Presoaking Treatment of Soybean [*Glycine max* (L.) Merrill] Seeds Using Fermented Plant Extracts and Commercial Liquid Fertilizer

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ABSTRACT

The yield of soybean in the Philippines is significantly low. Presoaking is one of the seed priming techniques to improve seed quality. This study aimed to determine the effects of selected fermented plant extracts and commercial organic fertilizer on germination and vigor of soybean seeds. The seeds presoaked in organic agriculture inputs such as ipil ipil FPJ (Fermented Plant Juice), squash FFJ (Fermented Fruit Juice), commercial organic fertilizer (PSPLOF), and water (control). The seeds were then subjected to seed quality tests to evaluate germination and vigor. Ipil ipil FPJ and squash FFJ significantly improved the percentage germination, first count, vigor index, seedling growth, and seedling emergence. Commercial fertilizer showed poor results in seed germination and vigor tests. Seeds soaked in water showed high percentage germination but have poor vigor. Data were arranged in factorial in Completely Randomized Design (CRD) and analyzed using Analysis of Variance (ANOVA). The initial seed quality results revealed significantly poor germination and vigor. Therefore, presoaking of soybean seeds in ipil ipil FPJ and squash FFJ is recommended to improve the germination and vigor of soybean seeds. The results could be useful to reduce the cost of soybean production and to enhance the yield of soybean.

Keywords — Agriculture, agronomy, soybean, seed germination, seed vigor, seed priming, seed presoaking, Asia, Philippines

INTRODUCTION

Soybean [*Glycine max* (L.) Merrill)] is an annual legume that belongs to Fabaceae family. United States of America (USA), Brazil, Argentina, China, and India are the top producers wherein they comprise about 90% of global soybean production (Dourado, Pascoal, Sakomura, Costa, & Biagiotti 2011). Due to the high protein content and low price of the soybean meal, it is a significant component of the livestock feed (Hartman, West, & Herman 2011). The crop is also widely grown for its vegetable oil and for human food consumption that is mostly in Asia. Some food uses of soybean include soya oil, soymilk, tofu, tokwa, miso, edamame, and soy sauce.

Since the 1970s, soybean has the highest increase in an area of production than any other major crop around the world due to increasing demand for meal and oil (Hartman, West, & Herman 2011). However, the volume of production of soybean in the Philippines is significantly low. According to the Bureau of Plant Industry (BPI) in 2012, the Philippines' area of production for soybean is just around 1,000 ha. The Philippines highly depends on soybean importation from USA, Brazil, Argentina, and China while the annual domestic needs have reached up to 300,000 MT. According to United States Department of Agriculture (USDA) Foreign Agricultural Service (2014), the Philippines are the second largest importer of soybeans from the United States. In the country, about 70%-90% of domestic production and imports are for animal feed while the remaining percentage is for human food consumption and other uses (Manuel, Huelgas, & Espanto 1987).

There is a need to increase the germination percentage and vigor of soybean since the demand for soybean would increase (Hartman, West, & Herman 2011).

Currently, the soybean in the country is primarily imported. In general, when the germination rate of the crop would increase, then the seeding rate would decrease which is favorable for the farmers. When the seed is vigorous, then the soybean seeds can combat diseases such as fungi and bacteria and also insect pests that injure the crop. Seed vigor would also determine if the seeds could withstand stressful environmental conditions.

Presoaking is one of the solutions to improve the seed germination and vigor of soybean. For the presoaking treatments, this experiment utilized inputs used in Organic Agriculture. Organic Agriculture in the Philippines has been gaining popularity and was officially recognized when the Republic Act No. 10068 or the Organic Agriculture Act of 2010 was enacted into law. Many organizations have sprouted gearing towards sustainable, organic, and ecological agriculture. Farmers who practice organic agriculture are also utilizing the principles and materials used in Korean Natural Farming (KNF) (Zamora and Calub 2016). KNF was introduced to farmers through the extension work of non-government organizations (NGOs) and State Universities and Colleges (SUCs). KNF inputs include Fermented Plant Juice (FPJ), Fermented Fruit Juice (FFJ) and other indigenous microorganisms. The fermented plant extracts can be used as fertilizers, inducing hormones, prevention of pests and diseases, and increasing plant vigor. They can also be good sources of nutrients that can boost crop growth. In this study, the FPJ and FFJ will be used as presoaking treatments. Besides, a liquid organic fertilizer product of a commercial organic enterprise that is also made through fermentation will be used.

OBJECTIVES OF THE STUDY

The general objective of the study was to determine the effects of selected fermented plant extracts and a commercial liquid organic fertilizer on the germination and vigor of soybean (*Glycine max*) seeds. The specific objectives of the study were (1) to evaluate the germination and vigor of soybean seeds presoaked in different fermented plant extracts and liquid organic fertilizer; and (2) to identify and compare which among the fermented plant extracts and the commercial liquid organic fertilizer will improve the germination and vigor of soybean seeds;

METHODOLOGY

Research Design

The experiment was laid out in factorial in a Completely Randomized Design (CRD) with four replicates. The seeds per treatment have undergone seed quality tests.

Research Site

The study was conducted in Seed Science and Technology Laboratory, Institute of Crop Science (ICropS), College of Agriculture and Food Science (CAFS), University of the Philippines Los Baños (UPLB), College, Laguna. The study was conducted from April to June 2017.

Data gathering and Procedure

Fermented fruit juice (FFJ) using squash (*Cucurbita maxima*) and a fermented plant juice (FPJ) using ipil-ipil (*Leucaena leucocephala*) leaves were prepared. Squash fruit and ipil ipil leaves were obtained from a backyard farm in Calinog, Iloilo. The plant materials were collected in the morning. Dirt from the plant materials collected was removed by shaking and without using water. The fruits and leaves were cut into small pieces of about four inches in width. Two kilograms of the plant materials were mixed with one liter of molasses. Each mixture was transferred to a plastic pail. The pails were covered with Manila paper and tied securely. The combinations in the container were kept away from sunlight undisturbed for seven days. The filtrate was strained into a plastic bottle using a filter. Two tablespoons of FPJ or FFJ were diluted in one liter of purified water to make 3% concentration of the solutions. The solutions served as the pre-soaking treatments.

Commercial Liquid Organic Fertilizer

The liquid organic fertilizer commercially known as Power Solution Premium Liquid Organic Fertilizer (PSPLOF) was obtained from YCRF Enterprises in Biñan, Laguna.). PSPLOF is included in the official list of Third Party Certified Organic Operators in the Philippines (Bureau of Agriculture and Fisheries Standards (BAFS), 2017). Two tablespoons of the liquid fertilizer were diluted in one liter of water and used as the presoaking treatment.

Presoaking Treatment

Soybean seeds (PSB Sy2) were obtained from Brown Gold Isabela Organic Enterprises. The initial percentage germination and vigor were determined.

Soybean seeds were first washed in running water for 5-10 minutes to remove dirt and other contaminants. The seeds were then rinsed with distilled water. Clean seeds were presoaked in different treatments at room temperature. The seeds were immersed entirely in the following treatments: Water, FPJ (Ipil ipil leaves), FFJ (Squash), and Liquid Organic Fertilizer. For each treatment, 600 soybean seeds were used (200 each for seed germination, vigor index, and seedling emergence). After presoaking, the seeds were blot-dried with a clean paper towel before performing seed quality evaluation.

Data collection

Percentage germination

For each treatment, 200 presoaked soybean seeds, divided into four replicates were sown in moistened paper towels. The paper towels were kept moist with an adequate amount of distilled water throughout the germination period. The first count was done five days after sowing (DAS) while the final count was done eight DAS. The percentage germination was determined using the formula:

% Germination =
$$\frac{\text{no.of normal seedlings}}{\text{total number of seeds sown}} \times 100$$

Seed Vigor

First count. The first count was obtained by determining the total number of germinated seeds on the standard germination set-up five DAS.

Vigor Index. The vigor index was determined through the speed of germination test. For each replicate, 50 seeds were sown on moist paper towels. Seedlings with 2 mm radicle protrusion were considered germinated. The number of seedlings with 2mm radicle protrusion was counted daily from the day after planting until the eighth day and was removed from the set-up. Summation of the germinated seedlings over the nth number of the day was used to determine the speed of germination or vigor index. The vigor index was computed using the formula:

Vigor index = $(n/1) + (n/2) + (n/3) + \dots + (n/8)$

Where n = number of germinated seedlings.

Seedling Growth Rate (Seedling Length, Root and Shoot Length, and Biomass). Randomly selected seedlings from the standard germination test were used. From the four replicates, ten (10) seedlings were selected for the test. The seedling length, and the root and shoot length (cm) of the chosen seedlings were measured using a ruler. The seedlings were oven-dried for 72 hours at 70°C. The oven-dried seedlings were weighed to determine the dry weight in grams (g).

Seedling Emergence. Fifty (50) seeds were sown to test the seedling emergence per replicate in unsterilized soil that was obtained from the ICropS, UPLB, College, Laguna. The set-up was supplied with an adequate amount of water throughout the experiment. The number of healthy seedlings that emerged was counted on the eighth day after sowing. The seedling emergence percentage was computed using the formula:

% Seedling Emergence = $\frac{no. of normal seedlings emerged}{total number of seeds sown} \times 100$

Data Analysis

The data was analyzed using Analysis of Variance (ANOVA) of STAR 2.0.1 software developed by the International Rice Research Institute (IRRI). The mean comparison was interpreted using the Least Significant Difference (LSD).

RESULTS AND DISCUSSION

Initial Seed Quality

Table 1 shows the initial seed quality of soybean, which included seed germination and vigor. The initial percentage germination, vigor index, shoot length, root length, seedling length, and seedling emergence were significantly lower than the results of presoaked seeds in different treatments for the same parameter. The standard germination percentage for soybean in generally higher than 80% (Egli and TeKrony, 1995). The initial seedling biomass was higher than the treated seeds.

Parameter	Value	
Percent germination	84%	
Vigor Index	42.58	
Shoot Length	7.51 cm	
Root Length	5.97 cm	
Seedling Length	13.48 cm	
Seedling Biomass	0.61g	
Seedling Emergence	72%	

Table 1. Initial Seed Quality of Soybean Seeds

Germination Percentage

The results of the ANOVA showed that the presoaking treatments were highly significantly different (Pr<0.01) from each other. There are various effects to soybean seeds among presoaking treatments. Results shown in Table 2 implies that ipil ipil FPJ, squash FFJ, and water treatments were effective in improving the percent germination of soybean seeds, from an initial of 84% to 97%, 97%, and 95%, respectively. Although ipil ipil FPJ showed the highest percentage germination, it should be noted that this was not significantly different from that of squash FFJ and water (control). The effect of commercial fertilizer was comparable to the results of the initial percentage germination.

Table 2. Percentage Germination of Soybean Seeds Presoaked in Different Presoaking Treatments.

Presoaking treatment	Percentage germination (%)
Commercial Fertilizer	85 b
Ipil ipil FPJ	97 a
Squash FFJ	97 a
Water (Control)	95 a

Means with the same letter are not significantly different.

Seed Vigor

First count

The number of normal seedlings five DAS were counted, and the result indicated the level of vigor of the seeds. The effect for the first count of soybean seeds (Table 3) was observed to be the same as the results in germination set up. Ipil ipil FPJ, squash FFJ, and water treatments showed significantly vigorous seeds than commercial fertilizer, which is not significantly different with the initial first count (Appendix 15).

At five DAS, there were no additional germinated seeds noted. Therefore, the soybean seeds that were presoaked in different treatments germinated on or before five DAS. Fungi affected most of the seeds that did not germinate specifically in those treatments that have low percent germination.

Table 3. The First Count of Soybean Seeds Presoaked in Different Presoaking Treatments.

Presoaking treatment	Number of seeds germinated
Commercial Fertilizer	43 b
Ipil ipil	49 a
Squash	49 a
Water (Control)	47 a

Means with the same letter are not significantly different.

Vigor Index

The ANOVA revealed a highly significant difference (Pr<0.01) for presoaking treatments concerning the vigor index.

The vigor index was measured through the speed of germination test. The vigor index of soybean seeds presoaked in different fermented extracts and commercial liquid fertilizer is shown in Table 4. Presoaking in ipil ipil FPJ proved to be most effective in increasing the vigor index. However, its result was not significantly different with water (control). Commercial fertilizer constantly showed poor performance.

Table 4. Vigor Index of Soybean Seeds Presoaked for 1 Hour and 12 Hours in
Different Fermented Extracts and Commercial Liquid Fertilizer.

Presoaking treatments	Presoaking treatment means
Comm. Fert.	38.78 с
Ipil Ipil FPJ	46.54 a
Squash FFJ	44.22 b
Water (Control)	44.37 ab

Means with the same letter are not significantly different.

Shoot Length

The ANOVA revealed that the presoaking treatments in shoot length have a highly significant difference (Pr<0.01) from each other

Table 5 shows the shoot length of soybean seeds presoaked in different presoaking treatments and commercial liquid fertilizer. The results of ipil ipil FPJ and squash FFJ showed no significant difference with that of water (control). Commercial fertilizer was observed to have the shortest shoot. The initial shoot length is significantly lower than the shoot length of soybean seeds presoaked in different presoaking treatments. Therefore, it is necessary to presoak soybean seeds to promote shoot growth.

Table 5. Shoot Length of Soybean Seeds Presoaked in Different Presoaking Treatments

Presoaking treatments	Presoaking treatment means
Comm. Fert.	8.96 c
Ipil Ipil FPJ	12.45 a
Squash FFJ	12.58 a
Water (Control)	11.90 b

Means with the same letter are not significantly different.

Root Length

The results of the ANOVA reveal that presoaking treatments in root length are highly significantly different (Pr<0.01). The root length of soybean seeds presoaked in different presoaking treatments (Table 6) reveals that ipil ipil FPJ has significantly longer root than all the other treatments followed by squash FFJ and water, respectively. Commercial fertilizer was noted to inhibit root growth substantially.

Table 6. Root Length (cm) of Soybean Seeds Presoaked in Different Presoaking Treatments

Presoaking treatments	Presoaking treatment means
Comm. Fert.	4.32 d
Ipil Ipil FPJ	7.62 a
Squash FFJ	6.61 b
Water (Control)	5.60 c

Means with the same letter are not significantly different.

Seedling Length

The ANOVA revealed a highly significant difference (Pr<0.01) for presoaking treatments in seedling length. The comparisons of different presoaking treatment mean in Table 7 show that ipil ipil FPJ and squash FFJ significantly improved the seedling growth of soybean seeds compared to water (control) treatment and the initial seedling length. Commercial fertilizer exhibited poor performance in seedling length and showed seedling growth inhibition when compared to the initial seedling length.

Table 7. Seedling Length (cm) of Soybean Seeds Presoaked in Different Presoaking Treatments

Presoaking treatment	Presoaking treatment means
Comm. Fert.	13.28 d
Ipil Ipil FPJ	20.08 a
Squash FFJ	19.18 b
Water (Control)	17.52 c

Means with the same letter are not significantly different.

Seedling Biomass

There are highly significant differences (Pr<0.01) in seedling biomass for different presoaking treatments. The results of the seedling biomass of soybean seeds presoaked in different presoaking (Table 8) show that ipil ipil FPJ, and squash FFJ significantly increased the seedling biomass of soybean compared to water (control) treatment. Seeds presoaked in commercial fertilizer have more massive seedling biomass than water (control), but the results between the two treatments are not significantly different.

Table 8. Seedling Biomass (g) of Soybean Seeds Presoaked in different Presoaking Treatments

Presoaking treatment means
0.54 bc
0.62 a
0.58 ab
0.49 c

Means with the same letter are not significantly different.

The initial seedling biomass value is relatively high. The result for the initial seedling biomass is not significantly different with ipil ipil FPJ and squash FFJ, which have massive seedling biomass among the treatments.

Seedling Emergence

The ANOVA for the seedling emergence of soybean seeds reveals that there is no significant difference in different presoaking treatments for seedling emergence.

Table 9 shows the seedling emergence of soybean seeds presoaked in different presoaking treatment. The different presoaking treatment showed almost no significant differences. The initial seedling emergence percentage is significantly lower than all the treatments. Therefore, to improve the seedling emergence percentage, presoaking of seeds in fermented extracts is necessary.

Presoaking treatment	Presoaking treatment mean
Comm. Fert.	91.75
Ipil Ipil FPJ	90.33
Squash FFJ	98.67
Water (Control)	80.63

Table 9. Seedling Emergence (%) of Soybean Seeds Presoaked in Different Presoaking Treatments

Means with the same letter are not significantly different.

Initial Seed Quality

The initial seed quality results for percentage germination, first count, vigor index, seedling growth rate, and seedling biomass were significantly lower than that of the treated seeds. The initial seedling biomass, however, was more massive than the pre-soaked seeds. This could be the effect of the attached fungi and other pathogens, which could have added weight to seedling biomass. It was observed that the seeds that were not presoaked in any treatment were significantly affected with fungi and other pathogens. The untreated soybean seeds showed poor performance in germination and vigor. This infers that the seeds that have not undergone presoaking will have a low percentage of surviving in the field where the soil has harmful bacteria and fungi, the pH may be detrimental to the plant, there are weeds to compete for nutrients, and other natural stresses that can injure and affect the growth of the crop. **Presoaking Treatments**

Ipil ipil FPJ significantly improved the performance of soybean seeds in percentage germination. It also showed vigorous seeds in different vigor tests such as first count, vigor index, seedling growth rate, and seedling emergence. Based on the results from various parameters, it was usually on top among the other treatments; therefore, ipil ipil FPJ was effective in enhancing the percentage germination and vigor of soybean seeds.

The efficiency of ipil ipil FPJ can be attributed to the different nutrients, vitamins, and microorganisms present in the fermented extract. Since ipil ipil contains high nitrogen content, the protein contents of soybean seeds may have been increased resulting in increased germination and vigor. This is on par with the study of Warraich, Basra, Ahmad, Ahmed, & Aftab in 2002 on wheat (*Triticum aestivum*) wherein seeds from plots that were fertilized with nitrogen resulted in increased final germination percentage.

Seeds that were pre-soaked in ipil ipil FPJ may have absorbed water faster during its germination resulting to significantly high first count and vigor index. Hara and Toriyama (1998) observed that seed lot of rice (*Oryza sativa*) with a higher amount of nitrogen applied showed faster water absorption, more rapid seedling emergence and more uniform emergence than the seed lot with low nitrogen content.

Seeds soaked in Ipil ipil FPJ were observed to have the best performance in seedling growth rate test (shoot length, root length, seedling length, and seedling biomass). Nitrogen that is abundant in ipil ipil is necessary to promote initial growth in soybean (Ohyama et al., 2013). Other effects of the essential nutrients present in ipil ipil FPJ may have interacted to produce positive results in seedling growth. Also, application of nitrogen results in increased biomass yield and protein yield (Blumenthal, Baltensperger, Cassman, Mason, & Pavlista 2008).

Ipil ipil FPJ, Squash FFJ, and commercial fertilizer significantly enhanced the seedling emergence percentage of soybean seeds. These fermented treatments contain Lactic Acid Bacteria (LAB) and also essential nutrients. Hamed, Moustafa, & Abdel-Aziz (2011) concluded that the LAB have growth promoting-effects. Therefore, seeds presoaked in the fermented treatments were able to combat fungi and bacteria, thus, resulted in successful and improved seedling emergence compared to the initial seedling emergence value. Also, the enzymes for metabolic processes may have been activated.

However, it was evident that ipil ipil FPJ has relatively lower seedling emergence percentage than the other fermented extracts. Some emerged soybean

seedlings presoaked in ipil ipil FPJ may have been affected by pathogens present in the unsterilized soil that resulted in decayed seedlings. Ohyama et al. (2013) reported that too much nitrogen applied to plants could make them more attracted to insects and diseases.

Squash FFJ

Squash FFJ was also effective in improving the performance of soybean seeds in percentage germination, first count, vigor index, seedling growth rate, and seedling emergence. Squash FFJ produced the most vigorous seeds in seedling emergence test.

Squash FFJ was also a good source of essential nutrients that resulted in high germination percentage and vigor of soybean seeds. Also, squash fruit has high vitamin A and phosphorus (P) content. Provision of a more considerable amount of P may have increased adenosine triphosphate (ATP) and deoxyribonucleic acid (DNA) that triggered metabolic processes resulting in high percentage germination and vigor. The results for squash FFJ are in agreement with the study of Zeļonka et al. (2005) in which seed coating with phosphorus of spring barley (*Hordeum vulgare*) resulted to higher germination percentage, greater chlorophyll content in the shoots and increased physiological activity.

Seeds pre-soaked in squash FFJ showed good performance on seedling growth rate test. The results were on par with the effect of phosphorus on shoot growth. The findings were also supported by the study of Shah, Ara, & Shafi (2011) on Okra (*Abelmoschus esculentus*) seeds that were soaked to P solutions which showed better seedling growth compared to seeds soaked in water. Inadequate P can affect shoot and root growth negatively.

Commercial Fertilizer

Commercial fertilizer showed poor results that are significantly lower or comparable with that of initial seed quality values in almost all of the parameters. However, its result for seedling emergence was considerably higher than that of control.

The commercial fertilizer is a mixture of water, molasses, sugar, fresh milk, lemongrass (*Cymbopogon citratus*), oregano (*Origanum vulgare*), malunggay (*Moringa oleifera*), garlic (*Allium sativum*), ginger (*Zingiber officinale*), chicken manure, and mill ash. The ingredients as mentioned earlier may have exhibited detrimental effects on soybean seeds resulting in poor performance.

The concentration of the commercial liquid fertilizer used in this experiment may have been too high. Szopinska (2013) stated that high levels of lactic acid

that is present in commercial fertilizer could reduce the germination and vigor of seeds. The concentration could have been moderated to achieve the optimum potential of the commercial liquid fertilizer.

Water (Control) Treatment

Seeds that were presoaked in water significantly improved the percentage germination compared to the initial seed quality values. Water treatment showed a relatively weak result in seedling growth rate.

Seeds soaked in water may have imbibed adequate amount of water needed for the seeds to germinate. The physiological process may have been triggered, thereby, resulting in a high germination rate and vigor index. Also, there was no possibility of allelopathic effects by water.

The seeds presoaked in water have no nutrients to acquire, therefore, regarding seedling growth rate, seeds soaked in water showed relatively poor performance compared to the fermented treatments and its performance in percentage germination and vigor index. In contrast, seeds presoaked in the fermented treatments have additional nutrients to absorb. Soybean seeds still need additional nutrients during germination for greater yield. To promote the initial growth of soybean, N is applied as a starter fertilizer (Ohyama et al., 2013). Water treatment has significantly longer seedling than the initial seedling length value but lower than the fermented treatments. Water may have supplied the seeds with an adequate amount of moisture. However, it lacked nutrients and other beneficial microorganisms that are present in fermented extracts. Seeds presoaked in water have the lightest seedling biomass among the treatments.

Water treatment showed significantly low seedling emergence percentage. Seeds soaked in water were not equipped with beneficial microorganisms to combat the harmful pathogens present in the unsterilized soil. Also, there are no essential nutrients supplied to the seeds to thrive and to grow vigorously in the stressful environment.

CONCLUSIONS

Presoaking of soybean seeds was necessary to improve the germination and vigor of soybean seeds. The result for the initial seed quality was significantly lower than the treated seeds. The untreated seeds were more prone to pathogens than the pre-soaked seeds. Ipil ipil FPJ and squash FFJ significantly enhanced the germination and vigor of soybean seeds. Therefore, it is recommended to use these fermented extracts as presoaking treatments since they contain microorganisms and essential nutrients.

Commercial fertilizer did not improve the germination and vigor of soybean seeds. The results for commercial fertilizer were either comparable to or lower than that of the initial seed quality. There might be a need to adjust the concentration of the commercial fertilizer when it is used for presoaking of soybean seeds.

Seeds soaked in water significantly improved the percentage germination of soybean seeds. However, the seeds were not vigorous since they showed low seedling growth and seedling emergence percentage.

This study recommends presoaking of soybean seeds in ipil ipil FPJ and squash FFJ to improve the percentage germination and vigor of soybean seeds significantly. However, further studies are necessary to determine the optimum presoaking period and concentration of the fermented extracts. Also, the floating seeds during the presoaking process should be removed and should not be included in seed quality testing. An adequate amount of water should be supplied in seedling emergence test. It would be better if the amount of water provided for each treatment would be uniform. Further studies could also include NPK analysis of the FPJ and FFJ.

TRANSLATIONAL RESEARCH

The findings of this study may be translated to the farmers who engage in Organic Farming and other farmers who opt to use less synthetic fertilizers. The effect of fermented plant extracts on soybean may give an idea to the farmers on what could be the effect to other crops that are related to soybean. Hence, wise decision making that could lead to higher yields and profits can be attained. The methodology of this study will also give an idea to the farmers and entrepreneurs on how they can optimize the product for achieving their purpose.

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