 ton/ha) in which only probiotic bacteria were applied. This result above all indicate clearly the efficiency of probiotic bacteria. However, the result in this treatment will require more investigations as none soil nutrients content was assessed before the implementation of this treatment. In this study where all the plots were subjected to the same soil, weather and irrigation water conditions, probiotic bacteria could be an alternative to chemical fertilizers and seem to be a promising achievement for rice cultivation in regard of previous works done on the probiotic's use [20], [4], [30]. Moreover, grain yields in the current study were high compared to those of [11], [17], [14] in SRI with the 3 cm water application. For example, they had [11], [14] found respectively a grain yield of 5.35 and 3.340 ton/ha with 3 cm depth weekly which were lower compared to our results. Our results reveal hence the efficiency of the probiotic bacteria under AWD and SRI practices. Results of the study imply that a reduction of fertilizers amount from 25 to 100% while replacing with a probiotic solution might not significantly affect grain yield. These results indicate also that when fertilizers and probiotic are well managed in SRI method, rice yield could be kept reasonably with a reduction of fertilizer cost from 25 to 100%. However, the application of probiotic should take into account climatic data and soil characteristics. For in this investigation, T3 (50% RDCF + 50% PS) might be suggested as reliable treatment while waiting for furthermore investigations.

5. Conclusion

The present study was conducted during wet season and aimed at investigating the effect of probiotic in rice cultivation. On the entire growth parameters results of five treatments, T3 (50% RDCF + 50% probiotic) has displayed high values. The probiotic bacteria's solution has therefore performed well in T3 when taking into account these parameters. The investigation specifies eventually the possibilities of the use of benefit bacteria to improve rice production system while reducing the use of chemical fertilizers in a context where the environment protection is a priority. In contrast, the analysis of yield attributes and grain yield has highlighted high yield components and grain yield in T1 (100% RDCF) without being statistically different to other treatments where probiotic has been applied. This result has been affected probably by the number of grains per panicle and the percentage of filled grains which sometime can affect the grain yield. However, reasonable and remarkable grains yields have been recorded in T2, T3, T4 and T5 where percentages of probiotic ranged from 25% to 100% have been applied instead of chemical fertilizers. Moreover, in T5 in which a consistent grain yield of 5.15 tons/ha has been recorded and where no fertilizer has been applied, the use of probiotic seems to confirm bacteria's activity and portend to attest the efficiency of these microorganisms in rice cultivation. The current results have been enhanced by soil characteristics, weather data and SRI practices with similar or high yields compared to previous SRI works in the same study area. The gain of these reasonable yield notwithstanding the reduction or the unused of fertilizers could be implied that probiotic bacteria are alternatives to the chemical fertilizers use in SRI and might open opportunities for farmers to reduce fertilizers cost between 25 to 100% even though probiotic solution cost has to be assessed. By applying T3 when considered the role of bacteria on phosphorus solubilization and nitrogen fixation, the current investigation suggests this treatment as efficient for SRI rice cultivation in rainy season and can be used to reduce as well chemical fertilizers cost and its drawbacks on the environment.

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