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ASSESSMENT OF THE SUSTAINABILITY OF RICE CULTIVATION PRACTICE OF FARMERS IN THREE CERTIFIED PROGRAMS COMPARED WITH CONVENTIONAL FARMERS IN UBON RATCHATHANI PROVINCE, THAILAND

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ABSTRACT

The sustainability of four types of rice cultivation practiced by farmers and the factors contributing to increased sustainability were assessed. The four types of rice cultivation consisted of organic rice production, Good Agricultural Practices (GAP), Sustainable Rice Platform (SRP), and conventional production. A total of 85 farmers were requested to respond to the questionnaires on farmer and farm characteristics, and farmer descriptions of 123 rice cultivation practices were assessed using SRP sustainability standards during September – October 2018. Four types of farmers were not significantly different for age, education, farming experience, level of farm integration and total income but they were significantly different for rice yield and labor size. Organic and conventional farming were highest for rice yield, whereas conventional farming system was highest for labor use. None of farming systems achieved an overall SRP score that enabled to claim as sustainable rice farm, but organic, GAP, and SRP farmers had significantly higher overall SRP scores and higher scores for farm management and pre-planting practice. The regression analysis showed that the factors promoting high SRP score were the SRP and GAP programs, and the characteristics which facilitated joining the program were the farm size, farm experience and rice yield.

Key words: organic, sustainable rice platform, good agricultural practice, certification

INTRODUCTION

Thailand is one of the world's top three rice exporting countries. In 2021, the area planted with rice was estimated at 9.12 million ha, and rice production was reported at 45.29 million tons (DFT 2021; OAE 2021). Most paddy fields are managed by family farmers. For decades, Thai government has played an encouraging role to increase productivity and raise farmers' income level by promoting intensive, chemically based agricultural systems. Nevertheless, 40% of Thai farmers are still poor (Chantatarat 2018). Rice production cost in Thailand was higher than those in other rice exporting countries (Poapongsakorn 2013; Pongsrihadulchai 2017). Yield loss is frequently affected by natural disasters including drought, flooding and high temperature, causing rice yields to vary considerably from year to year. Moreover, paddy fields are one cause of climate change due to emission of methane (Kawasaki 2010). Rice fields are responsible for 11% of global agricultural nitrous oxide (N_2O) emissions (US-EPA 2020). Thailand is also ranked 20th in the world in terms of agricultural CO_2 emissions (FAO 2018).

In 2018, the Thai government established a "20-year National Strategy Plan" that includes a 20-year agricultural strategy (2018-2037) (NSSO 2017). The principal objectives of this plan are to expand the area in high standard agriculture and increase the number of high-performance farmers, especially organic and sustainable rice farmers. The rice department of the Thai Ministry of Agricultural and Cooperatives is collaborating with GIZ (German International Co-operation) and Olam (Thailand) in implementing a sustainable rice project called "Market-Oriented Smallholder Value Chain (MSVC)". The objectives of MSVC were to promote and develop farmers' knowledge and use of Sustainable Rice Platforms (SRP) to reduce production costs, make appropriate use of local labor, stop the burning of paddy stubble, and use production practices that mitigate greenhouse gas (GHG) emissions from rice cultivation, promote climate-resilient rice production systems, and enhance the livelihoods of smallholders (Vichitlekarn 2018). The MSVC project established SRP Standards to enable farmers to assess the current level of sustainability of their rice production practices and, thereby, stimulate farmers' awareness and provide concrete improvement targets. The SRP project has first been implemented in Ubon Ratchathani province as a pilot area and will be expanded to other areas soon.

At present, not all farmers in Ubon Ratchathani participate in the SRP program. Some farmers produce organic rice based on Participatory Guaranteed System (PGS) that is promoted by the government and certified as organic rice. Other farmers follow Good Agricultural Practices (GAP) standards, a certification program for export. Farmers who do not participate in any certification program use conventional, chemical-based practices.

To determine the best way to increase the level of sustainability of rice production practices and overall farm sustainability in the future, data on rice production practices used on all types of farms are needed. For this purpose, the following two objectives were established for this study in the SRP project area:

1. To assess the sustainability of rice cultivation based on SRP standards for 4 types of rice practices: Conventional rice practices, organic rice practices (PGS standard), GAP rice practices, and SRP rice practices.
2. To identify the characteristics of rice farmers, farm management, and rice production practices that contribute to increased sustainability based on SRP standards of rice production practices.

METHODOLOGY

Description of study location and sample size determination. Det Udom district is part of Ubon Ratchathani province located in the northeastern part of Thailand near Laos and Cambodia (Fig.1). This is an area of rainfed agriculture, and the main crop is rice. Thai rice department selected Bua Ngam and Klang subdistricts as the pilot-testing area of the SRP sustainable rice project in 2016. Stratified random sampling was used to select farmers from each of four types, SRP, organic, GAP and conventional. Lists of farmers in each type were obtained from the Extension Service Center of Det Udom district. The resulting sample consisted of 22 SRP farmers from 30 active SRP farmers (73%), 22 GAP farmers from 300 GAP farmers (7%), 19 organic farmers from 60 PGS organic farmers (32%), and 22 conventional farmers from 400 farmers who did not belong to any certified program of the government (6%). The total sample size of 85 farmers was 11% of the farmers in the lists (790 farmers in total).

Data collection and analysis. We collected both quantitative and qualitative data from the 85 sample farmers during September – October 2018. We used questionnaires to obtain data on farmer and farm characteristics. Farmers gave their descriptions of 123 rice cultivation practices, which we assessed based on SRP sustainability standards as described below.

The SRP standards contain 8 themes, each with multiple requirements, for a total of 46 types of requirements for sustainable rice production. These requirements have environmental agronomic, economic, and social impacts (Table 1). This reflects a holistic conception of sustainability that not only limited to environment but also aspects of farmer's life

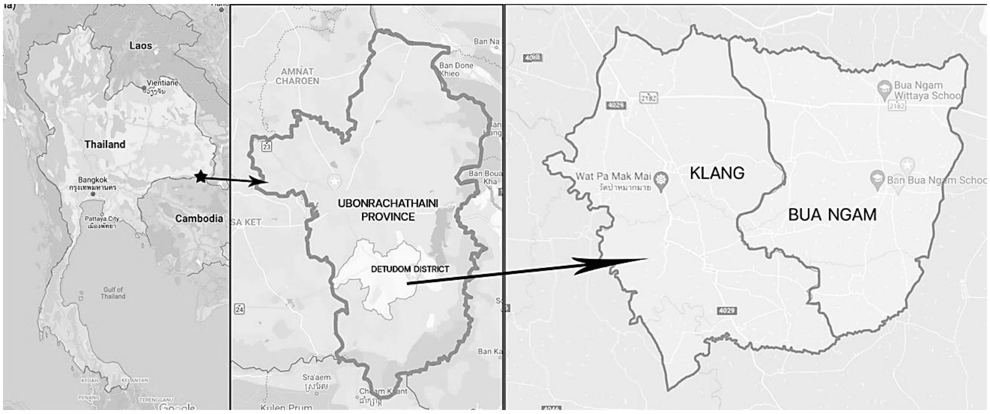

Fig. 1. Research location in Klang and Bua Ngam subdistricts in Det Udom district, Ubon Ratchathaini province, Thailand

Table 1. The SRP theme requirements and impacts (version 1.0)

Theme	Requirement
Farm management	1) Crop calendar 2) Record keeping 3) Training Impact to: Profitability, yield
Pre-planting	1) Heavy metal 2) Salinity 3) Land conversion 4) Invasive species 5) Leveling 6) Seed variety Impact to: Food safety, profitability, yield, water, GHG, biodiversity
Water use	1) Water management 2) Irrigation system 3) Inbound water quality 4) Water extraction 5) Drainage Impact to: Profitability, yield, water, GHG
Nutrient management	1) Nutrient management 2) Organic fertilizer 3) Inorganic fertilizer choices 4) Inorganic fertilizer use Impact to: Profitability, yield, nutrients, GHG, biodiversity
Pest management	1) IPM (Integrated pest management) – weed management / Insect management / Disease management / Mollusk management / Rodent management / Bird management 2) Pesticide selection 3) Targeted application 4) Label instruction 5) Calibration Impact to: Profitability, yield, food safety, pesticides, biodiversity, health and safety, community
Harvest and postharvest	1) Timing of harvest 2) Harvest equipment 3) Drying time 4) Drying technique 5) Rice storage 6) Rice stubble 7) Rice straw Impact to: Profitability, yield, food safety, nutrients, GHG, community
Health and safety	1) Safety instruction 2) Tools and equipment 3) Training of pesticide applicators 4) Personal protective equipment (PPE) 5) Washing and changing 6) Applicator's restrictions 7) Re-entry time 8) Pesticide storage 9) Pesticide disposal Impact to: Pesticides, food safety, health and safety
Labor rights	1) Child labor 2) Hazardous works 3) Education 4) Forced labor 5) Discrimination 6) Freedom of association 7) Wages Impact to: Child labor, Labor rights

Farmers, who joined the program, received training and were expected to develop their farm activities following the SRP standards. Within each requirement, the level of sustainability of each type of practice is shown by a score. The highest performance level for each requirement was indicated by a score of 3 points. Other practices with intermediate sustainability were indicated by scores of 1 or 2 points. Unsustainable practices were indicated by a score of 0 point. Scores were collected and assigned by the inspectors based on the guidelines of the first public version of the SRP standards (Ellis 2015).

Next, we calculated weighted scores for each requirement and average scores per theme and per farmer to obtain values on a common scale across themes with different numbers of requirements and unequal numbers of farmers in each type. Weighed scores for each requirement were calculated as a fraction of the maximum score of 3

Weighted score per requirement = Score of the requirement / maximum score.

The average score per theme was calculated relative to the number of requirements in the theme

Average score per theme = sum of weighted scores per requirement / no. of requirements

Average scores per farmer were calculated as the sum of all scores / maximum total score, where the maximum total score was 138 (46 requirements x 3, the maximum score per requirement).

All scores are shown as percentages. Scores for each requirement and theme were assigned to one of three levels by SRP:

1. Entry level : 10-67
2. Essential level : 68-90
3. Sustainable level : 91-100

Due to the complexity of comparing four rice programs, SRP themes were utilized to illustrate similarities and differences in the requirements of the four rice programs based on their objectives.

The information on (Table 2) showed the strictness of 4 rice programs according to SRP theme. The organic program did not allow chemical use on farm and focused on clean sources of water and natural system of soil. This program was the only one type that require farmer to pass the transition period. The SRP program was the only one program that mentioned about child labor, and other requirements were similar to those for organic program but still allowed chemicals on farms. The GAP program allowed farmers to use agricultural chemicals. For the conventional farm, there was no requirement to force the farmers to attain. However, the activities that farmers did on their farm would reflect the sustainable scores.

Table 2. The similarities and differences between the 4 rice programs

Program	ORGANIC	SRP	GAP	CONVENTIONAL
Requirement	Transition period 3 years after training	Transition period n/a	Transition period n/a	Transition period n/a
Farm management	Record keeping on seed variety, production sources, fertilizer use, cost, pest management and other operation on farm	Record keeping on seed variety, production sources, fertilizer use, cost, pest management and other operation on farm	Record keeping on seed variety, production sources, fertilizer use, cost, pest management and	N/A

Program	ORGANIC	SRP	GAP	CONVENTIONAL
			other operation on farm	
Pre planting	Farm not located in contaminated area, Not located in primary forest, Buffer zone needed, Document proof for heavy metals safe 100%, Organic seeds only from trustworthy sources, No GMO seed	Farm not located in contaminated area, Not located in primary forest, Cultivated on flat land or on terrace, Document proof for heavy metals safe and no risk of soil salinity, Use pure seed from trustworthy sources	Farm not located in contaminated area, Document proof for heavy metals safe and no risk of soil salinity, Use pure seed from trustworthy sources	N/A
Water use	Clean sources free from biological, saline and heavy metal and chemical substance	Clean sources free from biological, saline and heavy metal	Clean sources free from biological, saline and heavy metal	N/A
Nutrient management	Focus on natural system of soil fertilizer enhancement, Organic fertilizer only	Focus on natural system of soil fertilizer enhancement, Chemical fertilizer can be use from trustworthy source	Chemical fertilizer can be use from trustworthy source	N/A
Pest management	No chemical	IPM, Allowed chemical at safe level, avoid killing insects and pest animals	Allowed chemical at safe level	N/A
Harvest and Postharvest	Harvest at appropriate time, Clean harvest equipment, Separated milling to prevent contamination, Appropriate drying, Cleaned and separated store away from hazardous substance and pest damage, Not burning rice stubble	Harvest at appropriate time, Clean harvest equipment, Separated milling to prevent contamination, Appropriate drying, Cleaned and separated store away from hazardous substance and pest damage Not burning rice stubble	Harvest at appropriate time, Clean harvest equipment, Appropriate drying, Cleaned and separated store away from hazardous substance and pest damage	N/A
Health and Safety	Calibration, maintenance and cleaning tools,	Workers receive regular safety instructions,	Calibration, maintenance and cleaning tools,	N/A

Program	ORGANIC	SRP	GAP	CONVENTIONAL
	No chemical pesticide[1]	Calibration, maintenance and cleaning tools, Use personal protective equipment, Pesticide storage in safe place	Use personal protective equipment, Pesticide storage in safe place	
Labor right	N/A	No child labor, forced labor in hazardous work, workers free to join worker's organization	N/A	N/A

Source: As researched by the author (2018)

To ascertain the sustainability of the four programs, statistical analysis was performed using SPSS version 23 (2015). ANOVA and Tukey method was applied, significance was defined as p-value > 0.05. Data were represented as means and one standard deviation. Two regression models are used to determine the factors affecting SRP scores, and the characteristics of rice farmers that encourage higher sustainability based on SRP standards of rice production practices.

1) To identify the factors that contributed to the SRP score, multiple linear regression was used to analyze the correlation between the SRP score and selected factors. The use of organic program, use of GAP program and use of SRP program were applied as a dummy variable. The following independent variables hypothesized to contribute to variation in the dependent variable Y: overall SRP scores, were initially entered into a full model:

$$Y = \beta_0 + \beta_1X_1 + \beta_2X_2 + \beta_3X_3 + \beta_4X_4 + \beta_5X_5 + \beta_6X_6 + \beta_7X_7 + \beta_8X_8 + \beta_9X_9 + \beta_{10}X_{10}$$

Where:

Y : SRP score (sustainable rice cultivation score)
X_1 : Use of organic program (dummy: use of the program = 1)
X_2 : Use of SRP program (dummy: use of the program = 1)
X_3 : Use of GAP program (dummy: use of the program = 1)
X_4 : Years of education
X_5 : Level of integration (no component, one-component, 2 and more components)
X_6 : Source of income (dummy: both on-farm and off-farm)
X_7 : Number of on farm laborers
X_8 : Yield (kg/ha)
X_9 : Farm size (ha)
X_{10} : Total income (baht)

2) To identify the characteristics of rice farmers that encourage positive sustainability. The correlation between significant factors identified from the multiple linear regression model were examined by using logistic regression and the wald test. The full equation is expressed below:

$$Z = \beta_0 + \beta_1X_1 + \beta_2X_2 + \beta_3X_3 + \beta_4X_4 + \beta_5X_5 + \beta_6X_6 + \beta_7X_7 + \beta_8X_8$$

[1] Only insecticides made from natural extracts are allowed to be used. The following substances are permitted: Siamese neem extract, lemongrass extract, holy basil extract, and wood vinegar.

Where:

Z : A significant variable that influences boosting the sustainability scores
X_1 : Sex
X_2 : Age
X_3 : Years of education
X_4 : Number of on farm laborers
X_5 : Farm size
X_6 : Total income
X_7 : Experience on rice farm
X_8 : Yield (kg/ha)

RESULTS AND DISCUSSION

Socioeconomic characteristics of the farmers and farms. Most farmers are farmer descendants (98%) with inherited land (96%). The farmers' ages was between 40 and 59 years. Half of them (51%) completed their elementary schools and 28% of farmers graduated from high schools. The majority (88%) of farmers were full-time farmers with more than 10 years' experience. Half of the farmers (53%) never had the experience in another career, and learned rice cultivation techniques from their parents since they were young. The common agricultural system in this area was integrated farming system. Most grew vegetables and raised cows, some grew peppers and bananas, and others raised fish. Farmer's families in this study planted a variety of food crops on their farms such as weed grass, holy basil, chili, banana and other vegetables and raised some chickens or cows. Some of them obtained free chicken and cows from the extension agent by participating in a government project. Fifty five percent of the conventional farmers used 2 component integrations, whereas 42% of the organic farmers used mono crop rice system. The SRP farmers had the highest number of farmers (59%) who achieved 2 sources of income, whereas 72% of the GAP farmers relied on agricultural income. However, 4 groups of farmers were not significantly different for age, education period, farming experience, source of income and farm integration.

There were statistical differences in the number of laborers in the farms and yield (Table 3). The conventional rice farms used more labor than other farms and there was significant difference between the conventional farmer group and GAP farmer group (Table 4). According to the interviews conducted, conventional farmers did not use two-wheel tractors, whereas other types of farmers did.

Table 3. Farm size, farm labor, yield (kg/rai) and income per year (baht) by farm type.

	FARM TYPE				F	P value
	Conventional (n=22)	**Organic (n=19)**	**SRP (n=22)**	**GAP (n=22)**		
Farm size	4.32 ±1.76	3.68 ±2.24	4.16 ±2.56	4.8 ±3.52	0.65	0.58
Labor on farm	3 ±1	2 ±1	2 ±1	2 ±1	2.74	0.04*
Yield	363 ±24	366 ±33	348 ±28	335 ±26	5.60	0.00**
Agri income	89,795 ±84,872	101,210 ±125,936	124,372 ±209,223	54,318 ±37,234	1.09	0.35
Non-agri income	27,781 ±87,900	44,210 ±110,167	31,590 ±48,682	2,409 ±5,188	1.20	0.31
Total income	117,486 ±124,683	124,421 ±167,300	155,963 ±243,029	56,727 ±36,776	1.65	0.18

Source: Survey by the author (2018)
Note: The scores show the average scores per theme ± standard deviation
Note: * *p-value* > 0.05 / ** *p-value* > 0.01

Table 4. The comparison of farm labor and yield (kg/rai) between farm type.

	Mean difference	Std	P value	Confidence Interval (CI)	
				Lower	Upper
Labor on farm					
Conventional farm / GAP farm	0.68	0.26	0.05*	0.00	1.37
Yield					
Conventional farm / GAP farm	27.27	8.29	0.00**	5.51	49.04
Organic farm / GAP farm	30.86	8.61	0.00**	8.25	53.47

Source: Survey by the author (2018)
Note: * *p-value* > 0.05 / ** *p-value* > 0.01

Conventional farms and GAP farms were significantly difference for rice yield, and organic farms and GAP farms were also significantly different for this parameter (Table 4). While GAP farms had the lowest yields, the conventional, organic, and SRP farms had the rice yields exceeding the average yield of Ubon Ratchathani province (330 kg) (OAE 2020). However, SRP rice farms in this research still had the lower yield than the expectation of the program which was expected to obtain more than 400 kilograms per Rai.

GAP farmers had the lowest income among all farmers. GAP farmers did not receive any assistance from government special programs. Farmers, who participated in the SRP program, could sell their rice at a guaranteed price. Organic farmers also received a higher price from the government subsidy. While conventional farmers achieved higher yields based on the amount of fertilizer used, some of them used fertilizer three times per crop, which was more frequent than that used by GAP farmers. There was no difference in selling prices between conventional and GAP farmers. Therefore, the farmers, who produced and sold larger amount of paddy, earned higher income.

Sustainable rice cultivation score between 4 rice practice. The sustainable score from SRP depended on the activities in the rice farm. Means of percentage scores, standard deviation and the evaluation of scores were used to express the sustainability of farms on 8 themes of SRP as show in Table 5.

Table 5. The average percentage of SRP scores between 4 farm type.

	FARM TYPE				F	P value
	Conventional (n=22)	**Organic (n=19)**	**SRP (n=22)**	**GAP (n=22)**		
Farm management	71.71 ±22.14	83.04 ±21.07	86.86 ±13.55	92.92 ±11.13	5.73	0.00**
Pre-planting management	56.56 ±16.67	69.29 ±19.89	78.78 ±21.32	83.08 ±14.89	9.03	0.00**
Water use management	90.90 ±15.19	84.21 ±20.39	90.90 ±15.19	85.60 ±18.03	0.86	0.46
Nutrient management	70.60 ±19.04	73.68 ±21.59	76.06 ±20.84	68.48 ±25.79	0.50	0.68
Pest management	83.63 ±11.76	84.56 ±15.40	84.84 ±10.72	80 ±13.95	0.64	0.59
Harvest and post-harvest management	76.40 ±13.42	81.45 ±22.15	83.33 ±13.44	83.54 ±11.82	1.01	0.39
Health and safety	73.48 ±9.22	79.82 ±18.81	80.55 ±9.03	82.57 ±9.11	2.36	0.07

	FARM TYPE				F	P value
	Conventional (n=22)	Organic (n=19)	SRP (n=22)	GAP (n=22)		
Labor right	99.78 ±1.01	95.23 ±19.63	100	99.35 ±2.22	1.14	0.33
Total SRP scores	77.86 ±11.65	80.99 ±6.12	86.81 ±6.47	84.15 ±4.47	5.50	0.00**

Source: Investigation by the author (2018)
Note: The scores show the average scores per theme ± standard deviation
Note: * *p-value* > 0.05 / ** *p-value* > 0.01
Note: Entry level (10-67 points) / Essential level (68-90 points) / Sustainable level (91-100 points)

Four types of farmers were significantly different for sum of SRP scores and 2 themes including farm management and pre-planting. Other 6 theme scores among 4 rice practices were not statistically different. All farmer types did not achieve the level of sustainable rice cultivation, but they attained the essential level, "working toward sustainable rice cultivation". Among the four types, the SRP farmers achieved the highest overall scores. The lowest percentage scores were farmers using conventional rice practice.

Four types of farmers were compared for farm management including crop calendar throughout the production period, attending the training or regular receiving professional advice and farm record such as seed source, yield, pesticide, fertilizer, water use and machinery operation. Four types of farmers were significantly different for two requirements including crop calendar and the training. In the study area, farmers who joined in the certified rice standard programs (Organic, SRP and GAP) must keep records their farm accounting and daily tasks. Because these are the forms of data which are required for standard certification by the audits. From the interviews with conventional farmer's group, the conventional farmers made the crop calendar and recorded some data. On the other hand, some farmers recorded only their cost because it was not necessary for them to show the detail of their activities to the audit. In their opinion, they preferred to working freely, they did not want to write down a lot of information in complicated forms (Table 6). As the use of crop calendar is time-consuming for the farmers, extension personnel should first review the methods of crop calendar use of the 3 certified programs. Extension personnel might organize a workshop with key farmers from each program to compare the methods used in each program, discuss their advantages and disadvantages, and design a farmer-to-farmer methodology program. This approach has been effectively used in the Northeast of Thailand for farmer-to-farmer technology development (Sukchan et al. 2010; Taweekul et al. 2009).

In pre-planting, this theme expected farmers to check the heavy metals, salinity, invasive species, seed source, land conversion and land leveling prior to planting the crop. These requirements affected profitability, yield, biodiversity and greenhouse gas emission (GHG). Farmers in this study did not invade the forest area or planted rice in primary or secondary forest, and they did not use invasive species. The organic, GAP and SRP farmers planted their rice on flat land or terraces, especially SRP farmers, and they were financially supported by the program for soil leveling. The conventional farmers leveled their land for more than 3 years and some of them planted rice on sloping lands, but they used the conservation technique such as erosion barriers and cover crops. Four types of farmers were significantly different for heavy metal check, salinity and seed variety requirement (Table 5). Conventional farmers had the lowest scores for heavy metals and salinity (Table 6), reflecting that they did not have documents to prove the safety of soil from heavy metal such as arsenic, cadmium, chromium, mercury, lead and the document to proof that the soil salinity was in acceptable level. For seed source (or seed variety), some conventional farmers purchased seed from regional rice seed centers and rice research centers, but most of them used self-saved seeds for more than three crop cycles. Low scores in these activities brought conventional farmer's score down to the entry level, whereas farmers using the other 3 types of rice production practices attained the essential level (Table 5).

Table 6. The score on farm management and pre-planting themes between 4 farm type.

	Farm Type				F	P value
	Conventional (n=22)	**Organic (n=19)**	**SRP (n=22)**	**GAP (n=22)**		
Farm management						
Crop calendar	2.3±0.7	2.8±1	2.7±0.6	2.9±0.5	4.33	0.00**
Training	2.2±1	2.3±1	3	2.9±0.3	6.45	0.00**
Pre-planting						
Heavy metal check	0.50±1	1.5±1.	1.8±1	2.2±1	6.78	0.00**
Salinity check	0.6±1	1.6±1	1.6±1.5	2.2±1	6.35	0.00**
Seed variety	2.3±1	2.8±0.3	2.8±0.5	2.3±0.5	4.43	0.00**

Source: Survey by the author (2018)

Note: The scores show the average raw scores per requirement ± standard deviation, range of scores: 0-3

Note: * *p-value* > 0.05 / ** *p-value* > 0.01

The conventional farmers always attend the training program which was organized by the extension agent even the number of trainings is lower than other farmers and this led to the significantly difference scores between conventional farmers and other farm types (Table 6, 7). In this area, some of the conventional farmers desired to switch to the organic rice farm in the future. There is also has the significantly difference between SRP farmers and GAP farmers in training (Table 7). As a pioneer group, SRP farmers worked closer with government audits more than GAP farmers. The program required SRP farmers to join the training program, record their farm activities and submit documents to the audits to pass the certified SRP farming.

Table 7. The comparison of significant different scores on farm management, pre planting and total SRP scores between farm type.

Farm management	**Mean difference**	**Std**	**P-value**	**Confidence Interval Lower**	**Upper**
Crop calendar					
Conventional farm / GAP farm	-0.59	0.17	0.006**	-1.05	-0.13
Training					
Conventional farm / SRP farm	-0.77	0.21	0.003**	-1.33	-0.22
Conventional farm / GAP farm	-0.68	0.21	0.010**	-1.24	-0.13
Organic farm / SRP farm	-0.63	0.22	0.02*	-1.21	-0.05
Pre planting					
Heavy metal check					
Conventional farm / SRP farm	-1.27	0.40	0.01**	-2.34	-0.21
Conventional farm / GAP farm	-1.77	0.40	0.00**	-2.84	-0.71
Salinity check					
Conventional / SRP	-1.09	0.41	0.04*	-2.17	-0.02
Conventional /GAP	-1.72	0.41	0.00**	-2.80	-0.65
Seed variety					
Conventional / Organic	-0.52	0.18	0.02*	-1.01	-0.04
Total SRP score					
Conventional / SRP	-8.94	2.32	0.00**	-15.04	-2.84
Conventional /GAP	-6.28	2.32	0.04*	-12.39	-0.18

Source: Survey by the author (2018)

Note: * *p-value* > 0.05 / ** *p-value* > 0.01

Considering the water use theme, rice crop was planted at appropriate times resulted from an understanding on seasons and local climate of the farmers. The farmers also prepared strong rice bunds to collected rainwater. Most of the farmers in this study (94%) did not use the agrochemicals in their farms, and there was no negative impact to biodiversity.

Under the nutrient management theme, SRP focused on the use of organic and inorganic fertilizers by farmers. These activities affected nutrient management, yield, greenhouse gas emission and profitability of farmers. Most of farmers (77%) used many soil fertility enhancement methods such as crop rotation, green manure, animal manure that are available for their farms. 67% of farmers used only organic fertilizers. Inorganic fertilizers were used by conventional farmers and GAP farmers in the lowest volume by economic reason. All of fertilizers came from registered and trustworthy companies. Most of farmers in this study (82%) applied fertilizers 2 times per crop at sowing and at 55-60 days after sowing (DAS). The method of fertilizer application by farmers was different from that suggested by the agricultural extension agents, who recommend farmers to apply fertilizer 3 times per crop at sowing, at 30-45 DAS and at 55-60 DAS (Table 8).

Table 8. Fertilizer usage between farm type

	FARM TYPE				
	Conventional (n=22)	**Organic (n=19)**	**SRP (n=22)**	**GAP (n=22)**	**Percentage of all farmers**
	%	%	%	%	%
Type of fertilizer					
Organic only	86	100	50	36	67
Inorganic only	14	0	0	9	6
Mix	0	0	50	55	27
Time					
1 time/crop	14	21	9	5	12
2 times/crop	77	79	77	95	82
3 times/crop	9	0	14	0	6

Source: Survey by the author (2018)

For the pest management theme, SRP requires farmer to use integrated pest management (IPM) techniques rather than simply applying pesticides to control insects, rodents, birds and mollusk. In case of emergency, the application of pesticides was allowed but the farmers had to strictly follow the government recommendations and label instructions and not apply on non-target areas. The farmers participating in the certified programs (organic, SRP and GAP) never used the chemical substances for pest management in their farms. All farmer types found weed problem that affected crop yields especially the SRP farmers who had this problem since 2017 (Atthawit 2017). Land preparation and manual weeding were used to solve the problem, but the process was ineffective. Conventional farmers used herbicides with limited amount for weed control. They followed the suggestions from other farmers and the agrochemical shops in the area and worried about their personal health problem.

For harvest and postharvest management theme, most of farmers (71%) sold their paddy as rough rice (unhusked rice). They did not need the drying technique or the storage except the organic rice farmers who need the highly clean equipment and the specific rice mills that prevented the contamination in yield. After harvest, the organic rice farmers used sun drying method with shelters and stored their paddy in clean and safe storage. Most of farmers (79%) incorporated rice stubble and rice straw into the soil to allow the aerobic decomposition before the beginning of next rice crop.

For health and safety theme, the objective of this theme is to protect the labors in dangerous farm activities. Most of the farmers (99%) in this study did not use the herbicide or pesticide, which made them to receive the high score on this theme. However, four types of farmers were significantly different for two requirements including safety instruction and tools and equipment (Table 5). The conventional farmers did not frequently calibrate and maintain their farm equipment. Moreover, some rice farms did not have first aid supplies available on farms.

Lastly, for the labor right theme, SRP standard paid attention to the child labor, forced labor, discrimination, freedom of association and labor wages. Four farmer types had high scores for this theme that achieved sustainable level (over 91 points) (Table 5). From these scores, it may not imply that these farmer types had the good management in labor rights theme because all laborers were family members and there was no child laborer (Table 2). The results were in agreement with those reported in 2017. Labor rights and child labor were not an issue of concern in Thailand (Watcharapongchai 2017).

Although four farmer types had similarly score on 6 themes, but their overall SRP scores are different. In general, farmers' agronomic techniques will have an impact on the sustainable scores and refer to rice farm's sustainability. However, development or change in agronomic practices of the farmers could not be carried out immediately, and this should be carried our gradually point by point. The similar results were also reported for mountain potato production in Japan (Caldwell and Ueda 2016). Programs designed to increase the motivation of farmers based on their own self-determination have proved to be effective in various countries (Sayanagi et al. 2016). Moreover, the results obtained in this section can be used as basic information for developing an integrated collaborative approach which combines agricultural extension, the three sustainability programs, and agricultural researchers working in collaboration with farmers and other stakeholders (Inaizumi 2017)

The factors that contribute to the development of sustainable rice farm.

Backwards elimination retained X_2 ,use of SRP program and X_3 ,use of GAP program as the only variables contributing significantly to variation in overall SRP scores. All other independent variables did not contribute significantly. as shown in the following equation (Eq 1.).

$$Y = 72.466 + 9.838\,(X_2) + 6.999\,(X_3)$$

$$(3.923)^{*} \quad (2.674)^{*}$$

$$R^2=0.237,\ \text{Adjust } R^2=0.134 \quad F=2.29$$

The regression formula is significant at 5% (F-test) (Eq.1)

The use of SRP (X_2) and GAP program (X_3) had a significant effect on SRP scores. In this study, organic farmers had lower scores on the pre-planting and health and safety themes (Table 5, 7). These factors had an impact on overall scores and made the organic program significantly different from other types of practices although it was the most complex practice among the four rice programs. However, without the SRP and GAP programs, farmers would avoid keeping record of their farm finances, planning their crop calendars, participating training programs to strengthen their skill and checking their soil quality. These activities were important to develop a good farm management plan that directly affected their farm's financial situation and could cause the difference in their sustainability of their rice production.

The use of SRP and GAP programs were the factors contributing to the development of a sustainable rice farm. Logistic regression was applied to investigate the factors influencing the use of

SRP and GAP programs. No variable in this model had a significant effect on SRP farmer adoption. For GAP farmers, the enter model showed the Cox and Snell R^2 of 0.41 and Nagellerle R^2 of 0.61. The results of logistic regression and Wald test are shown in Table 8 and the logistic regression model obtained in this research is shown in (Eq 2).

$$Z = -75.292 + 0.09(X_5) - 0.03(X_8) \qquad \text{(Eq.2)}$$

Cox and Snell $R^2 = 0.41$ and Nagellerle $R^2 = 0.61$

Table 8. Logistic regression result and wald test of GAP farmer

Variable	B	S.E	Wald test	p-value	EXP (B)
Sex (X_1)	1.13	0.86	1.73	0.18	3.12
Age (X_2)	-2.49	1.08	5.30	0.21	0.08
Years of education (X_3)	0.07	0.10	0.62	0.42	1.08
Number of on farm labors (X_4)	-0.02	0.43	0.00	0.96	0.97
Farm size (X_5)	0.09	0.03	5.68	0.01	1.09
Total income (X_6)	0.00	0.00	3.91	0.48	1.00
Experience on rice farm (X_7)	22.98	8530.18	0.07	0.98	9.58
Yield (X_8)	-0.03	0.01	5.05	0.02	0.96
Constant	-75.29	34120.74	0.00	0.99	0.00

Source: Survey by the author (2018)
Note: The prediction performance's correct percentage is 72.7% (use of GAP:1) and 88.9% (use of other practice: 0)
Note: * *p-value* > 0.05 / ** *p-value* > 0.01

The significant factors that influenced a farmer's decision to adopt GAP rice program were 1) farm size and 2) yield. Farmers with larger farm sizes were more likely to practice GAP than others. Larger farm sizes allowed larger space for GAP-compliant activities such as fertilizer and chemical storage as well as a drying yard. Farmers with limited farmland may be concerned about this topic because the smaller the farmland, the lower the growing area available for rice production. For yield, farmers with lower yields were more interested in the GAP program than farmers with higher yields. Farmers, who had problems with their cultivation, were willing to try new methods to improve their rice production. In Thailand, GAP practice is widely promoted by extension agents, and it is a good choice for farmers who cannot adopt the organic program.

According to the complexity of rice programs, the organic program was most difficult to implement due to the requirements, especially the transition period of the program (Table 2). Although the use of the organic program did not show a significant difference in (Eq 1), we were still curious about the factors that led farmers to adopt this rice program. Table 9 and (Eq 3) below show the logistic regression and Wald test.

According to (Eq 3), the factors that effected the adaptation of the organic program were experience on rice farm (X_7) and yield (X_8). The new farmers or the less experienced farmers (regardless of age) would be eager to try new things and would be more likely to adopt complex rice practices such as the organic rice program than other farmers. According to the interviews, farmers with a lot of experience are doubtful of the new rice standard, as well as any government enterprise. Because they frequently have mistrust towards the government officers. Many government projects that are directed at farmers had problems in the past and the government officers frequently abandon farmers to their own problems. Farmers who have worked with government authorities for a long period have a negative attitude of government-led projects. In addition, farmers who had a high yield, appeared to be more interested in the organic rice program than others. Some organic farmers explained that farming is risky because farmers must invest their money without knowing if they will get it back. Choosing a new technique puts farmers under more stress than it provides them confidence. So, if they had a large number of rice plots and already had high yield, they would implement the organic program by dividing their farm plot and utilizing various rice practices. They were already aware that the beginning state of the organic rice program would have an impact on yield. They would adopt the organic program if they had other farm plots with higher yields that could help stabilize their income.

Table 9. Logistic regression result and Wald test of Organic farmer

Variable	B	S.E	Wald	p-value	EXP(B)
Sex (X_1)	-0.41	0.65	0.39	0.52	0.66
Age (X_2)	0.63	0.55	1.28	0.25	1.88
Years of education (X_3)	-0.08	0.08	1.16	0.28	0.91
Number of on farm labor (X_4)	-0.21	0.35	0.35	0.55	0.80
Farm size (X_5)	-0.02	0.02	1.14	0.28	0.97
Total income (X_6)	0.00	0.00	1.68	0.19	1.00
Experience on rice farm (X_7)	-1.38	0.62	4.89	0.02	0.25
Yield (X_8)	0.02	0.01	4.13	0.04	1.02
Constant	-3.80	4.57	0.69	0.40	0.02

Source: Survey by the author (2018)
Note: The prediction performance's correct percentage is 36.8% (use of organic:1) and 98.5% (use of other practice: 0)
Note: * *p-value* > 0.05 / ** *p-value* > 0.01

$$Z = -3.80 - 1.38(X_7) + 0.02(X_8) \quad \text{(Eq.3)}$$

Cox and Snell $R^2 = 0.17$ and Nagellerle $R^2 = 0.27$

CONCLUSIONS

The farmers who joined any of the three rice programs with certification were more likely to achieve a higher SRP score and reach a higher level of sustainability of rice cultivation. The significantly different activities among farmers were: 1) crop calendar 2) training 3) documents prove the heavy metal check and 4) documents that prove the salinity check on their rice farm. The factors that affected to increase the sustainability on rice farm were 1) use of GAP rice program and 2) use of Organic rice program. However, at present, half of the farmers in this study area did not participate in any of these programs.

To adopt GAP, farmers with larger farms and lower yield were more likely to use GAP rather than others. To adopt Organic, farmers with higher yield and less experience would be more willing to become organic farmers. Further research on the farmer’s motivation to overcome the physical farm constraints and the studies on the sustainable marketing channel of rice farmers are necessary. These researches might provide valuable data that extension agents and other stakeholders can use to create a support systems or training programs for sustainable rice farmer development in the near future.

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THE IMPACT OF AGRICULTURAL SECTOR GROWTH IN WEST JAVA ON THE INTER-PROVINCIAL ECONOMY

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ABSTRACT

The agricultural sector is the third-largest contributor to West Java's gross regional domestic product (GRDP), yet its growth tended to decrease in the last five years. Accordingly, this research sought to analyze the impact of agricultural growth on the economy of West Java and other regions, and analyze intra-regional and inter-regional linkages of West Java's agricultural sector. The method used in this study was a multi-regional input-output model. Interregional Input-Output tables of Indonesia for 34 provinces in 2016 were used for the analysis. The effect of West Java's agricultural sector growth has a slight intra-regional effect on regional output growth and a finite spillover effect in several provinces within Java Island. The agricultural sector in West Java has weak backward and forward intra-regional linkages, which means that the agricultural sector is less able to push the growth of the upstream and downstream sectors. The agricultural sector in West Java only has a strong forward linkage with the manufacturing sector in DKI Jakarta.

Key words: intraregional effect, intra and interregional linkages, multi-regional input-output, spillover effect

INTRODUCTION

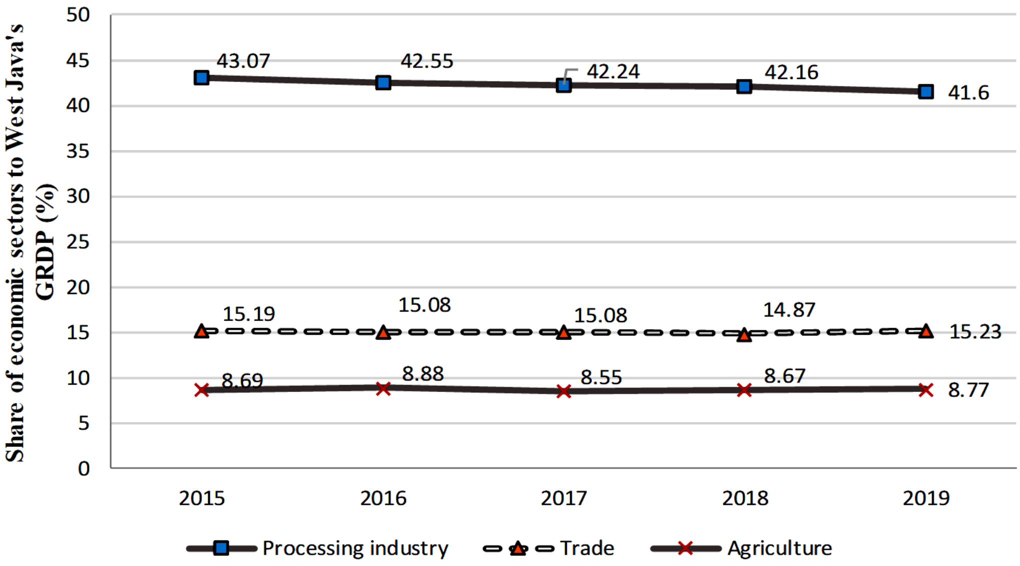
The agricultural sector is strategic in supporting the acceleration of economic development in Indonesia since its contribution to the national gross domestic product (GDP) is the third-largest contributor after the manufacturing and the trade sector during the 2016-2019 period (Statistics Indonesia 2021a). The role of the agricultural sector is indeed inseparable from the performance of regional economic development in 34 provinces in Indonesia, especially for provinces that put agriculture as a basis sector in economic development, such as West Java Province (Sulistyowati et al. 2021). The share of West Java's agricultural sector to Indonesia's GDP reaches 7.9 percent over the same period (Statistics Indonesia 2021a).

The agricultural sector is one of the leading sectors in the West Java economy, especially its contribution to the gross regional domestic product (GRDP) (Nugrahadi et al. 2010; Bank Indonesia 2020; Sulistyowati et al. 2021). The share of the agricultural sector to West Java's GRDP during 2015-2019 is the third-largest after the processing industry and trade sector (Statistics of Jawa Barat Province 2021). The share of the agricultural sector to West Java's GRDP during 2015-2019, on average, was about 8.72 percent (Fig. 1). In addition, the agricultural sector also absorbs a reasonably large workforce, which is around 40 percent (Statistics of Jawa Barat Province 2021). However, in the same period, the GRDP growth of the agricultural sector in West Java tends to decline. Based on Klassen's typology, this condition indicates a developed but depressed sector, where it has a considerable contribution to the economy but faces some challenges to grow (Widianingsih et al. 2015). The average GRDP growth rate of the agricultural sector was 2.48 percent, from 2015-2019, the third-lowest after the mining sector and the electricity and gas sector (Statistics of Jawa Barat Province 2021). This condition has direct implications for the economy of West Java, especially for regional output growth.

In an open economic system, the growth and development of an area will impact other areas around it, thus the growth that occurs in the agricultural sector in West Java will impact the economy of West Java itself (intraregional multiplier) and the economy of other regions around West Java (spillover effect), and vice versa. This research expected that there are linkages between the agricultural sector and other economic sectors in

West Java and with economic sectors outside West Java. There are interrelationships between economic sectors and regions in an economic system (Isard et al. 1998; Miller and Blair 2009). The existence of economic linkages between regions was also stated by Hoover (1985), Fujita et al. (1999), and McCann (2001).

However, the question is how significant the impact of the growth of West Java agricultural sector is on the economy of West Java itself and outside West Java, and which economic sectors inside and outside West Java have strong linkage with the agricultural sector in West Java. Thus, this study sought to analyze the impact of the growth of the West Java agricultural sector on the economy of West Java and outside West Java, and analyze the linkages of the different sectors using the Multi-Regional Input- Outputs (MRIO).



Source: Statistics of Jawa Barat Province (2021)

Fig. 1. Share of economic sectors to West Java's GRDP in the period of 2015-2019

METHODOLOGY

Data types and sources. The data used in this study is secondary data in Indonesian Inter-Regional Input-Output (IRIO) table data as a database. The IRIO table used is the Indonesian IRIO Table of Domestic Transactions Based on Producer Prices by 34 Provinces and 17 Economic Sectors in 2016 sourced from Statistics Indonesia (2021b).

MRIO Indonesia table structure. The Indonesian MRIO table in this study is based on data from the Indonesian IRIO Table of Domestic Transactions Based on Producer Prices by 34 Provinces and 17 Business Fields in 2016 (Statistics Indonesia 2021b). The Indonesian MRIO table in this study is classified into 17 economic sectors consisting of a matrix of intra-regional and inter-regional transactions in 34 provinces in Indonesia. Intra-regional is a transaction of trade in goods and services between sectors of the economy within the same province, while inter-regional is a transaction of trade in goods and services between sectors of the economy between provinces.

Intra-regional and inter-regional analysis of output multiplier. Intra-regional and inter-regional output multiplier in this study is used to analyze the impact of West Java's agricultural sector growth on the economy of West Java and outside West Java which is written as follows:

$$O_j^{LL} = \sum_{i=1}^{n} \hat{b}_{ij}^{LL} \quad (1)$$

$$O_j^{ML} = \sum_{i=1}^{n} \hat{b}_{ij}^{ML} \quad (2)$$

where:

O_j^{LL} = Intra-regional output multiplier (intra-regional effect) sector j in province L against province L

O_j^{ML} = Inter-regional output multiplier (spillover effect) sector j in province L against province M

j = agricultural sector

L = West Java Province
M = Provinces Other Than West Java
$\widehat{b_{ij}}$ = Leontief inverse matrix element MRIO

Interregional multiplier output shows the amount of change in the value of output in the province of M due to changes in final demand in sector j in province L. Inter-regional output multipliers show the magnitude of the spillover effect (Isard et al. 1998). At the same time, the intra-regional output multiplier shows the amount of change in the province of L's output value due to changes in final demand in sector j in province L. The output multiplier value is the sum of the columns in the Leontief inverse matrix, both intra-regional and inter-regional sum of the columns in the Leontief inverse matrix, both intra-regional and inter-regional.

Analysis of intra-regional and inter-regional linkages. Analysis of intra-regional and inter regional linkages, both forward and backward, is used to analyze the linkages of the West Java agricultural sector with other economic sectors in West Java and outside West Java. Intraregional linkages, both backward and forwards, are written as in equations (3) and (4), while interregional linkages, both backward and forwards, are written as in equations (5) and (6). According to Isard et al. (1998) and Miller and Blair (2009), if the value of backward and forward linkages is more significant than one, then the sector has strong linkages and can encourage the growth of its upstream, downstream sectors.

$$BL_j^{LL} = \frac{n\sum_{i=1}^{n} \hat{b}_{ij}^{LL}}{\sum_{i=1}^{n}\sum_{j=1}^{n} \hat{b}_{ij}^{LL}} \quad (3)$$

$$FL_i^{LL} = \frac{n\sum_{j=1}^{n} \hat{b}_{ij}^{LL}}{\sum_{i=1}^{n}\sum_{j=1}^{n} \hat{b}_{ij}^{LL}} \quad (4)$$

$$BL_j^{ML} = \frac{n\sum_{i=1}^{n} \hat{b}_{ij}^{ML}}{\sum_{i=1}^{n}\sum_{j=1}^{n} \hat{b}_{ij}^{ML}} \quad (5)$$

$$FL_i^{ML} = \frac{n\sum_{j=1}^{n} \hat{b}_{ij}^{ML}}{\sum_{i=1}^{n}\sum_{j=1}^{n} \hat{b}_{ij}^{ML}} \quad (6)$$

where:

BL_j^{LL} = Backward linkage of sector j in area L with sector other economies in the L
FL_i^{LL} = forward linkage of the sector I in area L to sector other economies in the L
BL_j^{ML} = Backward linkage of sector j in area L with sector other economies in the M
FL_i^{ML} = forward linkage of the sector I in area L to sector other economies in the M
j = agricultural sector in backward linkage
i = agricultural sector in forward linkages
L = West Java Province
M = Provinces Other Than West Java
$\widehat{b_{ij}}$ = Leontief inverse matrix element MRIO

RESULTS AND DISCUSSION

Impact of West Java's agricultural sector growth on the economy of West Java and outside West Java. The slowdown in growth in the agricultural sector in West Java, both directly and indirectly, will impact the economy in West Java itself (intra-regional) and the economy outside West Java (inter-regional). The analysis shows that the impact of agricultural sector growth on the economy in West Java itself can be seen based on the intra-regional output multiplier or often called the intra-regional effect, which is presented in Table 1.

The value of the intra-regional effect for the agricultural sector in West Java is 1.15. This value indicates that if there is growth in the agricultural sector due to an increase in final demand of 1 million rupiahs, it will increase the total output in all sectors of the West Java economy to IDR 1.15 million or 1.15 times. However, the value of the intra-regional effect for the agricultural sector is the smallest after the real estate sector compared to all other economic sectors in West Java. The intraregional effects of the growth of the agricultural sector are mostly felt only by the manufacturing sector and the agricultural sector itself (Statistics Indonesia 2021b). Notably, the value of the intra-regional effect shows that the growth that occurs in the agricultural sector has a negligible impact on increasing output in all sectors of the West Java economy compared to other economic sectors. Based on the output demand structure, the low value of the intra-regional effect of the agricultural sector was caused by agricultural output characteristics. A large part of agricultural output is fresh products and consumed directly in the form of final demand without being reprocessed by other sectors. Agricultural output in form of intermediate input share about 53,8 percent and only 35% percent of this amount is used by the manufacturing sector as raw materials (Statistics Indonesia 2021b).

Table 1. Intra-regional effect of West Java economic sector

No	Sector	Intra-regional effect
1	Electricity and gas supply	1.92
2	Processing industry	1.46
3	Construction	1.45
4	Transportation and warehousing	1.44
5	Provision of accommodation and drinks	1.41
6	Health services and social activities	1.37
7	Company services	1.36
8	Water supply, waste management, waste	1.35
9	Government administration, defense, and social	1.33
10	Information and communication	1.29
11	Other services	1.27
12	Wholesale and retail trade; motorcycle car repair	1.25
13	Education services	1.24
14	Mining and excavation	1.20
15	Financial services and insurance	1.18
16	**Agriculture**	**1.15**
17	Real estate	1.12

Source: Statistics Indonesia 2021b (processed)

The growth in the agricultural sector in West Java impacted the growth of other areas around West Java in the form of a spillover effect. The sum of the intra-regional effects and spillover effects becomes the total effect or the overall impact nationally. Table 2 shows that the total effect of the West Java agricultural sector is 1.26, which mean that if there is growth in the West Java agricultural sector due to an increase in final demand as much as IDR 1 million, it will increase the total output in all sectors of the West Java economy by IDR 1.15 million and have a spillover effect. In the form of an increase in output to other provinces by IDR 0.11 million or a national impact of IDR 1.26 million.

Table 2. Total effect of West Java's agricultural sector

No.	Effect	Mark
1	Intra-regional effect	1.15
2	Spillover effect	0.11
3	Total effect	1.26

Source: Statistics Indonesia 2021b (processed)

Economic development in a region does not only have an impact on the region itself but also has a spillover effect on the surrounding area. Economic development in one sector will have an impact on the region itself and the surrounding area. However, the development of the agricultural sector also will have an impact on the economy in the region and the surrounding area (Zhang et al. 2015; Wang et al. 2020). Moreover, there is a spillover effect of the development of the agricultural sector in Sulawesi on the economic sector in Kalimantan (Arman et al. 2016). Meanwhile, the construction of agricultural roads has a direct effect on agricultural output solely and also a spillover effect on the surrounding area (Tong et al. 2013)

The spillover effect in this study shows that if there is a growth in the agricultural sector in West Java due to an increase in final demand, then to produce output from the agricultural sector in West Java, goods and services are needed from other provinces so that it has an impact on increasing output in these other provinces. The magnitude of the spillover effect due to the growth of the agricultural sector in West Java is presented in Table 3. analysis results show that the most significant spillover effect is in DKI Jakarta, continued at Banten and East Java. The main economic sectors most affected by the spillover in the three provinces are the manufacturing, agricultural, financial services, and transportation and storage industry. The manufacturing industry sub-sector that receive the largest spillover effect are chemical industries, pharmaceutical industries, and traditional medicine industries where the outputs are the main inputs for agriculture sector such as fertilizers, concentrate feed, pesticides, and other inputs.

Table 3. Spillover effect of West Java agricultural sector growth.

No.	Province	Spillover effect	Economic sector most affected
1	East Java	0.034	Processing industry and agriculture
2	DKI Jakarta	0.017	Processing industry and financial services
3	Banten	0.012	Processing and transportation and warehousing Industry

Source: Statistics Indonesia 2021b (processed)

Linkage of West Java's agricultural sector with other economic sectors. Based on various studies, the development of an economic sector in a region has linkages with other economic sectors in the region as well as outside the region using the Multi-Regional Input-Output approach. There is a strong relationship between the growth of the agricultural sector and the growth of the non-agricultural sector in developing countries (Imai et al. 2016). Specifically, there is a spatial relationship between agriculture-based areas and manufacturing-based areas (Okomoto and Ihara 2016).

West Java's agricultural sector has trade links with other economic sectors in West Java (intra-regional linkages) and economic sectors outside West Java (inter-regional linkages). The agricultural sector in West Java has an intra-regional linkages value of less than one (<1), both forward linkages and backward linkages (Table 4). If the forward linkage index (<1) then the sector has a weak linkage in encouraging the growth of its downstream sector, while if the backward linkage index (<1), then the sector has a weak linkage in attracting growth in its upstream sector (Cella 1984; Clements and Rossi 1991).

Table 4. Intra-regional backward and forward linkages economic sector in West Java.

No.	Sector	Backward Linkages	Forward Linkages
1	Agriculture	0.86	0.97
2	Mining and excavation	0.90	0.99
3	Processing industry	1.09	2.29
4	Electricity and gas supply	1.43	1.34
5	Water supply, waste management, waste	1.01	0.75
6	Construction	1.08	0.86
7	Wholesale and retail trade; car repair, motorcycle	0.93	1.17
8	Transportation and warehousing	1.07	1.02
9	Provision of accommodation and drinks	1.05	0.78
10	Information and communication	0.96	0.97
11	Financial services and insurance	0.88	0.97
12	Real estate	0.84	0.80
13	Company services	1.02	0.99
14	Government administration, defense, and social	0.99	0.75
15	Education services	0.92	0.78
16	Health services and social activities	1.02	0.76
17	Other services	0.95	0.80

Source: Statistics Indonesia 2021b (processed)

The weak linkages of the agricultural sector in encouraging the growth of the upstream and downstream sectors are caused by the absence or small transactions of trade in goods and services between the agricultural sector and other economic sectors throughout the West Java region. The backward linkage of the agricultural sector in West Java is mostly (about 67 percent) with the agriculture sector itself and the processing industry, especially the chemical and pharmaceutical industries, as producers of fertilizers and pesticides (Statistics Indonesia 2021c). Meanwhile, the future linkages of the agricultural sector in West Java are mostly (about 65 percent) with the food accommodation provider sector and the processing industry, especially the food and beverage industry as a processed industry based on agricultural products (Statistics Indonesia 2021c).

Trade transactions in the agricultural sector with its upstream sector show that the output from the upstream is used as input by the agricultural sector. On the other hand, the agricultural sector's output is used as input by the downstream sector. Trade linkages between the agricultural and other economic sectors in West Java are described in Table 5 based on the flow of goods and services between sectors. The backward linkage is from using inputs from an economic sector by the agricultural sector. In comparison, the forward linkage is from inputs from the agricultural sector by an economic sector.

Table 5. Composition of input and output use of agricultural sector with other economic sectors in West Java (in percent)

No.	Sector	Input use of agricultural sector	Output of agricultural sector used by other sector
1	Agriculture	0.38	0.10
2	Mining and excavation	0.00	0.00
3	Processing industry	0.29	0.65
4	Electricity and gas supply	0.00	0.00
5	Water supply, waste management, waste	0.00	0.00
6	Construction	0.07	0.00
7	Wholesale and retail trade; car repair, motorcycle	0.14	0.00
8	Transportation and warehousing	0.03	0.00
9	Provision of accommodation and drinks	0.00	0.21
10	Information and communication	0.00	0.00
11	Financial services and insurance	0.03	0.00
12	Real estate	0.00	0.00
13	Company services	0.02	0.00
14	Government administration, defense, and social	0.00	0.00
15	Education services	0.00	0.01
16	Health services and social activities	0.00	0.01
17	Other services	0.02	0.01

Source: Statistics Indonesia 2021b (processed)

Inter-regional linkages describe inter-sector linkages between regions described by trade transactions between economic sectors in one region and other regions. These linkages also describe how the role of an economic sector in a region towards the output growth of the economic sector in other regions. This section discusses how the agricultural sector in West Java relates to the economic sector in other regions, especially with the provinces that have the most significant spillover effect from the growth of the agricultural sector in West Java, namely DKI Jakarta Banten and East Java.

The inter-regional backward linkages of the agricultural sector in West Java with its upstream sector in three regions, namely DKI Jakarta, Banten, and East Java, have weak linkages or linkage index of less than one (BL < 1) (Table 6). When compared with intra-regional linkages (Table 4), the agricultural sector in West Java, both intra-regionally and inter-regionally, is equally less able to encourage the growth of the upstream sector (downstream). The agricultural sector of West Java cannot attract the growth of its input supply sector both in West Java and in the provinces around West Java. This result is consistent with the small value of the spillover effect from the West Java agricultural sector. However, West Java's agricultural sector has strong inter-regional forward linkages with FL > 1 only with DKI Jakarta Province. That number means that the agricultural sector of West Java can encourage the growth of the downstream sector in the surrounding area but is limited to the province of DKI Jakarta. The future linkage of the West Java agricultural sector with DKI Jakarta can be seen from the number of intermediate inputs used by the West Java agricultural sector by the economic sector in DKI Jakarta.

The agricultural sector in West Java has the most significant linkage with the accommodation and food and drink provider sector in DKI Jakarta. The total output of the West Java agricultural sector used as input by the accommodation and food and drink provider sector in DKI Jakarta is around Rp. 1.13 trillion or about 70.5 percent of the total output of the West Java agricultural sector, which is used as input by all economic sectors in DKI Jakarta. DKI Jakarta, as the capital city of Indonesia as well as the center of the service and trade economy, the accommodation and eating and drinking sectors such as hotels, restaurants, and food stalls providing small and medium scale food, is growing and is urgently needed to meet the consumption demands of the people who live and work in DKI Jakarta.

Table 6. Inter-regional forward and backward linkages West Java agricultural sector

No	Inter-Regional Linkages	DKI Jakarta	Banten	East Java
1	Forward linkages	1.03	0.99	0.71
2	Backward linkages	0.40	0.55	0.71

Source: Statistics Indonesia 2021b (processed)

On the whole, trade linkages between areas of the agricultural sector of West Java, both forward and backward linkages, are still weak in attracting growth in the upstream sector and still limited in encouraging growth in the downstream sector. The only exception is the province of DKI Jakarta. Weak inter-regional linkages in the agricultural sector of West Java is caused by the small proportion of total input and output of the agricultural sector traded between regions, which is only 9.24 percent, the remaining 88.6 percent of input-output of the agricultural sector of West Java is traded within the province of West Java itself (Statistics Indonesia 2021b). Trade transactions between the output areas of the West Java agricultural sector are primarily concentrated in several provinces on the island of Java, namely DKI Jakarta, Banten, Central Java, and East Java, with a total trade proportion of 78.54 percent (Statistics Indonesia 2021b).

Table 7. Trade between regions for West Java agricultural sector output

No	Province	Percentage of Total Trade
1	East Java	2.49
2	Central Java	2.40
3	Banten	2.19
4	DKI Jakarta	1.84
	Total	**9.24**

Source: Statistics Indonesia 2021b (processed)

CONCLUSIONS AND RECOMMENDATIONS

The growth of the agricultural sector in West Java has a small intra-regional effect and a limited spillover effect. The intra-regional effects of the growth of the agricultural sector in West Java were felt mainly by the manufacturing industry sector and the agricultural sector itself in West Java. In contrast, the spillover effect is limited to only a few provinces around West Java, namely DKI Jakarta, Banten, and East Java. West Java's agricultural sector has weak intra-regional linkages with the upstream (backward) and downstream (forward) sectors. Agricultural sector in West Java only has a strong forward linkage with the manufacturing sector in DKI Jakarta. For this reason, the development of the agricultural sector in West Java is directed at increasing productivity to encourage growth in the upstream and downstream sectors and increasing added value to spur growth in the agricultural sector. In addition, the provision of agricultural infrastructure is essential to support the smooth distribution and trade with provinces that have strong links to the agricultural sector of West Java.

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VEGETATIVE PHASE EXTENSION FOR STEVIOL GLYCOSIDE ACCUMULATION IN STEVIA: PHOTOPERIOD, *IN VITRO* AND *EX VITRO* CULTURES MANIPULATION

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ABSTRACT

The growing consumers' concern over excessive sugar intake leading to obesity and diabetes has created a huge demand for an alternative, low-calorie non-synthetic sweetener. *Stevia rebaudiana* Bertoni is a good sugar alternative that produces sweet-tasting leaves owing to their steviol glycoside (SG) content. *In-vitro* and *ex-vitro* experiments were conducted to determine the effect of different photoperiods [24h/natural daylength (control), 11-h, 13-h and 15-h] on flowering and steviol glycoside (stevioside and rebaudioside A) accumulation in *Stevia rebaudiana* Bertoni. Under *in-vitro* conditions, 11-h photoperiod did not induce flowering of stevia except in a few cultures which exhibited flower bud formation (2%). *In vitro*-grown stevia had lower SGs (average of 0.88% stevioside and 0.28% rebaudioside-A) compared to the *ex vitro*-grown plants derived from tissue culture. Exposure to non-inductive 15-h photoperiod effectively inhibited flowering in tissue culture-derived stevia. Highest stevioside (6.62%) and rebaudioside-A (3.84%) content of *ex vitro*-grown stevia was obtained in the leaves under 15-h photoperiod.

Key words: tissue culture, stevia, photoperiod, steviol glycosides percentage, flowering

INTRODUCTION

Stevia (*Stevia rebaudiana* Bertoni), a perennial herb of the Asteraceae family, is known to contain steviol glycosides (SGs), which are reported to be about 300 times sweeter than sucrose at their concentration of 4% (w/v) (Kinghorn and Soejarto 1985). SGs are largely used as a natural sweetener and some of the compounds present in stevia are known to be therapeutic, non-toxic, non-carcinogenic and non-mutagenic (Brusick 2008). The huge demand for an alternative sweetener such as stevia increased because of growing concerns over excessive sugar intake leading to obesity. Commercially, stevia is consumed either fresh or in processed form as a sweetener for tea, chocolate, jam, cookies, ice cream, juice, soft drinks and yoghurt (Ibrahim et al. 2008).

Stevia growth and their production of secondary metabolites are known to be influenced by external and internal factors. SG accumulation pattern in stevia leaves is specifically shown to vary with cultivar (Serfaty et al. 2013; Bondarev et al. 2003), phenological stage (Brandle and Rosa 1992), growth conditions like photoperiod (Ceunen and Geuns 2013), temperature and available nutrients (Pal et al. 2013). The leaves are the most economically important part of stevia since the sweet compounds SGs are predominantly found in this organ, but when flowering commences, leaf production stops. Therefore, it is crucial for stevia growers to extend the vegetative phase of the plant so that more leaves will be produced. The maximum production of SGs in the leaves occurred just before or during the formation of flower buds (Kang and Lee 1981). Young leaves contained more SGs than senescent leaves (Jain et al. 2014). Moreover, rebaudioside-A and stevioside contents were highest when 50% of the plants harvested was at the flower bud stage (Kumar et al. 2011). Stevia has been established to be a short-day plant whose flowering is induced at photoperiods shorter than 13-h (Metivier and Viana 1979; Valio and Rocha 1977), thus altering the photoperiod becomes a useful means of prolonging the vegetative growth of the plant. The exposure of plants to long-day conditions was proven to delay flowering thus resulting in the increase leaf biomass and steviol glycoside accumulation in stevia by as much as 50% (Metivier and Viana 1979; Ceunen and Geuns 2013). In the past, research on stevia dealt with the effects of photoperiod on steviol glycoside accumulation (Ceunen and Geuns 2013; Zaidan et al. 1980; Metivier and Viana 1979; Valio and Rocha 1977).

The daylength under Philippine conditions renders a constantly favorable conditions for the flowering of stevia since it does not exceed 13 h. In this study, cultural intervention such as prolonging the day length to delay flowering to increase SG production was investigated under local conditions. It is known that growing this plant under long-day (LD) condition prolongs vegetative growth. If stevia plants will be exposed to long-day condition, flowering process can be delayed and its vegetative growth will continue, this could result to greater leaf mass and SG yield (Metivier and Viana 1979; Ceunen et al. 2013, Hossain et al. 2017). The supplementation of light was reported to easily modulate photoperiod by using additional electrical energy required to artificially extend the photoperiod to create a long-day environment (Ceunen et al. 2012, Yoneda et al. 2017). The study sought to determine the influence of different photoperiods on SG accumulation under *in vitro* and *ex vitro* conditions.

MATERIALS AND METHODS

The study was conducted at the Crop Physiology Division, Institute of Crop Science, College of Agriculture and Food Science, University of the Philippines Los Baños (UPLB), College, Laguna, Philippines from August 2015 to February 2016.

In vitro plants. Under *in vitro* conditions, *Stevia rebaudiana* Bertoni plantlets, from the Macapuno Laboratory, University of the Philippines Los Baños, were established initially in Murashige and Skoog's (MS) basal medium following the protocol of Zara et al. (2014). After one month of exposure to continuous light, these were grouped according to size and extent of shoot proliferation and then distributed to the different photoperiod treatments, namely 11-, 13-, 15- and 24-h light. The experiment was laid out in Complete Randomized Design (CRD) having three replications with 15 samples per replicate. The cultures were maintained in a room temperature at 25ºC for 5 months without subculturing. The number of days to first sighting of floral bud formation, flower opening, and percentage flowering were recorded. Data on the number, length, and weight (fresh and dry) of shoots as well as the number and length of roots were collected at the termination of the experiment.

Ex-vitro plants. For the *ex-vitro* experiment, stevia cultures maintained in 15-h photoperiod with well-developed roots were taken out of the culture room and were acclimatized by gradually exposing to ambient room conditions for five days; these were then transferred to the greenhouse gradually exposing to sunlight and removing the cover of the bottles. On the day of transplanting, plantlets were taken out of the culture bottles and the roots were washed thoroughly with tap water to remove traces of the agar medium. The plants were dipped for 2 seconds in 2g/L mancozeb fungicide (Dithane M-45) solution before planting in small polyethylene pots (4x4x7 in) containing garden soil, carbonized rice hull and coconut coir dust mixture (1:1:1 v/v/v). To prevent desiccation, the potted plants were covered with clear polyethylene bags and maintained under intermittent mist, thrice a day. The plastic cover was removed after three weeks and the plants were transferred to bigger polyethylene pots (10x10x17 in) containing the same soil medium. The experiment was laid out in Complete Randomized Design (CRD) having three replications with 10 samples per replicate.

Similar to the *in vitro* experiment, the plants were subjected to the different photoperiods: 11 hr-,13hr-, 15-hr light and natural daylength as control (equivalent to 12.6-h) (Dateandtime.Info.2108). To simulate the different photoperiods, the plants were placed under structures with opaque cover except for the control; during daytime, the cover was removed to allow the plants to be exposed to natural light. Artificial lighting provided by 40W Philips single fluorescent tubes per level of culture shelves was used. The plants were fertilized with 4.18 gram urea per 5 L water every month and watered when needed. Insect infestation (e.g leaf worms, aphids) was controlled manually through hand picking if needed.

The shoot length, number of leaves and biomass production of the plants grown *ex vitro* were determined after five months. The number of days for the appearance of the first flower bud and percentage flowering were also recorded.

Steviol glycoside (SG) analysis. Samples of the shoots, leaves and flowers from the *in vitro* and *ex vitro* experiments were collected, cleaned and air-dried under the shade for 24 h. The air-dried samples were dried further in an oven at 50 ºC for 16 h. The samples were pulverized immediately after drying using mortar and pestle and then stored in sealed polyethylene bags in the refrigerator at 4 ºC until further use. SGs in the powdered samples were extracted using ethyl alcohol (70%, v/v) (Kolb et al. 2001). The extracts were analysed for presence of stevioside and rebaudioside-A through HPLC according to the protocol established by the FAO (2010). Chromatographic conditions of the two columns (Capcell pak C18 MG II- Shiseido Co.Ltd. or Luna 5µ C18(2) 100A (Phenomenex) or equivalent (length: 250 mm; inner diameter: 4.6 mm, particle size: 5µm)} were used to test the most suitable for the analysis of SGs stevioside and reb-A present in the dried samples to be able to achieve a clear separation of the two SGs. Analyses were done at the National Institute of Molecular Biology and Biotechnology (BIOTECH), UPLB.

Statistical analysis. All data were analyzed by using the computer software Statistical Tool for Agricultural Research (STAR) 2013 software based on CRD design employing ANOVA then followed with a post hoc LSD test at 0.05 alpha level.

RESULTS AND DISCUSSION

Effects of photoperiod on *in vitro* grown plantlets. The effects on the flowering of stevia *in vitro* to the different photoperiod treatments became evident at two months exposure. The relatively few number of shoots developed under short photoperiods induced the elongation of these shoots as observed in plants exposed to 11-h photoperiod. The number and length of shoots were showed direct relationship, wherein under relatively short photoperiods (11-13hr), less number of shoots emerged but with higher shoot length. These responses were continuously observed for five months incubation (Table 1 and Fig 1). Continuous 24h photoperiod resulted in significantly higher fresh shoot weight biomass allocation (about 19g plant^{-1}). There were no significant differences for fresh weight of roots regardless of the photoperiod treatments, except for that the 13h of light caused the plant to have significantly low fresh root weight (below 0.4g plant^{-1}). All plants formed roots; the more profuse the shoots in a culture bottle, the fewer and shorter roots formation was observed.

Table 1. Effect of photoperiod on growth and flowering response of *in vitro* grown stevia after 5 mo of incubation in Murashige and Skoog's (MS) medium.

Parameter	Photoperiod (h)			
	11	13	15	24
Number of shoots	17 b	17 b	17 b	22 a
Shoot length (cm)	31.69 a	30.04 a	28.96 a	17.05 b
No. of roots	86 a	72 a	72 a	70 a
Root length (cm)	16.89 a	11.15 b	11.63 b	6.52 c
Fresh weight of shoot (g plant^{-1})	14.41 b	13.44 b	12.47 b	18.86 a
Fresh weight of roots (g plant^{-1})	0.45 a	0.35 b	0.48 a	0.44 a
Dry weight of shoot (g plant^{-1})	1.39 a	1.18 a	1.22 a	1.45 a
No. of days to flowering	79	-	-	-
Percentage flowering (%)	2	NF	NF	NF

* Means followed by the same letter per row are not significantly different at 5% level of significance based on LSD test
**NF-No Flower

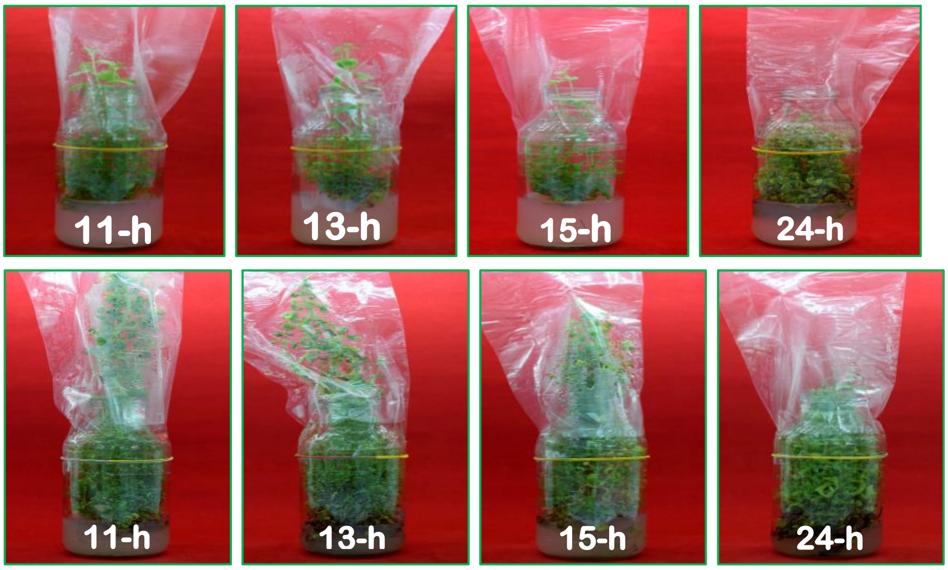

Fig. 1. Stevia shoots exposed to various photoperiods for 1 mo (top) and 4 mo (bottom) under *in vitro* conditions.

Only the shortest photoperiod of 11-h induced flower bud formation at 2% while the first sighting of floral bud formation was observed at 79 d following the photoperiod treatment (Fig. 2). The flowering shoots had noticeably shorter internodes and rosette arrangement of leaves than the non-flowering shoots. So far, no similar reported study on *in vitro* flowering of stevia by other researchers were found.

Fig. 2. Flower buds observed in stevia plants after 79 d of exposure to 11-h photoperiod under *in vitro* conditions. Photographed using camera macro lens (left) and microscope with 20x magnification (right).

Tissue culture-derived plants grown *ex vitro.* Tissue culture-derived plants were established in the greenhouse and exposed to the different photoperiod treatments. Tissue-cultured stevia exposed to the natural day length for 3 mo significantly attained the highest shoot length (44.68 cm) compared to the plants subjected to 11-h, 13-h and 15-h photoperiods (Fig. 3 and Table 2). In terms of biomass production, the fresh weight of stevia shoots grown under the natural daylength was statistically higher than those of the three photoperiod treatments; the lowest values for these parameters were recorded in plants maintained at 11-h photoperiod. All tissue-cultured stevia plants exposed to the different photoperiods flowered after 40-57 d except those kept at 15-h photoperiod where flowering was completely inhibited. This indicates that tissue cultured plantlets have the capability to develop into mature plants that can perceive both inductive and non-inductive photoperiodic condition. Flowering was earliest at the shortest photoperiod of 11-h.

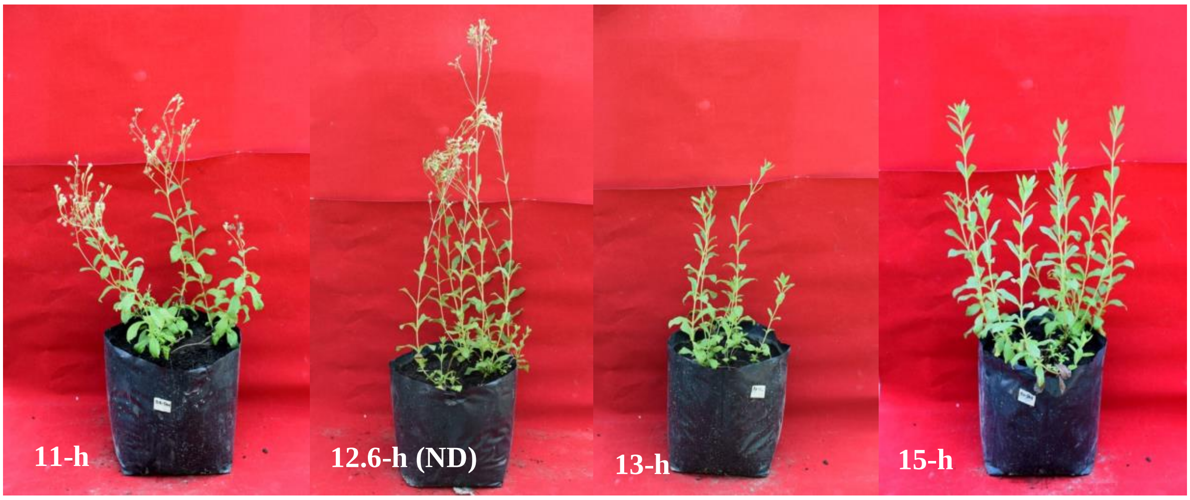

Fig. 3. Tissue culture-derived stevia plants exposed to different photoperiods for 3 months under greenhouse conditions. (*ND -natural daylength)

In matters of the number of flowers, the plants exposed to the shortest photoperiod of 11-h had the tendency to produce the most number of flowers, but statistically comparable to those maintained under the natural daylength (12.6-h), the least was at 13-h photoperiod (Table 2). The significantly low number of flowers obtained in 13-h photoperiod could be due to the few flowering shoots produced by the plants under this treatment. All three photoperiods, 13-h, 11-h and natural daylength (12.6-h) induced stevia plants to flower but not the 15-h photoperiod. Similarly, a photoperiod of 14 h inhibited floral initiation of stevia *(Piquerria trinervia*), with a 16 h photoperiod maintains the plants in a vegetative condition (Healy and Graper 1989). Under the local condition, the prevailing natural daylength is approximately 12.6-h, which is conducive for stevia flowering. Moreover, it was noted that the number of flowering shoot tips and percentage flowering per plant significantly decreased with increasing photoperiod up to 15-h. However, in the experiment, a tissue culture derived stevia was used.

Table 2 . Effect of photoperiod on growth and flowering response of tissue culture-derived stevia after 3 months of culture in pots under greenhouse conditions.

Parameter	Photoperiod (h)			
	Natural daylength (12.6)	11	13	15
Plant height (cm)	44.68 a	38.09 b	37.53 b	40.13 b
Number of leaves	178 b	143 bc	127 c	227 a
Fresh weight of shoot (g plant^{-1})	9.91 b	8.76 b	7.84 b	14.54 a
Fresh weight of leaves (g plant^{-1})	4.21 b	4.13 b	4.37 b	7.23 a
Dry weight of shoot (g plant^{-1})	2.49 b	2.10 bc	1.81 c	3.50 a
Dry weight of leaves (g plant^{-1})	1.23 b	1.15 bc	0.98 c	1.97 a
No. of days to flowering	54 b	40c	57 a	NF
Percentage flowering	97	100	100	NF
No. of flowers	53 a	59 a	24 b	NF
Fresh weight of flowers (mg plant^{-1})	610 a	490 b	300 c	NF
Dry weight of flowers (mg plant^{-1})	190 a	200 a	110 b	NF

* Means followed by the same letter per row are not significantly different at 5% level of significance based on LSD test
** NF - no flower

Steviol glycoside accumulation. Under local screenhouse conditions, the daylength conditions throughout the year is naturally inductive for the flowering of stevia and this limits leaf yield, which is responsible for the bulk of steviol glycosides (stevioside and rebaudioside-A) that may be harvested from the plant. The SG content of stevia shoots exposed to the different photoperiods after 5 months of incubation in MS medium was below 1% and comparable among treated plants (Table 3). Stevioside content ranged from 0.83% - 0.90% while rebaudioside A amounted to only 0.02% -0.42% in shoots exposed to 24h ,13-h, and 15-h photoperiod, respectively. Rebaudioside-A was not detected in the shoots maintained at 11-h. The result indicated that *in vitro* grown stevia had comparable level of stevioside and rebaudioside-A regardless of the length of photoperiod used in the experiment.

Table 3. Effect of photoperiod on steviol glycosides content (percent) of stevia shoots after 5 mo of incubation in Murashige and Skoog's (MS) medium.

Steviol glycoside	Photoperiod (h)			
	11	13	15	24
Stevioside	0.89 ± 0.014	0.90 ± 0.010	0.89 ± 0.010	0.83 ± 0.009
Rebaudioside A	ND	0.39 ± 0.026	0.42 ± 0.016	0.02 ± 0.003

* ± SD
** ND – none detected

When *in vitro* plantlets were potted out and allowed to develop under greenhouse conditions (Table 4), higher SG content of the leaves was obtained compared to the plantlets that were continuously maintained under *in vitro* conditions (Table 3). Furthermore, in this experiment, it was found that photoperiod influenced SG accumulation in stevia leaves and flowers. The highest leaf stevioside (6.62%) and rebaudioside-A (3.84%) contents were obtained from plants exposed to 15-h photoperiod while the lowest values (4.85% stevioside and 2.97% rebaudioside A) were recorded at 11-h photoperiod. The result indicated that exposure of tissue culture-derived stevia plants to 15-h photoperiod successfully inhibited flowering and increased level of the SGs. Ceunen and Geuns (2013) reported that long-day photoperiod (≥14h) prolonged the vegetative growth in *S. rebaudiana* resulting in a greater amount of leaf mass and total SG accumulation. Moreover, Rajasekaran et al (2006) also recorded a highest stevioside contents in 1-month-old greenhouse leaves (64.80g steviolbioside kg^{-1} dried plant material) and *in vitro* leaves (0.99g rebaudioside-A kg^{-1} dried plant material). As expected, the SG content of plants exposed to natural daylength (12.6h) and 13-h were statistically not significant.

Table 4. Steviol glycosides content of tissue-cultured stevia exposed to different photoperiods after 3 mo of culture under greenhouse conditions.

Steviol Glycoside Content (%)	**Photoperiod (h)**			
	Natural Daylength (12.6)	**11**	**13**	**15**
Leaves				
Stevioside	5.98 ab	4.85 c	5.12 bc	6.62 a
Rebaudioside A	3.19 b	2.97 b	3.08 b	3.84 a
Flowers				
Stevioside	3.24 b	2.57 b	5.64 a	NF
Rebaudioside A	1.40 ab	0.97 b	2.10 a	NF

*Means followed by the same letter per row are not significantly different at 5% level of significance based on LSD test
** NF – no flowers

None of the plants kept under 15-h photoperiod flowered until the 5th month. These results provide additional evidence that indeed stevia is a short-day plant with a critical daylength of 12-13h (Silva de Andrade et al. 2021). Stevia is indeed an obligate short-day plant, as suggested earlier by Mohede and van Son (1999) since the plants kept under 15-h, *in vitro* and *ex vitro,* remained vegetative up to five months.

CONCLUSION AND RECOMMENDATION

Overall, flowering of stevia under *in vitro* condition was not induced but if grown under *ex vitro* conditions, tissue culture-derived plantlets flowered under short day photoperiods at 11-h, 13-h and natural daylength (12.6-h). However, if plants are subjected to longer photoperiod (15-h), flowering was successfully inhibited and increased the number of leaves thereby increasing their SGs content. The best photoperiod period condition with the highest stevioside (6.62%) and rebaudioside-A (3.84%) content for *ex vitro*-grown stevia from tissue culture was in the stevia leaves grown under 15-h photoperiod.

Relevant studies assessing the presence of other SGs aside from stevioside and rebaudioside-A as well as antioxidants in both *in vitro* and *ex vitro* grown stevia may be considered. Moreover, night-interruption may be applied to the treatments used in the experiment to determine its effect on flowering and leaf biomass.

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DIVERSITY AND COLONIZATION OF ENDOPHYTIC MYCOFLORA AND STEM END ROT PATHOGEN, *Lasiodiplodia theobromae* [PAT.] GRIFF. AND MAUBL. IN MANGO cv. CARABAO

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ABSTRACT

The endophytic mycoflora harbors pathogenic and non-pathogenic fungi that affect the postharvest disease occurrence. This study explored the endophytic mycoflora in mango cv. Carabao that may affect the colonization of *Lasiodiplodia theobromae* [Pat.] Griff. and Maubl. Sequential sampling and isolation of pre-flowering, inflorescence, and fruit panicles of mango trees in dry and wet season production revealed the association of *L. theobromae* and eight other fungal genera with a total of 559 endophytic isolates. High endophytic mycoflora composition during the wet season by which *Penicillium* and *Aspergillus* exhibited the highest colonization frequency, possibly affected the endophytic colonization of *L. theobromae*. Fungal endophytes on 'Carabao' mango both for dry and wet seasons displayed low diversity (dry season-*H'*:1.93; wet season-*H'*:1.63). *L. theobromae* and endophytic mycoflora colonization showed positive correlation during the dry season and negative correlation during the wet season. Pathogenicity test revealed that endophytic *L. theobromae* isolates were pathogenic to mango fruits. This is the first report of endophytic colonization of *L. theobromae*, the dominant fungal pathogen causing stem end rot in mango cv. Carabao in the Philippines.

Key words: colonization rate and frequency, fruiting season, fungal diversity, pathogenicity

INTRODUCTION

Stem end rot (SER) is considered a major disease of mango similar to anthracnose. Preharvest fruit drop and postharvest rots are typical manifestations of SER disease. Infections remain latent at postharvest stage or until fruit senescence (Johnson et al. 1992b). The fungal rot is a postharvest disease with the typical rotting that initiates as brown to black discoloration at the stem end of the fruit. Rotting then progresses throughout the flesh of the fruit, thereby, affecting the quality, storage life, and transit of mango fruits leading to significant losses in mango production. Various fungal pathogens including *Dothiorella dominicana, D. mangiferae, Lasiodiplodia theobromae, Neofusicoccum* spp., *Phomopsis mangiferae, Cytosphaera mangiferae, Pestalotiopsis* sp., and *Alternaria alternata* are associated with SER disease (Alkan and Kumar 2018; Johnson et al. 1992a; Galsurker et al. 2018). In the Philippines, *L. theobromae* is known to be the main causal pathogen of SER. This fungal pathogen along with *Colletotrichum gloeosporioides* were consistently isolated in mango fruits sourced from major mango producing areas in the country (Lacambra 2005; Portales 2008). *L. theobromae* colonizes mango pedicels and inflorescences endophytically and eventually colonizes the fruit during ripening stage which results in SER infection (Johnson et al. 1992a; Diskin et al. 2017). Postharvest losses in mango do not result from infection at flowering or fruit set since fruitlets infected at this time are aborted (Johnson et al. 1992b).

Aside from the colonization of *L. theobromae*, several non-pathogenic microorganisms including fungal endophytes colonize mango stem endophytically. These endophytes reside inside healthy plant tissues without causing evident damage on the host (Stone et al. 2000). Promoting plant growth and providing protection against pathogens through different mechanisms are some of the benefits of these endophytes. A study on rice endophyte diversity reported that the frequency of colonization between sites, seasons, and varieties were found to differ significantly, which they attributed to the antagonistic properties of endophytes against fungal pathogens (Naik et al. 2009). Since the pathogenic *L. theobromae* and the fungal endophytes co-exist in the host tissues, the diversity of fungal endophytes might affect the endophytic colonization of *L. theobromae*. However, to the best of our knowledge, there has been no studies conducted on the relationship between *L. theobromae* and the diversity of fungal endophytes in the different phenological stages of ‘Carabao’ mango. Microbial population prior to harvesting may affect the establishment of pathogens impacting on fruit quality (Bill et al. 2021). Thus, this study aimed to determine the correlation of diversity of fungal endophytes isolated from different growth stages to *L. theobromae* endophytic colonization in mango cv. Carabao in two fruiting seasons. Likewise, a pathogenicity test was carried out to determine if the endophytic *L. theobromae* isolates was able to cause SER disease in mango fruits.

MATERIALS AND METHODS

Sample collection. Experiments were conducted from 2018 to 2019 in a mango orchard in Los Baños, Laguna. Dry season coincided within the months of March to June while wet season occurred from October to January. Samples were collected in mango trees (10-20 years) at regular intervals in a mango orchard with moderate to high SER incidence. These mango trees were not sprayed with fungicides throughout the mango production. Two mango trees with uniform flowering were randomly chosen for the sequential sampling. Five healthy plant samples per tree were randomly collected at every growth stage (1: pre-flowering; 2: flowering; 3: fruit set; 4: young fruit (mungbean size); 5: fruit fill I (quail egg size); 6: fruit fill II (chicken egg size); and 7: late mature). These plant samples or panicles were divided into three parts particularly the top, middle, and bottom and for each part, five tissue segments (5mm long) were obtained for isolation. Tissue segments were processed employing the procedure of Johnson et al. (1992a) as adapted from Petrini (1986).

Isolation and identification. The tissue segments were triple sterilized (immersion in 95% ethanol for 60s, 2.5% sodium hypochlorite for 3 min and 95% ethanol for 30s) and inoculated in potato dextrose agar (PDA) amended with streptomycin sulfate (40ug/ml). The frequency of isolation of *L. theobromae* on tissue samples was noted. The endophytic fungi that were isolated in sequential sampling were characterized morphologically. The size, shape, and cultural growth of these fungal isolates were described. Each isolate was identified based on published literatures.

Colonization rate and frequency. Their occurrences in the sequential sampling were noted and correlated with *L. theobromae*. To determine the frequency of specific endophyte, present across all growth stages of mango, colonization frequency (CF) was calculated by the number of endophyte detections divided by the total number of segments for all growth stages multiplied by 100%. Fungal growth from the mango tissue planted onto the agar plate was considered an endophyte detection. To calculate the rate of colonization of all endophytes in a given growth stage of mango, colonization rate (CR) was determined as the number of detections of all endophytes divided by the total number of segments in that growth stage multiplied by 100%.

Diversity analysis. To determine the diversity of endophyte species, Shannon-Wiener diversity index was utilized using the formula:

$$H' = -\sum[(pi) * \log(pi)].$$

where pi was the proportion of endophyte of i-th species in the mango sample collections and was computed as pi = n/N. The n in the pi formula was the endophytes of a given species and N was the total number of endophytes in the mango sample collection. To compute for the Evenness, the formula used was:

$$E = H / \ln(k);$$

where H was the diversity value and k was the number of endophyte species.

Correlation analysis. To determine the effect of *L. theobromae* colonization to the fungal endophytes, Pearson correlation was computed for both wet and dry seasons using the fungal detection per growth stages. The correlation of *L. theobromae* and *Colletotrichum* sp., which incites anthracnose, was also calculated to determine if there is a positive or a negative correlation between the two pathogenic endophytes. The correlation of endophyte colonization and weather factors were also analyzed. Weather factors were correlated to endophyte incidence using the weather data from the Department of Science and Technology - Philippine Atmospheric, Geophysical and Astronomical Services Administration (DOST-PAGASA). The average temperature and rainfall (dry season: March to June 2018; wet season: October 2018 to January 2019) for each growth stage duration were used for the computation.

Pathogenicity of *L. theobromae* isolates. The *L. theobromae* isolates were subcultured in PDA for pathogenicity test. Asymptomatic mature ‘Carabao’ mango fruits were surface sterilized in running water and 70% ethyl alcohol. Fruits were pin pricked before inoculation. After airdrying, fungal growth was inoculated in mature mango fruits at nearly ripe stage (more yellow than green color in the peel). Inoculated fruits were incubated in moist condition by placing the fruits in blotter set-up. Lesion length was measured. Three fruits were inoculated per isolate. Re-isolation of inoculated fungal isolates was done. Colony and morphological characteristics of the re-isolated fungus were compared with the original culture. Means were compared through analysis of variance using least significant difference (LSD) (p-value < 0.05).

RESULTS AND DISCUSSION

Diversity and colonization analyses. Isolation from healthy plant tissues obtained a total of 559 endophytic fungal isolates in two mango fruiting seasons (dry season: 178; wet season: 381) belonging to 9 fungal genera (Table 1). These fungal endophytes belong to 9 fungal genera which include *Aspergillus*, *Colletotrichum*, *Curvularia*, *Epicoccum*, *Fusarium*, *Lasiodiplodia*, *Penicillium*, *Pestalotia*, and *Phomopsis*, which were all present in the dry and wet seasons except for *Curvularia*, *Epicoccum*, and *Phomopsis.* Among these fungal endophytes isolated, 4 genera are reported to cause SER namely *Lasiodiplodia*, *Phomopsis*, *Pestalotiopsis*, and *Dothiorella*. *L. theobromae* is considered the main causal organism of SER in the Philippines (Lacambra 2005; Portales 2008; Johnson et al. 1993). In some mango producing regions particularly in Asia and Australia, the main causal fungal pathogens were *Lasiodiplodia* and *Dothiorella* (Johnson et al. 1993). In Israel, the main pathogens causing SER are *Alternaria* and *Lasiodiplodia* (Diskin et al. 2017). This study presented only the mycoflora, however, it should be noted that various species of microorganisms including yeast and bacteria which may not be pathogenic are also present in the plant tissues (Galsurker et al. 2018). In addition, recovered major pathogenic fungal groups in mango including *Penicillium*, *Alternaria*, *Botryosphaeria*, and *Fusarium* as well as beneficial bacteria were also previously detected in several fruits such as avocado and grapes (Bill et al. 2021).

Considering the CF, the wet season showed higher frequency value (36.29%) than the dry season (16.95%) (Table 1 and Fig. 1). In terms of individual endophytes isolated, *Penicillium* has the highest CF value (14.86%) followed by *Aspergillus* (14.19%) across two fruiting seasons. The most frequently isolated endophyte in the dry season was *Penicillium* with CF of 5.52% while the most prevalent fungal endophyte in the wet season was *Aspergillus* exhibiting 12.67% colonization. It is interesting to note that *Penicillium* and *Aspergillus* had high CF during the wet season. Ubiquitous nature of these endophytes accounted to this result. Isolation from multiple and various plant parts revealed various endophytes especially *Penicillium* (Nicoletti et al. 2014). *Curvularia, Epicoccum* and *Pestalotia* have the lowest CF (0.10%). Seasonal change might affect colonization rate thus, resulting to difference in CF between the two fruiting seasons (Lodge and Cantrell 1995).

In terms of fruiting season, the wet season showed higher CF of *Lasiodiplodia* (3.24%). than the dry season (2.38%) (Table 1). This finding contrasts with the common observation that the dry season normally has a higher incidence of *Lasiodiplodia* or SER incidence than the wet season. It has been reported that SER infection may be higher than anthracnose disease in drier areas (Department of Agriculture and Fisheries-Queensland Government 2021; CABI 2021). The result in this study, however, was based on endophytic colonization and not on disease incidence on mango fruits. Likewise, results also suggested a higher detection of *Colletotrichum* than *Lasiodiplodia* during the wet season. This result is consistent with the common observation that anthracnose infection is expected to be higher when mango production coincides with the wet season, although this study did not assess disease infection during the postharvest stage.

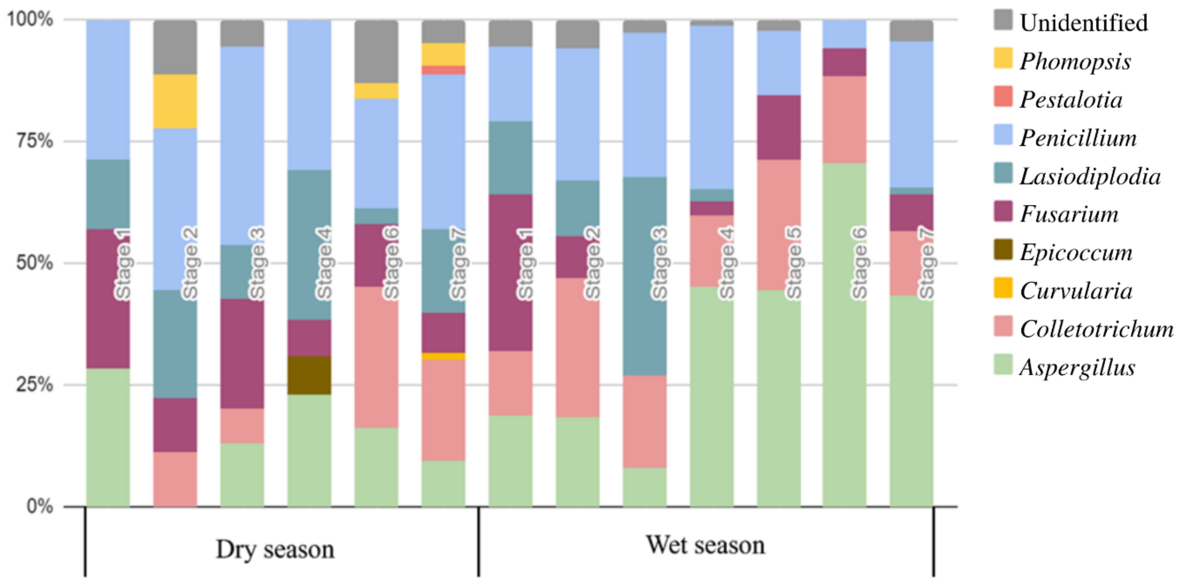

Fig. 1. Percentage of fungal endophytes detected during the dry and wet mango fruiting season 2018.

Table 1. Colonization frequency (CF) of endophytic fungi detected in mango cv. Carabao stem tissue from pre-flowering to late mature stages during the dry and wet seasons.

Endophytes	Dry Season									Wet Season								
	Growth stages*							Total detections	CF (%)	Growth stages*							Total detections	CF (%)
	1	**2**	**3**	**4**	**5**	**6**	**7**			**1**	**2**	**3**	**4**	**5**	**6**	**7**		
Aspergillus	2	0	7	3	0	5	6	23	2.19	10	13	3	34	20	24	29	133	**12.67*** **
Colletotrichum	0	1	4	0	0	9	13	27	2.57	7	20	7	11	12	6	9	72	6.86
Curvularia	0	0	0	0	0	0	1	1	0.10**	-	-	-	-	-	-	-	0	0.00
Epicoccum	0	0	0	1	0	0	0	1	0.10**	-	-	-	-	-	-	-	0	0.00
Fusarium	2	1	12	1	0	4	5	25	2.38	17	6	0	2	6	2	5	38	3.62
Lasiodiplodia	1	2	6	4	0	1	11	25	2.38	8	8	15	2	0	0	1	34	3.24
Penicillium	2	3	22	4	0	7	20	58	**5.52*** **	8	19	11	25	6	2	20	91	8.67
Pestalotia	0	0	0	0	0	0	1	1	0.10**	-	-	-	-	-	-	-	0	0.00
Phomopsis	0	1	0	0	0	1	3	5	0.48	-	-	-	-	-	-	-	0	0.00
Unidentified	0	1	3	0	1	4	3	12	1.14	3	4	1	1	1	0	3	13	1.24**
Total	**7**	**9**	**54**	**13**	**1**	**31**	**63**	**178**		**53**	**70**	**37**	**75**	**45**	**34**	**67**	**381**	

*Sequential sampling in different growth stages of mango cv. Carabao such as 1: pre-flowering; 2: flowering; 3: fruit set; 4: young fruit (mungbean size); 5: fruit fill I (quail egg size), 6: fruit fill II (chicken egg size), and 7: late mature.

**lowest CF

***highest CF

In terms of colonization rate (CR), stage 7 or the late mature stage of the mango fruit in the dry season and stage 4 or young fruit stage (mungbean size) in the wet season showed the highest CR value, 42% and 50%, respectively (Table 2). Stage 5 (fruit fill I - quail egg size) in the dry season (1%) and stage 6 (fruit fill II - chicken egg size) in the wet season (23%) exhibited the lowest CR value. These results imply that growth stages might not influence the colonization of fungal endophytes. Other factors including seasonal changes, weather factors, biochemical and physiological changes in fruiting stages, and mycoflora compositions possibly affect the endophyte colonization in mango.

Table 2. Colonization rate (CR) of endophytes isolated from various growth stages and fruiting seasons.

Growth Stages	Days After Flower Induction (DAFI)	Dry Season		Wet Season	
		Total detections*	CR (%)	Total detections*	CR (%)
Pre- flowering (1)	0	7	5.0 a**	53	35.0 a
Flowering (2)	27-28	5	6.0 a	70	47.0 a
Fruit set (3)	30-35	9	36.0 a	37	25.0 a
Young fruit (4)	40-45	54	9.0 a	75	50.0 a***
Fruit Fill I (5)	50-55	1	1.0 a	45	30.0 a
Fruit Fill II (6)	70-80	31	21.0 a	34	23.0 a**
Late Mature (7)	110-120	63	42.00 a***	67	45.0 a
Total	-	178	17.0	381	36.0

*Fungal isolates obtained from mango tissues planted on the agar media plates.
**Lowest CR
***highest CR

However, the differences in the CR for both seasons and for the seven (7) growth stages were not significant (Table 3). Endophytic outgrowth of SER fungi was reported in the inflorescence and mature stem tissues of mango (Johnson et al. 1993). Mango inflorescence had the richest fungal and bacterial communities at full bloom stage (Bill et al. 2021). Furthermore, a decline in fungal richness and diversity was lowest at the small size fruit stage similar to our observations. This finding can be attributed to the low sugar content in the pulp of immature fruit that gradually increases during fruit development (Quintana et al. 1984). Various physiological and biochemical changes during fruit development, such as activation of ethylene synthesis and other phytohormones, pH change, and decline of antifungal compounds might affect the mycoflora (Alkan and Fortes 2015). Likewise, this study presented an increase in fungal diversity at a late mature stage which is consistent with the findings of Bill et al. (2021).

The dry season (*H'*: 1.93) had a higher Shannon-Wiener diversity index (*H'*) value than the wet season (*H'*: 1.63) (Table 3). These diversity values were relatively low as species diversity commonly ranges from 1.5 to 3.5. Contrary to the species diversity value, wet season (0.84) has a higher Evenness (*E*) value than the dry season (0.91). These values strengthen the low diversity calculated for both fruiting seasons since evenness is inversely proportional to diversity. This contradicts to the previous findings that high fungal diversity is observed during wet or rainy seasons due to favorable conditions such as high humidity and low temperature (Mishra et al. 2012).

Table 3. Diversity of endophytes in the two fruiting seasons of mango cv. Carabao using Shannon-Wiener diversity index.

Indices	Dry season	Wet season
Shannon-Wiener diversity (*H'*)	1.93	1.63
Evenness (*E*)	0.84	0.91

Table 4. Correlation of *Lasiodiplodia theobromae* with fungal endophytes, and *Colletotrichum.*

	Lasiodiplodia	
	Dry season	**Wet season**
Endophytes	0.808	-0.461
Colletotrichum	0.675	0.095

Correlation analysis. The correlation between fungal endophytes and *L. theobromae* endophytic colonization showed contrasting results for both fruiting seasons. In the dry season, detection of *L. theobromae* showed positive correlation (0.808) to fungal endophytes colonization (Table 4). For instance, stage 7 (late mature stage) which showed the highest CR (42%) revealed that 11 isolates were *L. theobromae* and 52 isolates were endophytes. On the other hand, stage 1 (pre-flowering stage) having the lowest colonization rate (5%) obtained only one isolate of *L. theobromae*. All stages showed the same pattern in the dry season. However, the wet season showed a negative correlation (-0.461) between fungal endophytes and *L. theobromae* colonization. For instance, stages 4 (young fruit - mungbean size) to 7 (later mature fruit) showed relatively lower incidence of *L. theobromae* from 0 to 2 isolates, however, stages 4 (young fruit, mungbean size) and 7 (late mature fruit) had a CR of 50% (highest CR value) and 45%, respectively. Fruitlets of mango infected at flowering or fruit set are aborted (Johnson et al. 1992b). It is interesting to note that wet season displayed higher detection of endophytes than dry season. The negative correlation between *L. theobromae* and the fungal endophytes during wet season, and the high detection of fungal endophytes during this season probably is due to competition. Presence of dominating fungal endophytes including *Aspergillus* (CF: 12.67) and *Penicillium* (CF: 8.67) may have an adverse impact on *L. theobromae* endophytic colonization. Colonization by other endophytes and *C. gloeosporioides* in mango fruits from unsprayed trees could have delayed or prevented fruit colonization by SER fungi possibly due to competitive inhibition, antagonism, or induced host resistance (Johnson et al. 1992b).

Both fruiting seasons showed positive correlation (dry season:0.675; wet season:0.095) between the two pathogenic endophytes, *L. theobromae* and *Colletotrichum* (Table 4). This positive correlation indicates that both *L. theobromae* and *Colletotrichum* are co-colonizers and there is no competition between the two fungal endophytes. Both anthracnose and SER pathogens infect the stem end of mango fruit (Johnson et al. 1993).

Weather factors revealed consistent correlation of fungal endophytes to rainfall and temperature during the sample collection only for the dry season. Endophyte colonization and isolation rate showed positive correlation (0.21) to rainfall and temperature (0.22) during the dry season (Table 5). On the other hand, correlation of endophyte colonization to rainfall showed positive (0.32), however, its correlation (-0.32) to temperature revealed negative result during the wet season. These results signify that temperature and rainfall may influence the colonization and isolation rate of fungal endophytes especially during the wet season. However, considering other climatic conditions such as relative humidity, and moisture aside from temperature and rainfall might provide conclusive results as these conditions might affect also the endophytic colonization. Moreover, field experiments with various agro-climatic conditions is deemed necessary to further establish this assumption.

Table 5. Correlation of rainfall and temperature with endophyte colonization.

	Lasiodiplodia	
	Dry season	**Wet season**
Rainfall	0.21	0.32
Temperature	0.22	-0.32

Pathogenicity of *L. theobromae* isolates. Pathogenicity tests revealed that the *L. theobromae* isolates caused typical SER symptoms on mango fruits. Inoculation of these isolates caused dark brown to black rotting in ripe mango fruit (Fig. 2).

Fig. 2. Typical symptom of stem end rot during inoculation of endophytic *Lasiodiplodia theobromae* isolates in mango cv. Carabao fruits.

The mean lesion size induced by the isolates from various growth stages, ranged from 10.11mm to 32.08mm (Fig. 3). The dry and wet season isolates were confirmed to be pathogenic to mango fruit causing lesions ranging from 10.11 to 24.50mm and 12.50 to 32.08mm at 4 days after inoculation, respectively. No lesion size was indicated in the graph since no *L. theobromae* isolates were recovered during the fruit fill I in dry season as well as fruit fill I and II during wet season. *L. theobromae* isolates were successfully reisolated from these infected fruits. Re-isolated fungal cultures exhibited similar morphological and cultural characteristics with *L. theobromae.* This finding suggests that *L. theobromae* colonizes mango at various growth stages such that postharvest SER may occur if endophytic colonization reaches the stem end of the fruit. Results further confirmed reports that SER pathogens occur endophytically in mango. Fungicides and postharvest heat treatments may not easily eradicate the endophytic mycelium, however, the SER fungi occurring as pathogens could be limited by endophytes in the host plant (Johnson et al. 1993). The pathogenic capability of the isolates suggest that the fungus must be prevented or slowed down in reaching the stem end of the fruit before harvest to effectively control SER (Johnson et al. 1992b). This is the first report of endophytic colonization of *L. theobromae*, the dominant fungal pathogen causing stem end rot in mango cv. Carabao in the Philippines.

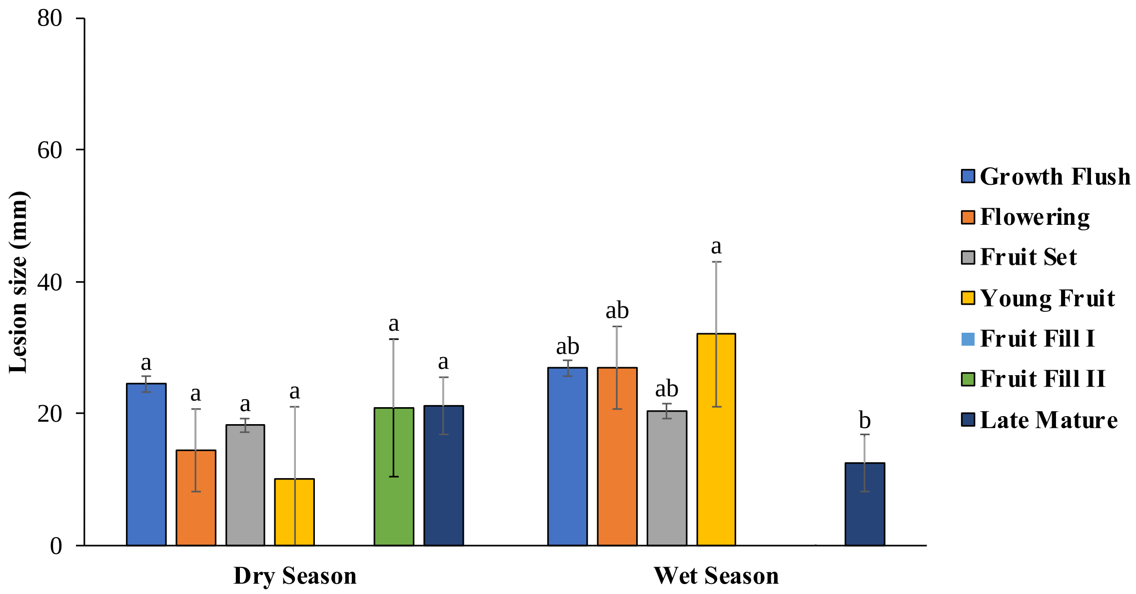

Fig. 3. Mean lesion size (mm) at 4 days after inoculation induced by endophytic *Lasiodiplodia theobromae* isolates from various growth stages in mango cv. Carabao fruits. Means with the same letter in each grouping are not significantly different in LSD (p-value = 0.05).

CONCLUSION AND RECOMMENDATION

Sequential sampling and isolation in pre-flowering, flower, and fruit panicles of mango trees in two fruiting seasons coinciding dry and wet season production obtained a total of 559 endophytic fungi belonging to 9 fungal genera. *Penicillium* (5.52%) and *Aspergillus* (12.67%) had the highest CF for the dry and wet seasons, respectively. Late mature fruit (dry season) and young fruit (wet season) displayed highest CR of 42% and 50%, respectively. Fruit fill I (dry season) and fruit fill II (wet season) exhibited the lowest CR value. Based on diversity index, both fruiting seasons obtained low diversity (dry season-*H'*:1.93; wet season-*H'*:1.63) of fungal endophytes.

Based on the data gathered in this study, wet season only affects the endophytic colonization of *L. theobromae* resulting to high endophytic mycoflora composition. Moreover, dry and wet seasons showed low diversity of fungal endophytes on 'Carabao' mango. Lastly, endophytic *L. theobromae* are pathogenic and can be a source of infection for SER in mango. The effect of application of systemic fungicide to the composition, diversity, and richness of fungal endophytes as compared to *L. theobromae* colonization is one of the future studies to explore. Lastly, the efficacy of fungal endophytes should also be studied to determine their potential as biological control agents against *L. theobromae* which can be utilized for disease management.

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OPTIMIZATION OF MYCELIAL CULTURE CONDITIONS AND FRUCTIFICATION OF *Ganoderma* SPECIES IN RICE STRAW-BASED SUBSTRATES

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ABSTRACT

Ganoderma (Basidiomycota, Ganodermataceae) species are saprophytic mushrooms that are widely known for their medicinal properties and are used in many Asian countries. This study focused on the effects of nutritional (culture media), environmental (pH, aeration, illumination, and temperature), and fructification of six *Ganoderma* species rescued from Luzon Island, Philippines. Mycelia of the tested *Ganoderma* species preferred coconut water agar as their basal culture media with pH levels ranging from acidic to slightly acidic medium (pH 5.0-6.0) while others were unaffected (pH 5.0-8.0). Aeration was not a major factor for three species whereas *Ganoderma applanatum* and *Ganoderma lucidum* strain 2 favored sealed conditions and *Ganoderma lucidum* strain 1 preferred unsealed. Light was proven to be beneficial for mycelial growth of *G. lucidum* strain 2 and dark conditions for *G. applanatum* though others were not affected. The majority of the *Ganoderma* species favored room temperature (30^{o}C) for optimum growth although *G. weberianum* preferred 23^{o}C. In conclusion, optimized and domesticated species/strains of *Ganoderma* showed variation among their fructification parameters and provide a data baseline for further studies.

Key words: conservation, domestication, Ganodermataceae, mushroom, sustainable production

INTRODUCTION

Diverse mushroom species can be found in temperate and tropical regions of the world and represent a large biodiverse group of organisms (Hawksworth 2001). Fungi play an important role as pathogens, decomposers, and symbionts in terrestrial ecosystems (Mueller et al. 2007). These are most visible at the start of the rainy season, but these can also be found in a variety of habitats and substrates, including rich soils, grassy ground, decaying plant litter, animal manure, and wooden logs in forests (López-Quintero et al. 2012). Over the years, mushrooms have been collected and consumed as a delicacy for their nutritional and medicinal properties (Nacua et al. 2018). These are low in calories and have a rich nutritional value with high protein content, vitamins, minerals, chitin, trace elements, cholesterol, and other bioactive mycochemicals (Wani et al. 2010). Medicinal activities can be observed among mushroom species, an example of which is *Ganoderma lucidum*. It contains triterpenoids, polysaccharides, mycins, steroids, and organic germanium (Ge) that contribute to its medicinal effects (Deepalakshmi and Mirunalini 2011; Taofiq et al. 2017).

The vast ecosystems in the Philippines provide a suitable habitat for various macrofungal species. Studies with regards to species listing, biodiversity, and distribution have been conducted on Luzon Island, the Philippines to identify the present mushrooms in the area. Common mushroom genera found in the wild include *Auricularia, Ganoderma, Lentinus, Marasmiellus, Phelinus, Pleurotus, Schizophyllum, Termitomyces,* and *Trametes* (Dulay et al. 2020; Licyayo 2018; Liwanag et al. 2017). *Ganoderma* (Ganodermataceae) are wood-rotting basidiomycetous fungi with a double-walled basidiospore that are common in tropical areas and have a close relationship to Asian traditional medicine (Donk 1946; Lin and Zhang 2004). The basidiocarp of this fungus has a laccate (shiny) or non-laccate (dull) pilear surface, and the basidiospores have an ovoid shape that is enlarged or truncated at the apex (Monclavo 2000). *G. lucidum* is a well-studied mushroom for its numerous bioactive compounds. It is mainly composed of polysaccharides, trace elements, fats, soluble proteins, and fiber, with domesticated varieties having similar nutritional values to wild types (Wasser 2005). Further studies show that several species of *Ganoderma* have antioxidant, antibacterial, and antidiabetic properties (Ma et al. 2015; Mohammadifar et al. 2020; Osińska-Jaroszuk et al. 2014).

Various *Ganoderma* spp. were documented, and identified from Luzon Island, the Philippines. Secondary mycelia of these mushrooms were successfully rescued and added to the culture collections of Philippine wild mushrooms at the Center for Tropical Mushroom Research and Development, Central Luzon State University. Knowing the bioactive compounds present in *Ganoderma*, it is of valuable interest to understand and determine the optimal culture conditions and fructification capabilities for mushroom biomass production that can lead to possible pharmacological utilization. This study sought to determine the optimal growth conditions of six collected *Ganoderma* species/strains.

MATERIALS AND METHODS

Evaluation of culture medium and pH. Four indigenous culture media namely: coconut water gelatin (CWG), potato sucrose gelatin (PSG), rice bran decoction gelatin (RBDG), and corn grit decoction gelatin (CGDG), and four commercially dehydrated media, such as potato dextrose agar (PDA), malt extract agar (MEA), Sabouraud dextrose agar (SDA) and mycological agar (MA) were used. Indigenous cultural media decoctions were prepared separately in 1L of distilled water using 250g of evenly sliced potato, 50g of rice bran, and 50g of corn grit. These decoctions were boiled separately until homogenized and strained to remove large particles and impurities. This mixture and coconut water were added discretely with 10% agar (local crude agar), 1% sucrose (for PSG, RBDG, and CGDG), and brought to boiling point to further homogenize the media. The dehydrated media were prepared according to the manufacturer's instructions: PDA; 39g/1000ml of distilled water (HIMEDIA), SDA; 65g/1000ml of distilled water (HIMEDIA), MA; 35g/1000ml of distilled water (HIMEDIA), and MEA; 33.6g/1000ml of distilled water (CONDA). All media were transferred into Erlenmeyer flasks with cotton plugs, sterilized in an autoclave at 121°C for 15 minutes, pour-plated, and solidified. Three replicates of the medium were prepared. A seven-day-old pure culture of secondary mycelia of the mushrooms was used. Each culture medium was aseptically inoculated with 10 mm-diameter mycelial discs centrally in the plated medium, sealed, and incubated at room temperature. The mycelial growth diameter of each mushroom species was measured every 24 hours for seven days and mycelial density was also recorded.

The optimum pH of the medium was determined once the optimal culture medium was established. Preparation of the media was done under the same procedure with the exception of adjusting the pH of the culture media from pH 5.0 to 8.0 at 0.5 intervals. Mycelial growth rate and thickness were also observed.

Evaluation of aeration, illumination, and temperature. The mycelial plate cultures with optimized medium and pH were subjected to three physical environmental factors, namely: aeration, illumination, and temperature, to elucidate the effects on the secondary mycelial growth and density. The plate cultures were incubated in two aeration conditions (sealed and unsealed), two illumination conditions [artificial light (322.92 lumens/m^2) and complete darkness], and three temperature conditions (6, 23, and 30°C). Three replicates were prepared for each condition. Mycelial growth rate and mycelial thickness were noted.

Grain spawn preparation. The substrate used for the spawn production of *Ganoderma* species was unmilled rice. Unmilled rice seeds were thoroughly washed and boiled in water until partial cracking of grains was observed, drained, and packed into 6 x 12 x 0.3 polypropylene bags (PP bags). The grains were then sterilized in an autoclave at 121 °C for 1 hour. An agar block of mushroom mycelia with the optimum culture conditions was then inoculated in sterilized grains and allowed for full mycelial ramification. After full ramification with mycelia, the grain spawns were used as inoculants for the production of mushrooms.

Fruiting body production. The main substrates used to determine the fruiting body production and bio-efficiency of *Ganoderma* species were rice straw and sawdust. Aseptically, 40 g of fully ramified mushroom grain spawns were inoculated into pasteurized fruiting bags (500g) using 6 x 12 x 0.3 (inches) PP bags containing the formulated rice straw-based substrate (7 rice straw: 3 sawdust v/v), composed of soaked rice straw for 5 to 7 days for partial degradation of lignin as well as other components and sawdust obtained from local lumber mills. These two were mixed and maintained a moisture level of 65% prior to pasteurization (60–100°C) for 8 hours, developed by the CLSU Center for Tropical Mushroom Research and Development (CLSU-CTMRD). Inoculated fruiting bags were then incubated at room temperature until the substrate is completely ramified by mycelia. The fully ramified bags were opened at one end to allow fruiting initials to develop into mature basidiocarps. Matured fruiting bodies were harvested, evaluated by measuring the pileus and stipe and weighed to determine the yield per bag and biological efficiency. Periods of incubation and primordial development were also recorded.

Statistical analysis. The data were analyzed using Analysis of Variance at a 5% level of significance and compared using Tukey's honestly significant difference and t-test.

RESULTS AND DISCUSSION

Culture media. In general, mushrooms require sources of carbon and nitrogen to facilitate normal metabolic processes essential for survival. The different *Ganoderma* species statistically favored CWG as their suitable culture media, with the capability to colonize the substrate rapidly (Table 1).

Table 1. Mycelial growth of *Ganoderma* species on different culture media, pH, aeration, illumination and temperature.

Factors	**Mycelial growth rate (day)**					
Culture media	*G. applanatum*	*G. gibbosum*	*G. australe*	*G. lucidum* strain 1	*G. lucidum* strain 2	*G. weberianum*
CWG	13.04±0.32^{ab}	15.00±0.00^{a}	15.00±0.00^{a}	12.28±0.55^{a}	12.86±0.00^{a}	15.00±0.00^{a}
PSG	8.21±2.34^{d}	10.74±0.60^{cd}	14.68±0.35^{a}	4.45±1.39^{f}	7.21±0.32^{cd}	8.83±1.20^{e}
CGDG	11.47±0.54^{bc}	10.01±0.17^{cd}	13.26±0.32^{b}	5.57±0.45^{ef}	6.33±0.35^{de}	12.34±0.52^{bcd}
RBDG	15.00±0.00^{a}	13.27±0.14^{b}	15.00±0.00^{a}	10.97±0.48^{ab}	9.53±0.85^{b}	13.76±1.47^{ab}
PDA	13.13±0.31^{ab}	11.05±0.38^{c}	14.44±0.45^{a}	8.37±0.49^{cd}	7.48±0.40^{cd}	10.65±0.11^{cde}
MEA	10.05±0.78^{cd}	7.58±0.28^{e}	13.05±0.72^{b}	7.01±0.75^{de}	3.69±0.76^{f}	10.25±1.10^{de}
SDA	14.54±0.40^{a}	9.82±0.78^{d}	14.61±0.39^{a}	10.03±0.91^{bc}	8.11±0.77^{bc}	13.13±0.66^{abc}
MA	9.75±1.20^{cd}	6.95±0.18^{e}	12.43±0.28^{b}	5.35^{e}±0.31^{f}	5.27±0.15^{e}	8.81±0.85^{e}

Factors	**Mycelial growth rate (day)**					
Culture media	*G. applanatum*	*G. gibbosum*	*G. australe*	*G. lucidum* strain 1	*G. lucidum* strain 2	*G. weberianum*
			pH			
5.0	13.50±1.49[ab]	12.84±0.41[c]	15.00±0.00[a]	11.87±0.44[a]	11.81±0.42[a]	12.30±0.03[ab]
5.5	12.37±0.49[bc]	14.27±0.44[ab]	13.47±0.38[b]	11.66±1.20[ab]	12.46±0.68[a]	12.55±0.15[a]
6.0	14.83±0.30[a]	13.97±0.04[abc]	13.32±0.32[b]	11.32±0.71[ab]	12.86±0.00[a]	12.29±0.05[ab]
6.5	9.58±0.00[d]	13.53±0.84[bc]	12.99±0.05[b]	8.75±0.84[ab]	11.98±0.97[a]	11.90±0.31[b]
7.0	11.23±0.39[cd]	14.77±0.11[ab]	13.42±0.19[b]	6.87±1.20[b]	12.27±0.46[a]	11.13±0.09[c]
7.5	10.42±0.71[cd]	14.46±0.56[ab]	11.26±0.13[c]	8.60±2.30[ab]	11.61±0.18[a]	10.75±0.33[c]
8.0	10.09±0.65[d]	15.00±0.00[a]	13.19±0.17[b]	8.86±3.36[ab]	11.52±0.08[a]	10.07±0.34[d]
			Aeration			
Sealed	18.00±0.00[a]	15.00±0.00[a]	12.78±0.14[a]	11.67±1.29[a]	15.00±0.00[a]	12.03±0.56[a]
Unsealed	14.58±0.75[b]	13.91±1.20[a]	12.39±0.81[a]	6.69±0.69[b]	13.01±0.56[b]	11.14±0.34[a]
			Illumination			
Light	4.73±0.77[b]	15.00±0.00[a]	12.86±0.00[a]	10.40±0.39[b]	15.00±0.00[a]	11.44±0.98[a]
Dark	18.00±0.00[a]	15.00±0.00[a]	12.86±0.00[a]	12.86±0.00[a]	13.50±0.15[b]	11.57±0.89[a]
			Temperature			
6°C	No Growth	No Growth	No Growth	No Growth	No Growth	No Growth
23°C	15.22±0.24[b]	14.26±0.66[a]	14.52±0.83[a]	14.93±0.12[a]	12.52±0.61[b]	12.51±0.08[a]
30°C	18.00±0.00[a]	15.00±0.00[a]	15.00±0.00[a]	15.00±0.00[a]	15.00±0.00[a]	11.90±0.27[b]

Mature coconut water contains minerals such as K, Ca, Na, Mg, P, Cu, and Fe (Uphade et al. 2008). These elements are necessary for enhanced optimum growth of mycelia and directly affect the yield of several mushrooms (Jonathan and Fasidi 2001; Siwulski et al. 2019). In addition, most of the soluble solids found in coconut water are sugars; glucose, fructose, and sorbitol (Shubhashree et al. 2014). These high concentrations of sugar provide the secondary mycelia with large amounts of energy for substrate colonization. While the aforementioned minerals and sugar constituents are present in other culture media used in the experiment, the distinct growth rates are accounted for by the concentration availability of these components from one medium to another.

Similar studies revealed *Ganoderma* species prefer coconut water gelatin as their basal medium for optimum growth and biomass production (Bellere 2018; Magday et al. 2014). The outcome of this experiment also showed that different species of the same genus can favor the same nutritional requirements. Likewise, *Pleurotus citrinopileatus, P. djamor,* and *P. salmoneostramineus* cultured in CWG had luxuriant growth and thick density (Jacob et al. 2015). The aforementioned findings support the results from this study that CWG is the best medium for the optimum growth of *Ganoderma* mycelia.

Effect of pH. Different species of *Ganoderma* in this study grew favorably in acidic to basic conditions. *G. applanatum, G. australe,* and *G. weberianum* had luxuriant mycelial growth on pH 5.0–6.0 indicating that these are pH sensitive while *G. lucidum* strain 1, *G. lucidum* strain 2, and *G. gibbosum* were not pH sensitive, hence having a broad pH range (Table 1). According to research, the optimal pH range for *Ganoderma* species is between 5.0 and 9.0 (Jayasinghe et al. 2008; Jo et al. 2009). Moreover, a Thailand strain of *G. australe* exhibited optimal growing conditions at pH 7.0–8.0 (Hyde 2017). This is not consistent with the pH 5.0 requirement of *G. australe* (Philippine strain) used in this study. This indicates that optimal pH conditions are species/strain dependent.

Influence of aeration. The presence or absence of air proves to be important for normal mycelial activity and growth. Table 1 shows no significant difference between *G. gibbosum, G. australe,* and *G. weberianum* under both aeration conditions (sealed and unsealed). Similar conditions were beneficial to three strains of *Volvariella volvacea* (Abon et al. 2020). *G. applanatum* registered the highest mycelial growth rate under sealed conditions. This response was observed in *G. lucidum*, *Lentinus strigosus* BIL 1324, and *Lentinus swartzii*, (Dulay et al. 2017; Dulay et al. 2021; Magday et al. 2014). Interestingly, the two strains of *G. lucidum* favored distinct aeration conditions, *G.lucidum* strain 1 under unsealed conditions and *G.lucidum* strain 2 under sealed conditions. This outcome is similar to the study of Kalaw et al. (2021) wherein sealed conditions was ideal for *Lentinus squarrosulus* strain 1 while *Lentinus squarrosulus* strain 2 grew luxuriantly in both sealed and unsealed conditions.

Influence of light. In the present study, mycelial growth of *G. lucidum* strain 2 statistically preferred lighted conditions while *G. gibbosum*, *G. australe*, *G. lucidum* strain 1, and *G. weberianum* were unaffected in both conditions shown in Table 1. *Chlorophyllum molybdites* and *V. volvacea* incubated under lighted and dark conditions displayed no discrepancies in their mycelial growth, indicating similar responses in other species as well (Abon et al. 2020; Garcia et al. 2020). Interestingly, *G. applanatum* favored dark environment as well as *Fomitopsis feei*, *L. swartzii*, and *L. strigosus* (De Leon et al. 2020; Dulay et al. 2020).

Influence of temperature. The results from this study imply that the *Ganoderma* species are grouped as tropical mushrooms due to statistically optimal growing conditions at 30°C. Likewise, two oyster mushroom species have the same optimal temperature of 28°C followed by 32°C and has improved growing conditions in autumn and summer seasons of tropical regions (Hoa and Wang 2015). However, *G. weberianum* has statistically optimal growth under air-conditioned (23°C). No growth was observed under refrigerated conditions (6°C) due to reduced enzymatic activity at low temperatures seen in Table 1.

The outcome of this study indicates that optimal temperatures are species/strain dependent. Other findings show that environmental temperatures between 20-30°C proved beneficial for the mycelial growth of *V. volvacea, P. salmoneostramineus, P. cornicopae, P. eryngii, P. giganetus, Laetiporus sulphureus* and *Lentinus conatus* (Akinyele et al. 2020; Karunarathna et al. 2014; Luangharn et al. 2014).

Fructification on rice straw and sawdust-based substrate. Different morphological parameters of the successfully cultivated *Ganoderma* species on rice-straw and sawdust-based formulation are shown in Table 2 and Figure 1. Various *Ganoderma* species respond differently upon cultivation on the same formulated substrate. *G. australe* and *G. gibbosum* registered the shortest incubation period with 21.3-21.7 days, followed by *G. applanatum*, *G. lucidum* strain 1, *G. lucidum* strain 2 and *G. weberianum* having 24.5-26.8 days. Although these mushroom species were grown under the same conditions, substrate colonization depends on the carbon and nitrogen concentration requirements of the species/strain (Hoa et al. 2015). Similar incubation periods can be observed in *Lentinus strigosus* (23.67 days) (Dulay et al. 2017). The variations may be due to the differences in temperature, species, and humidity (Suwannarach et al. 2022).

Fully ramified fruiting bags were opened at one end and the days to primordia formation or fruiting initials were observed. The two strains of *G. lucidum* displayed significant differences with strain 1 having the earliest primordia after 19.6 days and strain 2 after 41.1 days. This is followed by the primordia of *G. weberianum, G. australe, G. applanatum,* and *G. gibbosum* after 34.4, 37.9, 39.6, and 40.0 days, respectively. Subsequently, the opened fruiting bags with emerged fruiting initials were watered allowing the primordia to further develop into mature fruiting bodies. Matured fruiting bodies of the *Ganoderma* species grown on rice straw substrates were measured shown in Table 2. With regards to the cap diameter, *G. australe* measures 33.36 mm, *G. gibbosum* at 30.88 mm, *G. applanatum* at 28.10 mm, and *G. weberianum* at 25.6 mm. Interestingly, *G. lucidum* strain 1 had a wider cap diameter (32.70 mm) when compared with strain 2 (17.12mm) and was the narrowest among the species. In terms of the stipe, *G. lucidum* strain 1 demonstrated the longest stipe (14.21 mm) among the species. This is followed by *G. australe* (8.82 mm), *G. gibbosum* (6.96 mm), *G. lucidum* strain 2 (3.56 mm), *G. applanatum* (3.72 mm), and lastly, *G. weberianum* (3.14 mm).

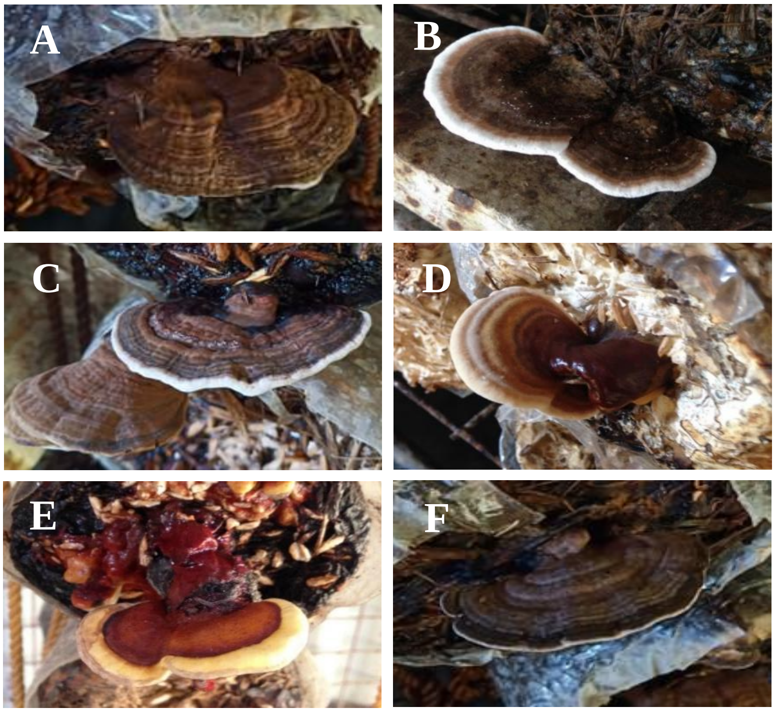

Fig. 1. Successfully domesticated *Ganoderma* species from Luzon Island, Philippines, *Ganoderma applanatum* AS4 (A), *Ganoderma gibbosum* AS10 (B), *Ganoderma australe* SS54 (C), *Ganoderma lucidum* strain 1 CPS6 (D), *Ganoderma lucidum* strain 2 C006 (E), *Ganoderma weberianum* DQS74 (F).

The yield and biological efficiency (BE) of the six culturally optimized *Ganoderma* species grown on rice-straw and saw dust formulation are displayed in Table 2. It can be perceived that *G. australe* had the lowest yield and a conforming BE while the highest recorded yield and BE among the *Ganoderma* species tested was that of *G. applanatum*. This was followed by *G. weberianum, G. lucidum* strain 1, *G. lucidum* strain 2, and *G. gibbosum*. These outcomes are very low when compared to a different cultivation method of *Ganoderma leucocontextum, G. resinaceum,* and *G. gibbosum* in soil casing layer (60.43% BE and 253.82 g/kg; 26.94% BE and 7.02 g/kg; 73.80% and 284.15 g/kg, respectively) and non-casing layer cultivation methods (13.60% BE and 58.18 g/kg; 109.26% BE and 27.75g/kg; 40.26% BE and 172.08g/kg, respectively) (Luangharn et al. 2020). *G. lucidum* grown in three different sawdust as basal substrates, on the other hand, yielded 15.05g/400gm with 15.69% BE, 1.5g/400gm with 0.512% BE, and no yield due to poor mycelial growth (Gurung et al. 2012). This indicates that the basal substrate affects greatly the development and morphology of fruiting bodies in mushrooms. The variation in the growth performance and morphology can be attributed to the genotype and physiological differences among the species and strains (Kalaw et al. 2021). Furthermore, different species of mushrooms require different carbon/nitrogen ratios in the substrate to obtain the highest yield possible (Kumla et al. 2020). Prevailing environmental conditions also affects the yield and morphological characteristics of the mushroom species (Chang and Miles 2004; Sher et al. 2010).

Table 2. Morphological characteristics of successfully cultivated *Ganoderma* species on rice straw-based substrate.

Mushroom Species	Incubation Period (days)	Days to Primordia Initiation (days)	Cap Diameter (mm)	Stipe Length (mm)	Yield per bag (g)	Biological Efficiency (%)
Ganoderma applanatum (AS4)	24.5 ±3.10	39.6 ±2.36	28.10 ±2.75	3.72 ±1.35	10.61 ±3.32	2.12 ±0.66
Ganoderma gibbosum (AS10)	21.7 ±1.25	40.0 ±2.0	30.88 ±3.73	6.96 ±1.69	7.00 ±1.15	1.40 ±0.23
Ganoderma australe (SS54)	21.3 ±1.25	37.9 ±2.38	33.36 ±6.96	8.82 ±1.68	4.58 ±1.69	0.91 ±0.34
Ganoderma lucidum strain 1 (CPS6)	24.6 ±1.51	19.6 ±0.97	32.70 ±2.96	14.21 ±2.71	8.7 ±1.63	1.74 ±0.33
Ganoderma lucidum strain 2 (C006)	25 ±2.44	41.1 ±1.66	17.21 ±2.36	3.56 ±1.13	7.3 ±2	1.46 ±0.4
Ganoderma weberianum (DQS74)	26.8 ±0.91	34.4 ±1.63	25.6 ±1.82	3.14 ±1.46	9.7 ±2.21	1.94 ±0.44

CONCLUSIONS

The optimal nutritional (culture media) and physical (pH, aeration, illumination, and temperature) requirements for the secondary mycelial growth of *Ganoderma* species and cultivation capacities using rice straw and sawdust-based substrate has been established. These findings prove that mycelia of *Ganoderma* species/strains respond differently when cultured under various conditions and favor different factors per species/strain. The incubation period, primordia initiation, cap diameter, stipe length, yield, and biological efficiency varied in performance among the species. *G. applanatum* exhibited the best cultivation performance among the species evaluated. However, further studies on the effect of substrate supplementation need to be investigated to improve the yield and biological efficiency of each species, especially for *G. australe* and *G. gibbosum*.

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DEVELOPMENT OF A PRACTICAL METHOD TO PRODUCE GABA RICH GREEN TEA BY VAPOR TREATMENT WITH trans-2-HEXENAL

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ABSTRACT

A practical method to produce GABA (γ-aminobutyric acid) rich green tea was developed using vapor treatment of *trans*-2-hexenal from 2017 to 2018 in Kanto district, Japan. Although the treatment with *trans*-2-hexenal in a closed plastic bag at 5°C for 4 hours failed to enhance GABA concentration in tea leaves, *trans*-2-hexenal vapor treatment in a ventilation system succeeded in enhancing GABA concentrations up to 8 to 10 times higher, or around 2 μmol/g. Flavor profiling by GC-O (gas chromatography-olfactometry) and GC-MS (gas chromatography-mass spectrometry) showed GABA rich tea produced by this method was found to possess slight strawberry and bean-like odors in addition to the original green tea flavor. Since *trans*-2-hexenal is a ubiquitous green odor generated from plant leaves and originally rich in tea leaves, this method can be regarded as a highly safe and environmentally friendly technique and might well contribute to the tea industry and tea market of Southeast Asian countries. It could also play an important role in promoting local public health as a functional tea to cope with stressful mental conditions.

Key words: amino acid composition, functional tea, metabolome analysis, ventilation system

INTRODUCTION

GABA (γ-aminobutyric acid) is known as one of the major inhibitory transmitters of the central nervous system (Bowery and Smart 2006) and has the potential to inhibit diabetic brain abnormality (Huang et al. 2013), promotes sleep (Cheng, et al. 2009), regulates blood pressure (Abe et al. 1995) among others. In Southeast Asian countries, tea cultivation has been one of the main industries historically, and easy and effective GABA rich tea production technique has long been earned, because GABA rich product market is now expanding all around the world (Horie et al. 2019).

Gabaron tea has been developed as a GABA rich semifermented tea produced by anaerobic treatment (Tsushida and Murai 1987), but it needs refinement to suppress unfavorable offensive odor (Hakamata et al. 1988). The vapor treatment of *trans*-2-hexenal treatment could enhance the amount of GABA in tomato fruit, thus the chemical was used to develop a new method to produce GABA rich green tea. Since *trans*-2-hexenal is a main natural constituent of tea leaf volatiles (Hatanaka and Harada 1973), it is quite acceptable to use this plant odor to produce GABA rich green tea.

MATERIALS AND METHODS

Treatment of tea leaves with *trans*-2-hexenal. Fresh leaves of tea, Yabukita cultivar of *Camelia sinensis* L., were harvested at Ishigami tea garden (112 Yoshikawa, Shimizu ward, Shizuoka city, Shizuoka prefecture) on May 2, 2017 at 10 o'clock in the morning and immediately treated with 0, 1, 10 and 100 ppm *trans*-2-hexenal vapor in an air-tight 600 ml glass jar which was prepared by evaporation from a filter paper on which a calculated amount of *trans*-2-hexenal was applied. After 1, 3, and 6 hours of incubation, tea leaves were exposed to liquid nitrogen and GABA was measured by GC-MS.

Method development. Two application methods were assessed to develop a practical method to produce GABA rich green tea with external application of *trans*-2-hexenal. This sought to determine whether GABA content in the tea leaves was enhanced, using a GC-MS. This was conducted at Okutomi-en on August 4, 2017 (36 Kazashi, Sayama, Saitama prefecture), and another experiment was conducted at Yoshida Cha-en on June 23, 2018 (1181 Ōtsutsumi, Koga, Ibaraki prefecture).

Fresh harvested tea leaves were placed inside a 70 liter polyethylene plastic bag and 7 g *trans*-2-hexenal were applied on paper filter to make the inside *trans*-2-hexenal concentration to set 100 ppm (w/v). After incubation for 3 or 4 hours, which was carried out at 5 ℃ to avoid browning, leaves were processed by steam heating, cooling, pressing, rolling and twisting, and drying. GABA content was determined by GC-MS with 5 replications.

Fresh tea leaves were harvested and treated with 0, 10 and 100 ppm *trans* -2-hexenal for 1, 3, or 6 hours inside a 3 m^3 steel container with ventilation system and covered with a blue plastic sheet. After the treatments, green tea leaves were processed in the same manner. The metabolome analysis including GABA was conducted with a GC-MS with 3 replications and flavor profiling was done with GC-MS and GC-O system.

GABA measurement and metabolome analysis with GC-MS. Metabolome analysis including GABA was conducted with GCMS-QP2010 Plus (Shimadzu, Japan), using an electron ionization, on a nonpolar phase column (DB-5, Agilent Technologies, USA) according to Yin et al. (2010) and Ijima and Aoki (2009) with some modifications. Each sample (0.1 g of frozen tissue powder) was extracted with 250 µl of methanol and chloroform, one after another. After adding 50 µl of 2.0 mg/ml ribitol solution as an internal standard and 175 µl of ultrapure water, the samples were vigorously mixed. These samples were centrifuged at 12000 rpm for 10 min at room temperature. Then 80 µl of the supernatant fluid of each sample was corrected into a 1.5 ml plastic tube. These samples were evaporated to dryness for 3 hours in a centrifuge evaporator (CVE-200D, Tokyo Rikakikai Co, Ltd, Japan). These samples were freeze-dried overnight using a lyophilization container (Modulyo 4K, Edwards, USA). For methylation, 40 µl of methoxylamine (20 mg/ml pyridine) was added to the samples and incubated for 90 min at 37℃ using a dry block bath (EB603, AS ONE company, Japan). Trimethylsilylation was performed by adding 50 µl of N-methyl-N-(trimethylsilyl)-trifluoroacetamide (MSTFA) solution for 30 min at 37 ℃. Helium gas was used as carrier gas at 2.0 mL/min. The initial column oven temperature was set at 100 ℃ for 4 minutes, then increased by 4 ℃ per minute until 320 ℃ for 10 minutes. Metabolites can be identified by comparing fragment patterns and retention indices with those of standard compounds in databases. The principal compound analysis (PCA) was done using the software Pirouette (GL science)

GC−O (gas chromatography-olfactometry) analysis. This analysis was performed using an Agilent 7890 A gas chromatograph (Agilent Technology, CA, USA) equipped with an olfactory port (OP275, GL Science). DB-FFAP and DB-5 columns (30 m x 0.32 mm i.d., 0.25 µm film thickness, J&W Scientific) were used. The samples (1.0 µL) were injected by the cold on-column method. The column temperature was held at 40 °C for 2 min, increased at a rate of 6°C/min to 230 °C, and then held at 230 °C for 5 min. Helium was used as carrier gas at a flow rate of 2.0 mL/min. The flow split ratio between the flame ionization detector and the olfactory port was 1:1. Linear retention indices (RI) of each odorant were calculated using n-alkanes C6−C26. The odors were determined by retention time and each separated odor was individually characterized by a trained specialist.

GC−MS (gas chromatography−mass spectrometry) analysis. An Agilent 7890 A gas chromatograph equipped with a 5975C mass spectrometer was used to identify each odorant. DB-FFAP and DB-5 columns (30 m x 0.32 mm i.d., 0.25 μm film thickness, J&W Scientific) were used. Samples (1.0 μL) were injected by the cold on-column method at 40 °C. The column temperature was held at 40 °C for 5 min, increased at a rate of 6 °C/min to 230 °C, and then held at 230 °C for 5 min. Helium was used as carrier gas at a flow rate of 2.0 mL/min. The ion energy for electron impact was 70 eV. The mass scan range was m/z 33–450. The compounds were identified by their GC RIs, which were calculated from the retention times in relation to those of a series of C6–C26 n-alkanes on DB-FFAP and DB-5 columns capillary column. The identification of compounds was performed by comparing their fragmentation patterns and RIs and co-injection with authentic compounds, if available.

RESULTS AND DISCUSSION

Vapor applications of *trans*-2-hexenal in a glass jar enhanced GABA content, around 8 -10 times, when treated with 10 or 100 ppm for 3 or 4 hours; the control tea leaves contained less than 0.1 μmol/g but 100 ppm *trans*-2-hexenal treatment achieved up to 2.0 μmol/g (Fig. 1). According to the PCA analysis (Fig.2), GABA, alanine, pyruvic acid and α-ketoglutaric acid were positively enhanced by *trans*-2-hexenal, while glutamic acid, glutamine and aspartic acid were decreased (proportion of the variance showed 86.4%). The metabolome shift (Fig. 3) indicated a similar tendency with the case of tomato fruit maturation under low O_2 conditions (Mae et al. 2012) and tea leaves treated by repeated anaerobic and aerobic incubation (Sawai et al. 2001). GABA is biosynthesized from α-ketoglutaric acid via glutamate by the activities of glutamate dehydrogenase and glutamate decarboxylase and catalyzed by the activity of GABA transaminase which is coupled with the formation of alanine. The enhancement of GABA by *trans*-2-hexenal vapor treatment might be derived by the activation of glutamate dehydrogenase, glutamate decarboxylase and the deactivation of GABA transaminase (Mei et al. 2016).

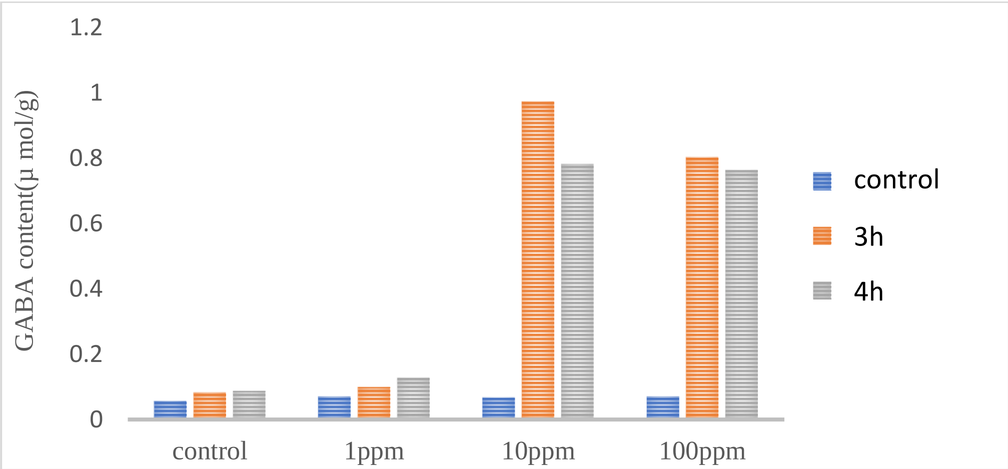

Fig. 1. Effect of *trans*-2-hexenal vapor treatment on GABA content in green tea leaves.

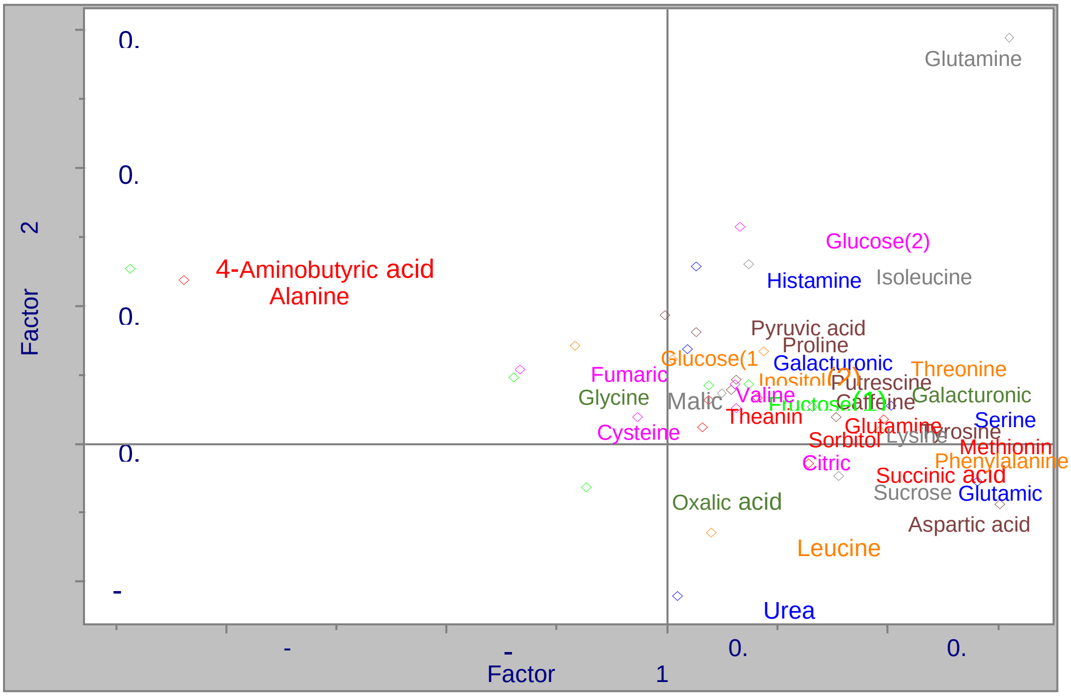

Fig. 2-1. Score plots on principal component analysis (PCA) of physicochemical parameters derived from metabolome analysis

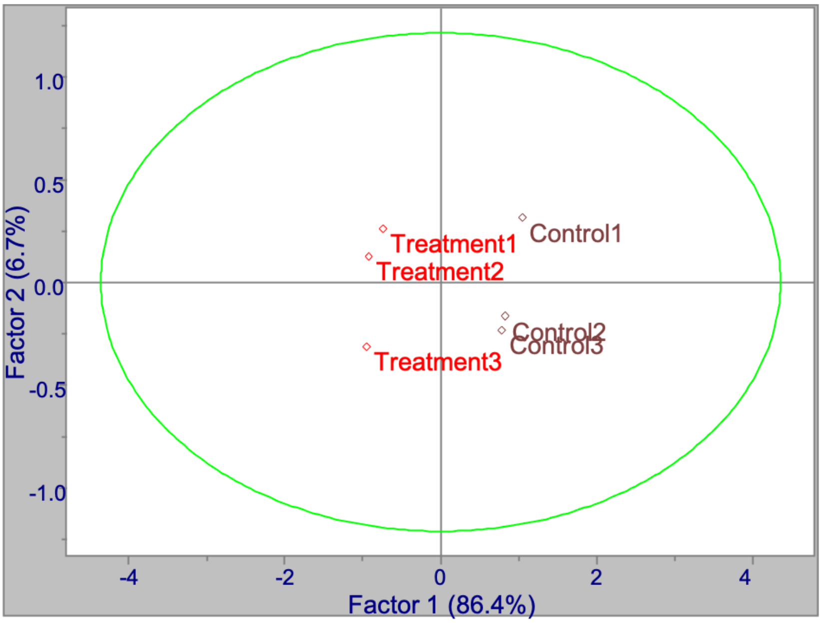

Fig. 2-2. Principal component analysis of control and 100 ppm *trans*-2-hexenal treated green tea leaves.

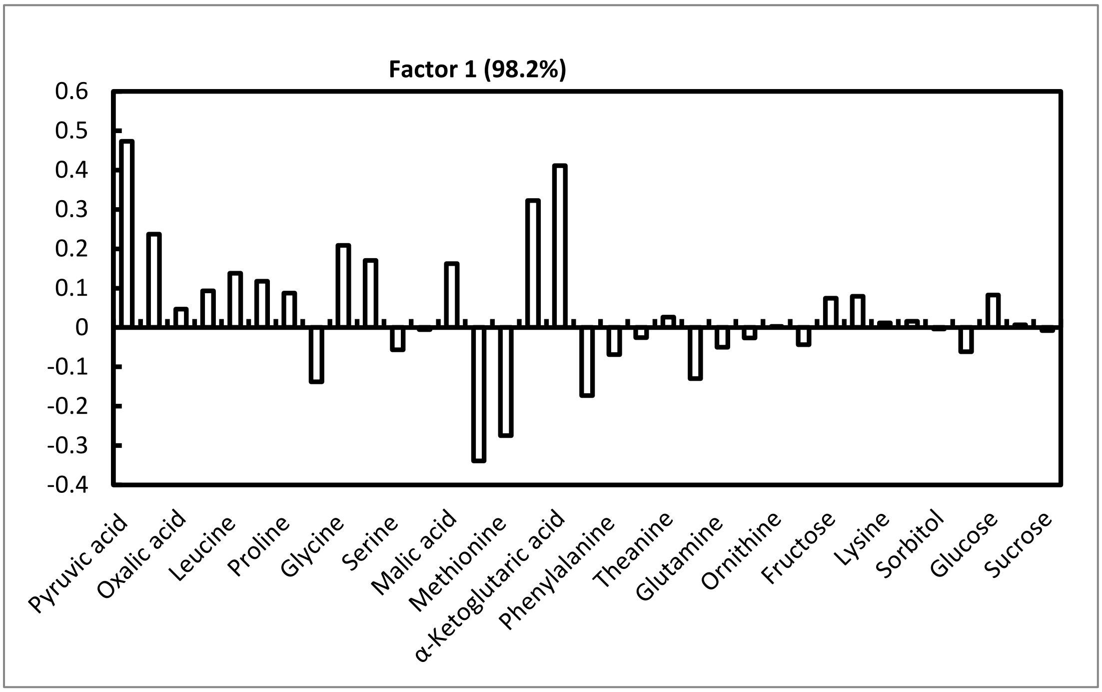

Fig. 3. Effect of 1 hour vapor treatment with 10 ppm *trans*-2-hexenal on metabolome of green tea leaves

As for the practical method to make GABA rich tea, the use of a closed system under low temperature failed to increase GABA (Fig. 4). The data suggested that a closed system might increase GABA content like a procedure to make Gabaron tea by repeated anaerobic and aerobic treatments. The failure might be due to the low temperature treatment which inhibited enzyme activities involved in GABA accumulation. When stored at ambient conditions, harvested tea leaves immediately lost water and started to brown, thus the closed method was not found to be practical.

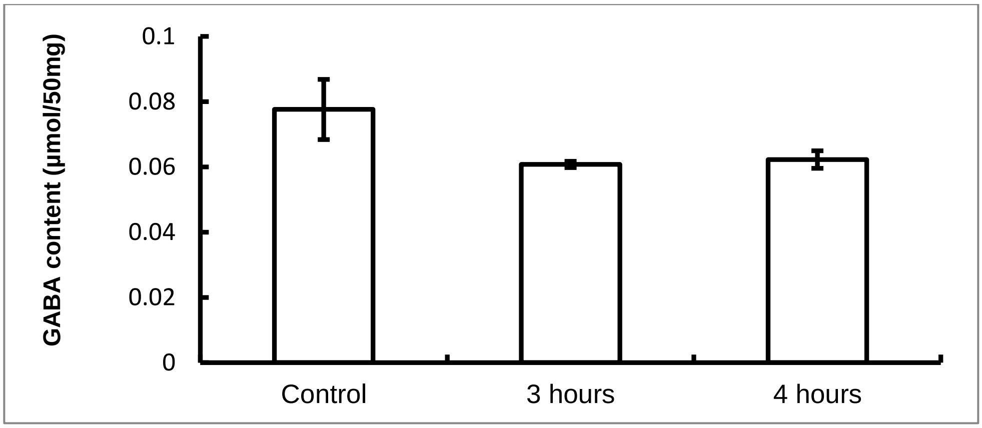

Fig. 4. GABA content of green tea in closed polyethylene plastic bag exposed to 10 ppm *trans*-2-hexenal vapor treatment.

On the other hand, the vapor treatment of 10 ppm *trans*-2-hexenal in a closed ventilation system succeeded in enhancing GABA content up to 2μmol/g, in which enzyme activities could be maintained (Fig. 5).

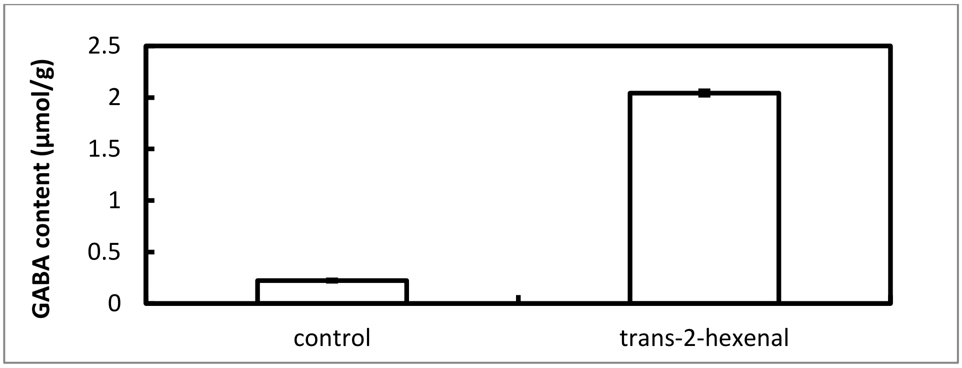

Fig. 5. GABA content of green tea exposed to10 ppm *trans*-2-hexenal vapor treatment in a closed ventilation system.

About the sensory characteristics of GABA rich green tea produced by this method, flavor profiling by GC-MS and sensory data are listed in Table 1 and Table 2. With the treatment with *trans*-2-hexenal, the odors like aldehyde, ketone, ester and acid groups increased, adding slight strawberry and bean-like odor on the basic green tea aroma, which might be acceptable to consumers judging from our taste test, judging from the specialists' reaction during the GC-O olfactory test.

The closed ventilation system produced more aldehydes than control which might be derived from the deactivation of alcohol dehydrogenase. This enzyme converts a variety of aldehydes into correspondent alcohols, but in cases when a substantial amount of *trans*-2-hexenal exists, other aldehydes might not be catalyzed, since the affinity of the enzyme is specifically strong for *trans*-2-hexenal (Eriksson 1968). Among such prominent aldehydes, *trans*-nonadienal might be the main attribute to exert bean-like smell (Kaneko et al. 2011). On the other hand, the strawberry-like odor might be attributed to γ-decalactone which only exists in *trans*-2-hexenal treated tea (Larsen et al. 1992).

Table 1. Flavor profile of GABA rich green tea exposed to 10 ppm *trans*-2-hexenal vapor treatment using ventilation method.

Retention time	Compound	Control	10 ppm *trans*-2-hexenal
12.886	undecane	○	○
14.956	1-methylethylbenzene	n.d.	○
15.976	2-hexenal	n.d.	○
17.442	tridecane	n.d.	○
17.756	1,2-diethyl-benzene	n.d.	○
17.978	1,4-diethyl-benzene	n.d.	○
19.178	1-methyl-2-benzene	n.d.	○
19.241	tetradecane	○	○

Retention time	**Compound**	**Control**	**10 ppm *trans*-2-hexenal**
19.763	(*E*)-2-hexen-1-ol	n.d.	○
20.784	1-ethenyl-4-ethyl-benzene	n.d.	○
21.209	pentadecane	○	○
23.076	hexadecane	○	○
24.851	heptadecane	○	○
25.712	4-ethyl-benzaldehyde	n.d.	○
26.268	4-ethyl-benzaldehyde	n.d.	○
26.539	octadecane	○	○
	heptadecane	n.d.	○
27.453	4-ethyl-benzoic acid	n.d.	○
27.618	hexanoic acid	n.d.	○
27.749	1-(4-ethylphenyl)-ethanone	n.d.	○
27.869	4-ethylbanzoic acid	n.d.	○
	3.5-dimethyl-benzoic acid	n.d.	○
28.160	eicosane	○	n.d.
	nonadecane	○	n.d.
28.406	1-(4-ethylphenyl)-ethanone	n.d.	○
28.948	phenylethylalcohol	n.d.	○
29.320	(*E*)-2-hexenoic acid	○	○
	2-hexenoic acid	n.d.	○
29.698	eicosane	○	○
30.738	*E*-15-heptadecenal	n.d.	○
33.020	14-methyl-pentadecanoic acid	○	n.d.
	hexadecanoic acid	○	○
33.717	2-(tetradecyloxy)-ethanol	n.d.	○
	2-(hexadecyloxy)-ethanol	n.d.	○
35.318	2-methoxy-4-(1-propenyl)-phenol	n.d.	○
36.101	16-methyl-heptadecanoic acid	○	○
	octadecanoic acid	○	○
36.377	19-methyl-heptadecanoate	n.d.	○
36.808	*trans*-13-octadecanoic acid	n.d.	○
	cis-13-octadecanoic acid	n.d.	○
36.808	16-octadecanoic acid	n.d.	○
36.812	indole	○	○
37.103	4-methyl-benzonitrile	n.d.	○
37.291	2H-1-benzopyran-2-one	○	○

Retention time	Compound	Control	10 ppm *trans*-2-hexenal
38.268	1,4,7,10,13,16-hexaoxacyclooctadecane	○	n.d.
39.187	vanillin	○	n.d.
39.889	1,4,7,10,13,16-hexaoxacyclooctadecane	○	○
42.370	tetradecanoic acid	n.d.	○
42.853	1.2-benzenedicarboxylic acid	n.d.	○

○ = detected; n.d. = not detected

Table 2. Sensory characteristics of GABA rich green tea exposed to 10 ppm *trans*-2-hexenal vapor treatment using the ventilation method.

Retention Index	Compound	Control	Treated	Aroma description
975	methylbutanoate	n.d.	○	yogurt
1110	methyl-3-methylbutanoate	n.d.	○	sweet
1130	2- methyl-pentane-1-thiol	n.d.	○	green
1240	(*E*)-6-decenal	n.d.	○	green, Matcha
1275	unidentified	○	n.d.	peanut
1290	1-octene-3-one	n.d.	○	mushroom
1300	propyl-2-methylhexanoate	n.d.	○	lemon
1330	Hexyl-2-methylpropanoate	○	n.d.	green
1360	dimethyltrisulfide	n.d.	○	pungent
1380	(*Z*)-3-hexenol	n.d.	○	green cut grass
1440	2-methyl-2,3-dimethylpyrazine	○	○	earthy
1440	hexyl-3-methylbutanoate	○	○	sweet
1470	5-nonene-7-one	n.d.	○	green, green tea
1510	2-isobutyl-3-methoxypyrazine	n.d.	○	cucumber, green pepper
1530	(*Z*)-4-decenal	○	○	green
1570	(*E*,*Z*)-2,6-nonadienal	n.d.	○	cucumber, green
1635	1-terpinene-4-ol	○	n.d.	green tea
1665	3-methylbutanoicacid	n.d.	○	sweaty
1700	geranial	n.d.	○	black tea
1735	(*E*)-2-undecenal	n.d.	○	green, oily
1800	(*E*,*E*)-2,4-decadienal	n.d.	○	green, fatty
1887	unidentified	n.d.	○	green tea
1900	2-phenylethanol	○	n.d.	honey, rose
1927	β-ionone	n.d.	○	rose, flowery

Retention Index	Compound	Control	Treated	Aroma description
1947	(*E*)-2-tridecanal	○	n.d.	green
1967	maltol	○	n.d.	sweet, cotton candy
1980	1-mercaptoctane-3-ol	n.d.	○	sweaty
2033	4-hydroxy-2,5-dimethyl-3(2*H*)-furanone	○	○	sweet, cotton candy
2120	4-hydroxy-5-methyl-3(2*H*)-furanone	○	○	sweet
2147	γ-decalactone	n.d.	○	peach, coconuts
2180	γ-(Z)-decenolactone	○	○	peach
2240	3-propylphenol	○	○	phenol, leather
2280	δ-undecalactone	○	n.d.	flowery,
2313	α-sinensal	n.d.	○	green, metallic
2333	2-furfuryl-2-methyl-3-furyldisulfide	○	n.d.	smoky
2461	coumarin	○	○	sakuramochi
2500	unidentified	○	n.d.	sweet
2550	phenylacetate	○	○	honey, rose
2580	vanillin	n.d.	○	vanilla
>2600	unidentified	n.d.	○	flowery
>2600	unidentified	n.d.	○	green tea

CONCLUSION

A method for GABA rich tea production was developed using vapor treatment with *trans*-2-hexenal. The vapor application of *trans*-2-hexenal could enhance GABA content 8-10 times higher if treated in a closed system with ventilation. GABA rich green tea produced by this method exerted slight strawberry and bean-like flavor. It has the potential acceptability to the market in Southeast countries.

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VALUE CHAIN ANALYSIS OF SALT IN THE VISAYAS REGION, PHILIPPINES

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ABSTRACT

Salt is important as food, food and feeds ingredient, and many more. With its economic benefits to the entire chain participants, it is imperative to study the value chain of salt in the Visayas Region, a major salt producer. A total of 222 supply chain participants (107 salt producers, 12 assembler-wholesalers, 6 wholesalers, 13 wholesaler-retailers, 24 retailers, 3 institutional buyers, and 57 household consumers) were interviewed in May to June of 2019. Four sets of pre-tested structured interview schedules and a key informant interview guide were used. Value chain mapping was performed along with quantitative and descriptive analyses. Results revealed that the solar evaporation of seawater as the common method of salt production caused its seasonal availability which is aggravated by the lack of appropriate storage facilities. Class A salt is more profitable for farmers to produce during peak months, but Class B was the more profitable during lean months. The retailers gained the highest profit relative to their marketing costs during peak and lean months and for both Class A and B salt, although they do not sell Class B during the lean months. There is strong horizontal relationships among chain participants, but farmers had a weaker relationship with their peers along the areas of price determination, seeking buyers, and knowledge on new technologies as they tend to compete with each other, limiting their ability to benefit from collective initiatives. Non-adherence to ASIN Law hampers the movement of salt to the target markets that are located beyond municipal boundaries.

Key words: Value chain mapping, solar evaporation, salt-making, salt quality specification, horizontal and vertical integration

INTRODUCTION

Sodium chloride or salt is an abundant, readily available, and inexpensive commodity found to be a basic requirement for life (Feldman 2011; American Chemistry Council 2020). It can be found throughout the world as mineral halite or as mixed evaporates in saline water. Its composition varies from lower values near the north and south poles to higher values closer to the earth's equator, and that, it is generally gray to white, but color varies depending upon the impurities present (Feldman 2011). Three techniques are being used in salt production, namely: (1) solution mining or the production of dry, crystalline sodium chloride from underground deposits using water followed by evaporation of the brine; (2) dry mining method or the direct extraction of the mineral halite from beneath the ground; and (3) solar salt harvesting (Morton Salt 2015). Salt-making in the Philippines is a pre-colonial industry with variations in the methods used throughout the island (Alcina 2004). However, generally, salt is produced through solar salt harvesting by the fisher folks and by coconut farmers who live in the country's shorelines (Yankowski 2019). Fisher folks are reported to be among the poorest in the country (Cervantes 2012) and among those who have the greatest number of children. Fishermen normally go out in the sea to fish while the women are left at home to do household chores and tend to the needs of the children. Thus, in salt making, it is highly possible that women and child labor are resorted to in the absence of the father-fisherman. The major requirements to produce salt are large areas of flat lands located near the brine source (e.g., the coast) with low rainfall rate, abundant sunshine, some winds to support optimum yields, and road network that is accessible to facilitate transportation of the produce (Feldman 2011).

Salt plays an important role in the Philippine economy as: (1) contributor to labor generation and poverty reduction; (2) having high local value-added product due mainly to its use of inputs like free sun and salt water, low-cost soil in shores and only the salaries and wages of salt farmers as the major cost item; (3) saver of foreign exchange; (4) key ingredient to major Philippine industries like food, coconut, dried fish, and other mixed-use industries; and (5) a key player in the elimination of iodine deficiency disorder (Philippine Chamber of Salt Producers 2009). Despite its important contribution, the domestic salt industry is losing its competitiveness. Industry reports that since 2000, the share of imported salt vis-a-vis the salt consumed in the country is increasing (Philippine Chamber of Salt Producers (2009), Yankowski (2019), Hontucan and Acedo (2017), Sadongdong (2017), Arnaldo (2017). The International Trade Center reported that the Philippines imported 80 percent or $24.4 M worth of salt in 2016 alone (Moran 2018). Among the perceived immediate effects of declining domestic salt industry because of increased salt importation are those related to the loss of employment and income redounding to escalation in poverty. It also would result to losses in foreign currency and may jeopardize salt-dependent industries like fish preservation, the coconut industry, food, and many other industries. It is only with the understanding of the interactions of participants in the domestic salt industry can the negative effects of declining industry competitiveness be mitigated and its eventual impact to salt farmers be reversed.

There is a dearth of literature concerning the Philippine salt industry. Its technology and organization have a deep-rooted relationship with the pottery industry, i.e., in Bohol, Philippines (Yankowski 2019). Profitability of small and medium scale enterprises in Misamis Oriental, Philippines was explored by Delos Reyes, et al. (2021). Further, salt farms and supply chain actors in the Visayas and Mindanao, Philippines were documented using GIS Maps (Bartolome et.al 2022). Also, the Nutrition Center of the Philippines surveyed the salt importers, producers, and traders in the Philippines for evaluation of internal and external quality assurance and control (NCP 2010). Other pioneering works document the industry status like the works of Alcina (2004), Philippine Chamber of Salt Producers (2009), and Sadongdong (2017). Given its many problems, several industry initiatives were recorded (Verdey and Abilay 2017). The response of the Philippine national government agency to calls from local government units (LGUs) asking for support for the declining salt industry in Mindoro Occidental were also documented (Verdey and Abilay 2017 and Tan 2020). An optional exemption from Salt Iodization Law that will benefit sea salt farmers who have lost their livelihood, industries that require sea salt in their recipes and products, and consumers who want to be able to choose between iodized and sea salt was proposed (Tan 2020).

With low supply of and high demand for salt (Neo 2019), potential for village-level salt processing business is very high making it imperative that a careful examination of the industry be done to see where the country has gone wrong and what can be done to improve the current situation, and maybe competitiveness. A jump-off to increasing the level of competitiveness of the domestic salt industry is to analyze it using the value chain approach. Value chain (VC) is defined as "the full range of activities which are required to bring a product or service from conception, through the different phases of production (involving a combination of physical transformation and the input of various producer services), delivery to final consumers, and final disposal after use (Morris 2000)." VC analysis can expose strategic and operational misalignments within chains, and the consequential misallocation of resources, hence, provides opportunities for improvement which create value and promote sustainability (Fearne & Martinez 2012). In development works, the value chain approach has been extensively used as a mechanism to foster economic growth. It probes on modalities where poverty groups can benefit from the increase in income through strengthening of markets that are relevant to the poor, improving the poor's access to markets, and/or by influencing the distributive outcome of the market processes (Springer-Heinze 2018). There is the necessity for firms to be competitive by becoming market-oriented (Grunert et. al. 1996) but to be effective in this endeavor, their value delivery network must be coordinated by a suitable governance structure (Elg 2008).

The value chain documentation of the Philippine salt industry is basically unknown. Given the importance of salt as food, as food and feeds ingredient, and the economic benefits it brings to the entire supply chain participants (from farmers to retailers), it is imperative to study the marketing structure, the supply chain, and the value specifications vis-à-vis the demand specifications by the market. Detailed data on the salt supply and demand situation including the value-adding activities and corresponding business enabling environment and support services will help a lot in upgrading the value chain and capacitating the chain participants. From the existing literature, little is known where value is gained or lost along the salt value delivery network and how value shares as well as the risks are distributed along the different nodes of the value chain. This study is an attempt to fill-in these existing gaps with its output seen as helping enhance the growth of the industry through market-led, reconfiguration. This will be useful for policy makers, regulatory agencies, industry participants, and other stakeholders.

METHODOLOGY

The study utilized both quantitative and qualitative approaches to achieve its objectives. The following key production areas were considered: Iloilo in the Visayas Region (Philippine Chamber of Salt Producers 2009), Guimaras, and Negros Occidental (Nutrition Center of the Philippines 2010). Representative municipalities from these regions were chosen based also on volume of production. Key officials from the Offices of the Provincial Agriculturist, Municipal Agriculturist, and the local government units (LGU) were consulted regarding industry participants to form the sampling frame.

Sampling size and selection of a total of 222 respondents varied by respondent type. For the 107 producers, simple random sampling of at least 30 per area (with substitution) was done, however, if the total population in one area is less than 30, complete enumeration was resorted to. The number of traders was determined by accumulating 80 percent of the total volume traded, which in this case was achieved for 58 traders. On the other hand, the number of household respondents was determined using the Slovin's formula at 95 percent confidence level:

$n = N/(1 + Ne^2)$ where:
n = number of samples
N = total population
e = margin of error (5%)

Respondents per demand area were proportionately allocated. Institutional buyers in each area were identified using snowball technique or tracing approach. These buyers purchase large volume of salt directly from the salt producers or traders and use it as an input for their businesses such as production of fish sauce (*patis*) and fish paste (*bagoong*), processing of salted dried fish (*daing*), among others. Ten of these buyers per demand area and non-food and food users of salt were interviewed (Table 1).

Primary data were gathered using pre-tested structured interview schedules and key informant interview guide. Three sets of interview schedules were developed: producers, traders, and household users. Key informant interviews of representatives from the local government units, business sector, and NGOs directly engaged in the management, production, regulation, policy development and implementation relevant to the salt industry were conducted.

The horizontal relationship was evaluated along the following five parameters: information sharing; collaboration to sell in bulk (e.g., prices, buyers, and practices); competition level; trust (e.g. management and selling); and benefits from collective initiative. Meanwhile, vertical relationships were evaluated for the following: procurement of supply; information sharing on technology and prices; quality control (e.g., texture, color, and cleanliness); and presence of value-added services. The strength of vertical and horizontal relationships among the participants in the value chain were assessed using four scales: 0 - No Relationship; 1 – Weak; 2 - Moderate; and 3 - Strong. Chain mapping was extensively used for visual representation of the value chain. Finally, quantitative data were analyzed using descriptive statistics, i.e., means, modes, frequencies, and percentages.

RESULTS AND DISCUSSION

Profile of respondents. The demographic profile of the study participants is shown in Table 2. Participants were grouped based on their role in the salt value chain - as producers, assembler-wholesalers, wholesalers, wholesaler-retailers, retailers, institutional buyers, and household consumers. Characteristics gathered include the participant's sex, age, civil status, educational attainment, and their respective number of years in the salt industry.

The salt industry is dominated by female players (63% vs. 37% for males). Males play a more leading role in production (56%) and wholesaling (67%) whereas the rest of the processes in the value delivery network are performed principally by female players, suggesting the critical role of women in the salt industry.

The age distribution of participants varied across the nodes in the chain. Their modal age was 41 to 50 years. Among producers, the modal age group was 51 to 60 years. For those involved in salt distribution, the modal age cuts across a wide range-- assembler-wholesalers (41-60 years old), wholesaling (31-60 years old), wholesaler-retailer (51-60 years old), and retailers (41-50 years old). Age range of institutional buyers is bimodal (41 to 50 years and 61 to 70 years).

Table 1. Distribution of 222 salt supply chain participants, Visayas, Philippines, 2018

Location	Type of Supply Chain Participant														TOTAL	
	P		A-W		W		W-R		R		IB		HC			
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Iloilo	18	12	1	6	1	14	4	24	5	17	2	33	57	38	88	40
Antique	20	13	-	-	-	-	1	6	11	37	-	-	-	-	32	14
Guimaras	30	20	2	13	1	14	-	-	4	13	-	-	-	-	37	27
Negros Occidental	39	26	9	56	4	57	8	47	4	13	1	17	-	-	65	29
TOTAL	107	48	12	5	6	3	13	6	24	11	3	1	57	26	222	100

P=producer; A-W=assembler-wholesaler; W=wholesaler; W-R=wholesaler-retailer;
IB=institutional buyer; HC=household consumer

Household consumers of salt in Western Visayas are composed mostly of purchase-decision makers in their 30s and 40s. Over-all, the salt industry players are generally married (70%) and such a civil status holds through across players in the entire value chain.

Most of the respondents have limited education with approximately 70 percent not reaching college. When analyzed by education by node in the chain, most salt producers (83%) have not entered any tertiary school. Participation of players with college degrees increased in the salt distribution/trading although modal educational attainment is high school graduate among institutional buyers (33%) and household consumers (31%). Modal years of experience in the salt industry among participants is 1 to 15 and it cuts across players in all nodes of the salt value chain.

The salt industry value chain. The core processes involved in the salt value delivery network, the tasks involved at each core process, the players, and the different enablers are mapped in Figure 1. Generally, the process involved in the salt value chain in the Visayas Region starts with the provision of inputs, salt production, marketing, then consumption. It is interesting to note that provision of inputs is generally performed by external providers such as the local government units, the Bureau of Fisheries and Aquatic Resources, and by the UNICEF. There are two dominant methods of producing salt: the solar evaporation method, and the cooking method. A large proportion of producers are women (44%), are less educated (88% have not entered college) and are in their advanced life stage (76% are 40 years and older).   The critical role of government agencies as enablers is evident and among those cited include the LGUs and the DA-BFAR (for input provision), the Office of the Provincial Agriculturists (during the production stage) and DTI (in the marketing of produce).

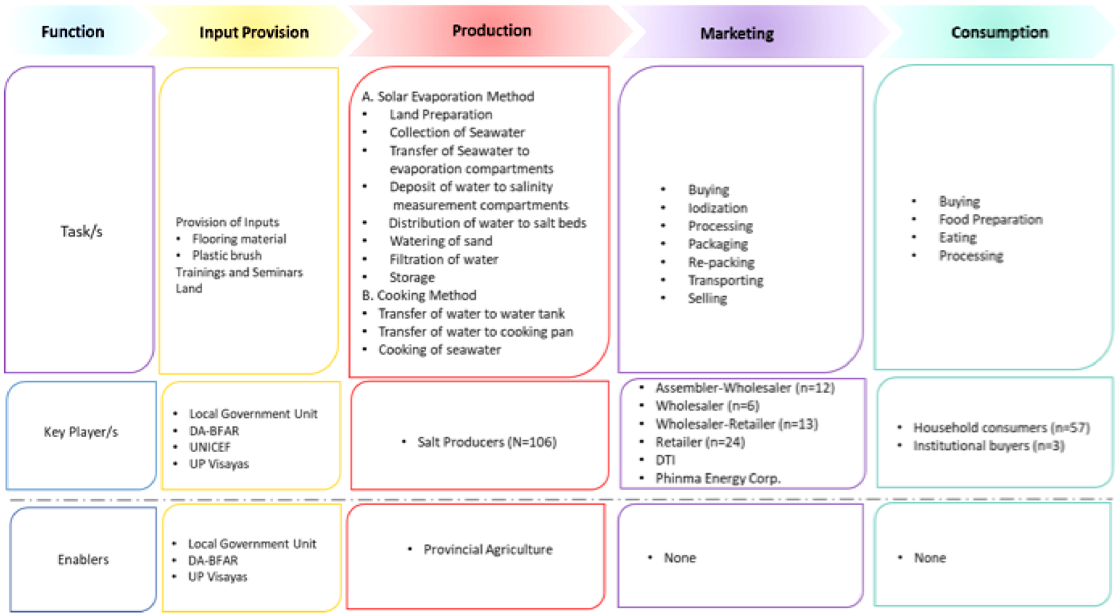

Fig. 1. Salt supply/value chain map, Visayas, Philippines, 201

Table 2. Distribution of socio-demographic characteristics, 22 supply chain participants, Visayas, Philippines, 2018.

Socio-Demographic Characteristics	Type of Supply Chain Participant							Total
	P (n=107)	A-W (n=12)	W (n=5)	W-R (n=14)	R (n=24)	IB (n=3)	HC (n=57)	
	Percent							
Sex								
Male	63	42	60	29	29	33	18	44
Female	37	58	40	71	71	67	82	56
Age (years)								
21-30	5	8	-	7	9	-	14	9
31-40	11	17	20	29	9	33	26	19
41-50	18	25	40	14	24	33	32	27
51-60	23	25	-	43	21	33	14	27
61-70	9	25	20	-	9	-	11	12
71-80	7	-	20	7	-	-	4	6
AVERAGE	51	49	52	50	49	46	45	
Civil Status								
Single	8	8	-	21	25	-	19	14
Married	81	75	60	50	67	100	67	73
Widowed	3	17	40	29	4	-	14	9
Separated	7	-	-	-	4	-	-	4
Educational Attainment								
Elementary Level	16	-	-	-	-	-	2	8
Elementary Graduate	22	8	-	-	17	-	5	14
High School Level	14	8	-	14	13	33	4	11
High School Graduate	28	25	40	36	58	33	33	33
College Level	7	17	20	14	-	33	14	10
College Graduate	5	25	40	21	4	-	35	15
Vocational	7	17	-	14	8	-	7	8
Years of Experience in Salt Business								
1-15	46	67	40	64	75	100	-	54
16-30	37	8	40	14	21	-	-	30
31-45	17	25	-	21	-	-	-	15
46-60	-	-	20	-	4	-	-	1
AVERAGE	20	14	16	15	14	10	0	15

P=producer; A-W=assembler-wholesaler; W=wholesaler; W-R=wholesaler-retailer; R=retailer; IB=institutional buyer; HC=household consumer

End-consumer specification for salt quality. The various salt quality specifications of the 57 end-consumer-respondents in the Western Visayas region are shown in Table 3. The top attributes preferred are the salt's color, texture, and purity. Salt had to be white and without impurities such as small stones. For texture, fine textured was preferred over the coarse ones. Least in the preference attribute ranking is being iodine-fortified despite the fact that the country has already implemented the ASIN law which mandates the addition of iodate in all salts traded and consumed locally.

Table 3. Salt characteristics preferred by 57 end-consumers, Visayas, Philippines, 2018.

	Salt Attributes								
Rank	**Color**	**Texture**	**Purity** (no dust/small stone)	**Taste** (tinge of bitterness)	**Moisture Content** (dry)	**Iodine-Fortified**	**Packed**	**Branded**	**Modal Attribute**
	Percent								
1	86	5	7	-	-	-	-	2	Color
2	5	26	54	5	2	5	2	-	Purity
3	5	51	18	9	7	5	2	4	Texture
4	-	7	5	12	61	9	2	4	Moisture Content
5	-	7	5	58	14	4	9	4	Taste
6	-	-	11	4	9	18	49	11	Packed
7	-	4	-	7	5	9	18	58	Branded
8	4	-	-	5	2	51	19	19	Iodine-Fortified

The product flow. The product flows show the volume produced, volume bought and sold per season of the key players involved in the salt supply chain from the point of production until the final product reaches the end markets (Fig. 2 & 3). Salt produced in the Philippines can be categorized into three based on quality. Class C salts are those type of salt that are harvested at the early stage of production. It is characterized by a light-brown color attributed to the presence of impurities and is usually priced the lowest among the three salt quality categories. Class B on the other hand, is characterized by off-white crystals and is harvested at the middle stage of salt production. Class A are the whitest crystals formed and are harvested at the peak of salt production when the sun is at its hottest (summer months). On the average, the Visayas Region produces only Class A (664,912.5 kg per season) and Class B salts (705,850 kg per season).

The number of channels for Class A salt varied by season with five during peak months (assembler-wholesalers, wholesalers, wholesaler-retailers, retailers, and institutional buyers) which becomes four during lean months due to the non-participation of wholesalers (Fig.2). For both seasons, the assembler-wholesalers absorb most of the produce. Majority of the Class A salt finds its way to the household consumers (94.62%). On the other hand, the lowest volume sold by the farmers was 60,350 kilograms (9.08%) to the retailers mainly because retailers buy and sell only in small quantities. These retailers are the neighbors of the farmers who own stalls in the public market and used tricycle as their mode of transportation for picking-up their purchased salt. It was also found that farmers do not sell directly to their neighbors for consumption as the latter usually ask for a handful of salt for their daily consumption. A total of 15,275 (2.30%) kilograms of salt produced by the farmers were given away to their neighbors and relatives. Institutional buyers source salt directly from the farmers during lean months to avail of lower prices.

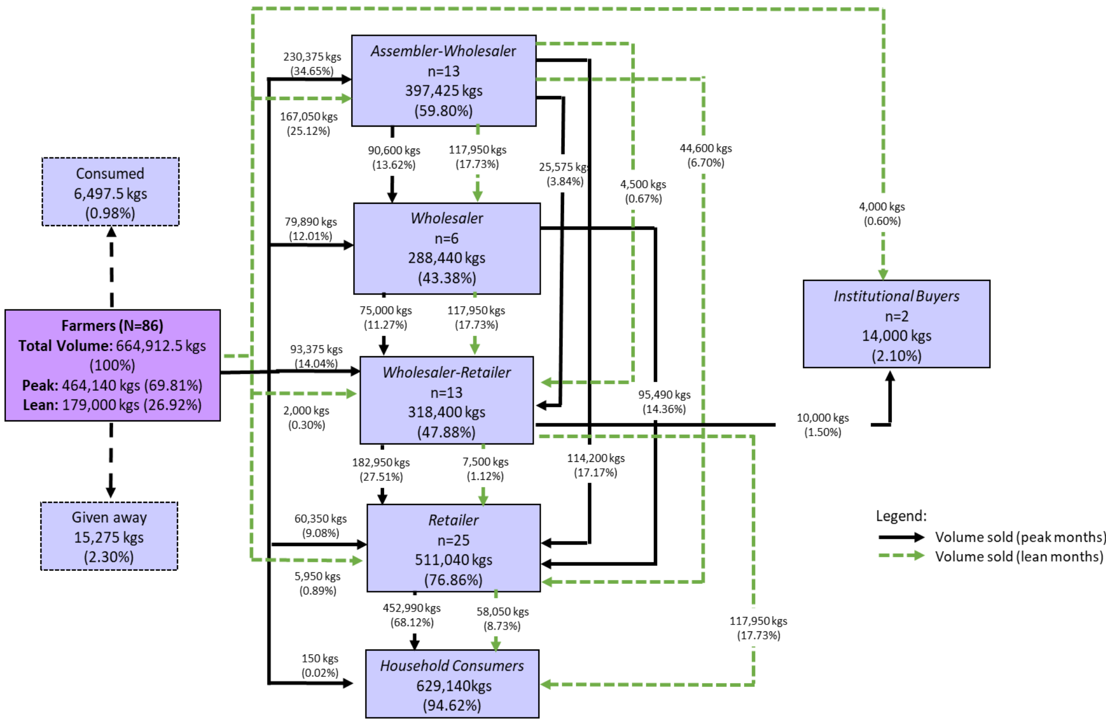

Fig. 2. Product flow of Class A salt, Visayas, Philippines, peak and lean months, 2018

Channel length for Class B salt is relatively shorter and tends to vary with season (Fig. 3). The Class B salt produced in the Visayas passes through five channel levels during peak months (assembler-wholesaler, wholesaler, wholesaler-retailers, retailers, and institutional buyers) but all the produce goes directly only to assembler-wholesalers during lean months. A large chunk of Class B salt produced in the region goes to the wholesalers (35.57%). It should also be noted that among the salt producing areas in the Visayas, only those in Iloilo were able to sell Class B salt to institutional buyers during peak months. This is because Class B salt are also bought and consumed by the household consumers in the region due to insufficient supply of Class A salt.

Based on the narratives of the traders, the seasonal reduction in channel levels is attributed to insufficient Class B salt. Also, the assembler-wholesalers have the capacity to buy in bulk and have established personal relationships with the farmers being their regular buyer or "*suki*" thus they are more preferred as an option. There were no losses reported as the salt moves across the nodes. Volume sold during the lean months are stored inventories which were purposely kept to hedge on seasonal price differences.

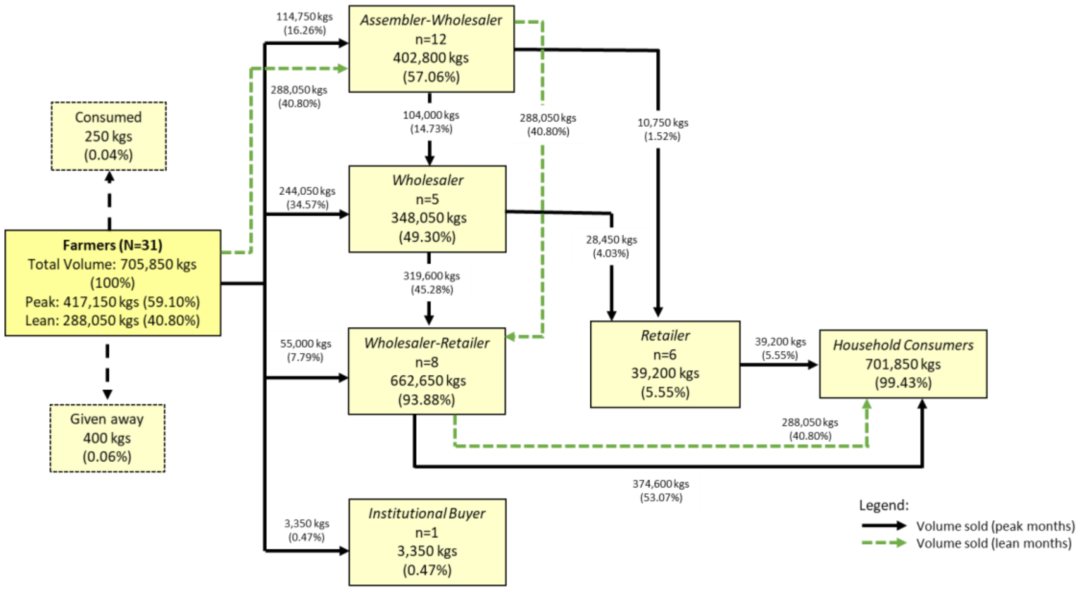

Fig. 3. Product flow of Class B salt, Visayas, Philippines, peak and lean months, 2018.

The geographical flow of salt. The geographical flow of salt shows its trail from the inputs until it reaches the market (Fig. 4). It also reflects the nature of salt being traded, whether it is iodized rock or non-iodized rock salt, and its classification (Class A, Class B, and Class C). Section 2-b of the Philippine Republic Act No. 8172 also known as *An Act Promoting Salt Iodization Nationwide and for Related Purposes* requires all producers/manufacturers of food-grade salt to iodize the salt that they produce, manufacture, import, trade or distribute (FDA-Philippines 1995). Sources and destinations of salt produced and traded are also shown.

It can be noted that salt is consumed where it is produced except in Guimaras which finds its way to Negros Occidental. Majority of salt produced in the Visayas Region is traded as non-iodized. Trading continues to focus more on non-iodized salt as it is more in demand even with the presence of ASIN Law. ASIN Law 'requires the addition of iodine to salt intended for animal or human consumption to eliminate nutrient malnutrition in the country'. As previously cited, the Visayas Region produced and distributed Class A and Class B salt. Of the volume produced in the area, about 11,700 kilograms representing 0.86 percent of the total salt produced and sold within Antique was iodized. The rest (99.14%) was sold as non-iodized. This indicates that there is no strict implementation of ASIN law within Visayas. In other areas, non-compliance to RA 8172 may also explain why the salt produced in major producing provinces are not being traded even to nearby provinces. According to the narratives of some residents (i.e., in Carles, Iloilo), locally produced salt contains enough iodine, and its fortification implies only the addition of synthetic chemicals, hence consumers do not like it.

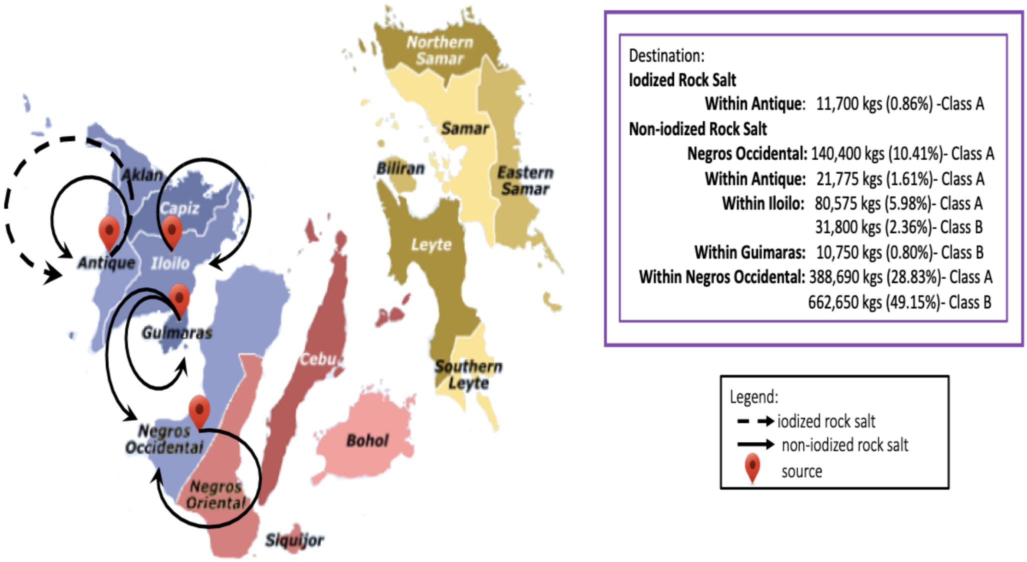

Fig. 4. Geographical flow of salt produced in the Visayas, Philippines, 2018

Value-added at the different levels in the value chain. The costs, prices, marketing margins, marketing costs, nominal profits, and profits expressed as percentage of the marginal costs for each value chain actor during peak and lean seasons for both Class A and Class B salts are presented in Tables 4 to 7. Highest cost is incurred by farmers as they transform the sea water into salt crystals. They were followed by the retailers who bulk-break, package, and sell salt to consumers. Nominal profit per sack is highest among retailers (PhP365.38/sack) across classes of salt and across seasons. Farmer-producers came next with PhP287.14/sack and least profit was generated by assembler-wholesalers (PhP5.47/sack). When profit was expressed as return to cost, the same trend is observed with retailers having the highest profits (1,358.80%) but the least was for wholesalers (101.79%) (Table 4). Producing Class A salt will generally result in a higher profit for the farmer due to the premium price paid for its high quality. Also, profits are generally higher during lean months as salt producers and sellers took advantage of low supply and therefore higher selling price (Table 5).

Consistent with the observation in the case of Class A salt, profit is generally higher during the lean season for Class B salt. Cost of good or production of Class B is lower than that for Class A salt for both production seasons. Nominal profitability was highest among retailers (PhP638.09/sack) during peak season, followed by salt farmers (PhP99.49/sack in the same season) (Table 6). The lean season is characterized by a shorter value delivery network and nominal profitability is highest among wholesale-retailers at PhP647.85/sack and then for farmers at PhP180.96/sack. With the absence of retailers during lean season, marketing profit shifted from the hands of retailers to wholesaler-retailers. Notable also is the close to the 82 percent increase in the profit share of farmers when Class B salt is sold during lean season (from PhP99.46/sack during peak season to PhP180.96/sack during lean season) (Table 7).

Table 4. Price structure of Class A salt (50kgs/sack), selected production areas, Visayas, Philippines, peak months (February-May), 2018.

Parameter	Type of Participant					
	Farmer	**Assembler-wholesaler**	**Wholesaler**	**Wholesaler-retailer**	**Retailers**	**Institutional Buyers**
Buying Price (PhP)		472.29	483.29	494.55	606.67	998.64
Selling Price (PhP)	472.29	483.29	494.55	606.67	98.64	-
Cost of Goods (COG, PhP)	185.15	-	-	-	-	-
Marketing Margin (PhP)	-	10.00	11.26	111.82	392.27	-
Value-Added (PhP)	-	4.53	5.58	23.46	26.89	-
Profit (PhP)	287.14	5.47	5.68	88.36	365.38	-
Profit as % of COG	155.09	-	-	-	-	-
Profit as % of MC		120.75	101.79	376.64	1,358.80	-

Table 5. Price structure of Class A salt (50kgs/sack), selected areas, Visayas, Philippines, lean months (June-November), 2018

Parameter	Type of Participant					
	Farmer	**Assembler-wholesaler**	**Wholesaler**	**Wholesaler-retailer**	**Retailers**	**Institutional Buyers**
Buying price (PhP)		523.76	535.09	545.17	620.02	1,000.00
Selling price (PhP)	523.76	535.09	545.17	620.02	1,000.00	-
Cost of Goods (COG, PhP)	192.56	-	-	-	-	-
Marketing margin (PhP)	-	11.42	10.08	74.85	379.98	-
Value-Added (PhP)	-	5.16	4.41	15.34	30.14	-
Profit (PhP)	351.11	6.26	5.67	59.51	349.84	-
Profit as % of COG	171.95	-	-	-	-	-
Profit as % of MC	-	121.31	128.52	387.94	1,160.72	-

Table 6. Price structure of Class B salt (50kgs/sack), selected areas, Visayas, Philippines, peak months (February-May), 2018.

Parameter	Type of Participant					
	Farmer	Assembler-wholesaler	Wholesaler	Wholesaler -retailer	Retailers	Institutional Buyers
Buying price (PhP)		195.83	207.30	216.00	330.51	998.64
Selling price (PhP)	198.53	207.30	216.00	330.51	998.64	-
Cost of Goods (COG, PhP)	96.34	-	-	-	-	-
Marketing margin (PhP)	-	11.47	8.70	114.51	668.13	-
Value-Added (PhP)	-	4.71	4.21	24.17	30.04	-
Profit (PhP)	99.49	6.76	4.49	90.34	638.09	-
Profit as % of COG	103.26	-	-	-	-	-
Profit as % of MC	-	143.52	106.56	373.76	2,124.13	-

Table 7. Price structure of Class B salt (50kgs/sack), selected areas, lean months (June-November), Visayas, Philippines, 2018

Parameters	Type of Participant			
	Farmer	Assembler-Wholesaler	Wholesaler-Retailer	Institutional Buyers
Buying Price (PhP)		261.00	280.00	950.00
Selling Price (PhP)	261.00	280.00	950.00	-
Cost of Goods (COG, PhP)	80.04	-	-	-
Marketing Margin (PhP)	-	19.00	670.00	-
Value-Added (PhP)	-	6.56	22.15	-
Profit (PhP)	180.96	12.44	647.85	-
Profit as % of COG	226.08	-	-	-
Profit as % of MC	-	189.63	2,924.83	-

Relationships and linkages among the value chain actors. Horizontal relationship pertains to relationship existing among participants in the same node of the value chain (e.g., among salt producers). Vertical relationship on the other hand pertains to relationship existing between participants in the different nodes of the chain (e.g., between salt producers and traders).

Of the five parameters under horizontal relationship, salt farmers exhibited strong relationships with co-farmers along the areas of information sharing, collaboration to sell in bulk, and trust (Table 8). Farmers demonstrated a weaker relationship among their peers along the areas of competition level and benefits from collective initiative. In contrast, traders demonstrated a strong relationship with peers across all horizontal relationship parameters except collaboration to sell in bulk. This is because each trader has his/her own set of buyers who have different schedules of delivery requests depending on convenience. Moreover, traders emphasized that they do not ask who the buyer of the other traders are to prevent vying for customers.

The degree of vertical relationship across the different players was found to be strong for verbal agreement. All transactions made across the salt value chain are done verbally. There is no written contract or agreement that market participants along the chain must comply with when trading salt. As long as salt producers can produce salt regardless of the demand of their buyers, traders will still buy these because they practice the "*suki system"* and because quantity demanded is always higher than quantity supplied. *"Suki system"* is a business relationship existing in the Philippines which happens when the buyer and seller informally commits to become each other's regular customer and supplier. This system fosters trust among the market participants. With the exclusion of input providers to farmers, a strong relationship across all members of the salt value chain was noted along the areas of information sharing on prices and quality control. Weak relationship across all value chain participants was noted along the areas of written contracts and sharing of quantity and technology.

Constraints mapping. The salt industry participants in the Visayas Region face several constraints at the different value chain levels and these are categorized based on the following segments: procurement of input assembly; selling of output; finance; training/seminar; market information; and technology. It is apparent that constraints are localized in a specific sector except in finance which happens to be a problem for all value chain participants (Table 9).

For the farmers, the main constraint is the high cost of flooring materials and the implements needed to heap and consolidate the salt crystals. There are two types of flooring materials that are being used - bricks, and clear plastics. Plastic liners can be used for the whole season and if one is careful enough its useful life can be extended up to two seasons or two years. Not many farmers though can afford the cost of required flooring materials preventing them from expanding and/or venturing into the salt making business without assistance from the government.

The problem of the traders on the lack of salt supply is the result of the farmers not having enough volume to supply them especially during the wet season when there is no production at all. This goes to show that if the farmers can supply more, then this problem of the traders will be addressed also. On the other hand, the problem on lack of markets for some farmers is due to non-iodization. Non-iodized salts are not permitted to be brought out of the municipality hence the available markets are only those within the area and yet the traders are experiencing a lack of supply for transporting to outside markets.

While purity is one of the top attributes sought for by the end-consumers, salt currently being produced is characterized by the presence of impurities thus posing a problem. Impurities come from being exposed to the open air environment where wind brings with it dust and other unwanted particles.

Table 8. Horizontal and vertical relationships among the identified key players in the salt value chain, Visayas, Philippines, 2018.

Parameter	Horizontal Relationship		Vertical Relationship				
	Farmer to Farmer	**Traders to Traders**	**Input Provider to Farmer**	**Farmer to Assembler-Wholesaler/ *Viajero***	**Assembler-Wholesaler / *Viajero* to Wholesaler**	**Wholesaler to Wholesaler-Retailer**	**Wholesaler-Retailer to Retailer**
Horizontal Relationship							
Information sharing							
Prices of salt	Strong	Strong					
Problems	Strong	Strong					
Practices	Strong	Strong					
Collaboration to sell in bulk	Strong	Weak					
Competition level							
Prices	Weak	Strong					
Buyers	Weak	Strong					
Practices	Weak	Strong					
Trust							
Management	Strong	Strong					
Selling	Strong	Strong					
Benefits from collective initiative	Weak	Strong					
Vertical Relationship							
Procurement or supply							
Written contract			Weak	Weak	Weak	Weak	Weak
Verbal agreement			Strong	Strong	Strong	Strong	Strong
Information sharing on technology and prices							
Price			Weak	Strong	Strong	Strong	Strong
Quantity			Weak	Weak	Weak	Weak	Weak
Technology			Weak	Weak	Weak	Weak	Weak
Quality control							
Texture			Weak	Strong	Strong	Strong	Strong
Color			Weak	Strong	Strong	Strong	Strong
Cleanliness			Weak	Strong	Strong	Strong	Strong
Presence of value-added services			Weak	Strong	Strong	Strong	Strong

Table 9. Problems encountered by the identified key players in the salt value chain, Visayas, 2018.

Segment	**Farmers**		**Traders**		**End Consumers**	
	Constraints	Opportunities	Constraints	Opportunities	Constraints	Opportunities
Procurement of Flooring Materials and Implements	High Cost	Availability of low-cost flooring materials and implements will encourage more farmers to venture in salt production	Lack of salt supply	There is unmet demand for salt in the market, for farmers to fulfill.	-	-
Selling of Output	Limited choice of market	There are many markets that can still be tapped if only there will be concerted effort to link with them.	Low selling price of salt	Decreased salt importation and storage during peak production months can help stabilize local salt price.	-	-
Finance	Lack of capital	There are existing laws that mandate the provision of low-interest capital for the farmers can take advantage of (e.g., Agri-Agra Law, etc.)	Lack of capital	Presence of local banks and/or microfinance institutions for the provision of low-interest loans for increased capital.	-	-
Training/Seminar	None	-	None	- -	-	-
Market Information	None	-	None	-	-	-
Technology	None	-	None	-	-	-
When Buying	-	-	-	-	Presence of impurities	Best practices in Class A salt production can be popularized and upscaled for its increased availability.
During Consumption/Use	-	-	-	-	Rock salt is hard to dissolve	There is existing technology that produces refined salt for consumers who desire to use easily dissolved salt.

CONCLUSIONS AND RECOMMENDATIONS

It can be concluded that the farmers remain the dominant actor in the salt value chain suggesting that any improvement at their level resonates in the whole value chain emphasizing the need to address the concern of the farmers for enhanced production. The solar evaporation of seawater as the common method of salt production results to its seasonal availability which is aggravated by the lack of appropriate salt storage facilities. Also, while producing Class A salt is more profitable for farmers during peak months, Class B was the more profitable during lean months. Among all the value chain participants, the retailers gained the highest profit relative to their marketing costs during peak and lean months and for both Class A and B, although they were not among the market participants for Class B salt during the lean months. In terms of governance, there is strong horizontal relationships among chain participants, but farmers had a weaker relationship with their peers along the areas of price determination, seeking buyers, and knowledge on new technologies as they tend to compete with each other, limiting their ability to benefit from collective initiative. Non-adherence to ASIN Law hampers the movement of salt to the target markets that are located beyond municipal boundaries.

While producing Class B salt was found more profitable for the farmers during lean months, it should be highly encouraged that during peak months, they upgrade to producing Class A salt because it is more profitable. Makeshift and simple seawater filtration process during production, as done in other areas of the country can help address this. To deal with the seasonal availability of salt which is the result of the seasonal nature of production, it is recommended that the farmers form an association that would facilitate bulk buying of the material inputs. On top of this, the association could set up a "farmer's piggy bank" or sustainability fund where they will be required to set aside a certain portion of their income as savings in preparation for the next season's purchase of flooring material which at the least is every two seasons or two years. In this way, they will not be dependent on non-assured dole-outs from the government and other donors. In line with this, financial literacy along with simple profitability analysis training should be provided to the producers. This should, eventually help address the problem of lack of supply of salt resulting from the expanded production areas. Also, with profitability per sack both for nominal and as percentage of marketing cost being highest downstream of the value chain (i.e., among retailers), farmers as a formed association can venture into retailing. Forming an association has an added advantage due to the possibility of farmer-members enjoying increased bargaining power for higher prices, enhanced market niching, and improved access to capital resulting from reduced transaction/loans processing cost. Improved access to capital is critical because across the salt value chain, financial constraint is the main limiting concern. This should promote economic sustainability and continuous and expanded salt farming operations. Once this is assured, setting up of storage facilities in optimal areas could assure the availability of salt even during the wet season. In the short- and in the mid-term, the local government units should be able to provide these and for maintenance and upkeep charge a minimal fee per unit of stored salt. In the long-term, there is a need to scout for salt production technologies that are suitable in the local setting and yet not wholly dependent on sunlight, availability of which is becoming highly unpredictable due to climate change. Benchmarking with other salt-producing countries would help a lot in this endeavor.

In addition, since some of the reasons for lack of salt market among producers and inadequacy of salt supply among traders are some provisions mentioned in the ASIN Law (An Act Promoting Salt Iodization Nationwide and for Related Purposes), it is recommended that this law be revisited. In particular, it should be reviewed in the light of present developments (e.g., marketing, etc.) in the salt industry and the environment where salt is being produced.

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PHYSICO-CHEMICAL CHANGES IN GARLIC *(Allium sativum L.)* AFTER PROLONGED STORAGE AT AMBIENT CONDITIONS IN THE PHILIPPINES

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ABSTRACT

Garlic bulbs from Batanes, Ilocos Norte, Nueva Vizcaya and Occidental Mindoro in the Philippines were collected to determine their physico–chemical characteristics including changes in pungency levels over time. Samples were taken during the harvest season (February–March, 2019) and stored for 6 - 8 months at ambient conditions (~30^0C). The following were evaluated: bulb size and weight, total soluble solids (TSS), and pungency in terms of pyruvate content. Garlic bulbs from major production areas in the Philippines would pass the premium ASEAN size standards (> 3.5cm), although actual size classification varies by production area. TSS values averaged 35°Brix but slightly declined after 6 – 8 months. Bulbs from Ilocos Norte and Nueva Vizcaya were initially more pungent, with higher pyruvate content (1.39 – 1.48 umol/g fresh weight (FW)) than those from Mindoro (1.13 umol/g FW) and Batanes (1.01 umol/g FW). After about 6 - 8 months, pyruvate level generally increased by 0.07 – 0.25 umol/g in most samples, except those from Batanes. Correlation between bulb size and pyruvate content showed that bigger bulbs had higher pyruvate content, except those from Occidental Mindoro. TSS values of Philippine garlics were comparable with those reported by other countries; but its pyruvate content was very low.

Key words: bulb size, bulb weight, weight loss, TSS level, pyruvate content

INTRODUCTION

Garlic is a popular spice in the Philippines and throughout the world. It is an ingredient in many food preparations, as well as in food supplements and herbal medicinal products (Petropoulos et al. 2017; 2018; Batiha et al. 2020). Hence, it is important that garlic is available the whole year round. However, being a bulb crop, garlic can be grown only during the dry months of the year. In developed countries, it is usually cold stored and thus it is available throughout the year. Such is not usually done in the Philippines, however, due to high cost of refrigeration. In garlic and other allium vegetables, total solids or dry weight (DW) is considered a quality trait positively associated with flavor intensity, length of postharvest life, and suitability for dehydration (Sance et al. 2008; Gelleta et al. 2013). An evaluation of a germplasm collection in Greece showed 26 - 42% DW of its garlic varieties (Avgeri et al. 2020). Those with > 40% DW had the longest shelf life; while those with low DW had short postharvest life, some of which had purple skin color.

Perception of garlic flavour is closely associated with its pungency level. The main bioactive compounds in garlic are saponins, flavonoids, organic acids, and various organosulfur compounds **(**Petropoulos et al. 2017). The latter are present in intact bulbs as peptides and sulfoxides (*i.e.* alliin). Alliin is synthesized in the leaves and translocated to the bulb in vesicles within the cytoplasm of mesophyll cells in cloves (Lawson 1996); while the enzyme allinase is said to be localized in the vacuoles of the bundle sheath cells, surrounding the phloem vessels (Ellimore and Feldberg 1994). Due to this difference in subcellular localization, allinase and alliin cannot interact until there is mechanical damage to the bulb, such as crushing (Yamazaki et al. 2002). In a way, this separation of the enzyme and its substrate contributes very well to the preservation of the bulb's pungency, as long as the bulb is intact.

When the bulb is crushed, alliin is metabolized to allicin by the enzyme allinase, producing ammonia and pyruvic acid (Yamaguchi and Kumagai 2019; Abe et al. 2020**)**. Hence, pyruvic acid is used as an indirect measure of pungency (Natale and Camargo 2005). It is reported to constitute up to 61% of total organic acids in garlic (Petropoulos et al. 2017). Those with high pyruvate content (plus organosulfur compounds) have the most intense flavor (Avgeri et al. 2020). Organo-sulphur compounds are responsible for the flavour and pungency of garlic. Of these, thiosulfinate**s** are the most abundant constituent (Barboza et al. 2020), and allicin is the predominant thiosulfinate (Block 2020). Pyruvate level is an estimator of total thiosulfinate content of garlic as they are positively correlated (Wall and Corgan 1992).

A local maturity study on garlic reported that when harvested 90 days after planting, it resulted in high weight loss, faster softening, and low TSS and pyruvate content, because the bulbs were still considered immature (Nuevo 1996). Hence, these bulbs were recommended to be harvested at least 105 days after planting. Bulbs were also found to be more pungent than its corresponding scapes, which are used in some food preparations (Gonzales et al. 2012).

Allicin content of garlic increased when bulbs were stored in low temperature (4 - 10^0C, and 60 - 90% RH) for 120 days (Sukkaew and Tira-umphon 2012). Maximum allicin level (36.5 mM/g DW) was found in bulbs stored at 4 - 6^0C, 80 - 90% RH after 60 days. The activity of allinase, the enzyme responsible for converting alliin to allicin yielding pyruvate, decreased at low temperature storage (-1.5^0C); while ambient storage (22^0C) maintained higher allinase activity up to six months after harvest (Ludlow et al. 2021). Similarly, onions stored for eight months at ambient conditions showed high allinase activity (Hanum et al. 1995). Pyruvate levels ranged from 3.69 to 72.47 umol/g fresh weight (FW) basis among 34 Greek genotypes (Avgeri et al. 2020). Pyruvate content was found to be strongly correlated with organosulfur compounds that had the most intense flavor among the Greek samples. The total phenolic content (12 - 82 mg GA /100g FW) was also found to be strongly and positively correlated with garlic's antioxidant capacity. Argentina, the second garlic exporter in the world, has garlic with 64 - 97 umol/g Pyruvic acid among its cultivars (Natale and Camargo 2005).

Aside from being an important ingredient in various food preparations, garlic has also many medicinal benefits. Its alliin and alliicin content can prevent and treat illnesses such as cancer (Nicastro et al. 2016), cardiovascular diseases (Banerjee and Maulik 2002), high blood pressure (Ried and Fakler 2014), diabetes (Ashral et al. 2005), Alzheimer's disease and dementia (Ray et al. 2011), as well as colds and infections (Josling 2001). Such medicinal benefits are attributed mainly to garlic's organosulfur and phenolic contents which are responsible for its flavor and pungency (Amagase et al. 2001; Rahman and Lowe 2006), of which thiosulfinates are the most abundant constituent (Block 2010). Its pyruvate content has been found to correlate positively with thiosulfinates, flavor intensity (Wall and Corgan 1992), and antioxidant activity (Soto et al. 2016; Beretta et al. 2017). Garlic has antimicrobial properties also (i. e. antifungal, antiparasitic, antiviral and antibacterial) due to its allicin content (Ankiri and Mirelman 1999; Harris et al. 2001).

In the Philippines, garlic can only be grown during the dry months of the year and also in limited areas of the country where there are distinct dry and wet seasons. Most popular of these areas is the Ilocos region in northern Philippines, where it is dry and hot during the summer season. After harvest, garlic is simply stored commercially at ambient temperatures. Hence, its price increases much toward the end of the year when it is off-season already.

Locally, there is no known study yet on the comparison of postharvest characteristics of garlic grown from various areas in the country. Hence, this study was conducted to document the size and weight of garlic, and the drying practices from key production areas in the country; to determine the TSS and pyruvate levels of garlic taken from key growing areas in the country; and to measure the pyruvate changes after prolonged storage under ambient condition.

MATERIALS AND METHODS

Garlic was collected during the harvest season (February–March 2019) from the following production areas of the country: Itbayat, Batanes; Pasuquin, Ilocos Norte; Bambang, Nueva Vizcaya and Lubang Island, Occidental Mindoro (Table 1). Bunched samples of various sizes of garlic, based on bulb diameter classified as extra large, large, medium, small, and extra small were collected from each area. These were further dried at ambient condition in the laboratory of the Postharvest Horticulture Training and Research Center, UP Los Banos for a month before analyses were done. The laboratory has an open window, with a temperature of 29 - 33^0C and 75 - 85% RH throughout storage. The diameter and weight of 10 individual bulbs for each size category were taken.

Table 1. Sources of garlic that were stored and analyzed for pungency, 2019.

Source	Harvest Month	Initial Analyses	Final Analyses	Storage Period
Batanes	February	April	November	7 months
Ilocos Norte	March	May	November	6 months
Nueva Vizcaya	March	May	November	6 months
Occidental Mindoro	February	April	December	8 months

The TSS content of the bulb was determined using a refractometer (Atago PR-1, Japan): 10 gm of garlic pulp was homogenized with 100 ml distilled water and filtered using cotton prior to placing the filtrate drop on the refractometer. The reading was multiplied with dilution factor and expressed in ^{0}Brix. Ten garlic bulbs from each size served as replicates.

Pungency was determined according to Schwimmer and Weston (1961) with some modifications (Gonzalez et al. 2012). Garlic cloves were peeled, weighed and blended in cold water. The sample was then filtered using cotton. One ml of the filtrate was mixed with 1 ml water and 1ml of 0.125% DNPH with 2N HCl. The tube content was mixed using a vortex mixer for 2 minutes, and then incubated at 37C water bath for 10 min. Afterwards, 5ml of 0.6N NaOH was added to the tube and the content was mixed again in a vortex mixer. It was left with cover against light for 10 min at room temperature for further reaction. Absorbance of the sample was measured using UV Vis spectrophotometer (Hitachi UH 5300, Japan) at 420 nm wavelength. A standard curve of pyruvic acid at various concentrations was prepared. Pyruvate levels of the bulbs were measured initially and after about 6 – 8 months at ambient conditions (29 - 33C). Analyses were done using 10 garlic bulbs of each size serving as replicates.

Data were analysed using SAS system (version 9). Means were compared using least significant difference (LSD) or Tukey's test (HSD) at 5% level of significance.

RESULTS AND DISCUSSION

Garlic size and weight. The size classification of the bulbs is variable by area. Actual measurement of bulb diameter differed in terms of extra small, small, medium, large and extra large bulbs (Fig.1). Only Batanes did not have the extra small (x-small) size bulbs, while only Ilocos Norte had the extra-large (x-large), the size of which (4.2 cm in diameter) however, was even smaller than the large bulbs of Nueva Vizcaya (4.5 cm) and of Occidental Mindoro (4.4 cm). Nevertheless, based on ASEAN standard for garlic size (https://www.asean.org/wp-content/), all sources had samples that would pass the ASEAN standard size 1 for garlic (Table 2). Hence, based on size, Philippine garlics can be marketed in the ASEAN countries.

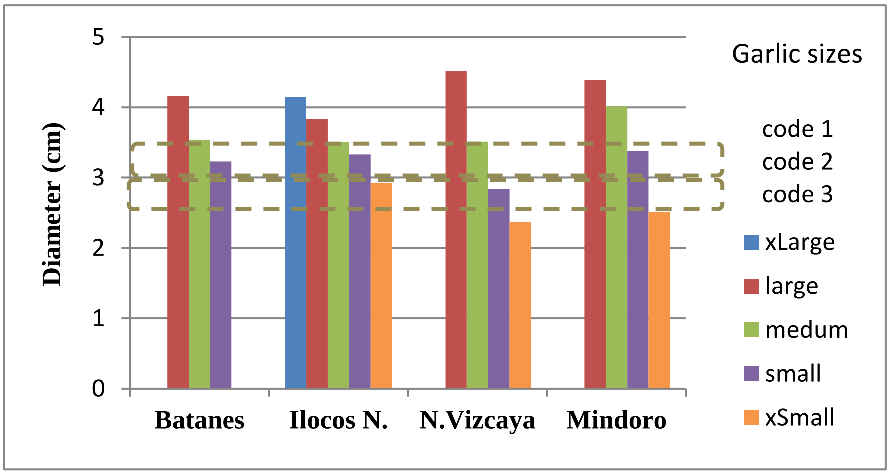

Fig. 1. Size classification of garlic according to source area compared with ASEAN standard codes 1-3. Each bar is the average of 10 bulbs. (Ilocos N.= Ilocos Norte; N.Vizcaya = Nueva Vizcaya; Mindoro = Occidental Mindoro).

Table 2. ASEAN Standard for garlic[1]

Code	Bulb diameter (cm)
1	> 3.5
2	3.0 < 3.5
3	2.5 < 3.0
4	1.5 < 2.5
5	< 1.5

[1]Based on ASEAN Standards 13.2009.

Garlic from Ilocos Norte, Nueva Vizcaya, and Occidental Mindoro had similar weights (8 - 24 g) across various size categories (Fig. 2). Bulbs from Ilocos Norte and Nueva Vizcaya used the same variety (Ilocos white), but those from Ilocos Norte had more categories having extra large and extra small sizes. Those from Batanes had the lowest bulb weight (<15 g).

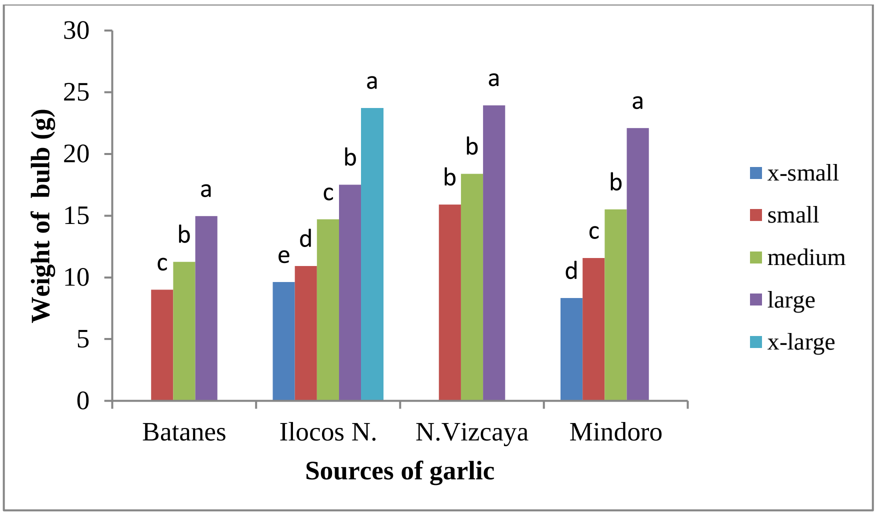

Fig. 2. Bulb weight (g) of garlic according to size classification in each production area. Each bar is the average weight of 10 bulbs. Mean comparison within each area is by LSD, 5%. (Ilocos N.= Ilocos Norte; N.Vizcaya = Nueva Vizcaya; Mindoro = Occidental Mindoro).

The bulbs from most areas were pure white (i.e. Ilocos Norte, Nueva Vizcaya and Occidental Mindoro), but those from Batanes had a slight red tinge in its skin. This coloring indicates that the Batanes variety likely belonged to cluster 2 or 3 of garlic like in Argentine cultivars, while the white variety belongs to cluster 1 (Barboza et al. 2020).

A very high regression coefficient (R^2) was obtained from all sources of garlic with respect to bulb weight at each size classification **(**Table 3). This means that farmers from each source of garlic were able to segregate well the bulbs even if based only on diameter estimation

Table 3. Correlation coefficient between garlic size and weight

Source of garlic	**Linear equation**	**R^2**
Batanes	2.98x + 2.81	0.98
Ilocos Norte	3.48x + 4.86	0.95
Nueva Vizcaya	4.02x + 7.34	0.95
Occidental .Mindoro	4.52x + 3.07	0.97

Drying and weight loss. In general, garlic farmers dried their harvest in bundles for about two weeks in their own areas (Table 4). Newly harvested garlic bulbs were laid flat on the ground for sun-drying and/or hung under the shade to air-dry. In case of Batanes, however, bulb samples were dried for one week only. Based on average monthly weight loss during storage, the samples from Batanes had the highest weight loss (Batanes Red: 4%; Batanes White: 12%). Samples from other places that were sun-dried or air-dried for two weeks had minimal weight loss (1-2%) in the next months. Hence, a minimum of two weeks drying under Philippines's ambient condition is necessary before storing garlic to have minimal weight loss later.

Garlics from Indonesia stored at room temperature (29 - 31^{0}C) for four months showed also similar weight loss (1.0 - 6.0%), while those kept at 5^{0}C had relatively higher weight loss (0.5 - 8.0%) toward the end of storage (Nurmalia et al. 2019).

In the Philippines, garlic is marketed based on bulb size, not weight, which is practiced as well in other ASEAN countries. However, since garlic is a fresh produce, weight loss is expected. Table 5 shows the weight loss of garlic samples from various production areas within six months. In general, garlic incurred 8 - 25% weight loss after six months. For Batanes, both varieties had minimal weight loss in the first four months, but from fifth month onwards, Batanes Red incurred a very high weight loss (37%), possibly due to disease that was manifested later.

Table 4. Drying practices of garlic farmers by production area.

Production area	Variety	Drying duration in the area	Storage method	Monthly rate of weight loss[1]
Itbayat, Batanes	Batanes white Batanes red	1 week air drying	Detopped, packed in net bags, piled	3.84 b 11.67 a
Pasuquin, Ilocos Norte	IIocos white	2 weeks sun drying	Bundled, piled	2.12 bc
Barat, Bambang, Nueva Vizcaya	IIocos white	1 week sun drying +1week air drying	Braided, hung	0.94 c
San Leonardo, Bambang, Nueva Vizcaya	IIocos white	4-5days sun drying + 1 week air drying	Bundled, piled	1.32 c
Tangal, Lubang, Occidental Mindoro	'Mindoro var.'	2 weeks sun drying	Bundled, hung	1.04 c
Tagbac, Lubang, Occidental Mindoro	'Mindoro var.'	2 weeks sun drying	Bundled, hung	1.05 c

[1]Weight loss in the last 3 months of storage. Comparison of means by HSD, 5%.

Table 5. Weight loss (%) of garlic during storage at ambient room (29 - 33^{0}C)[1].

Source	Color /Size	Length of Storage (months)		
		2	4	6
Batanes	Red	0.7a	5 a	37 a
	White	0.4 a	3 a	13 b
	Ave.	**0.5**	**4.0**	**25.0**
Ilocos Norte	x-Large	1.8 a	4 a	9 a
	Large	1.5 b	3 b	9 ab
	Medium	1.4 bc	3 b	8 ab
	Small	1.2 cd	2 c	7 c
	x-Small	1.2 d	2 c	8 b
	Ave.	**1.42**	**2.8**	**8.2**
Occidental Mindoro	Large	18 b	20 b	22 b
	Medium	18 b	20 b	22 b
	Small	20 b	21 b	24 b
	x-Small	26 a	27 a	29 a
	Ave.	**20.5**	**22.0**	**24.2**

[1]Each value is the average of 10 bulbs. Comparison of means within columns per area is by LSD, 5%.

For the first four months, the average weight loss of the various sizes of garlic from Batanes and Ilocos Norte was low (0.5-2.8%); whereas those from Occidental Mindoro were very high (18 - 26%). After six months, both Batanes Red and those from Occidental Mindoro exhibited a high weight loss (37%, 24%); whereas those from Ilocos Norte incurred only 8%. This may be due to immaturity of the Mindoro bulbs, where the peel or outer skin is not well developed, or the bulbs were not properly dried yet by the time garlic samples were set up. Weight loss of garlic was found to be lower after 120 days under ambient conditions (3 - 4%) than under low temperature storage ranging from 4 to 10^{0}C (4 - 11%) (Nurmalia et al. 2019; Sukkaew and Tira-umphon 2012). Moreover, moisture content of garlic was reported to remain constant (59 - 68%) during storage for 12 months, both at 22^{0}C and at -1.5^{0}C (Ludlow et al. 2021).

Total soluble solids (TSS). Initial TSS levels of garlic ranged from 28 to 39 ^{0}Brix. Samples from Mindoro had the highest initial TSS levels (37.6 ^{0}Brix), followed by Batanes (35.2 ^{0}Brix), Nueva Vizcaya (34.1 ^{0}Brix) and Ilocos Norte (31.8 ^{0}Brix). After 6 – 8 months of storage (final), the TSS levels in all samples slightly decreased (Fig. 3). Those of garlic from Occidental Mindoro decreased the most (6 ^{0}Brix); those from Batanes had the lowest decrease (1 ^{0}Brix). The TSS decrease was due to garlic's continuous respiration as it uses energy for its cellular maintenance.

The pattern of changes in TSS values with respect to bulb sizes was not consistent for all sources of garlic (Table 6). Initially, TSS values decreased as bulb size increased except for samples from Occidental Mindoro. However, only garlic from Ilocos Norte and Nueva Vizcaya showed the same pattern, after 6 - 8 months in storage. Those from Batanes and Occidental Mindoro have increasing TSS values with increase in bulb size. Hence, bulb size of garlic seems not a factor of TSS level in this case. Possibly, more samples must be taken to verify such correlation.

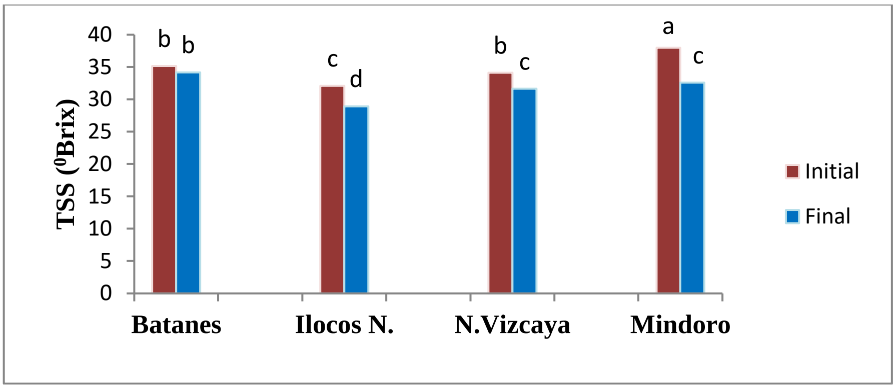

Fig. 3. Changes in TSS content of garlic after 6 - 8 months storage at ambient conditions. Each bar represents the average of 30 garlic samples. Mean comparison is by LSD, 5%. (Ilocos N.= Ilocos Norte; N.Vizcaya = Nueva Vizcaya; Mindoro = Occidental Mindoro).

Table 6. Correlation between garlic sizes and TSS values from each production source over 6 - 8 months at ambient conditions[1].

	Initial		Final	
Garlic source	**Linear equation**	**R^2**	**Linear equation**	**R^2**
Batanes	-0.25x + 35.92	0.03	0.5x + 32.77	0.99
Ilocos Norte	-0.51x + 33.33	0.54	-0.26x + 29.66	0.73
Nueva Vizcaya	-0.98x + 37.04	0.9998	-0.75x + 33.88	0.70
Occidental Mindoro	0.73x + 35.8	0.92	1.445x + 28.3	0.95

[1]Each value represents the average of 30-50 garlic bulbs of various sizes (x-large, large, medium, small, x-small)

In the present study, after six to eight months of ambient storage, most of the garlic samples that were stored, were still of marketable quality. The TSS values obtained here (28 - 39 ^{0}Brix, raw data not shown) were higher than garlic samples from Spain (25 - 29 ^{0}Brix) (Pardo et al. 2007), but slightly lower than some samples (32-40 ^{0}Brix) from Greece (Petropoulos et al. 2018). TSS level of garlic from another Greek study (26 - 42%) indicated long shelf life (Avgeri et al. 2020). In comparison, the TSS levels of some tropical fruits at the edible ripe stage are usually lower than that of garlic: banana (20 - 24 ^{0}Brix), mango (17 - 19 ^{0}Brix), citrus fruits (8 - 9 ^{0}Brix) and papaya (9 ^{0}Brix) (Agillon et al. 1987; Lado et al. 2014; Khandaker et al. 2018).

Pyruvate content. The initial pyruvate levels (Table 7) were highest in samples from Ilocos Norte (123 ug/g FW or 1.39 umol/g FW) and Nueva Vizcaya (130 ug/g FW or 1.48 umol/g FW), and lowest in samples from Batanes (89 ug/g FW or 1.01 umol/g FW). Values obtained were comparable with local samples analysed before (Nuevo 1996); but were much lower than samples from Greece (0.95 – 1.91 g/100 g FW) (Petropoulos et al. 2018).

After 6 - 8 months of storage at ambient conditions (Fig. 4), most samples had an increase in pyruvate content (6 - 22 ug/g FW or 0.02 - 0.49 umol/g FW), except those from Batanes which showed a slight decline (8 ug/g FW or 0.09 umol/g FW). Those from both Batanes and Nueva Vizcaya samples did not change much after six months. Ilocos Norte and Nueva Vizcaya samples, which are actually of same variety (Ilocos white), had the highest pyruvate content. It is possible that samples from Occidental Mindoro were not properly dried before its collection. Hence, the big difference in pyruvate content over time due to changes in bulb weight, on which pyruvate computation is based.

Storage of local garlic at ambient conditions is comparable with other countries, where garlic or onion is usually stored at low temperature (-1.5 ^{0}C) to have an extended shelf life for 6 - 8 months (Hanum et al. 1995; Ludlow et al. 2021).

Table 7. Pyruvate content of different sizes of garlic from various areas in the Philippines.[1]

Source	Size	Pyruvate content			
		ug/g FW		umol/g FW	
		Initial	Final	Initial	Final
Batanes	Small	78 ns	72 b	0.88	0.82
	Medium	91	79 b	1.03	0.90
	Large	98	93 a	1.11	1.06
	Ave.	**89.0**	**81.0**	**1.01**	**0.93**
	c.v.	40	9.8	0.12	0.12
Ilocos Norte	x-Small	115 d	122 b	1.31	1.39
	Small	125 c	142 a	1.42	1.61
	Medium	128 bc	134 ab	1.45	1.52
	Large	134 ab	139 a	1.52	1.58
	x-Large	138 a	142 a	1.57	1.61
	Ave.	**122.7**	**135.9**	**1.39**	**1.54**
	c.v.	7.7	11.8	0.10	0.09
Nueva Vizcaya	Small	128 ns	134 b	1.45	1.52
	Medium	130	132 b	1.48	1.50
	Large	133	144 a	1.51	1.64
	Ave.	**130.3**	**136.5**	**1.48**	**1.55**
	c.v.	11.3	6.7	0.03	0.08
Occidental Mindoro	x-Small	97 ab	140 a	1.10	1.59
	Small	98 ab	126 ab	1.11	1.43
	Medium	91 b	125 ab	1.03	1.42
	Large	108 a	114 b	1.23	1.29
	Ave.	**99.3**	**121.7**	**1.13**	**1.38**
	c.v.	13.8	13.6	0.08	0.12

[1]Each value is the average of 10 bulbs. Mean comparison within each area and period is by LSD, 5%.

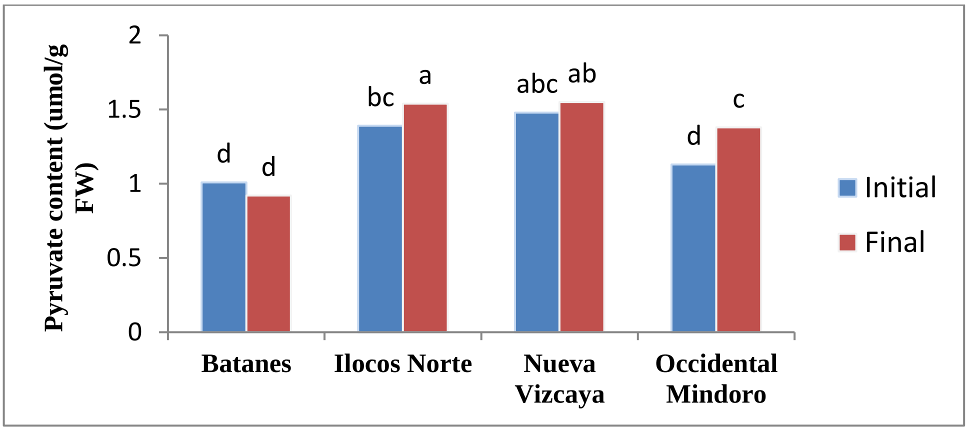

Fig. 4. Changes in pyruvate content of garlic from various areas over time. Only small, medium and large bulbs were considered in getting the average (n=30). Comparison is by LSD test at 5%.

Samples from Ilocos Norte (northwest Philippines) and Nueva Vizcaya (northeast Philippines), which planted the Ilocos white variety, showed almost the same pyruvate content levels, both initially and after storage. These areas are on opposite sides of the northern Philippines archipelago and have different topography and climate. Likewise, Petropoulos (2018) reported that in Greece, various garlic genotypes from the same or different regions showed similarities in terms of chemical composition and morphology. A study of garlic from America also showed that pyruvate levels after storage in normal air (33.9 umol/g) or under controlled atmospheres (35.4 umol/g), were almost similar after six months (Cantwell et al. 2003a).

Sizes of garlic from Batanes, Ilocos Norte and Nueva Vizcaya were found initially correlated with its pyruvate levels (Table 8). Pyruvate content increased with increase in bulb size consistently, except those from Occidental Mindoro. After 6 - 8 months of storage (final), garlic from the same three areas still showed positive correlations between size and pyruvate content, although lower values were shown in Ilocos Norte and Nueva Vizcaya samples

(R^2 = 0.5, 0.6). Possibly, pyruvate content of garlic later becomes stable, or garlic had minimal changes in weight loss after prolonged storage.

Whereas samples from Batanes consistently showed nearly the same pyruvate levels (89, 81 ug/g FW or 1.01, 0.93 umol/g FW) with respect to storage time; only garlic from Occidental Mindoro had decreasing pyruvate content with increase in bulb size after 6 - 8 months of storage. Hence, the effect of bulb size on pyruvate content is conclusive only for garlic samples from Batanes, Ilocos Norte and Nueva Vizcaya, which are all located in northern Philippines.

Both Ilocos Norte and Occidental Mindoro have Type I climate, while Nueva Vizcaya has Type III. Batanes which is located in the northernmost part of the country has also dry months before July (~Type I). During the growing season of garlic which only takes about 110 days to mature, all areas are dry between January to May (https://discover the Philippines.com/Climate of the Philippines/).

Table 8. Correlation between size of garlic and pyruvate content from each production source over 6 - 8 months at ambient condition[1].

Source of garlic	Initial		Final	
	Linear equation	R^2	Linear equation	R^2
Batanes	10.0x + 59	0.9709	10.0x + 51	0.9709
Ilocos Norte	5.5x + 111.5	0.9634	3.88x + 124	0.507
Nueva Vizcaya	2.5x + 122.8	0.9868	5.0x + 121.5	0.5714
Occidental. Mindoro	2.6x + 92.4	0.2165	7.9 x + 146	-0.9158

[1]Each equation considered 30-50 bulbs of garlic taken from each area.

The pyruvate levels obtained from local garlic (1.01 – 1.55 umol/g FW) were way below than those reported in Argentina, which were about 60 - 97 umol/g FW (Natale and Camargo 2005), as well as those of the Greek genotypes (4 - 73 umol/g FW) (Avgeri et al. 2020). Hence, Philippine garlics were less pungent than most cultivars from other countries, in terms of pyruvate content. However, local garlics may also be stored up to eight months under ambient conditions, resulting in slight increase in its pyruvate content.

Great diversity in organic acid content (i.e. pyruvic acid) was observed between genotypes regardless of the growing area, indicating that other than genotypes, other factors may affect its chemical composition; as well as the assays used (Petropoulos 2018). It was also reported in Argentinian garlics that significant differences in pyruvic acid content was observed between different cultivars grown in the same area; as well as between the same cultivar grown in different areas (Soto et al. 2010). Preharvest practices such as sulfur fertilizer application may have significant effect on pyruvic acid content as well (Poldma et al. 2011).

CONCLUSION

This evaluation of garlic from major production areas in the Philippines shows that all sources are producing sizes that pass the ASEAN standard for garlic. There is a need, however, to realign the local size classifications with the ASEAN standard in order to be able to export. The shelf life extension of garlic through drying and ambient storage practices of local growers in the Philippines is at par with that of low temperature storage in other countries. However, in terms of pyruvate levels, the quality of local garlic under ambient conditions is inferior to garlics from Argentina and Greece, which are stored under low temperature. Although production sources and/or varieties yielded some differences, pungency levels of garlic from the Philippines did not dramatically decline even after 6 – 8 months under ambient conditions (29 - 33^{0}C).

What needs attention is the control of postharvest diseases during prolonged storage, beginning from its field production. Future work may also focus on evaluation of garlic from other production areas in the country.

Moreover, other active components of the local garlic varieties like alliin, allicin, phenolic content, thiosulfinates and antioxidant activity, and their antimicrobial properties need to be established so that their potential for medicinal use can be harnessed.

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FACTORS DRIVING TRACTORIZATION OF MAIZE FARMS IN THE PHILIPPINES

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ABSTRACT

Maize farming in the Philippines is generally characterized by relatively slow adoption of mechanization technologies. With the more aggressive effort to promote the extensive use of mechanical power as a means of improving land and labor productivity, information is needed as guide in program implementation. This paper analyzed the factors that influence the decision of maize farmers to adopt machines in land preparation. Using the data gathered by PHilMech and UPLB-AMDP in 2013 from 13 major maize-producing provinces in the country, the determinants of tractor adoption and ownership by 1,018 maize farmers were established by applying logistic regression. Empirical estimates revealed that the farmer's level of education, age, availability of credit, land area and topography influence their decision to use or acquire tractors. Higher education and older age increases the likelihood of tractor adoption while farmers availed credit are more likely to adopt tractors. Moreover, area and land topography are significant variables that affect tractor use and ownership. Farmers with larger landholdings and situated in the lowlands have higher propensity to mechanize their farms. With these findings, it is recommended that mechanization intervention should be more focused in the low-lying areas, increase access of maize farmers to low-cost credit and review policies on land reform, farm consolidation or clustering.

Key words: Adoption, logistic regression, maize, mechanization, tractorization

INTRODUCTION

Maize plays a significant role is sustaining the livelihood and nutritional requirements of significant number of Philippine population. Yellow maize is an essential ingredient in the formulation of animal feeds and raw materials for the manufacture of various industrial products. On the other hand, white maize is a main staple or rice substitute in some parts of Visayas and Mindanao. While maize is widely grown in almost all parts of the country, large number of maize farmers still employs manual labor and animal power (Dela Cruz and Malanon 2017). For decades, mechanization has been limited to land preparation and shelling operations. Planting and harvesting operations have been predominantly performed using manual labor while furrowing and in-field hauling activities are done with the aid of draft animals such as carabao, cattle and horse.

The decision of farmers to mechanize their farm is influenced by various factors but primarily economic in nature, driven by changes in costs of labor relative to capital. The increasing wage rate or rising cost of labor indicates diminishing labor supply and this situation rationalizes the use of labor-replacing technology. The declining local labor supply could be due to ageing population, structural change that caused transfer of farm workers to other sectors of the economy and the waning interest of youth in farming. Moreover, the declining population of draft animals also contributes in the rising costs since there are farm operations that require both human and animal power. The cultivation of idle lots previously devoted for grazing land for animals make it more difficult and costly to maintain farm animals for draft purposes. Another factor is the constrained calendar of farm activities that creates bottlenecks as affected by synchronous planting and scarce labor and/or draft animals. While studies did not indicate the direct effect of tractors on yield, it allows fast/timely completion of farm activities, increase arable land, redress scarce labor amidst the dwindling labor supply and decreasing draft animal population.

In the Philippines, early study showed that large irrigated farms were mechanized first, especially the laborious farm operations such as land preparation (AMIC 1988). The study added that the decision to mechanize farm operation is influenced by farmer's educational attainment, farm size and distance of the farm to the nearest market centers. The study further showed that accessibility of the farm as indicated by the distance of the farm to the market was essential in mechanization, especially for large machines. Amongo et al. (1996) also identified socio-economic factors that affect mechanization of rice farms. These include educational attainment, income, tenurial status, type of irrigation, availability of family labor and access to credit. Education and availability of family labor had negative effect while income had positive effect.

Compared to rice farms where mechanization of tillage operation is already predominantly practiced with the proliferation of more affordable hand tractors, maize mechanization has been sluggish although tractors were already introduced decades ago. The indivisibility of large capital investment hinders small farmers from acquiring large tractors suitable for maize farms. Moreover, government programs on mechanization focuses on rice as rice farmers are more organized to serve as conduits of government facility assistance.

While studies on the determinants of tractor adoption were already well studied for other crops such as rice, there were limited investigations done for maize, both in terms of focus and geographical scope. This paper aims to provide empirical information on the determinants of tractor adoption by maize farmers. The research findings could provide important insights on how to devise strategies in accelerating the adoption of mechanization technologies as a means of increasing land and labor productivity and ultimately enhance the competitiveness of the maize subsector.

METHODOLOGY

Locale of the study. The study covered 13 major maize producing provinces in the Philippines, representing about 40% of the total national area planted to maize. One province per region was chosen based on the yield of the province that approximates the regional crop average. The areas of the study include the provinces of Pangasinan, Isabela, Tarlac, Ifugao, Occidental Mindoro, Camarines Sur, Iloilo, Leyte, Bukidnon, Davao del Sur, South Cotabato and Agusan del Sur. After the selection of sample provinces, representative municipalities from each provincial district were also chosen based on the provincial average crop yield.

Sampling size and sampling procedure. The total respondent was determined by applying the Yamane equation:

$$n = \frac{N}{1 + N * e^2}$$

Where:

n = Sample size
N = Population size
e = Acceptable sampling error ranging from 1-10%

The respondents composed of 1,018 maize farmers drawn from 1,235 total respondents surveyed using multi-stage sampling. For each province, 95 maize farmers were randomly selected and the sample size for each municipality was determined using proportional allocation.

Data collection and research instrument. Data were taken from the survey conducted by PHilMech and UPLB-AMDP in 2013. Key informant interview, actual field observation and secondary data collection were also done to validate gathered information and gain deeper knowledge on the details of issues surrounding corn mechanization.

Analytical Framework

The Use/Ownership of Tractor General Model. To determine which variables were important in affecting the decision-making process of maize farmers towards the adoption of tractors in land preparation, a binary choice model was applied. These models are generally used when economic decision makers choose between two mutually exclusive outcomes. The farmer's choice of maize land preparation technique is represented by the dummy variable:

$$z = \frac{1 \ \ if maize\ farmer\ uses\ tractor}{0\ if\ maize\ farmer\ still\ use\ draft\ animal\ or\ practices\ zero\ tillage}$$

The probability that a maize farmer adopts tractor can be expressed as:

$$[z = 1] = P_i$$

Drawing from the equation, the probability that the farmer still use utilize draft animal in land preparation or not practice tillage, hence, does not adopt tractor can be illustrated as:

$$[z = 0] = 1 - P_i$$

The specific binary choice model that used was the logistic regression analysis or the logit model. In this model, the probability P_i that the farmer adopts tractor is specified as:

$$P_i = P(z) = f\left(\beta_0 + \sum_{i=1}^{N} \beta_i X_i\right) = \frac{1}{1 + e^{-z}}$$

$$P_i = \frac{1}{1 + e^{-\left(\beta_0 + \sum_{i=1}^{N} \beta_i X_i\right)}}$$

Where:

P_i= probability of adopting tractor in land preparation
Z = measure of the total contribution of all the independent variables used in the model
β_0= constant
N= number of independent variables
β_i= coefficients of the independent variables
X_i= independent variables (factors that influence tractor adoption)
e= base of the natural logarithm

This equation can also be expressed as:

$$P_i = \frac{e^{(Z_i)}}{1 + e^{(Z_i)}}$$

$$Z_i = \beta_0 + \sum_{i=1}^{N} \beta_i X_i$$

Both sides are then multiplied by $1 + e^{(Zi)}$,

$$P_i + e^{(Zi)}P_i = e^{(Zi))},$$

The factor $e^{(Zi)}P_i$ is then transposed and distribution is performed, $P_i = e^{(Zi)} + e^{(Zi)}P_i = (1 - P_i)\, e^{(Zi)}$

This equates to: $\frac{Pi}{1-Pi} = e^{(Zi)}$

The natural logarithm of both sides is then taken, $\ln \frac{Pi}{1-Pi} = Z_i$

The dependent variable in the formula is represented by the logarithm of the probability that a particular decision was made. Because P_i represents the probability of adopting tractor and $1 - P_i$ represents the probability of using draft animal in land preparation, the ratio $\frac{Pi}{1-Pi}$, also known as the odds ratio will determine whether the farmer would adopt tractor. If P_i is equal to zero, then $\frac{Pi}{1-Pi}$ would also be equal to zero.

The regression probability is:

$$\ln \frac{P_i}{1 - P_i} = z = b_0 + b_1X_1 + b_2X_2 + \cdots + b_nX_n$$

Therefore, variable Z was defined in this study as:

$$Z = \beta_0 + \beta_1X_1 + \beta_2X_2 + \beta_3X_3 + \beta_4X_4 + \beta_5X_5 + \beta_6X_6 + \beta_7X_7 + \beta_8X_8 + \beta_9X_9 + \beta_{10}X_{10} + \beta_{11}X_{11} + \varepsilon$$

Where:

Z= adoption of tractor in land preparation (1 = using tractor, 0 = not using tractor)
X_1 = age of farmer-respondent, in years
X_2 = farmer's educational attainment: 1 = college level; 0 = below college level
X_3 = membership in farm organizations: 1 = yes; 0 = otherwise

X_4 = attendance to seminars/trainings related to mechanization: 1 = yes; 0 = otherwise
X_5 = credit access: 1 = yes; 0 = otherwise
X_6 = tenurial status: 1 = land owner; 0 = tenant
X_7 = topography: 1 = lowland; 0 = hilly/upland
X_8 = landholding, in hectare
X_9 = cost of prevailing practice to be replaced by machines
X_{10} = labor situation during peak period of planting or harvest: 1 = sufficient; 0 = scarce
X_{11} = ownership of draft animals: 1 = yes; 0 = no
ε= disturbance term

The specific variables included in the adoption or use of agricultural machineries, data measurement and the hypothesized signs are presented and described below. Numerous studies have established significant association between human capital and technological adoption. Higher human capital accumulation as indicated by age of farmer (Bootinger et al. 2010), educational attainment (AMIC 1988; Amongo et al.1996), affiliation in farm organizations, attendance to seminars and trainings and access to credit facilities (Amongo et al. 1996) were hypothesized to increase the likelihood of mechanization technology adoption. These variables were expected to have positive signs. In addition, tenurial status of farmers was included in the model, with owners hypothesized to use or even own machineries (Amongo et al.1996).

Most adoption studies likewise included variable reflecting farm size or volume of production (AMIC 1988; Pingali 2007). Larger farms allow more efficient use of machineries and economies of scale operation. It can also better spread the fixed costs of a given technology over a larger output compared to smaller farmers, thereby lowering average fixed costs. In this study, bigger farms were hypothesized to be more likely to use agricultural machineries. Farm size was measured in hectares and was hypothesized to have a positive sign. Farm characteristics such as agro-ecology and topography were also expected to have positive signs as irrigated farms and farms in lowland plains more likely to utilize or own machineries.

Other factors hypothesized to be influencing use or ownership of machineries were the cost of prevailing practice which machineries replace, labor situation in the area during peak periods and ownership of draft animals (Pingali 2002; Ulluwishewa 1987; Tsuchiya 1972).

For the dependent variable use and ownership of agricultural machineries in corn production, a value of one was assigned for farmers who utilized or own agricultural machineries while a value of zero was assigned for farmers who did not use mechanical power in tillage operations.

Where β_{is} are estimated coefficients and X_{is} are the independent or explanatory variables. The logistic coefficient is interpreted as the change in the log-odds associated with one unit change in the independent variable. The coefficients do not measure marginal effects of independent variables but only show if any variable has significant influence on the dependent variable. The significance of the estimated coefficients may be shown in terms of Wald Statistic, t-ratios, correlation coefficients or $E(\beta_i)$, i.e exponentiation value of β_i. Among these, $E(\beta_i)$ gives a more direct interpretation of β_{is} and it is derived by rewriting the equation in terms of odds rather than log odds as follows:

$$\frac{Prob\ (event)}{Prob\ (noevent)} = e^{\beta 0+\ \beta 1X1+\cdots+\ \beta nXn}$$

Now, e raised to the power β_i is the factor by which the odds change when the $_ith$ independent variable increases by one unit. If β_i is positive, $E(\beta_i)$ is more than1 which means that the odds are increased. If β_i is negative, $E(\beta_i)$ is less than1 which means that the odds are decreased. If $\beta_i = 0$, $E(\beta_i) = 1$ which leaves the odds unchanged**.**

RESULTS AND DISCUSSION

Characteristics of maize farmers. The profile of maize farmers revealed that adopters and non-adopters of tractors did not differ in terms of age but varied significantly in terms of education and experience in maize farming (Table 1). Moreover, majority of adopters were owners of the land they planted with maize while high percentage of non-adopters were tenants. While membership to farm organizations were almost the same for adopters and non-adopters of tractors, lesser numbers of non-adopters were not members of farm organizations. This was also noted for the attendance to seminars and trainings. Meanwhile, about two-thirds of adopters applied for loans used for maize farming compared to 52% for non-adopters.

Table 1. Characteristics of maize farmers in 13 major producing provinces, Philippines

Item	Adopter	Non-Adopter	*Mean Difference*
Age	49.09	49.06	*0.03*^{ns}
Education, years	9.19	8.16	*1.03****
Farming experience, years	21.31	22.97	*1.66***
Tenurial status			
Owner	66%	62%	
Tenant	21%	29%	
Leaseholder	13%	9%	
Membership to farm organizations			
Yes	51%	41%	
No	49%	59%	
Attendance to trainings or seminars related to mechanization			
Yes	49%	41%	
No	51%	59%	
Availed credit			
Yes	66%	52%	
No	34%	48%	

***Significant at 1%
**Significant at 5%
^{ns} Not significant at 10%

Farm characteristics. Generally, the adopters of the tractors operated larger farms, with an average of 2.25 hectares (Table 2). This was significantly larger than their non-adopter counterparts. Most of the farms of adopters were also located in the lowlands and near riverbanks while 38% of non-adopter maize farms were in the uplands. Both adopters and non-adopters reported that they encountered problem on scarcity of labor during the conduct of labor-intensive maize farm operations. This was mentioned as primary reason in the adoption of tractors. While non-adopters also recognized the need to mechanize their farms, barriers to adoption are more difficult to overcome so they continue to utilize traditional method of land preparation.

Table 2. Characteristics of maize farms, 13 major producing provinces, Philippines

Item	Adopter	Non-Adopter	*Mean Difference*
Area of farm, ha.	2.25	1.88	*0.37***
Land topography			
Plain/lowland	83%	62%	
Slopping/upland	17%	38%	
Labor availability			
Sufficient	84%	84%	
Insufficient	16%	16%	
Cost of land preparation, Php/ha.	1,671.54	1,922.75	*251.21**

**Significant at 5%
*Significant at 10%

Determinants of tractorization in maize farms. The model was fitted for the use of land preparation machineries in maize production. Important variables include topography, area, credit access, education and age (Table 3). The result suggests that corn farms in the plains, lowland and gently sloping areas are more likely to use tractors by 3.08 times compared to farms in hilly areas with more steep gradients. In terms of area, a one-hectare increase in farm area cultivated increases the likelihood of tractor adoption by 1.35 times. This is consistent with the findings of Pingali (2007) and AMIC (1988).

Availability of credit is another significant variable influencing use of machines for land preparation. It should be noted that tractor rental is usually paid on a cash basis, hence, farmers lacking capital but can easily borrow money from various sources are more likely to avail tractor services to cultivate their farms. Farmers who availed credit are more likely to use tractors by 1.51 times compared to farmers with no source of loans or borrowings.

Age is also an important variable influencing adoption or use of tractors. As farmers grow older and their physical strengths start to decline, labor-replacing technologies become a necessity. As the age of maize farmer increases by a year, the likelihood of using tractor increases by 1.01 times. This finding is contrary to most literatures asserting that technology adoption decreases with age, as older farmers are usually more reluctant to change (Bootinger et al.2010, Awotide et al.2014).

Another significant variable affecting farmers' decision to adopt tractor is the farmer's level of education. This implies that farmers with college level education are more likely to adopt tractors by 1.09 times compared to farmers with no college education.

Meanwhile, other variables such as tenurial status, membership in farm organizations, attendance to trainings and seminars related to corn mechanization, cost of land preparation using draft animals, ownership of draft animal dummy and labor situation dummy do not influence the decision of maize farmers to use tractors. It is important to note that landowners make all farm decisions including the use of farm power. Moreover, there was limited number of cooperatives or farm organizations engaged in maize production and marketing so the data did not show large variations in terms of cooperative membership. This was also observed for trainings or seminars related to maize production, postharvest or mechanization.

The Hosmer and Lemeshow goodness-of-fit p-values of 7.843, with 8 *df* is not significant, indicates that the model fit the data well.

Table 3. Regression results showing determinants of mechanizing land preparation by maize farmers in the Philippines

Independent Variables	β_i	***S.E.***	$E(\beta_i)$
Age	0.011^{*}	*0.006*	1.011
Educational attainment	0.084^{***}	*0.023*	1.088
Tenurial status	-0.087^{ns}	*0.140*	0.917
Membership in farm organizations	0.202^{ns}	*0.141*	1.223
Attendance to seminars/trainings	0.127^{ns}	*0.141*	1.135
Credit access	0.413^{***}	*0.142*	1.511
Area	0.299^{**}	*0.136*	1.349
Topography	1.125^{***}	*0.161*	3.080
Man-animal land preparation cost	*0.000*	*0.000*	1.000
Owned draft animals	-0.077^{ns}	*0.143*	0.926
Labor situation during peak periods	0.168^{ns}	*0.190*	0.183
Hosmer and Lemeshow Test			
χ^2		7.843^{ns}	
df		8	
Nagelkerke Pseudo R^2 = .140			

*Significant at 10%
**Significant at 5%
***Significant at 1%
^{ns}Not significant

Determinants of agricultural machinery ownership. For the ownership of agricultural machinery model, important determinants are topography, area of the farm, education and age (Table 4). Farmers with farms situated in the lowlands are more likely to purchase agricultural machinery by 1.92 times compared to farmers in the uplands. For an enterprising farmer, investment in agricultural machineries such as tractors in the lowland areas has higher potential of faster recovery of capital as low-lying areas are more productive because of higher cropping intensity and generally better yield. Moreover, the area of the farm is significant variable influencing ownership or purchase of agricultural machineries. One-hectare increase in farm area cultivated increases the likelihood of acquiring farm machinery by 1.35 times.

For demographic variable age of farmers, a one-year increase in the age of farmers increases the likelihood of purchasing agricultural machinery by 1.014 times. This is even more essential for farmers who are already old, who can no longer perform tedious activities. With regard to education, rice farmers who obtained higher education are more likely to purchase agricultural machines by 1.65 times compared to farmers with low level of education. While education is considered influencing productivity by affecting farmers' ability to understand the complicated information related to different technologies and to adjust quickly to new practices, educated farmers are more likely to have higher or other sources of incomes so they have the capability to buy or invest in agricultural machineries.

The Hosmer and Lemeshow goodness-of-fit p-values of 4.466, with 8 *df* is not significant, hence, the model fit the data well.

Table 4. Regression results showing determinants of tractor ownership by maize farmers in the Philippines.

Independent variables	β_i	*S.E.*	$E(\beta_i)$
Age	0.014^{***}	*0.004*	1.014
Educational attainment	0.503^{***}	*0.133*	1.653
Tenurial status	0.108^{ns}	*0.099*	1.114
Membership in farm organizations	-0.049^{ns}	*0.101*	0.952
Attendance to seminars/trainings	0.005^{ns}	*0.101*	1.005
Credit access	-0.118^{ns}	*0.099*	0.889
Area	0.299^{**}	*0.136*	1.349
Topography	0.653^{***}	*0.106*	1.921
Owned draft animals	-0.011^{ns}	*0.102*	0.989
Labor situation during peak periods	-0.105^{ns}	*0.114*	0.900
Hosmer and Lemeshow Test			
χ^2		4.466^{ns}	
df		8	
Nagelkerke Pseudo $R^2 = .103$			

**Significant at 5%
***Significant at 1%
^{ns}Not significant

CONCLUSION

This study provides empirical information concerning the factors that determine the adoption of tractors by maize farmers in the Philippines. The adoption of tractors is higher among older farmers and with higher level of education. As the age of farmers increases, the likelihood of using tractors also increases.. Availability of credit also increases tractor adoption, as farmers who are able to avail of loans are more likely to use tractors. As the area of the farm increases, the likelihood of tractor adoption also increases. Moreover, corn farms in the plains, lowland and gently sloping areas are more likely to be mechanized compared to farms in hilly areas with steep gradients.

With the findings, it is recommended that the factors and barriers to adoption be considered in devising intervention and promotion strategies to stimulate wider adoption of mechanization technologies such as tractors. The Department of Agriculture should intensify maize mechanization efforts in order to allow small farmers to collectively own tractors. However, the physical attributes of maize farms should be considered in the development and promotion of machines suitable for the hilly areas. The provision of low-cost credit is also important to increase adoption of tractors. Furthermore, there is a need to reconsider early consolidation or clustering efforts to enable small fragmented farms to avail of capital intensive machines and allow economies of scale operation.

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HOST PLANTS OF *MYTHIMNA SEPARATA* (WALKER) (LEPIDOPTERA:NOCTUIDAE) IN THE PHILIPPINES AND INVENTORY OF WORLD RECORDS

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ABSTRACT

Mythimna separata (Walker) is an invasive species and a serious pest of corn (cereals), pasture and forage crops in the Philippines. Previous studies on the biology, ecology, and management of this important pest were conducted in the Philippines in late 60's. At present, the host plants of *M. separata* were studied based on fragmental bibliographic information, reports from local government units, as well as field observations in different areas, particularly in Luzon islands where corn is commonly grown. Host plants for mass rearing of the larvae under laboratory conditions were likewise studied at the Biocontrol Laboratory of the National Crop Protection Center, College of Agriculture and Food Science, University of the Philippines Los Baños from November 2019 to May 2022. The literature review and actual field and laboratory observations resulted in a total of 95 larval host plant records belonging to 18 families, majority of which belong to Poaceae (44 species), Brassicaceae (10), Cucurbitaceae (9), and Fabaceae (8). A native open-pollinated variety of corn (IPB var 6) was evaluated to be useful in mass rearing of *M. separata* for about 19 generations because of its suitability and ease of planting and maintenance.

Key words: invasive species, host plant range, paddy armyworm, polyphagous pest

INTRODUCTION

The true armyworm, more commonly known as paddy armyworm, *Mythimna separata* (Walker), is a serious and invasive pest of corn in the Philippines (Cadapan and Sanchez 1972). It is a polyphagous pest which attacks a whole range of host plants, including vegetables, root crops, cucurbits, legumes, cereals, sugarcane, solanaceous crops, fruits, ornamentals, cut flowers, and weeds, with several reports citing varying number of host plants. It was reported that hectares of plants in the field such as corn, sugarcane, and pasture grass, were entirely consumed in a single day (Cadapan and Sanchez 1972). Severe damage during outbreaks were reported in rice, wheat, sorghum and millets in India, Japan, China, Australia, and New Zealand (Sharma and Davies 1983; Jiang et al. 2011; Koyama and Matsumura 2019). In a more recent report, it is a major migratory pest of cereal crops in East Asia, South Asia, and Australia, resulting in significant losses (Li et al. 2021). The difficulty in predicting and preventing outbreaks was attributed to powerful flight capacity, high reproductive potential of females and the high voracity of older larval instars of *M. separata*. Earlier, host plants were identified that sustain adult moths during long-distance migration (Liu et al. 2017). Using core barcode markers and pollen morphology, 13 plant species belonging to nine families, mainly from Angiosperma, Dicotyledonae were identified. Recently, *M. separata* was reported as widespread in 21 countries in Asia and present in Europe (Russia) and Oceana (Australia, Cook Islands, Fiji, New Caledonia, Norfolk Island, New Zealand, Papua New Guinea, Samoa, Solomon Islands, Tonga and Vanuatu (CABI 2022).

Other researchers reported on the responses of *M. separata* to three plant volatiles (Hou et al. 2019), a modified synthetic diet consisting of corn leaf powder – casein, albumin, peptone and cellulose (Jian et al. 2019), cross resistance studies to insecticides (Rasul et al. 2021), application of CRISPR – Cas9 genome editing (Tang et al. 2021), and its biological control using entomopathogenic fungi (Mantzoukas et al. 2022).

Information on the host plants of *M. separata* in the Philippines have been published in the works of Deang (1969), Cadapan and Sanchez (1972), Catindig et al. (1994), and Gabriel (1997). In other countries, literature on host plants covering the periods 1961 to 1981 were reviewed and 33 species in eight families were reported (Sharma and Davies 1983). The Plantwise Knowledge Bank cited 31 species in eight families (CABI 2022).

The study aimed mainly to produce a consolidated list of host plants from the Philippines and abroad and to find host plants suitable for mass rearing of *M. separata* in the laboratory. This new comprehensive and updated list of host plants will improve the understanding of *M. separata*, its biology, and its management. It can also be useful as a reference for future studies of the pest.

MATERIALS AND METHODS

Survey and field observation of damage on host plants of *M. separata.* Field collection and observation of host plants were conducted from November 2019 to May 2022 in different provinces in Luzon, Philippines, in areas where corn and other crop hosts of *M. separata* are commonly grown. These included the provinces of Tarlac, Pangasinan, Isabela, Cagayan, Laguna, Batangas, and Quezon (Fig. 1). Plants and weeds in and around the corn field were surveyed following the method of van Strien et al. (2013) which is opportunistic in nature, observation of species collected without standardized field protocol and without explicit sampling design, to cover the most extensive area possible in a limited time.

Inclusion of a plant in the list of host plants was based on the presence of *M. separata* larva or larvae observed feeding on the plants and subsequently identified after rearing in the laboratory until adult emergence. The host plants tested in the laboratory as potential host for mass rearing were eggplant, sweet potato, Johnson grass, spring onion, black pigweed, rice, and corn as standard host plant. Host plants were also photographed and collected for identification.

Fig. 1. Survey site in Barangay Bocohan, Lucena City, Quezon Province, Philippines. (Note: The larvae on the lower left picture were collected by the farmer, which were killed by drowning in a pail of water.)

Evaluation of host plants from published literature and databases. Host plants of *M. separata* were identified using illustrations and descriptions from books, global databases, and journal articles. Assistance from systematists of the Museum of Natural History of the University of the Philippines Los Baños was also sought. Scientific names and family of host plants were validated through The Plant List (http://www.theplantlist.org/). Likewise, English names were validated through the Center for Agriculture and Bioscience International (CABI, https://www.cabi.org).

Systematic gathering and review of published journal articles, books, handbooks, global databases, and IEC (information, education and communication) materials from the Philippines and abroad were conducted to come up with an updated comprehensive list of reported host plants of *M. separata.*

RESULTS AND DISCUSSION

Based on reports from literature, larvae of *M. separata* feed on at least 94 species and varieties/cultivars of host plants. The list enumerated the host plants reported by earlier workers, mostly citing Deang (1969), Cadapan and Sanchez (1972), Sharma and Davies (1983), Catindig et al. (1994), Gabriel (1997) and CABI Plantwise Knowledge Bank (2022). Additional species were observed and collected as host plants of *M. separata* from the local survey conducted, bringing the total hosts to 95 species/varieties (Table 1).

Table 1. List of host plants of *Mythimna separata* (Walker).

Host Plants			
Family	**Scientific name**	**English Name**	**References**
Aizoaceae	*Trianthema portulacastrum* L.*	Black pigweed	New record (tested in the laboratory)
Amaranthaceae	*Achyranthes bidentata* Blume***	Ox knee	Koyama and Matsumura 2019; Yooboon et al. 2020
	Beta vulgaris L.***	Sugarbeet	Sharma and Davies 1983; Plantwise Knowledge Bank 2019
Amaryllidaceae	*Allium fistulosum* L.***	Green onion	Koyama and Matsumura 2019; Yooboon et al. 2020
Asteraceae	*Lactuca sativa* L.**	Lettuce	Deang 1969
Brassicaceae	*Brassica juncea* (L.) Czern.**	Mustard	Deang 1969; Gabriel 1997
	Brassica napus L.***	Rape / Rapeseed	Sharma and Davies 1983; Koyama and Matsumura 2019; Yooboon et al. 2020
	Brassica oleracea var. botrytis L.**	Cauliflower	Deang 1969; Gabriel 1997
	Brassica oleracea var. capitata L.**	Cabbage	Deang 1969; Gabriel 1997; Koyama and Matsumura 2019; Yooboon et al. 2020
	Brassica oleracea var. italica Plenck**	Brocolli	Deang 1969
	Brassica rapa L.***	Turnip	Sharma and Davies 1983; Plantwise Knowledge Bank 2019
	Brassica rapa subsp. chinensis (L.) Hanelt**	Chinese cabbage	Deang 1969; ; Cadapan and Sanchez 1972; Sharma and Davies 1983; Gabriel 1997; Plantwise Knowledge Bank 2019
	Brassica rapa subsp. Oleifera***	Turnip rape	Plantwise Knowledge Bank 2019
	Brassica rapa subsp. pekinensis (Lour.) Kitam.***	Napa Cabbage	Plantwise Knowledge Bank 2019
	Raphanus raphanistrum subsp. sativus (L.) Domin**	Daikon	Deang 1969; Gabriel 1997; Kuramitsu et al. 2016; Koyama and Matsumura 2019; Yooboon et al. 2020
Cannabaceae	*Cannabis sativa* L.***	Hemp	Sharma and Davies 1983; Plantwise Knowledge Bank 2019
Commelinaceae	*Commelina diffusa* Burm.f.**	Spreading dayflower	Catindig et al. 1994
Convolvulaceae	*Ipomoea batatas* (L.) Lam.**	Sweet potato	Deang 1969; Cadapan and Sanchez 1972; Gabriel 1997
Cucurbitaceae	*Benincasa hispida* (Thunb.) Cogn.**	Wax gourd	Deang 1969, Gabriel 1997
	Citrullus lanatus (Thunb.) Matsum. & Nakai**	Water melon	Deang 1969; Gabriel 1997; Koyama and Matsumura 2019; Yooboon et al. 2020
	Cucumis melo var. cantalupo Ser.**	Sweet melon	Deang 1969; Gabriel 1997

Host Plants			
Family	**Scientific name**	**English Name**	**References**
	Cucumis sativus L.**	Cucumber	Deang 1969; Cadapan and Sanchez 1972; Gabriel 1997
	Cucurbita maxima Duch**	Squash	Deang 1969; Cadapan and Sanchez 1972; Gabriel 1997
	Lagenaria siceraria (Molina) StandL.*	Calabash	Deang 1969; Gabriel 1997
	Luffa acutangula L.**	Ridged gourd	Deang 1969
	Momordica charantia L.**	Bitter gourd	Deang 1969; Cadapan and Sanchez 1973; Gabriel 1997
	Sechium edule (Jacq.) Sw.**	Chayote	Deang 1969; Gabriel 1997
Cyperaceae	*Cyperus brevifolius* (Rottb.) Hassk.**	Shortleaf Spikesedge	Catindig et al. 1994
	Cyperus difformis L.**	Smallflower umbrella-sedge	Catindig et al. 1994
	Cyperus iria L.**	Rice flatsedge	Catindig et al. 1994
	Cyperus rotundus L.**	Nutgrass	Sharma and Davies 1983; Catindig et al. 1994; Plantwise Knowledge Bank 2019
	Fimbristylis quinquangularis (Vahl) Kunth**	Grasslike fimbry	Catindig et al. 1994
	Rhynchospora colorata (L.) H.Pfeiff.**	Starrush Whitetop	Catindig et al. 1994
Eriocaulaceae	*Eriocaulon sexangulare* L.***		Sharma and Davies 1983
Euphorbiaceae	*Manihot esculenta* Crantz**	Cassava	Cadapan and Sanchez 1972
Fabaceae	*Arachis hypogaea* L.**	Peanut	Cadapan and Sanchez 1972
	Cajanus cajan (L.) Millsp.***	Pigeon pea	Plantwise Knowledge Bank 2019
	*Glycine max (*L.) Merr.**	Soybean	Catindig et al. 1994; Koyama and Matsumura 2019; Plantwise Knowledge Bank 2019; Yooboon et al. 2020
	Phaseolus vulgaris L.***	Beans	Sharma and Davies 1983; Kuramitsu et al. 2016; Plantwise Knowledge Bank 2019
	Pisum sativum L.***	Pea	Sharma and Davies 1983; Plantwise Knowledge Bank 2019
	Vigna radiata (L.) R.Wilczek**	Mungbean	Catindig et al. 1994
	Vigna unguiculata (L.) Walp.**	Bush sitao	Cadapan and Sanchez 1972; Catindig et al. 1994
	Vigna angularis (Willd.) Ohwi & H.Ohashi***	Adzuki bean	Koyama and Matsumura 2019; Yooboon et al. 2020
Linaceae	*Linum usitatissimum* L.***	Linseed	Sharma and Davies 1983; Plantwise Knowledge Bank 2019
Malvaceae	*Gossypium arboreum* L.**	Cotton	Gabriel 1997
Poaceae	*Avena sativa* L.***	Oat	Sharma and Davies 1983; Plantwise Knowledge Bank 2019
	Brachiaria distachya (L.) Stapf**	Armgrass millet	Cadapan and Sanchez 1972; Catindig et al. 1994
	Brachiaria mutica (Forssk.) Stapf**	Para grass	Cadapan and Sanchez 1972; Sharma and Davies 1983; Plantwise Knowledge Bank 2019

Host Plants			
Family	**Scientific name**	**English Name**	**References**
	Chloris barbata Sw.**	Swollen finger grass	Catindig et al. 1994
	Chrysopogon aciculatus (Retz.) Trin.**	Love grass	Cadapan and Sanchez 1972
	Cynodon dactylon (L.) Pers.**	Bermuda grass	Sharma and Davies 1983; Catindig et al. 1994
	Dactyloctenium aegyptium (L.) Willd.**	Crowfoot grass	Cadapan and Sanchez 1972; Catindig et al. 1994
	Digitaria ciliaris (Retz.) Koeler**	Habit of summer grass	Catindig et al. 1994
	Digitaria sanguinalis (L.) Scop.**	Hairy crabgrass	Catindig et al. 1994
	Echinochloa colona (L.) Link**	Jungle rice	Gargav et al. 1972; Sharma and Davies 1983; Catindig et al. 1994; Plantwise Knowledge Bank 2019
	Echinochloa crus-galli (L.) P. Beauv.**	Barnyard grass	Catindig et al. 1994; Plantwise Knowledge Bank 2019
	Echinochloa crus-pavonis (Kunth) Schult.**	Gulf cockspur grass	Cadapan and Sanchez 1972
	Echinochloa esculenta (A.Braun) H.Scholz***	Japanese barnyard millet	Sharma and Davies 1983
	Eleusine coracana (L.) Gaertn.***	Finger millet	Sharma and Davies 1983; Plantwise Knowledge Bank 2019
	Eleusine indica (L.) Gaertn.**	Goose grass	Cadapan and Sanchez 1972; ; Sharma and Davies 1983; Catindig et al. 1994
	Eriochloa procera (Retz.) C.E.Hubb.**	Rice weeds	Catindig et al. 1994
	Hordeum vulgare L.***	Barley	Sharma and Davies 1983; Kuramitsu et al. 2016; Plantwise Knowledge Bank 2019
	Imperata cylindrica (L.) Raeusch.**	Cogon grass	Catindig et al. 1994
	Isachne globosa (Thunb.) Kuntze**	Swamp millet	Catindig et al. 1994
	Leersia hexandra Sw.**	Southern cutgrass	Catindig et al. 1994
	Leptochloa chinensis (L.) Nees**	Red sprangletop	Catindig et al. 1994
	Oryza sativa L.**	Rice	Cadapan and Sanchez 1972; Sharma and Davies 1983; Catindig et al. 1994; Gabriel 1997; Chen and Hu 2000; Wang et al. 2006; Kouassi et al. 2009; Kuramitsu et al. 2016; ali et al. 2017; Zhao et al. 2018; Koyama and Matsumura 2019; Plantwise Knowledge Bank 2019; Yooboon et al. 2020
	Panicum maximum Jacq.**	Guinea grass	Cadapan and Sanchez 1972; Catindig et al. 1994
	Panicum miliaceum L.***	Millet	Sharma and Davies 1983; Chen and Hu 2000; ali et al. 2017; Koyama and Matsumura 2019; Plantwise Knowledge Bank 2019; Yooboon et al. 2020

Family	Host Plants Scientific name	English Name	References
	Panicum proliferum Lam.***	Little millet	Sharma and Davies 1983
	Panicum repens L.**	Torpedo grass	Cadapan and Sanchez 1972
	Panicum setigerum Retz.***	Little millet	Sharma and Davies 1983
	Panicum sumatrense Roth***	Little millet	Sharma and Davies 1983
	Panicum antidotale Retz.***	blue panicgrass	Plantwise Knowledge Bank 2019
	Paspalidium flavidum (Retz.) A.Camus**	Yellow Watercrown Grass	Cadapan and Sanchez 1972; Catindig et al. 1994
	Paspalum conjugatum P.J.Bergius**	Carabao grass	Cadapan and Sanchez 1972; Catindig et al. 1994
	Paspalum dilatatum Poir.**	Dallis grass	Cadapan and Sanchez 1972
	Paspalum distichum L.**	Knotgrass	Catindig et al. 1994
	Paspalum scrobiculatum L.**	Kodo millet	Catindig et al. 1994
	Pennisetum glaucum (L.) R.Br.**	Pearl millet	Cadapan and Sanchez 1972; ; Sharma and Davies 1983; Plantwise Knowledge Bank 2019
	Pennisetum purpureum Schumach**	Napier grass	Cadapan and Sanchez 1972; Sharma and Davies 1983; Plantwise Knowledge Bank 2019
	Rottboellia exaltata (L.) L.f.**	Itch grass	Cadapan and Sanchez 1972
	Saccharum officinarum L.**	Sugarcane	Cadapan and Sanchez 1972; Sharma and Davies 1983; Gabriel 1997; Plantwise Knowledge Bank 2019
	Secale cereale L.***	Rye	Sharma and Davies 1983; Plantwise Knowledge Bank 2019
	Setaria italica (L.) P.Beauv.***	Foxtail millet	Sharma and Davies 1983; Koyama and Matsumura 2019; Plantwise Knowledge Bank 2019; Yooboon et al. 2020
	Sorghum bicolor (L.) Moench**	Sorghum	Cadapan and Sanchez 1972; Sharma and Davies 1983; Gabriel 1997; Kouassi et al. 2011; Plantwise Knowledge Bank 2019
	Sorghum halepense (L.) Pers.**	Johnson grass	Cadapan and Sanchez 1972; Sharma and Davies 1983; Plantwise Knowledge Bank 2019
	Triticum aestivum L.**	Wheat	Sharma and Davies 1983; Catindig et al. 1994; Gabriel 1997; Chen and Hu 2000; Wang et al. 2006; ali et al. 2017; Zhao et al. 2018; Koyama and Matsumura 2019; Plantwise Knowledge Bank 2019; Yooboon et al. 2020

Host Plants			References
Family	**Scientific name**	**English Name**	
	Zea mays L.**	Maize	Cadapan and Sanchez 1972; Sharma and Davies 1983; Catindig et al. 1994; Gabriel 1997; Chen and Hu 2000; Wang et al. 2006; Kouassi et al. 2010; Kuramitsu et al. 2016; ali et al. 2017; Zhao et al. 2018; Plantwise Knowledge Bank 2019
Polygonaceae	*Fagopyrum esculentum* Moench***	Buckwheat	Koyama and Matsumura 2019; Yooboon et al. 2020
Solanaceae	*Capsicum annuum* L.**	Pepper	Deang 1969
	Nicotiana tabacum L.***	Tobacco	Sharma and Davies 1983; Plantwise Knowledge Bank 2019
	Solanum lycopersicum L.**	Tomato	Deang 1969; Cadapan and Sanchez 1972
	Solanum tuberosum L.**	Potato	Koyama and Matsumura 2019; Yooboon et al. 2020
	Solanum melongena L.**	Eggplant	Deang 1969; Koyama and Matsumura 2019; Yooboon et al. 2020

Legend: * - new record; ** - previous records for the Philippines: *** - plants reported from other countries and occurring in the country but were not encountered in the field survey including those reported only the genus having representative species in the country.

The host plants in the list belong to 61 genera and are classified under 18 families as follows: 44 species under Poaceae (=Graminae); 10 under Brassicaeae; nine under Cucurbitaceae; eight under Fabaceae (=Leguminosae); six under Cyperaceae; five under Solanaceae; two under Amaranthaceae; and one each under Aizoaceae, Asteraceae, Amaryllidaceae, Cannabaceae, Commelinaceae, Convulvuceae, Eriocaulaceae, Euphorbiaceae, Linaceae, Malvaceae and Polygonaceae.

The host plants of *M. separata* in the Philippines, particularly rice (*Oryza sativa*) and corn (*Zea mays*), were consistently reported by local workers and also observed in this study.

Twenty species in six families were listed as host plants of *M. separata* (Deang 1969)   A total of 28 species in 8 families were listed, including 17 preferred and 11 eaten only under stressed conditions. It was reported that host plants, cawitcawitan, *Anchaus echinatus* was not found in the Plant List (plantlist.org), hence, these were omitted on the list (Cadapan and Sanchez 1972). The scientific names of para grass, *Panicum purpurascens* was updated to *Brachiarria mutica* (Forssk) Staph; yellow water crown grass, *Paspalidium flavidum* (Retz.) A. Camus; bittergourd, *Momordica chantia* L. to *M. charantia* L.; sweet potato, *Ipomea batatas* (L.) Poir to *I. batatas* (L.) Lam; tomato, *Lycopersicon esculentum* L. Karsten to *Solanum lycopersicum* L.; pechay, *Brassica chinensin* (L.) to *B. rapa subsp. Chinensis* (L.) Hanelt; cassava, *Manihot maritima* Bohl to *M. esculenta* Crantz, and bush sitao, *Vigna sinensis* L. to *V. unguiculata* (L.) Walp. Ten other host plants listed by Cadapan and Sanchez (1972): carrot, arum, asparagus, hyacinth bean, lima bean, okra, roselle eggplant, cane grass and Egyptian paspalidum which were reported as host plants of *Spodoptera litura*, based on the list of Deang (1969), were excluded in the list of host plants of *M. separata*. The list of Gabriel (1997) included mustard, radish, wax gourd, cucumber, bitter gourd, chayote, cotton, and Johnson grass.

In other countries, Sharma and Davies (1983) reviewed the literature on host plants of *M. separata* covering the periods 1961 to 1981 and 33 species in eight families were reported. All of the host plants were included in the list (Table 1) but the nomenclature was updated using The Plant List (theplantlist.org). Sugar beet listed under family Chenopodiacedae was corrected to Amaranthaceae; rape or rapeseed, *Brassica campestris* was changed to *Brassica napus* L.; Chinese cabbage, *Brassica campestris* var. capitata to *Brassica rapa* subsp. chinensis (L.) Hanelt; jungle rice, *Echinochloa colonum* to *Echinochloa colona* (L.) Link; japanese barnyard millet, *Echinochloa crusgalli* to *Echinochloa esculenta* (A.Braun) H.Scholz; finger millet, *Eleusine coracana* to *Eleusine coracana* (L.) Gaertn.; goose grass, *Eleusine indica* to *Eleusine indica* (L.) Gaertn.; little millet, *Panicum miliare* to *Panicum miliaceum* L.; little millet, *Panicum serigerum* to *Panicum setigerum* Retz.; pearl millet, *Pennisetum americanum* to *Pennisetum glaucum* (L.) R.Br. *Panicum scrobiculatum* was not found in the Plant List, hence, was omitted in the list.

A total of 31 plant species were reported to support complete larval development of *M. separata* pupation and adult emergence (Catinding et al. 1994). Larval survival to pupation was highest on *Leptochloa chinenis* (58%), *Isachne globose* (54%), *Paspalum paspalodes* (53%), and rice (51%). Larval development was shortest on rice (19.2 days) and longest on *Imperata cylindrica* (34.8 days) and *Brachiaria distachia* (37.8 days).

In the present study, of the seven host plants tested under laboratory conditions, *M. separata* when reared on young leaves of corn (IPB var 6) and laid eggs which hatched in 4-5 days, larval period of 17-19 days, pupal period of 10 days, 94% larval survival, 100% pupation of surviving larvae and adult emergence of 96% (Fig. 2).

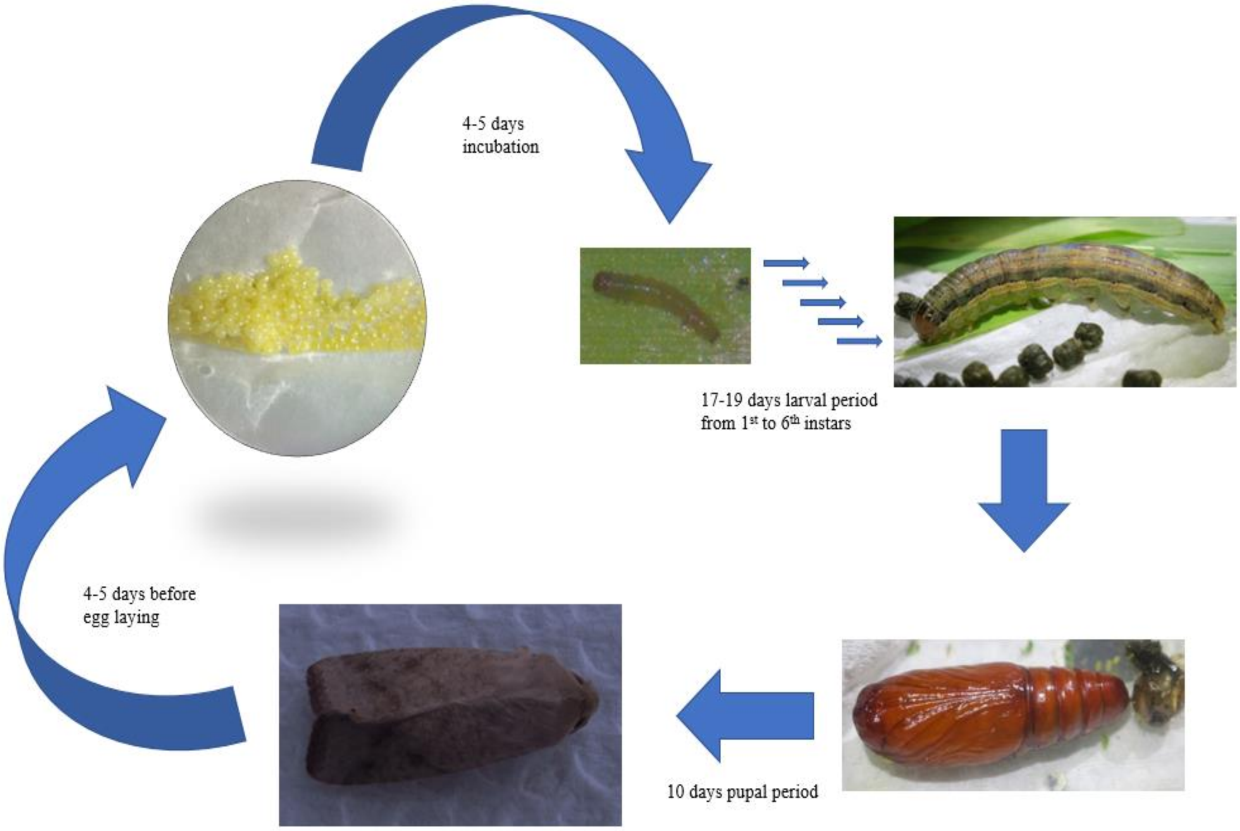

Fig. 2. Life cycle of *Mythimna separata* on young leaves of corn (IPB var 6) under laboratory conditions.

The consolidated global list now has a total of 95 plant species belonging to 61 genera in 18 families, indicating that the pest is highly polyphagous. This polyphagous feeding behavior allows *M. separata* to build or maintain populations on non-crop plants adjacent to or within crop fields, then move to cultivated crops, maximizing crop damage potential. In addition, polyphagous feeding behavior allows *M. separata* to build or maintain its population outside of primary cropping season or outside of cropping areas, contributing to increased pest pressure.

Effective *M. separata* pest management practices must consider the presence of host plants within and around crop fields throughout the year. Knowledge of potential hosts for *M. separata* is an essential component of this approach. This study emphasizes the importance of basic biological information, such as host plant lists, in the development of pest management strategies.

CONCLUSION

The global list of host plants of *M. separata* now has a total of 95 plant taxa belonging to 61 genera in 18 families, indicating that the pest is highly polyphagous. Young leaves of corn (IPB var 6) were found suitable for sustaining 19 generations under laboratory conditions without loss of vitality and vigor of the original population. The information on the host plants is particularly important in the development of pest management strategies against the *M. separata* .

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