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Project ECHHO (Electrolysis Complex with Hyper-Hydrogen Output): A Device Prototype for Inducing *Oryza sativa*'s Seed Germination and Growth

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***Abstract*—**The economic need for rice has prompted many agriculturists to innovate methods on hastening its growth to supply the increasing demand with limited agricultural space for farming. To present, fertilizers and soaking of rice grains were the common treatments to induce rice germination and growth. In this study, the innovated device called Project ECHHO, produces increased levels of Hydrogen gas through electrolysis of water tested via quasi-experimental approach with control set-ups of Hydrogen peroxide and water (seed germination), and NPK fertilizer and no fertilizer treatment (seed growth). This study primarily aims to test the effectiveness of the developed Project ECHHO in inducing rice grains' germination and growth through Hydrogen misting. Hydrogen misting of rice grains via Project ECHHO revealed statistical no difference to Hydrogen peroxide on seed germination ($p>0.05$) and NPK fertilizer on seed growth ($p>0.05$). Furthermore, these findings were supported by the statistical difference on the seed germination of rice grains between Project ECHHO and water ($p<0.05$), and seed growth of rice grains between Project ECHHO and no fertilizer treatment ($p<0.05$). It was concluded that the use of Project ECHHO holds promising future in the field of agriculture for inducing rice grains' germination and growth with minimal costing for development, and reusable green device for successive seed treatment. The device can be used as alternative to fertilizers and other chemicals used for farming. It was recommended to alter the design of Project ECHHO by improving the module of electrolysis complex to harness greater amounts of Hydrogen in water. Also, the need for testing the Project ECHHO in other seeds is recommended.

Keywords—Germination and Growth Inducer, Agriculture, Device Innovation, Electrolysis, Oryza Sativa

I. INTRODUCTION

The archipelago of Philippines is extensively known for its agricultural farmlands with rice as the mostly grown crop planted about 30% of the total agricultural area [1]. The fact that rice is the most important staple food of the country, its annual yield cannot suffice the need of the country's growing population relying on rice imports from nearby agricultural countries. In constant debates regarding the self-sufficiency of the Philippines in rice production, geography, urbanization, investment, and lack of researches were seen as the root causes for the country's failure in establishing independence in meeting the rice demands of its people [2].

Reference [3] noted in *The Future of Food and Agriculture* that increasing the harvest of a crop can be attained through (1) providing more farmlands as plantations, or (2) developing effective fertilizers and techniques to hasten crop growth and production. The latter was considered as the current research's entry point in addressing the economic issue on self-sufficiency to improve rice yield through the use of techniques in hastening plant growth, thus meeting too soon the demand for food.

The heart of agriculture is a seed; growth starts from a seed. Seeds break its dormancy and start to germinate when environmental conditions become favorable for its growth and survival [4]. Many folk practices consider soaking the rice grains in water until it germinates. The underlying principle holds to the scarification of seed coat until it softens for the young shoot to sprout. More advanced technique includes soaking the rice seeds/grains in Hydrogen peroxide (H_2O_2) as research articles have long proven that Hydrogen is an effective agent in seed scarification and essential for seedling development [5]. Though water also contains Hydrogen, Hydrogen peroxide is often used since it has a reactive oxygen species (ROS), and a more effective seed scarifying agent though solid understanding on the role of the molecule in seed germination is still unknown [6]. As to seed growth, fertilizers were used to ensure the overall health of the seedlings through enhancing soil quality by supplying nutrients needed for plant development. One common fertilizer used for rice growth is NPK complete fertilizer (14-14-14). A recently documented study revealed a significant effect in the growth of rice treated with the new NPK complete fertilizer (15-15-15) [7]. Aside from fertilizers, one study also showed that hydrogen gas is effective for seed growth [8].

This literature review holds significant amounts of evidences on the potential use of Hydrogen in seed germination and growth. Hydrogen in water, on the other hand, can be utilized through reversing the bonding of its molecules. Electrolysis separates water molecules into its elemental components. Harnessing the Hydrogen from this process is a potential source of an effective agent in inducing rice grains' germination and growth. This research primarily aims to test the effectiveness of the created prototype device (Project ECHHO) that generates pure and induced levels of Hydrogen gas in improving and hastening rice grains' germination and growth, which in the long run can improve rice yield too soon. Specifically, this research was conducted in 2 successive studies sought to identify the answers on the following research objectives:

Study 1: Rice Grain Germination

S1.1. Determine the number of days it takes for Hydrogen misting of Project ECHHO to initiate germination of rice grains in comparison to positive (soaking in Hydrogen peroxide) and negative control (soaking in water) set-ups for three trials; and

S1.2. Determine if the number of days for rice grains to germinate among set-ups vary for three trials.

Study 2: Rice Grain Growth

S2.1. Measure the height of the rice seedlings treated by Hydrogen misting from Project ECHHO versus positive (NPK fertilizer) and negative (no treatment) control set-ups in 10 consecutive days; and

S2.2. Determine if the height of rice seedlings among set-ups vary for 10 consecutive days in three trials.

II. RELATED WORK

Numerous techniques were employed in the alteration of the electrolysis model to harness Hydrogen to be used as fuel. To name a few, methods include electrolysis of brine solution at low temperatures, and oxidative electro-synthesis using aqueous electrolyte [9], whereas reference [10] developed an experiment for obtaining Hydrogen.

In agriculture, earliest reports on the study of Hydrogen gas was documented on its significant effect on the germination of rye seeds [11]. Recent articles also have proven the power of hydrogen-rich water (HRW) in modulating plant growth particularly in rice [12]. Another study also testified for the effectiveness and role of Hydrogen gas in promoting plant growth and development [13]. Also, studies revealed that Hydrogen gas stimulates the development of roots in plants [14-15]. The role of Hydrogen gas was presumed to be the underlying scientific principle why Hydrogen peroxide is an effective agent to boost seed germination and growth.

In fact, studies revealed the effect of Hydrogen peroxide in promoting seed germination and growth particularly to eggplants [16], tomatoes [17], corn [18], and rice [19].
The consolidated review of related works revealed the active role of Hydrogen gas in stimulating seed germination and growth. Accounts also showed that the harnessing of Hydrogen gas through electrolysis can be done in various methods to utilize the maximum Hydrogen production. This review led to the hypothesis that the use of Hydrogen gas from Project ECHHO significantly improved the rice grains' germination and growth.

III. METHODOLOGY

A. Materials

For the development of the Project ECHHO, materials used include 12V DC AVR power source, 4 large containers with a maximum fluid capacity of 1.5L, 8 pieces of 200ml bottles, 8 pieces of 500ml buffer (reservoir), 4L of tap water, 1kg of salt, 12in of tin metal plate, 12in of steel metal plate, metal cutter, 10m vinyl tube, and an inflatable plastic container as hydrogen incubator.

For the experimental procedure, 2 studies were conducted. For study 1 (germination), materials used include the developed Project ECHHO prototype, water, 3% Hydrogen peroxide, *Oryza sativa* grains (1kg), and petri dishes. These materials were used in 3 consecutive trials for the testing of rice grains' germination.

For study 2 (growth), materials used include the developed Project ECHHO prototype, 3 tbsp. of NPK fertilizer (14-14-14), *Oryza sativa* grains (1kg), petri dishes with moistened loam soil, and caliper. These materials were used in 3 consecutive trials for the testing of rice grains' growth.

The quality of rice grains used for the study was carefully established by soaking the grains in water and stirred for 1 minute. Rice grains that subsided at the bottom were collected and used for the study while floating rice grains were removed. This was done to ensure that all rice grains have healthy seed structures while reassuring that the grains have equal chances of growing in the treatments. After which, collected rice grains were sun-dried.

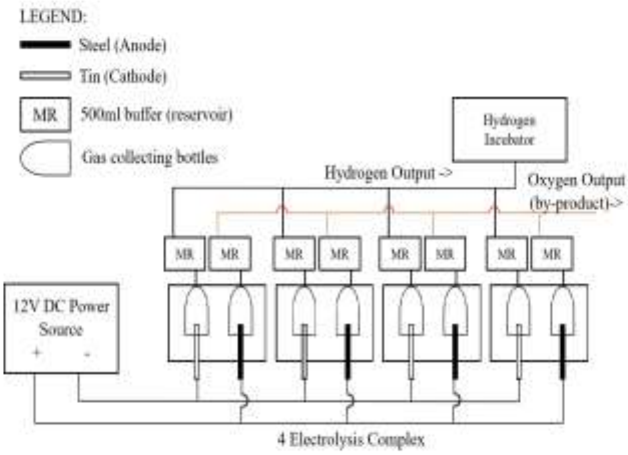

Figure 1. The Schematic Diagram of Project ECHOO (Industrial Design Patented)

B. Development of Project ECHHO

In the prototype design, 4 electrolysis modules were developed. This was done to boost the Hydrogen gas production. Transparent containers with a maximum fluid capacity of 1.5 L were used. These 4 containers were filled with 1L of water each with 0.25kg of dissolved salt to hasten the electrolysis of water (brine solution). Three inches of Tin metal was used as the cathode while same length of steel was used as anode. These metal plates were connected to a 12V DC AVR power source. Positive terminal of the power source was connected to the steel while the negative terminal was connected to the tin. This was done to all electrolysis modules. For every electrolysis module, 2 smaller bottle containers were used to harness the Hydrogen and Oxygen outputs. Also, 2 500ml buffers(reservoirs) were used to void backflow of the collected Hydrogen and Oxygen gases.

Figure 2. The electrolysis module.

The buffers (reservoirs) collecting the Hydrogen gas from the tin metal plate (cathode) were connected to series of capillary tubes that lead to the Hydrogen incubator. On the other hand, bottles collecting the Oxygen gas from steel were considered as by-product of the prototype. The Figures 1 and 2 show the electrolysis module and schematic diagram of the developed Project ECHHO.

C.Experimental Set-Up for Project ECHHO in Inducing Oryza sativa's Germination and Growth

The study employed quasi-experimental approach testing the effectiveness of Project ECHHO for inducing rice grains' germination and growth. This was tested in 2 successive studies: Study 1 (seed germination); and Study 2 (seed growth).

Study 1: Rice Grain Germination

Three set-ups were prepared. These include experimental set-up (Project ECHHO), the positive control set-up (Hydrogen peroxide), and negative control set-up (water). Sources of data were the number of days it took for rice grains to germinate across all set-ups. Germination was illustrated by the appearance of rice grains' radicle.

In experimental set-up, 20 pieces of moistened healthy rice grains were placed in a petri dish. Project ECHHO was previously activated for 30 minutes to inflate the Hydrogen incubator with the harnessed Hydrogen gas from the electrolysis of water. Moistened rice grains in petri dish were placed inside the Hydrogen incubator. A small hole was punched on the surface of incubator to serve as exhaust of excess Hydrogen gas. Project ECHHO was allowed to operate until the appearance of rice grains' radicle. In positive control set-up, 20 pieces of healthy rice grains were soaked in a petri dish of 3% Hydrogen peroxide. Also, 20 pieces of healthy rice grains were soaked in a petri dish of water for negative control set-up.

These set-ups were placed in the same lighted room to control other extraneous variables like amount of sunlight and air. The experiment was conducted in 3 trials.

Study 2: Rice Grain Growth

Three set-ups were also prepared. These include experimental set-up (Project ECHHO), the positive control set-up (NPK complete fertilizer), and negative control set-up (no fertilizer). Height of the rice seedlings' primary leaf in coleoptile was the source of data. This was measured using a caliper for 10 consecutive days.

Prior to testing, all rice grains were allowed to germinate in the same environmental conditions (soaked in water). Utilizing surface sowing, 20 pieces of germinated rice grains were sowed on the surface of equally moistened soil in 3 petri dishes. For experimental set-up, the germinated seeds in petri dish were placed in the inflated Hydrogen incubator (transparent). Project ECHHO was allowed to operate for 10 days. Positive control set-up was treated with 1 tbsp. of NPK fertilizer (14-14-14), while negative control set-up was not treated with any fertilizers. These set-ups were placed in a well-lighted room to control other extraneous variances that might contaminate the result of testing like amount of sunlight, and air. Daily measurements of primary leaf in coleoptile was determined to record changes in height for 10 days. The experiment was conducted in 3 trials.

C. Data Analysis

Descriptive and inferential statistics were used in treating the data. Specifically, it used mean to represent the measurements on the number of days for seed germination, and height of seedlings for seed growth. Analysis of variance (ANOVA) and post-hoc Bonferroni were used to determine the significance of difference in the means of the data in seed germination and growth.

IV. RESULTS AND DISCUSSION

Study 1: Rice Grain Germination

The first stage of the study tested the effectiveness of Project ECHHO in rice grains' germination in comparison to positive (Hydrogen peroxide), and negative (water) set-ups for three trials. The number of days it took for the seeds to germinate was recorded via the appearance of radicle.

S.1.1. Number of days for Rice Grains to Germinate in Project ECHHO

Table 1 below was the summary of results for rice grains' germination.

Table 1. The number of days for the rice grains to germinate in comparative experimentation for three trials between Project ECHHO (experimental), water (negative control group), Hydrogen peroxide (positive control group).

	SET-UPS		
	Experimental Set-Up (Project ECHHO)	Positive Control Set-Up (Hydrogen peroxide)	Negative Control Set-Up (Water)
1st trial	2.5	2	3.5
2nd trial	2	2	3.5
3rd trial	2.5	2.5	3
Mean	**2.33**	**2.17**	**3.33**

Result revealed that it took longer days for the rice grains to germinate when water was used as medium for its germination. On the other hand, the use Hydrogen peroxide recorded the fastest germination in rice grains followed by the experimental Project ECHHO at a mean difference of 0.16. The result presumed that the quantity of reactive hydrogen (the hydrogen peroxide and Project ECHHO) is an indication of fast seed germination where these hydrogen atoms can easily react to the seed coat of the rice grains, thus leading to scarification. Though the mean of results showed minute differences across trials, inferential statistics was therefore used to determine its significance.

S.1.2. Significance of Difference in the Number of Days for Rice Grains to Germinate in Project ECHHO and Control Set-ups for 3 Trials

Result of inferential statistics through ANOVA showed a computed *p*-value of 0.00 indicating a difference in the cohort of data ($p<0.05$). To determine which data set caused the difference in ANOVA testing, post-hoc analysis through *Bonferroni* was performed. Table 2 shows the summary of ANOVA and post-hoc testing results.

Table 2. Comparison of seed germination using ANOVA and post-hoc (*Bonferroni* correction).

	ANOVA (p=0.05)	Post-hoc (*Bonferroni* correction)[a]
Comparison of data across 3 set-ups.	0.00 (S)	p>0.017 between Experimental and Positive Control (Not Significant)[b]
Comparison of data across 3 trials	0.94 (NS)	-

[a] *Bonferroni* correction alpha is equal to 0.017 for 3 comparative events (p=0.05/3).

[b] One-tail and two-tail t-test assuming equal variances.

The result of *Bonferroni* post-hoc analysis revealed a statistical similarity ($p>0.017$) in the number of days for rice grains to germinate between Project ECHHO and Hydrogen peroxide. This suggested similar effectiveness of 2 treatments in inducing the germination rate of rice grains despite the minute mean difference in Table 1. Furthermore, a statistical difference was observed through *Bonferroni* post-hoc analysis between water and Project ECHHO ($p<0.017$), and water and Hydrogen peroxide ($p<0.017$). These statistical differences revealed that the use of water treatment to induce rice grain germination is less effective compared to other 2 treatments. This was supported by the result on longer day duration for rice grains to germinate in negative control set-up shown in Table 1. Generally, the experimentation on the effectiveness of Project ECHHO was proven effective in inducing rice grain germination, and a comparable substitute to expensive Hydrogen peroxide treatments.

To test the consistency of trials, ANOVA was ran revealing a *p*-value of 0.813. The result showed a statistical similarity among trials ($p>0.05$) demonstrating result consistency and uniformity with no intervention of contamination or other extraneous variances.

Discussion

Parallel to the findings in the literature review, the result of current study revealed that the use of Hydrogen gas treatment to induce rice grain germination is effective. This result can be attributed to the accounts in the literature body indicating the role of Hydrogen gas in softening the seed coat to allow for seed structures to sprout. This was assumed following the difference in results of rice grain germination between Hydrogen misting of Project ECHHO and water.

Table 3. Rice Grain Growth across 3 set-ups for 3 trials

SET-UPS (mm)										
Experimental Set-Up (Project ECHHO)				Positive Control Set-Up (NPK Complete Fertilizer)				Negative Control Se (No treatment)		
Trial 1	Trial 2	Trial 3	Ave.	Trial 1	Trial 2	Trial 3	Ave.	Trial 1	Trial 2	Trial 3
0.00	0.00	0.00	**0.00**	0.00	0.00	0.00	**0.00**	0	0	0
6.33	7.00	6.67	**6.67**	5.67	6.33	5.00	**5.67**	5.67	4.67	5.67
15.67	15.33	14.67	**15.22**	13.67	14.67	13.67	**14.00**	6.23	6.56	6.11
36.33	36.67	36.33	**36.44**	33.33	34.00	33.33	**33.55**	14.67	15.09	14.56
58.00	57.33	58.33	**57.89**	49.33	48.67	48.00	**48.67**	18.45	18.21	18.14
70.33	71.33	70.00	**70.55**	61.33	62.67	61.33	**61.78**	21.23	21.39	20.89
85.67	86.00	85.00	**85.56**	73.00	73.67	74.00	**73.56**	25.97	26.18	26.31
98.00	97.33	98.67	**98.00**	89.67	88.00	89.33	**89.00**	29.12	28.98	28.76
111.67	112.00	111.00	**111.56**	100.00	103.00	100.67	**101.22**	32.56	33.45	33.83
125.67	127.67	125.67	**126.34**	112.67	115.00	113.33	**113.67**	48.21	47.56	48.53
60.77	**61.07**	**60.63**	**60.82**	**53.87**	**54.60**	**53.87**	**54.11**	**20.21**	**20.21**	**20.28**

Figure 3. Rice Grain Growth across 3 set-ups for 3 trials

Moreover, the testing of Project ECHHO showed that its efficacy is similar to the known advanced techniques for inducing seed germination like the use of Hydrogen peroxide. This innovation was deemed helpful in agriculture since the prototype is reusable, and development is comparatively cheaper than the use and procurement of expensive non-renewable chemical consumables like Hydrogen peroxide. This study recommends to adjust certain properties of Project ECHHO like inducing voltage input, adding electrolysis complexes, adapting methods in the literature to hasten the harnessing of pure Hydrogen gas, and testing the prototype to other seeds.

Study 2: Rice Grain Growth

A set of germinated rice grains were used in the testing of Project ECHHO for seed growth and other control set-ups. These seeds were germinated under the same condition to eliminate variances that might possibly affect the result of testing. The summary of rice grain growth across three set-ups in 3 trials was presented in Table 3.

S.2.1. Height of Primary Leaf in Coleoptile Across 3 Set-Ups

Data on each trial were computed by the average of 20 seedlings' height for each set-up. Ranking the height of primary leaf of coleoptile revealed that the use of Hydrogen misting through Project ECHHO yielded the most improved seedling growth of rice grains. This was followed by the use of the NPK fertilizer (14-14-14) at a mean difference of 6.71mm on the average scale. Meanwhile, poor seedling growth was shown in the seedlings not treated with fertilizer. These data connoted the effectiveness of Hydrogen misting in boosting seedling growth and development. Moreover, the 10^{th} day of the experimentation exhibited the comparatively taller rice seedlings on Project ECHHO compared to 2 other set-ups (126.34>113.67>100.56). Nevertheless, the same descriptive result was recorded for Project ECHHO since day 2 of the experimentation (see average in Table 3). To further visualize the growth of seedling following the data presented in Table 3, Figure 3 was presented.

S.2.2. Significance of Difference in the Height of Primary Leaf in Coleoptile for Project ECHHO and Control Set-ups for 3 Trials

ANOVA testing revealed varying measures of height of primary leaf in coleoptile across set-ups ($p<0.05$). This means that the treatments caused varying effects to the growth of the rice grain seedlings. Table 4 shows the summary of the statistical testing using ANOVA.

Table 4. Comparison of seed growth using ANOVA and post-hoc (*Bonferroni* correction).

	ANOVA (p=0.05)	Post-hoc (*Bonferroni* correction)[a]
Comparison of data across 3 set-ups.	0.039 (S)	$p>0.017$ between Experimental and Positive Control (Not Significant)[b]
Comparison of data across 3 trials	0.999 (NS)	-

[a] *Bonferroni* correction alpha is equal to 0.017 for 3 comparative events ($p=0.05/3$).
[b] One-tail and two-tail t-test assuming equal variances.

Since ANOVA revealed that seed growth varies per set-up, a post-hoc analysis through *Bonferroni* correction was conducted ($p=0.017$) to determine the data set that caused the difference in the cohort of data. Post-hoc showed that seed growth between experimental (Project ECHHO) and positive control set-up (NPK Fertilizer) was statistically not different ($p>0.017$) suggesting similarity in the degree of effectiveness for boosting seedlings' development. Moreover, the post-hoc test revealed difference in seed growth between Project ECHHO and no fertilizer treatment ($p<0.017$), and NPK fertilizer and no fertilizer treatment ($p<0.017$). The result of post-hoc showed that the seed growth from negative control set-up (no fertilizer treatment) caused the statistical difference result in ANOVA testing. This further suggests that rice grains not treated with fertilizers were lagging behind in growth and development. Nevertheless, the statistical testing demonstrated the effective use of Project ECHHO as a green tool and alternative means in boosting seedlings' growth and development.

To test the consistency and potential contamination of data across trials, ANOVA was directed. ANOVA testing revealed statistical no difference ($p>0.05$) suggesting no contamination in experimentation, stability of data, and rawness of derived information.

Discussion

Literature has also showed the positive effect of Hydrogen gas in seed growth and development. This study verified the existing accounts by culturing the Hydrogen gas from the electrolysis of the water and used for Hydrogen misting of the rice grain seedlings. In the findings, the use of Hydrogen gas was presumed to effect the signaling molecules to regulate the genes of rice grain for growth and development [20].

Though the present study showed statistical no difference in the height of seedling growth between Project ECHHO and the NPK fertilizer, the prototype innovation is deemed to cause significant impact in chemical agriculture through green chemistry. The by-product of electrolysis is oxygen while commercialized fertilizers result to the build-up of heavy metals in soil, pollution, carcinogenic properties

[21], and its indirect effect to fish kill by eutrophication [22]. Nevertheless, this testing of Project ECHHO showed its potential in promoting seed growth and development, and can be used as an effective alternative to non-organic fertilizers that are lethal to environment and humans.

V. CONCLUSION AND FUTURE SCOPE

In agriculture, the use of synthetic chemical fertilizers has been widely practiced to improve growth and development of plants to sustain the demand for food. The innovated Project ECHHO was proven to be as effective as the commercialized chemical consumables for farming by harnessing Hydrogen gas through electrolysis of water. This was testified by the result of experimental testing showing its effect in inducing rice growth and germination through Hydrogen misting. This green innovation can be used as an alternative tool to accelerate seed germination and growth for meeting food demand while promoting ecologically safe method for farming. However, the result of the testing is limited only to the use of seeds and juvenile rice seedlings. Project ECHHO's effect to mature rice plant was not tested.

Therefore, certain considerations need to be met to fully improve and understand the full potential of the device. Future studies may venture on using the Project ECHHO on sustaining growth of mature rice plants. Also, the need to determine its effect in grain-bearing rice plants is needed to study. Since rice is not the only highly demanded staple food, studies on using the Project ECHHO on other seedlings are also recommended. As to engineering of the Project ECHHO, future researchers may alter the design by adding more electrolysis and using effective chemical solutions to collect Hydrogen gas efficiently. Future researchers may also add up a Hydrogen bubbulator to produce a hydrogen-rich water which was proven to induce plant growth and development. Therefore, the effectiveness of the alterations made in Project ECHOO must be tested again to agricultural plants. The prototype of Project ECHHO was proven to be an effective avenue for green agriculture.

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