Enhanced Assimilation Rate due to Seaweed Biostimulant Improves Growth of Indigenous Rice bean (*Vigna umbellata*)

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Abstract. Rice beans are traditionally planted as intercrop to Corn or as the main crop during dry season where corn production is difficult. The use of biostimulants are widely studied to ameliorate the negative effects of biotic and abiotic stresses. Three possible fermented biostimulants: seaweed, bamboo shoot and Japanese snail, were compared to a commercial organic liquid fertilizer (10ml/l) based on morphological and photosynthetic responses. The treatments were arranged in Randomized Complete Block Design and photosynthetic parameters were measured using LI-6800 Portable Photosynthesis System. Data were subjected to analysis of variance (ANOVA) and Tukey's test at $p<0.05$. Fermented seaweed treated rice beans registered the greatest average vapor pressure deficit at 4.33 KPa on the first month and is comparable to the highest average VPD of 4.39 KPa registered by plants applied with fermented Japanese snail on the second month. This, interestingly, did not result to differences in transpiration rate (µmol H2O m-2 s-1). Such could be attributed to the plants reduced stomatal aperture when applied with fermented seaweed at 406.80 µmol CO2 mol stomatal conductance and 38.59 Pa total conductance on the second month. Despite this, average assimilation rate of rice beans was still increased in both the first (15.26 µmol CO2 m-2 s-1) and second (16.51 µmol CO2 m-2 s-1) month. This increased assimilation rate of fermented seaweed treated rice beans resulted to approximately 12 cm difference in height at 128.53±3.91 cm (R=0.894**) when compared to those applied with the commercial liquid organic fertilizer. Thus by limiting stomatal conductance, despite the differences in VPD, transpiration rate was not affected while significantly increasing assimilation rate thereby improving the growth of rice beans.

Key words: Japanese snail liquid fertilizer, morphological responses, Organic foliar fertilization, photosynthetic responses, seaweed liquid fertilizer

INTRODUCTION

Rice Bean is a neglected crop, cultivated in small areas by subsistence farmers in Nepal, northern India, and parts of Southeast Asia. It can be grown in diverse conditions and is well known among farmers because of its wide adaptation and production even in marginal lands, drought-prone sloping areas, and flat rainfed tars (Joshi et al., 2007). The nutritional quality of rice beans is higher than many other legumes of the Vigna family (Katoch, 2012). The plant has appreciable levels of crude protein with 59–93% protein digestibility and all essential amino acids (especially methionine, tryptophan, lysine, tyrosine and valine), minerals, vitamins, and a relatively high proportion of healthy, unsaturated fatty acids make it a nutritious health package.

Farmers commonly use inorganic fertilizers because it is known to increase yield, which also increases income. However, it is observed to have severe and detrimental effects on soil, the environment, and other species. For that reason, many farmers turn back to using organic fertilizers.

Seaweed biostimulant is one of the known organic fertilizers. It is a beneficial marine resource that is affordable and this is also rich in various bioactive compounds. One of those are lipids, proteins, carbohydrates, mineral nutrients and antimicrobial compounds (Raghunandan et al., 2019). It increases the biochemical constituents of every plant, and this possesses environmental stress mitigating potential. Application of seaweed liquid fertilizer to soils improves soil health by enhancing micronutrient quantity and quality and microbial activity.

Net assimilation rate is used to determine the photosynthetic or growth efficiency in plants. NAR has been used as a growth predictor for some woody and herbaceous plants (Shipley, 2006; Poorter and Nagel, 2000). Photosynthesis results from the assimilation of water and carbon dioxide. The plant absorbs them and converts them into a plethora of organic molecules directly in the plant's numerous cells. A reduced assimilation rate meant that CO2 and water are less utilized for the production of essential biomolecules. Carbon dioxide assimilation in plants is the most critical key for crop production (Lawlor, 2002).

This study is deemed necessary considering that the University is engaged in seaweed blends production, a health drink that utilizes seaweed and generates waste that can easily be used as a biostimulant for crop production.

METHODOLOGY

Collection, Preparation, and Fermentation of Organic Fertilizers

The materials needed for fermentation were collected at the farm of Cebu Technological University – Barili Campus. Only seaweeds were bought at the Barili Public Market. All of the materials were washed and cleaned to remove dirt and the seaweed pulp by-product were added with molasses. A ratio of 1:1 was used following the procedure of Pascual et al., 2013. After seven days of fermentation, all materials were squeezed and filtered through a strainer. Lastly, it was stored in a cool dry place.

Bamboo shoots were collected at the farm of Cebu Technological University – Barili Campus. The outer covering of Bamboo Shoots was peeled off and was then chopped into pieces. Water was added at a 1:1 ratio. After 24 hours of soaking, it was squeezed and filtered through a strainer. Lastly, it was stored in a proper place.

Experimental Site and Plant Samples

The research was conducted in one of the greenhouses of Cebu Technological University – Barili Campus (10°7’53” N, 123°32'45" E) with an area of 8.95 m x 6 m. The research was conducted from August to November 2020. Rice bean seeds used as planting material was secured from a local farmer. A 2.5 kg garden soil placed in a pot was used as growing media.

Experimental Design and Treatments

This research used a randomized complete block design with three replications and four treatments. These include T0 commercial natural liquid fertilizer, T1 fermented seaweed, T2 fermented bamboo shoot, and T3 Japanese snail. The rate of 10ml/L used was based on the study of Pascual et al., 2020. Data were analyzed using Analysis of Variance (ANOVA), and a further test was done using Tukey's test at $p<0.05$ to test for differences between treatment means.

Data Gathered

Li-6800 Portable Photosynthesis System was used to measure photosynthetic parameters. Five fully expanded leaves were measured on the top, bottom, and middle of the plant. Morphological parameters as plant height, leaf length, and the number of leaves were also recorded.

Result and Discussion

Morphological Responses

Based on Table 1, seaweed biostimulant was significantly different from the other treatments at 128.53 ± 3.91SD cm. This resulted by a 12 cm difference in height with the commercial liquid organic fertilizer. This result coincides with Pascual et al., (2020) study that length of shoots outperformed other treatments due to the auxins present in the seaweed extracts that have an effective role in cell division and enlargement. In the study of Das and Prasad (2015), 18% of the total indole-3-acetic acid (IAA) was present in Kappaphycus alvarezii seaweed that is responsible for the enhanced growth of the plant. This is the essential auxin in plants, which controls many important physiological processes that include cell enlargement and division, tissue differentiation, and responses to light and gravity (Leveau and Lindow, 2005).

Bamboo shoot resulted to have significantly enhanced the leaf length of rice bean at 12.10±0.62SD cm on the sixth week of the study. The study of Carabio et al. supported this result, (2021) where bamboo shoot-based liquid fertilizer alone produced the longest leaves that which is also comparable with the control. It was reported by Gamuyao et al., (2017), that the young tissues of bamboo shoot were reported to have an enrichment of genes associated with synthesis of DNA and DNA precursors, the cell cycle, cell division and cell organization like kinesins and microtubules. The leaf primordium in plants grows mainly through cell proliferation which will be replaced with an alternative mode of cell cycle activity, endoreduplication, which enhances endopolyploidy linked to increased cell size (Gonzalez et al., 2010). This might be the reason why bamboo liquid fertilizer has increased leaf length compared with the other treatments.

For the number of leaves, Japanese snail fertilizer has the highest among all the treatments with 116.73 ± 5.22SD on the eight week. In the study of Jatto et al., (2010), Japanese snail's shell has a chemical composition that includes proteins, carbohydrates, fats, and minerals such as iron, zinc, and copper, all of which are essential for the growth as well as the number of leaves. This is also found to be rich with phosphorus, an important element affecting plants' growth like plant height, leaf number, and shoot dry biomass (Malhotra et al., 2018).

Table 1. Effects on Plant Height (cm), Leaf length (cm), and Number of Leaves (± SD) on Rice beans with the Application of Different Foliar Fertilizers.

Parameters	Treatments	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8
Plant Height	T0	12.30 ± 0.12	21.09 ± 0.80	27.90 ± 2.60 b	34.60 ± 1.22 b	92.40 ± 10.23 b	106.33 ± 10.44	107.60 ± 6.92 a	116.00 ± 6.97 a
	T1	12.09 ± 0.13	19.03 ± 1.10	23.40 ± 2.08 ab	30.47 ± 0.70 a	67.27 ± 5.22 a	96.13 ± 3.74	123.80± 3.67 b	128.53 ± 3.91 b
	T2	12.41 ± 0.64	20.82 ± 0.88	26.37 ± 1.59 b	39.93 ± 0.42 c	97.60 ± 2.88 b	113.40 ± 7.8	114.60 ± 1.56 ab	117.33 ± 2.23 a
	T3	11.95 ± 0.50	18.78 ± 0.83	20.67 ± 0.31 a	28.27 ± 2.58 a	66.53 ± 2.87 a	100.33 ± 5.60	113.27 ± 3.70 ab	118.30 ± 1.31 ab
Leaf Length	T0	6.61 ± 0.53 b	7.73 ± 0.37	9.79 ± 0.69 ab	11.27 ± 0.29	11.40 ± 0	11.70 ± 0.35 ab	12.77 ± 0.86	12.97 ± 1.20
	T1	6.05 ± 0.15 ab	6.88 ± 0.19	8.43 ± 0.32 a	10.46 ± 3.00	10.60 ± 0.56	10.87 ± 0.15 a	11.28 ± 0.48	11.43 ± 1.21
	T2	6.49 ± 0.03 ab	7.14 ± 0.19	9.99 ± 0.57 b	10.60 ± 0.56	11.63 ± 0.55	12.10 ± 0.62 b	12.37 ± 0.25	12.63 ± 0.29
	T3	5.97 ± 0.19 a	6.85 ± 0.54	8.60 ±0.57 ab	10.50 ± 0.55	10.64 ± 1.17	10.70 ± 0.44 a	11.36 ± 0.61	11.23 ± 0.83
Number of Leaves	T0	4.73 ± 0.23	10.73 ± 0.31	16.87 ±1.70	25.53 ± 1.81 ab	33.33 ± 1.70	59.13 ± 3.31 ab	66.13 ± 5.03 a	69.27 ± 5.61 a
	T1	4.47 ± 0.50	9.73 ± 0.90	15.60 ±3.47	24.33 ± 2.66 ab	34.60 ± 3.49	61.33 ± 3.20 ab	83.00 ± 1.56 b	85.00 ± 3.49 b
	T2	4.87 ± 0.23	9.33 ± 1.14	24.40 ± 12.41	28.53 ± 1.72 b	35.93 ± 3.10	71.47 ± 3.52 c	103.27 ± 2.53 c	97.07 ± 7.40 b
	T3	4.40 ± 0.53	8.93 ± 0.92	15.13 ±2.66	22.27 ± 2.73 a	31.53 ± 2.72	51.13 ± 8.33 a	72.57 ± 4.10 a	116.73 ± 5.22 c

Physiological Responses

On Figure 1, fermented seaweed treated rice beans registered the greatest average vapor pressure deficit at 4.33 KPa on the first month and is comparable to the highest average VPD of 4.39 KPa registered by plants applied with fermented Japanese snail. This, interestingly, did not result in differences in transpiration rate (µmol H2O m-2 s-1) (Figure 1A). However in the study reported by Schoppach et al. (2017) that limited whole-plant transpiration under high VPD in wheat has resulted in advantageous water conservation and crop yield increase which relates to this study's result in which plant treated with seaweed biostimulant increased the plant height with high VPD. This was also supported by Gholipoor et al., (2010) in which studies in species also indicate that limited transpiration at high vapor pressure deficit in water-limited environments results in yield increases. Limiting transpiration in this situation would help the plant to conserve water for use later in the crop growing seasons when drought develops Mura and Vangimalla, (2018).

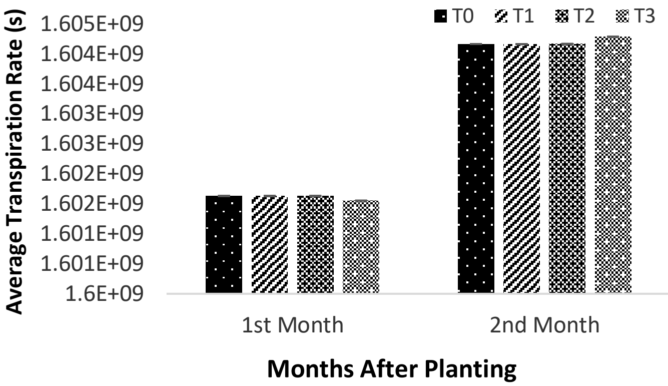

Figure 1. Average assimilation rate of rice bean after application of liquid organic fertilizer

Such could be attributed to a reduced stomatal aperture of rice beans applied with fermented seaweed at 406.80 µmol CO2 mol stomatal conductance (Figure 2) and 38.59 Pa total conductance (Figure 1D) the second month. It was stated that stomatal conductance decreases as vapor pressure deficit increases because of an increase in transpiration (E) that lowers leaf water potential. The results that are available for wheat are not consistent with stomatal closure at high vapor pressure deficit being a response to an increased whole leaf transpiration rate or lower leaf water potential. The lack of conductance response to VPD in CO2- free air suggests that ABA may mediate the response (Medina et al., 2019). And this was also supported by Grossiord et al., (2020), where it was stated that there is an abundance of evidence that suggests that stomatal conductance declines under high VPD, just the same with the total conductance to CO2.

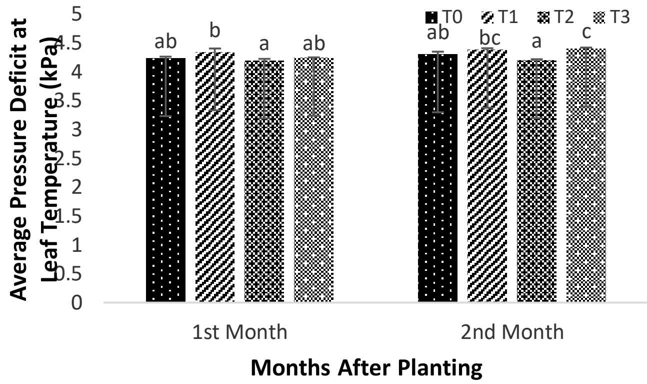

Figure 2. Average pressure deficit at leaf temperature of rice bean after application of liquid organic fertilizer

Photosynthetic carbon assimilation (A) is directly related to stomatal conductance (Figure 1C). Still, this relationship is mediated by the intrinsic water-use efficiency (iWUE = A/gs). The response of photosynthesis to vapor pressure deficit depends on the stomatal sensitivity to VPD and the extent to which iWUE itself changes as VPD rises. In this case, this study's assimilation rate increases because the vapor pressure deficit was also higher in this treatment. The average assimilation rate of rice beans was increased in both the first (15.26 µmol CO2 m-2 s-1) and second (16.51 µmol CO2 m-2 s-1) when applied with fermented seaweed. This increased assimilation rate of fermented seaweed treated rice beans resulted in about 12 cm difference in height at 128.53 cm (R=0.894**) compared to those applied with the commercial liquid organic fertilizer. In the study conducted by González et al., (2013), Oligo-carrageenans stimulate the growth of 3-year-old Eucalyptus globulus trees by increasing photosynthesis, nitrogen assimilation, and basal metabolism. It was also stated that fast-growing individuals always had high net assimilation rates and individuals with high assimilation rates always grew fast. The study of Al-Hamzawi, (2019) supported this, that seaweed extract and microelements significantly increase plant height and number of leaves of Chinese carnation and the increase of vegetative growth also lead to increase assimilation.

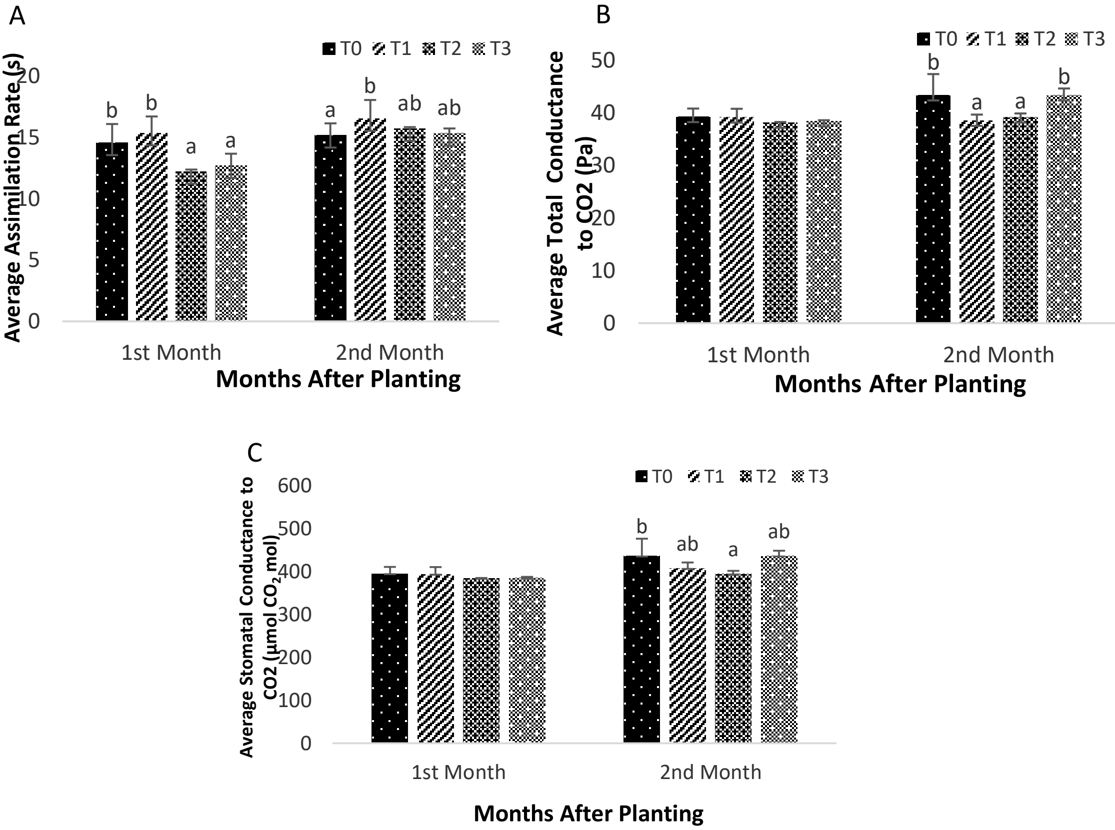

Figure 3. Photosynthetic Responses after application of different treatments on Rice Bean: (A) Average Assimilation Rate (B) Average Stomatal Conductance to CO2 (C) Average Total Conductance to CO2

As shown in Figure 2, plant height and assimilation rate have a strong correlation with each other at R=0.894. This means that the application of seaweed liquid organic fertilizer increases assimilation rate, which is the reason for the increase of plant height on Rice Bean. In the study of Li et al., (2016), forest trees always had high net assimilation rates and the individuals with high assimilation rates always grew fast. It is one reason why it was reported that the net assimilation rate and sustainable growth rate were strongly positively associated with maximum photosynthetic rate and leaf N concentration, especially when expressed on the basis of leaf area.

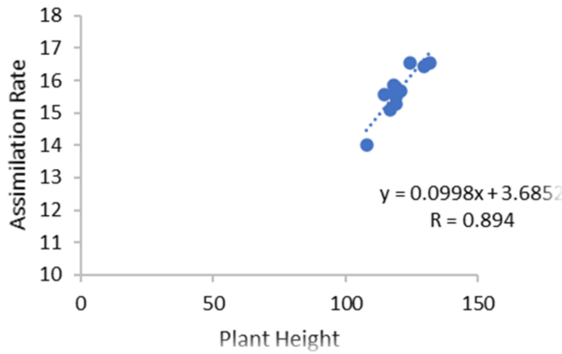

Figure 4. Relationship of Assimilation rate with plant height after the application of Seaweed liquid organic fertilizer.

Conclusion

Application of Seaweed biostimulant enhanced the plant growth of Rice Bean. This treatment proves to enhance the assimilation rate, which would be vital to the plants.

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