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CONTENTS

GROWTH AND YIELD-RELATED TRAITS OF KHAO DAWK MALI 105 (KDML105) RICE IN PADDY AND UPLAND CONDITIONS

Wattanadatsaree Vatanee[1]*, Lisawadiratanakul Sumetee[1], Kenji Irie[1], and Chakhatrakan Somchai[2]

[1]Graduate School of Agriculture, Tokyo University of Agriculture, 1-1-1 Sakuragaoka, Setagaya-ku, Tokyo 156-8502, Japan.

[2]Office of the Vocational Education Commission (OVEC), Institute of Vocational Education in Agriculture-Center Region, 59 M.1 Potang, Bang sai, Phranakhonsi Ayutthaya, Thailand 13290.

*Corresponding author: 46319003@nodai.ac.jp

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ABSTRACT

Drought is a major factor limiting the production and quality of rice. In recent years, damage caused by drought in rainfed lowland rice areas has become a serious problem in Thailand. The study sought to clarify Thai Jasmin rice's growth and yield-related traits compared with three other rice cultivars under paddy and upland conditions. The effects of different water supply levels, corresponding to paddy and upland conditions on four rice cultivars, i.e., KDML105, Dro1-IR64, Kinandang Patong (KP), and IR64 were examined. All four cultivars under upland conditions demonstrated decreased growth (plant height and number of tillers per plant), photosynthesis rate, transpiration rate, stomatal conductance, number of panicles per plant, percentage of filled grains, number of spikelets per panicle, 1000-grains weight and the number of spikelets per plant of rice, whereas the 50% days to heading (50% DTH) increased. The 50% DTH of KDML105 indicated that this cultivar's flowering time occurs later even under paddy conditions, and the longer days (218.5 days) under dehydration stress conditions. Compared to the paddy conditions, the 50% DTH under upland conditions increased by 10.91% for KDML105, 19.51% for Dro1-IR64, 18.91% for KP, and 17.81% for IR64. Regarding yield-related traits, the percentage of filled grains under upland conditions was reduced by 3.77% in KP, 3.97% in IR64, 9.10% in Dro1-IR64, and 12.42% in KDML105 compared with the paddy conditions. KDML105 rice is less resistant to drought compared to the other varieties, and KDML105 is not significantly affected by yield-related traits under water stress conditions. However, this cultivar's long growing season as a photosensitive variety exposes it to drought risk for a long period, and this will affect rice production in the long term. To avoid this unexpected feature, the improvement of non-sensitive varieties is a challenge that should be addressed in the near future against climate risks.

Key words: drought tolerance, photosynthetic trait, Thai Jasmine rice

INTRODUCTION

Rice (*Oryza sativa* L.) is one of the world's most important food crops (Liu et al. 2006). The world's population has doubled since the early 1960s, and the existing yields of the major cereal crops are projected to be insufficient to meet the food needs of the future (Somchai 2005). Among the many varieties of rice, Khao Dawk Mali 105 (KDML105) is the most important rice in Thailand's economy because of its ease of preparation and features (e.g., stickiness, softness, and pleasant fragrance); these characteristics made KDML105 rice popular with consumers. However, KDML105 can be planted only once a year during the rainy season because of cultivation constraints, including the seasonal change in

day length. KDML105 is not a well-known drought-tolerant variety, but its adaptability in a rainfed lowland ecosystem was ascribed to the plasticity of the root system under abiotic stresses (O'Toole and Bland 1987).

The total rice-growing area of Thailand is 9.47 million ha, and the northeast region has the highest production of rice, accounting for 5.22 million ha (63.10%) of Thailand's rice-growing area, followed by the north, central, and south regions, which account for 22.49%, 14.38%, and 1.33% of the area where rice is planted, respectively (Office of Thai Agricultural Economics 2019). Thailand's rice crops are commonly grown under rainfed lowland conditions without irrigation water (Kamoshita et al. 2008). If water deficit problems or inadequate precipitation occurs during the rainy season, the changes in the rice's physiological and biochemical properties will inhibit plant growth (Fukai and Cooper 1995), thus affecting rice development and reducing rice production (Wang et al. 2019). Drought stress affects water metabolism in plants, preventing nutrient uptake, restricting the transpiration rate and plant growth, interfering with physiological and biochemical processes, and decreasing the yield and quality (Maghsoudi et al. 2016).

In Thailand, most of rice-growing areas in the rainfed regions have a relatively limited amount of water, which affects the plants' growth and decreases rice production (Hanson et al. 1990). Drought is a severe factor that limits rice production and quality. Lack of water can cause a reduction in the number of panicles and developed seeds (Liu et al. 2006). When rice plants are subjected to drought during the growing season, the yield is reduced by approx. 17% plus by another 30% during the reproductive stage, when the formation of the young grains takes place (Thawatchai 1992). This is consistent with earlier findings showing that higher temperatures cause water shortages that in turn increase the water evaporation in plants' anthers, resulting in abnormal male pollen (Mohammed and Tarpley 2011). A drought can thus severely limit the quantity and quality of rice.

The present study sought to evaluate growth characteristics and yield of the rice cultivar Khao Dawk Mali 105 (KDML105) and its ability to resist drought stress in comparison with three other rice cultivars under different water regimes, toward the goal of using information obtained to develop and improve rice varieties in the future.

MATERIALS AND METHODS

Plant materials and growth conditions. Thai rice cultivar KDML105 was evaluated alongside the shallow-rooting cultivar IR64, the deep-rooting cultivar Kinandang Patong (upland rice), and the cultivar Dro1-IR64, which is a near-isogenic line homozygous for the Kinandang Patong (KP) allele of Deeper Rooting 1 (DRO1) in the IR64 genetic background. Seeds of each cultivar were sown in nursery boxes to germinate in a greenhouse and transplanted after 30 days: one seedling per hill in a concrete tank under both paddy and upland conditions. The soil moisture content was controlled at 2.0–2.2 kPa at 20 cm from the soil surface using a pF-meter (TAKEMURA Electric Works Ltd., Tokyo, Japan) to achieve a water deficit in the upland condition. Fertilizer (N-P_2O_5-K_2O) was applied at 30 and 60 days after transplanting at 25:25:25 kg/ha and 25:0:0 kg ha^{-1}, respectively. The experiments were conducted from May 2019 to October 2019 at the Tokyo University of Agriculture, Tokyo, Japan.

Data measurement. The plant height and tiller number per plant as growth parameters were recorded. The photosynthetic rate, transpiration rate, and stomatal conductance were recorded by using the plant photosynthesis analyzer (LCi-SD, ADC BioScientific Ltd., UK) equipped with blue and red-light sources to measure photosynthesis under 1000 mmol m^{-2} s^{-1} photosynthetic photon flux density (PPFD) and a 500 mmol s^{-1} flow rate at 70 days post-transplantation (the booting stage). The photosynthetic parameters of the rice plants were analyzed between 9:00 and 11:00 a.m. at the middle part of the most developed leaf (Zhang et al. 2017). The following yield-related traits were recorded: 50% days to

heading (50%DTH), the number of panicles per plant, the percentage of filled grains per panicle, the number of spikelets per panicle, the 1000-grains weight, and the number of spikelets per plant.

Statistical analysis. Statistical differences were determined using SPSS Statistics ver. 22 (Chicago, IL, USA). Mean differences were compared by Duncan's multiple range test (DMRT) and t-test at a probability level of 0.05. A principal component analysis (PCA) of the mean-centered average results from the four rice cultivars and the two conditions (paddy and upland) was conducted. The PCA axes 1 and 2 were tested with the SPSS software.

RESULTS AND DISCUSSION

Plant growth. The growth parameters of the rice cultivars under paddy and upland conditions are summarized in Table 1. Under upland conditions, all traits were influenced by the agronomic characteristics of KDML105 and the other three rice cultivars. Compared to paddy conditions, the upland condition decreased significantly plant height in all cultivars by 7.01% in KDML105, 17.70% in Dro1-IR64, 15.32% in KP, and 17.95% in IR64. These data demonstrated that KDML105 had the least decrease compared to other rice cultivars, and this decrease was due to the specific characteristics of the KDML105 cultivar, which has a plant height at 140–150 cm (ORDPB 2012). At the same time, the tiller number per plant differed significantly between the cultivars KP and IR64. This is consistent with the study reported by Manal et al. (2014) showing that rice grown under a decreased water supply exhibit reduced plant height, leaf area, root length, and root biomass (Rodriguez et al. 2005; Kang and Futakuchi 2019). In addition, drought hinders leaf and tiller development at the vegetative stage of rice (Pantuwan et al. 2002). Sunflowers grown under dehydration, showed decreased plant height, root length, total leaf area, and total dry weight compared to sunflowers grown under normal conditions (Manivanna et al. 2007)

Relative chlorophyll content (SPAD). The comparison of the water resistance of the four rice varieties revealed that the environmental conditions and rice cultivars significantly affected the soil and plant analyzer development (SPAD) value, which is a measure of the relative chlorophyll concentration of leaves (Netto et al. 2005). The rice variety with the highest SPAD value was KP (44.48), followed by KDML105 (40.40 units) and Dro1-IR64 and IR64, the values of which were not significantly different, 39.22 and 39.24 units, respectively (Table 1). Compared to the paddy condition, the upland condition significantly increased the cultivars' SPAD values; the SPAD value of IR64 increased by 26.17%, that of KP by 25.98%, KDML105 by 25.90%, and Dro1-IR64 by 20.40% (Table 1). Darunee et al. (2017) reported that dehydration affects the increase in SPAD because chlorophyll density was increased under drought conditions (Arunyanark et al.2008; Zokaee-Khosroshahi et al. 2014) causing higher chlorophyll content per unit leaf area (Songsri et al. 2009). The present findings reveal a significant increase in SPAD values in rice grown in water-shortage conditions, which has also been reported for potato (Rolando et al. 2015) and snap beans (Nemeskéri et al. 2017). This phenomenon may be due to the alterations of the water and chlorophyll content in the leaves, accompanied by decreased photosynthetic active light absorbance; the large reflectance is thus manifested in high SPAD values (Rolando et al. 2015).

Photosynthetic rate, transpiration rate, and stomatal conductance. The comparison of the four rice cultivars under different irrigation conditions demonstrated that the rate of photosynthesis (Pn), transpiration rate (T), and stomatal conductance (Sc) were all significantly affected ($p \leq 0.01$). Compared to the paddy condition, the photosynthetic rate was lower for all cultivars grown under the upland condition. The photosynthetic rates under the upland condition were significantly decreased in Dro1-IR64 and IR64 by 41.48% and 39.07%, compared to the paddy condition while those of KDML105 and KP were relatively smaller at 19.37% and 20.09%, respectively (Table 1).

Table 1. Effects of water deficit on the growth, photosynthetic rate, transpiration rate, and stomatal conductance of the four rice cultivars.

Cultivar	Condition	Plant height (cm)	Tiller number/ plant	SPAD	Photosynthetic rate (μmol m^{-2} s^{-1})	Transpiration rate (mmol m^{-2} s^{-1})	Stomatal conductance (mmol m^{-2} s^{-1})
KDML105	Paddy	123.34 **	12.17 ^{ns}	35.45 **	5.99 ^{ns}	3.07 **	0.073 ^{ns}
	Upland	114.69 (−7.01)	10.42 (−14.38)	44.63 (+20.57)	4.83 (−19.37)	1.16 (−62.21)	0.050 (−31.51)
Dro1-IR64	Paddy	103.30 **	15.19 ^{ns}	35.59 **	7.69 **	5.85 *	0.344 **
	Upland	85.02 (−17.70)	15.10 (−0.59)	42.85 (+16.94)	4.50 (−41.48)	2.27 (−61.20)	0.082 (−76.16)
KP	Paddy	141.35 **	4.72 **	39.73 **	7.29 ^{ns}	4.94 **	0.191*
	Upland	119.70 (−15.32)	2.74 (−41.95)	50.03 (+25.92)	5.76 (−20.99)	2.07 (−58.10)	0.065 (−65.97)
IR64	Paddy	97.91 **	14.93 *	34.70 **	7.96 **	4.41 *	0.143 *
	Upland	80.34 (−17.95)	11.67 (−21.84)	43.78 (+20.74)	4.85 (−39.07)	2.51 (−43.08)	0.090 (−37.06)
Cultivar		**	**	**	ns	**	**
Condition		**	**	**	**	**	**
Cultivar × Condition		**	*	*	ns	*	*

Values in the parentheses indicate an increase (+) and decrease (-) in each parameter under upland conditions.
*p<0.05, **p<0.01, ns: not significantly different by t-test.

Drought affects various steps of the photosynthetic pathway by impairing pigments, photosynthesis, gas exchange, and photosynthetic enzymes in the plants (Asharf and Harris 2013). A similar trend was observed in this study but the degree of reduction was smaller for KP and KDML105 suggesting that KP grown in upland cultivation and KDML105 grown in rain-fed lowland cultivation are adapted to dry conditions. The transpiration rate (T) and stomatal conductance (Sc) in the upland-grown cultivars were significantly reduced compared to those grown in the paddy condition. Reductions in Sc of the shallow-rooted KDML105 and IR64 were smaller than those of the deep-rooted Dro1-IR64 and KP. Stomatal conductance is a major factor in plant photosynthesis (Reddy et al. 2004; Farooq et al. 2009), because a decrease in Sc decreases carbon dioxide (CO_2) fixation.

Generally, chloroplasts are an essential part of the photosynthesis process damaged by reactive oxygen species (Smirnoff 1995). When stomata conductance decreases, the level of photosynthesis also decreases. Drought-tolerant rice cultivars also exhibit intense stomatal closure by reducing stomatal conductance more effectively compared to drought-susceptible cultivars (Ji et al. 2012).

Heading and yield-related traits in a water-deficit condition. Table 2 provides the 50% days to heading (50% DTH) data of the four rice cultivars under paddy and upland conditions. Both the cultivar and the treatment (paddy vs. upland) significantly affected the number of days to heading ($p \leq 0.01$). The water deficit was associated with a substantial delay in the heading date (Table 2). The heading was delayed by 21.5–24.0 days in the upland condition compared to the paddy condition (KP had the lowest 50%DTH, followed by Dro1, IR64, and KDML105, respectively). The heading date or rice grown in a short-day (SD) condition was earlier than that in the long-day (LD) condition, and the heading date of a mutant was earlier than that of the wildtype (Saikumar et al. 2016; Luan et al. 2009). It was observed that KDML105 was the wildtype and very sensitive to environmental factors, especially the photoperiod and temperature. For this reason, it takes more time to flower.

The heading was delayed by water-deficit stress. Although KDML105 showed a longer growing season due to the changing day length in this study conducted in Japan, the delay in heading due to the water-deficit condition was relatively small compared to the other three cultivars. KDML105 is a strong photosensitive variety, and this characteristic suppresses heading under LD conditions and promotes it under SD conditions (Izawa 2007). Rice heading is well known to be delayed under low-temperature conditions (Luan et al. 2009). The most important quantitative trait loci (QTLs) for the flowering process in rice are Hd1 and Hd3a (Kojima et al. 2002); Hd1 promotes the expression of Hd3a under SD conditions and inhibits it under LD conditions (Yano et al. 2000; Izawa et al. 2002; Zhang et al. 2012; Nemoto et al. 2016). Even within a genotype, drought stress strongly affects heading delays (Kang and Futakuchi 2019).

Table 2 provides the data of the yield-related traits, i.e., the number of panicles per plant, the % of filled grains per panicle, the number of spikelets per panicle, the 1000-grains weight, and the number of spikelets per plant. Under the upland condition, all of these traits except the number of spikelets per panicle of KP were lower than the corresponding values under the paddy condition (Table 2). The number of panicles per plant was decreased in all cultivars under the upland condition, with KP showing the greatest reduction (−51.50%), followed by IR64, KDML, and Dro1-IR64 (−27.82%, −19.47%, and −5.70%, respectively). Compared to the paddy field condition, the percentage of filled grains per panicle decreased under the upland condition. The most significant decrease was observed in KDML105 (12.42%), followed by Dro1-IR64, KP, and IR64, (9.10%, 3.97%, and 3.77%, respectively) (Table 2).

Table 2. Effect of water deficit on days to 50% days to heading (50%DTH) and yield components of the four rice cultivars

Cultivar	Treatment	50% DTH, days	No. of panicles per plant	% Filled grains per panicle, %	No. of spikelets per panicle	1000-grains weight, g	No. of spikelets per plant
KDML105	Paddy	197.00 **	6.42 *	56.50 **	87.35 **	25.60 ^{ns}	563.79 **
	Upland	218.50 (+10.91)	5.17 (−19.47)	49.48 (−12.42)	65.75 (−24.73)	24.12 (−5.78)	339.49 (−39.78)
Dro1-IR64	Paddy	123.00 **	11.58 **	85.71 ^{ns}	154.21 *	27.05 *	1789.78 *
	Upland	147.00 (+19.51)	10.92 (−5.70)	77.91 (−9.10)	142.71 (−7.46)	25.25 (−6.64)	1556.45 (−13.04)
KP	Paddy	119.00 **	4.33 **	83.68 ^{ns}	280.39 ^{ns}	23.22 ^{ns}	1212.28 **
	Upland	141.50 (+18.91)	2.10 (−51.50)	80.52 (−3.77)	281.70 (+0.47)	21.07 (−9.26)	591.55 (−51.20)
IR64	Paddy	123.50 **	12.58 *	80.27 ^{ns}	149.90 **	25.53 ^{ns}	1885.22 **
	Upland	145.50 (+17.81)	9.08 (−27.82)	77.08 (−3.97)	133.92 (−10.66)	23.77 (−6.89)	1215.31 (−35.53)
Cultivar		**	**	**	**	**	**
Condition		**	*	**	**	**	**
Cultivar × Condition		**	**	ns	ns	ns	*

Values in the parentheses indicate an increase (+) and decrease (-) in each parameter under upland conditions.
*p<0.05, **p<0.01, ns: not significantly different by t-test

The low temperature during the ripening period may have affected the percentage of filled grains per panicle of KDML105 since the heading date in Japan was late (October). In addition, it was observed that drought affects rice yields (Mohammed and Tarpley 2011). Higher temperature is one of the reasons for dehydration, which influences the ability of the pollen to cause abnormalities (Mohammed and Tarpley 2011; Rang et al. 2011) another study indicated that the amount of pollen that can be pollinated was reduced under higher temperatures (Rang et al. 2011). In addition, drought stress at the vegetative stage caused a 50% reduction in the grain yield because the drought hindered the leaf and tiller development, subsequently affecting the development of panicles and thus causing an approx. 17% yield loss (Swain et al. 2017; Thawatchai 1992; Pantuwan et al. 2002); a 30% reduction at the reproductive stage with the formation of the young grains due to a decrease in the number of filled grains per panicle was also described (Thawatchai 1992; Pantuwan et al. 2002). When stress was applied between panicle initiation and pollen meiosis, the decreased yield was also affected by the flowering delay and a delay in panicle excretion (Saikumar et al. 2016) due to the delay in floral development (Lafitte et al. 2004).

The relationship between environmental conditions and rice cultivars. To evaluate the effects of the rice cultivar under water deficit conditions, a PCA was conducted using a correlation matrix of seven traits: 50% DTH, SPAD value, photosynthetic rate, transpiration rate, stomatal conductance, number of panicles per plant, and number of spikelets per panicle. The contribution of the first principal component was 57.27% for all variations, with high positive correlations with the transpiration rate, photosynthetic rate, and stomatal conductance, and a high negative correlation with 50% days to heading and SPAD value, indicating that these factors are related to photosynthesis and earliness. The second principal component, with a contribution rate of 27.01%, SPAD value was highly positively correlated with the number of panicles per plant and strongly negatively correlated with the number of spikelets per panicle, and the SPAD value can thus be considered a factor related to yield-related traits in terms of the number of panicles per plant and the number of spikelets per panicle (Fig. 1).

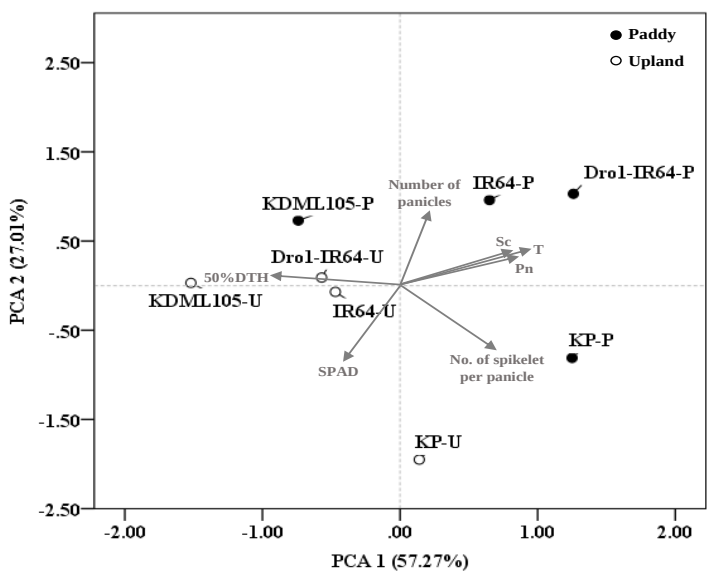

Fig. 1. The principal component analysis (PCA) biplot for the ordination of growth and yield from the rice cultivars KDML105, Dro1-IR64, KP, and IR64 under two paddy and upland conditions. Ps: photosynthesis rate, Sc: stomatal conductance, T: transpiration rate.

Scatter plots for the scores of the first and second principal components demonstrated that under the upland condition, all four rice cultivars showed reduced photosynthetic traits of transpiration rate,

photosynthetic rate, and stomatal conductance, longer 50% DTH periods, and reduced numbers of panicles per plant and numbers of spikelets per panicle. Compared to the other cultivars, KDML105 showed lower photosynthesis-related factors under both paddy and upland conditions. Water stress reduced the standard pace of Pn, Sn, and T (Farooq et al. 2009) while the days to heading increased (Luan et al. 2009).

The correlation coefficient between PCA1 and the number of spikelets per plant was r=0.981, a significant correlation (Fig. 2). These results indicate that water deficit factors and yield have relatively little effect on KDML105 compared to the other tested cultivars, even under a water-deficit condition. The KDML105 has adapted to rainfed cultivation and irregular droughts and water deficits. On the other hand, KDML105 is a strong photosensitive variety and inhibits flowering under LD conditions (Izawa 2007). Late flowering and exposure to low temperatures during the ripening period of KDML105 affected the percentage of spikelets (Fig. 2).

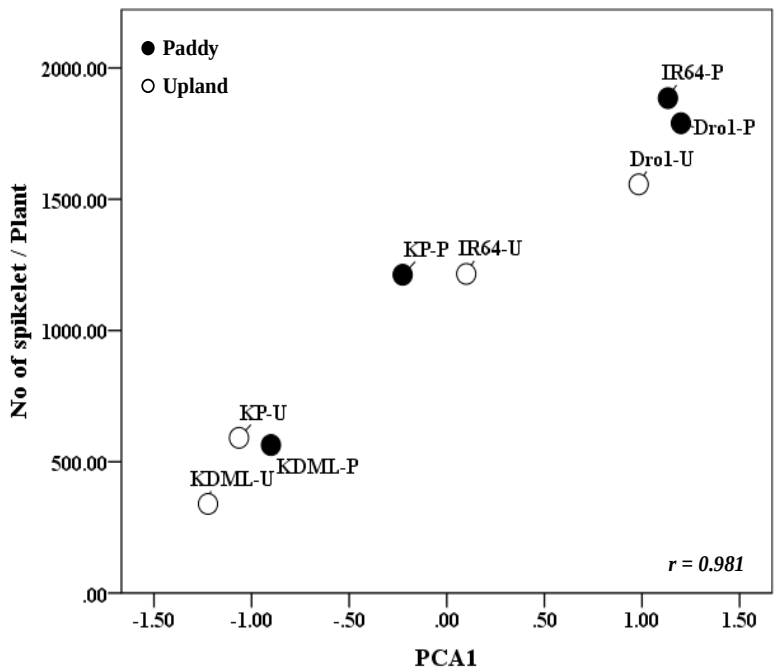

Fig. 2. The ability to adapt to dehydration conditions of all four rice cultivars by using the relationship between principal component 1 (PCA1) and the number of spikelets per plant under paddy and upland conditions.

CONCLUSION

The result of this study can be concluded that the water deficit conditions resulted in decreased growth and yield components. Moreover, when exposed to drought conditions, KDML105 was less drought-tolerant than other rice varieties. In contrast, Dro1 had the best drought tolerance, followed by IR64 and KP. However, the long growing season of KDML105, as it is a photosensitive variety, exposes it to drought risk for an extended period, affecting rice production in the long term. To avoid this unexpected feature, improving non-photosensitive varieties is a challenge that should be addressed shortly against climate risks.

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METABOLOME ALTERATION OF GABA RICH GREEN TEA PRODUCED BY VAPOR TREATMENT WITH *trans*-2-HEXENAL

Kazuomi UCHIDA[1], Keisuke TANAKA[2], Naoki TERADA[1], Atsushi SANADA[1], Takashi Shinohara[1], Takao MYODA[3] and Kaihei KOSHIO[1]

[1]Graduate School of Agriculture, Tokyo University of Agriculture, 1-1-1 Sakuragaoka, Setagaya-ku, Tokyo 156-8502, Japan

[2] NODAI Genome Research Center, Tokyo University of Agriculture, 1-1-1 Sakuragaoka, Setagaya-ku, Tokyo 156-8502, Japan

[3]Department of Food, Aroma and Cosmetic Chemistry, Tokyo University of Agriculture, 198 Yasaka, Abashiri, Hokkaido 099-2493, Japan

Corresponding author: ku207861@nodai.ac.jp

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ABSTRACT

GABA (γ-aminobutyric acid) rich green tea produced by vapor treatment of *trans*-2-hexenal was examined for its possible metabolic pathway using metabolome and transcriptome analysis. Metabolome analysis showed enrichment of GABA (15.1 times higher than control) was accompanied with the increased amount of .pyruvic acid (11.9 times higher than control), alanine (8.5 times higher than control), α-ketoglutaric acid (2.4 time g higher than control), as well as decreased amount of succinic acid (0.8 times as control), aspartic acid (0.4 times as control) and glutamine (0.2 times as control). Based on transcriptome analysis, it was determined that glutamate decarboxylase activity was highly activated (4.1 times higher than control), with the decreased activity of GABA transaminase (0.6 times as control), succinic semialdehyde dehydrogenase (0.7 times as control) and glutamate dehydrogenase activity (0.6 times as control), respectively.

Key words: GABA shunt, glutamate decarboxylase, metabolome, transcriptome

INTRODUCTION

GABA (γ-aminobutyric acid) rich products attract the interests of health conscious people both in developed and developing countries, such as Southeast Asian countries, since GABA 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), and regulates blood pressure (Abe et al. 1995), among others. The GABA rich product market is now expanding all around the world (Horie et al. 2019) including Southeast Asian countries.

A new method was developed to produce GABA rich tea by *trans*-2-hexenal vapor treatment, which succeeded in enriching GABA content almost 10 times higher than when treated in a ventilation system (Uchida et al. 2022). The metabolome alteration caused by *trans*-2-hexenal vapor treatment, however, is not yet clear. In this article, the study sought to clarify the possible physiological mechanism of GABA enrichment, using metabolome and transcriptome analysis, focusing on the GABA shunt.

MATERIALS AND METHODS

Treatment of tea leaves with *trans*-2-hexenal. Fresh leaves of young green tea, Yabukita cultivar of *Camelia sinensis* L., were harvested at Yoshida Cha-en on June 23, 2018 (1181 Ōtsutsumi, Koga,

Ibaraki prefecture). These were treated with 0, 10, 100 ppm *trans*-2-hexenal for 1, 3, or 6 h with two biological replications, and used for the following experiments.

GABA measurement and metabolome analysis with GC-MS. Each sample (0.1 g of frozen tissue powder) was extracted with 250 µl of methanol and chloroform, one after another vigorously and centrifuged at 12000 rpm for 10 min at room temperature. The supernatant fluid (80 µl) of each sample was corrected into a 1.5 ml plastic tube and evaporated to dryness for 3 h in a centrifuge evaporator (CVE-200D, Tokyo Rikakikai Co, Ltd, Japan). The 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 ℃. 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. Helium gas was used as carrier gas at 2.0 mL/min. The initial column oven temperature was set at 100 ℃ for 4 min, then increased by 4 ℃/min until 320℃ for 10 min. Metabolites were identified by comparing fragment patterns and retention indices with those of standard compounds in databases.

Transcriptome analysis

1. RNA isolation, library construction, and sequencing. The total RNA was isolated from fresh green tea leaves treated with 0, 10, 100 ppm *trans*-2-hexenal for 1, 3, or 6 h with two biological replications. The RNA was extracted with a Trizol reagent (Invitrogen). RNase free DNase (Qiagen, Germany) was used to eliminate genomic DNA contamination. To check the purity of the RNA, gel electrophoresis, nanodrop, and the Agilent 2100 bioanalyzer were used. Highly pure Messenger RNA (mRNA) was isolated from the total RNA using oligo (dT) beads. The Illumina TruSeq RNA Library Prep Kit v2 was used to synthesize the second strand cDNAs library. The Illumina HiSeq 2500 platform was used to sequence the constructed cDNA libraries. Sequencing results were obtained as paired-end reads (2 × 100 bp each) in the FASTQ format.

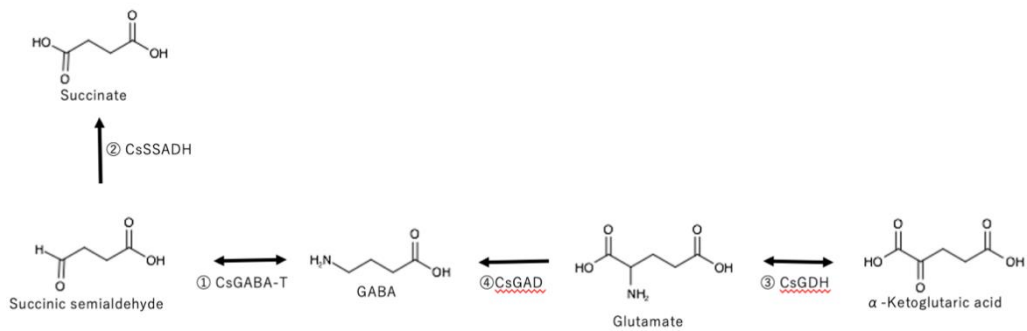
2. *De novo* assembly, ORF detection, and clustering. Raw reads were subjected to quality control by fastaQC (an online tool). Any poor-quality reads and adaptor sequences were filtered by the Trimmomatic and the FASTX-toolkit (Bolger et al. 2014; Gordon et al. 2014). The clean reads were deposited in the sequence read archive (SRA) of the National Center for Biotechnology Information (NCBI) under the accession number SRP128956. The obtained clean reads were assembled into transcriptome, *de novo*, by Bridger (Chang et al. 2015). After the transcriptome was assembled, a TransDecoder was used for the identification of long open reading frames (ORFs) within the transcripts and to score them according to their sequence similarity (Haas et al. 2013). In .order to filter redundancies and to reduce noise in the generated contigs, clustering was performed by the CD-HIT program (Li and Godzik 2006).

3. Gene annotations. Contigs generated by the *de novo* assembly were regarded as the products of tea genes and used as queries for the BLASTX search (Altschul et al. 1990) to examine which protein they encode. For the BLASTX search, non-redundant protein sequences were used as the database. On the basis of the results of the BLASTX search, functional annotations were assigned to the contigs.

4. Differential gene expression and pathway enrichment analysis. To obtain the lists of differentially expressed genes (DEGs) in the control and *trans*-2-hexenal treated green tea, the CLC genomic workbench version 9.5 (QIAGEN) was used. The Empirical Analysis of Differential Gene Expression (EDGE) test was implemented to calculate the p-values and the false discovery rate (FDR). The DEGs were filtered as upregulated and downregulated based on the FC (fold change) ≥ 2 or $FC \leq -2$

respectively with FDR-corrected p values < 0.01. KEGG (Kyoto Encyclopedia of Genes and Genomes) analysis was performed on the upregulated and downregulated differentially expressed genes in order to map them with various biological pathways (Ogata et al. 1999; Kanehisa, 2002). The PlantTFcat online tool (http://plantgrn.noble.org/PlantTFcat/) was used to identify genes encoding transcription factors (Dai et al. 2013).

5. Validation of the RNA-Seq results by the real-time PCR. Ten randomly selected DEGs were chosen for validation by qRT-PCR. The reaction was performed using TB Green™ Premix Ex Taq™ II Kit (Tli RNaseH Plus) (TaKaRa, Tokyo, Japan) in a volume of 20 µl containing 10 µl of TB Green premix Ex Taq II (2X), 0.4 µl of ROX Reference Dye II, 200 ng of cDNA template, and 0.4 µM of each of the primers. Amplification was performed as follows: 95 °C for 30 s followed by 40 cycles of 95 °C for 5 s and 60 °C for 30 s. All the experiments were performed in biological triplicate. The tea gene *Css* and *Csa* were used as a reference gene as previously described (Zhang et al. 2021). Relative gene expression was calculated using the ΔΔCT method (Livak and Schmittgen 2001). The primers used for real-time PCR study are listed in the following Fig. 1.



① CsGABA-T 1 F 5'-TTCACAGATAACAAGTCACCTAAT-3'
R 5'-TCTCACACTCTGCTCCAA-3'
2 F 5'-TAGTATGTTGGCACCATTCAC-3'
R 5'-ACCATAGACCAGCGAGAG-3'
3 F 5'-CGCAGTAGAAGTAGCAGTTG-3'
R 5'-GTCGGTTGTAAGAGATGTGAAT-3'
② CsSSADH 1 F 5'-CCACCAAGTTCCAGAGATAC-3'
R 5'-GCAAGTCCACAGGTAAGG-3'
2 F 5'-ACATTCGCTATAACTTCACCAT-3'
R 5'-TCCTCTCTCGGCAGATTAG-3'
③ CsGDH 1 F 5'-AGGTGGAGTTACGGTTAGTTA-3'
R 5'-GCACGAGCAACACGATTA-3'
2 F 5'-TTGTCCTCCTATTACCTCCAT-3'
R 5'-GAATCCAAGCCGAGAATGT-3'
④ CsGAD 1 F 5'-AGTGACATCCAGAAAGTCTTGCT-3'
R 5'-CACCATTAGTCTTCTTCCTACTGAG-3'
2 F 5'-TTCGACA TCTGCAAGGTGCTCCA-3'
R 5'-ACTTGTGTTTTCCTAGCCAAGAC-3'
3 F 5'-TTTCACATAACAAATGCAACGTC-3'
R 5'-CTCCCTTTGTCTTACCACCCATA-3'

Fig. 1. Primers of GABA related enzymes used for real time PCR

6. Pathway analysis. GABA pathway assignments were carried out using online KEGG mapper (http://www.genome.jp/kegg/tool/map_pathway2.html).

7. RNA Seq-data submission. The obtained RNA-Seq data were submitted to the DNA Data Bank of Japan (DDBJ) under the following accession numbers:

Submission: DRA013348 (tomohiro-0491_Submission)
BioProject: PRJDB12914 (PSUB016606)
BioSample: SAMD00439333-SAMD00439341 (SSUB020458)
Experiment: DRX327085-DRX327093 (tomohiro-0491_Experiment_0001-0009)
Run: DRR338129-DRR338137 (tomohiro-0491_Run_0001-0009)

RESULTS AND DISCUSSION

The metabolome alteration caused by 10 ppm *trans*-2-hexenal vapor treatment on green tea leaves is shown in Table 1. Three hour vapor applications of *trans*-2-hexenal to green tea leaves in ventilation system enhanced GABA content up to 0.98μmol/g, which was 15.1 times higher than control (0.06μmol/g). Increased amount in pyruvic acid (11.9 times higher than control), alanine (8.5 times higher than control) and α-ketoglutaric acid (2.4 times higher than control), and decreased amount of succinic acid (0.8 times as control), aspartic acid (0.4 times as control) and glutamine (0.2 times as control) were accompanied with GABA increase.

Table 1. Metabolome alteration caused by 10 ppm *trans*-2-hexenal vapor treatment on green tea leaf

	Control (0 h)	Treatment (3 h)	Ratio
GABA	0.06	0.93	15.1
Pyruvic acid	4.85	57.5	11.9
Alanine	0.12	1.02	8.5
α-Ketoglutaric acid	0.45	1.10	2.4
Succinic acid	0.22	0.18	0.8
Aspartic acid	0.53	0.20	0.4
Glutamine	1.62	0.39	0.2

Data are shown in μmol/g and the ratio is calculated by division of treatment value by control value.

As for the transcriptome analysis, the changes in the activities of main enzymes concerning to GABA metabolism are shown in Table 2. The data compares the values of control and 10 ppm *trans*-2-hexenal treatment for 3 h. The increase in GABA could well be attributed to the increased gene expression of glutamate decarboxylase (4.1 times higher than control), and decreased expression of GABA transaminase (0.6 times as control), succinic semialdehyde dehydrogenase (0.7 times as control) and glutamate dehydrogenase (0.6 times as control).

Table 2. Transcriptome alteration caused by 10 ppm *trans*-2-hexenal vapor treatment on green tea leaf.

	Control (0 h)	Treatment (3 h)	Ratio
GABA transaminase①	3.22	1.78	0.6
Succinic Semialdehyde dehydrogenase②	1.34	0.91	0.7
Glutamate dehydrogenase③	0.77	0.50	0.6
Glutamate decarboxylase④	0.93	3.67	4.1

Data are shown in TPM (Transcripts Per Million) and the ratio is calculated by division of treatment value by control value.

The possible physiological mechanism of the enhancement of GABA by *trans*-2-hexenal vapor treatment is based on the results of both metabolome analysis (Table 1) and transcriptome analysis (Table 2) as shown in Figure 2 .

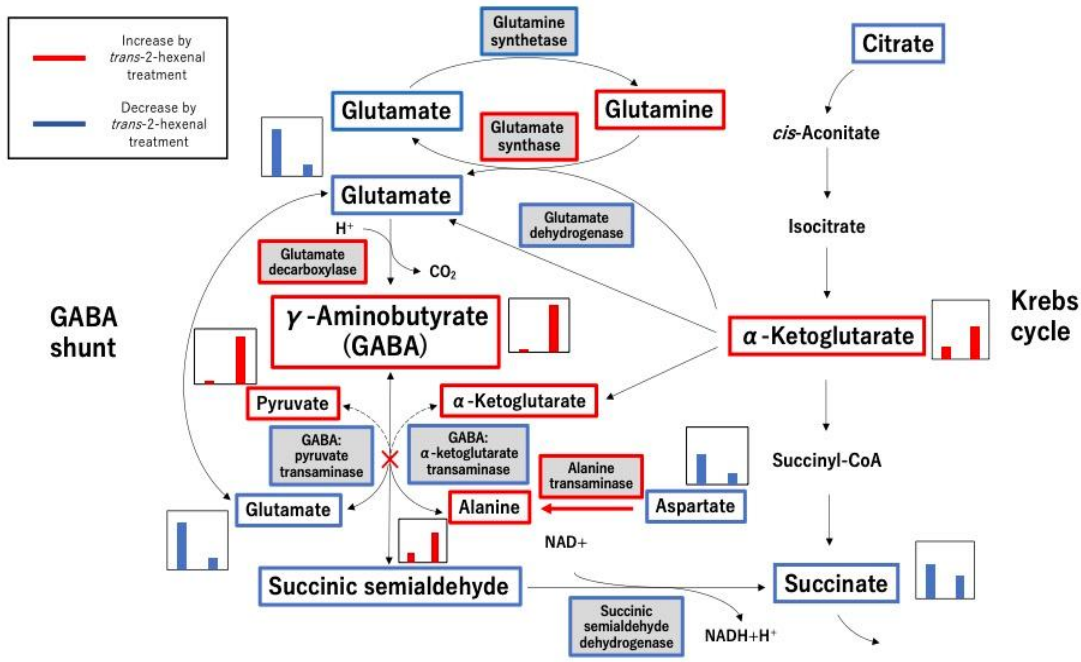


Fig. 2. Possible mechanism of GABA increase in *trans*-2-hexenal vapor treated green tea leaves

Considering the biosynthesis of GABA, the increased GABA content might primarily be derived from the activation of glutamate decarboxylase (4.1 times higher than control) which directly changes glutamate into GABA. This result can be well supported by the reports of Tsushida and Murai (1987), which mentioned the activation of the identical enzyme under anaerobic conditions through the process of Gabaron tea production. The mechanism of stress reaction of tea is different from the one of Arabidopsis, characterized by the accumulation of GABA which is accompanied with the activation of CsGAD1 (tea glutamate decarboxylase 1) through the regulation by calmodulin as well as the accumulated mRNA of CsGAD2 (tea glutamate decarboxylase 2) (Mei et al. 2016). Glutamate, a direct precursor of GABA, could be provided from the enriched amount of glutamine mainly by the activation of glutamate synthase (3.9 times higher than control, although not shown in Table 2), rather than by glutamate dehydrogenase, which was deactivated down to 0.7 times as control. The enriched α-ketoglutaric acid could also serve as a rich precursor of glutamate.

As for the catabolism of GABA, the deactivation of both GABA transaminase (0.6 times as control) and succinic semialdehyde dehydrogenase (0.7 times as control), might well contribute to the higher amount of GABA which remained without catabolism. The activation of glutamate decarboxylase was observed under anaerobic conditions in tomato fruit (Mae et al. 2012), and it was considered to be caused by the activation of glutamate decarboxylase as well as the deactivation of α-ketoglutaric acid dependent GABA transaminase. The present experimental results also support the similar metabolic alteration.

CONCLUSION

A new method to produce GABA rich green tea was recently developed by *trans*-2-hexenal vapor treatment and a possible metabolome alteration concerning the GABA shunt. From the analysis of metabolome and transcriptome, the main reason for enriched GABA by *trans*-2-hexenal vapor treatment could be attributed to the activation of glutamate decarboxylase with decreased activity of GABA transaminase and succinic semialdehyde dehydrogenase.

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ARE COOPERATIVES READY FOR THE DIGITALIZATION OF SMALLHOLDER PALM OIL PLANTATIONS? ANALYSIS OF FARMER'S PARTICIPATION IN DIGITALIZATION OF A VILLAGE COOPERATIVE UNIT

A Faroby Falatehan[1], Yusman Syaukat[1], Sriwulan Ferindian Falatehan[2] and Hariyadi[3]
[1] Department of Resource and Environmental Economics, Faculty of Economics Management, IPB University, Bogor 16680, Indonesia
[2] Department of Communication and Community Development Sciences, Faculty of Human Ecology, IPB University, Bogor 16680, Indonesia
[3] Department of Agronomy and Horticulture , Faculty of Agriculture, IPB University, Bogor 16680, Indonesia
Corresponding author: alfaroby@apps.ipb.ac.id

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ABSTRACT

The cooperative behavior of palm-oil smallholders at Village Cooperative Organization (VCU) as participation is the primary perspective to examine the implementation of digitalization institutions by evolving the normative and cultural-cognitive pillars. Feedback from the organization, such as incentives and sanctions, becomes prominent as a context for members' attitudes and identity formation in the middle of a values exchange with the VCU's principles, such as market-oriented, memberships fees, annual business meetings, *sharia*, and solidarity bonds. This study observed how the organizational feedback renders cooperative behavior of members through *Group Engagement Model* in fortifying the digital readiness of palm oil smallholders at VCU. The surveys were conducted in August-September 2021 at 46 VCU's members in Riau Province. The data analysis technique applied was descriptive, correlation, and causality. Members' cooperative behavior as participation in digitalization correlated with their general attitudes toward the organization. Social identity was the factor that elevated the digitalization institutions at VCU, which correlated with members' general attitudes and was affected by the fairness perception of the VCU's performance. This finding verified that the organization's feedback could invoke the emergence of social identity and attitudes as the principles of reorganizing for the digitalization transformation of palm oil smallholders in VCU.

Key words: cooperative behavior, digitalization, identity, palm-oil smallholders, village cooperative unit

INTRODUCTION

Revolution 4.0 is interpreted as a transformation towards process improvement by integrating production lines and the cyber world, where all production processes go online through an internet connection as the primary support. Three instruments to prepare for digital transformation have been critically reviewed: the form of digital organization, the infrastructure of digital institutions, and digital institutional building blocks (Hinings et al. 2018). The digital economy is a sharing economy with the ability to boost many small and medium enterprises to enter the world of business. The wave of the digital economy is inclusive, comes with a sloping topography, and spreads equal opportunity. This characteristic has a competitive concept that becomes the industry spirit that is easily raised by startup players who focus on collaboration and synergy.

The palm oil plantation is the prime sector in Indonesia which cannot be separated from the effect of the rapid development of digitalization. Economic development in Indonesia, particularly in the palm oil subsector, is highly supported by the increasing productivity achievement, mainly due to the role of smallholder palm oil farmers. Crude Palm Oil (CPO) production from smallholder farmers is 15.5 million tons, 27 million tons from the large scale private-owned plantation, and 2.3 million tons from the large scale state-owned plantation (National Statistics Bureau 2020)

Palm oil digitalization includes efforts to meet the needs for information and capacity building for palm oil farmers related to palm oil production and marketing. The data reveal that the number of internet users in Indonesia increased in 2020 to 196,7 million and 171 million in 2019 (Ministry of Communication and Information Technology 2020). The power of digital institutionalization to digitize work processes with a digital basis will eventually strengthen institutions to digital transformation (Savic 2019). Digitalization institution in smallholders' palm oil plantation is still growing as an innovation to contribute to the productivity of smallholder palm oil plantations through the Farmer Group Association or Village Cooperative Unit (VCU). Even though it affects changes in members' digital culture, such as farmers accessing information about fresh fruit bunch (FFB) prices, it is not yet related fully to the input and production aspect (Falatehan et al. 2020; Falatehan et al. 2021).

This background leads this study to strengthen the normative and cultural-cognitive pillars of digitalization institutions in local organizations. Three pillars of institutions are: (1) the regulative, (2) normative, and (3) cultural-cognitive (Scott 2013). The less functionality of regulative pillars was found when VCU launched a digital program at the VCU's Annual Meeting. Still, it can not dispose of every member involved in the digitalization institution of the VCU (Falatehan *et al.* 2020). This study challenges the readiness of the VCU to provide the normative and cultural-cognitive pillars of digitalization institutions. Farmers identified less digital culture than the other groups and built the digital divide in rural areas (Mazya 2022).

Improvement in these pillars of digitalization institutions may be extended with a cooperative behavior perspective. It is member attitude and behavior underneath the readiness to participate in digitalization in the palm oil business at VCU management. Attitude, as favorable to an object that can predict a consistency with behavior when a social context permits an individual to express it (Branscombe and Baron 2017). Attitudes among VCU members exist in the middle of social exchange and interactions with other members and are influenced by the VCU's performance. A more favorable attitude of members to VCU performance is assumed to lead a proper cooperative behavior which reflects a behavioral intention to be involved in the VCU's digitalization.

The predicted factor that evolve in the cooperative behavior of members in VCU, which is influenced by feedback from organization namely incentives and sanction, in responding to the digitalization of palm oil smallholders is social identity. Social identity is one predictors of collective action at the group and individual levels (van Zomeren *et al.* 2008). Cooperative behavior in the organization would pursue mandatory and discretionary behavior from their members (Tyler and Blader 2003). *The Group Engagement Model* proposes that people will engage in the organization if they maintain their favorable identity, which is underneath by fairness perception (Tyler and Blader 2003).

Fairness perception becomes the issue of digitalization at VCU because member participation needs to challenge the feedback from the organization where integrate socio-economic benefits, membership fees, business rewards annually, risk-seeking, and also maintaining collective solidarity, namely *kebersamaan*, to gain collective welfare. Fairness is an essential part of *The Group Engagement Model* states that identity formation is based on resource/distributive and procedural assessment and will lead to an attitude to VCU and finally lead the cooperative group behavior, especially digital

participation. The integrated model of fairness contributed to satisfaction in Indonesia's context built from distributive, procedural, and interactional fairness (Faturochman 2002; Tyler 1994).

This study sought to investigate smallholder palm oil farmer's participation in digitalization in VCU and its correlation with a general attitude toward VCUs' performance; identify the correlation between the general attitude of farmers to VCUs and social identity as a form of feedback from an organization; and determine the role of perception of fairness in the social identity.

METHODOLOGY

Location and time. This study was conducted in one VCU at Dayun Subdistrict, Siak Regency, Riau Province, from August to September 2021. This cooperative was selected as the model at the national level for palm oil smallholders, which changed its management system to Sharia in 2019.

Types of data. The data used in this study are primary data from several variables in a group through interviews and questionnaires. Several active members were mostly palm oil farmers. In total, the number of VCU's active members consisted of 100 members with ordinary status and 50 with extraordinary status. Membership system of the cooperative is based on the Law of The Republic of Indonesia number 25 of 1992. Based on this law, cooperative member's statuses are categorized into ordinary and extraordinary. Extraordinary members are people who want to receive services and become members of the Cooperative but do not fully meet the requirements as stipulated in the Articles of Association. They could become the member without paying for collateral or principal contribution. Extraordinary members did not have both principal and mandatory savings. The determination of respondents was proposed by applying the procedure of snowball sampling to 46 people from the cluster of ordinary status and extraordinary status, thus resulting in 26 and 20 respondents, respectively.

Research instrument. Four sets of questions were used to examine the behavior of the member: their participation in the last five years, social identity, attitude, and assessment of fairness concerning cooperative management during the period. Answer options used in cooperative behavior as part of the participation of mandatory and non-mandatory activities in the organization were Yes and No. Moreover, answer options for social identity, attitude, and fairness assessment were expressed on the Likert scale using six scales, including: strongly disagree, disagree, slightly disagree, slightly agree, agree, and strongly agree. The analysis method used was descriptive analysis using means and percentages. In contrast, mediation analysis on variables tested in this study was a correlation technique with Pearson and mediation analysis with an indirect effect.

Participation in VCU. General participation was constructed to operationalize cooperative behavior. In total, there were eight items used to measure participative behavior, such as saving for replanting program, sales of FFB, agricultural production facility, annual meeting, paying for a voluntary contribution, savings and loans, purchase of basic needs in cooperative store, and the digital participation (use of Sharia cooperative mobile application/social media namely *Whatsapp group*).

Attitude towards VCU performance. The questionnaire on attitude consisted of 19 statements covering cognitive, affective, and conative components. One of the reference tools for analysis to question assessment about cooperative performance is the Development Ladder Assessment which includes the dimension of vision, capacity, resource, and network (Firdaus and Baga 2019). Organizational performance should also measure the dimensions of behavior towards an organization, including existence, process, and result accepted by members because of organizational membership.

One of the dimensions of attitude and behavioral measurement is the extent of dimension to be measured from the behavior object (Azwar 2016; Sax 1980). The entail of digitalization in attitude measurement to cooperative behavior was extended through 27 items from 8 dimensions of cooperative

performance, namely (1) Program; (2) Accessibility; (3) Facilitation provided by cooperative management to fulfill the needs of the member; (4) Social relation in program services; (5) Benefit from participating in the VCU; (6) Digital performance; (7) Memberships financial obligation; and (8) Managerial capacity of VCU's management.

Social identity. This study used the identification of members to VCU with an organization to explore a social identity.

Perception of fairness. There are four items used to measure the evaluation of fairness which contain procedural (2 items), distributive (1 item), and interactional (1 item) fairness assessment. In the field context, the fairness aspect was investigated by profit sharing according to the contribution, organizational controls to propose an activity, and relational response when members experienced problems.

Data analysis. The statistical analysis techniques were applied to deliver the aims of this study. The Spearman rank correlation was used to identify the correlation between general attitudes toward organizations and participation in digitalization. The correlation between social identity and attitude to VCUs was analyzed using a Pearson Product-Moment. In contrast, regression analysis was used to identify the influence of the perception of fairness on social identity.

RESULTS AND DISCUSSION

Based on the membership status, respondents were Ordinary (56%) and Extraordinary (44%). The data presented characteristics of respondents according to their status and gender, the length of membership in Village Cooperative, and age (Table 1). The length of membership consisted of less than 20 years (52%) and more than 20 years (48%). It is seen from the data that female respondents from the age group of less than 40 years old have extraordinary status.

Table 1. Distribution of the number of respondents (%) according to length of membership in village cooperative, age group, gender, and membership status in VCU

Length of Membership	Age Group	Gender	Membership Status				Grand Total	
			Ordinary		Extraordinary			
			N	%	N	%	n	%
Less than 20 years	Less than 40 years old	Male	3	50.0	3	50.0	6	100
		Female	1	9.1	10	90.9	11	100
	More or equal 40 years old	Male	1	33.3	2	66.7	3	100
		Female	1	25.0	3	75.0	4	100
	Total		6	25.0	18	75.0	24	100
More than 20 years	More than 40 years old	Male	15	100	0	0.0	15	100
		Female	5	71.4	2	28.6	7	100
	Total		20	56.5	2	43.5	22	100

The Group Engagement Model describes the cooperative behavior of members in an organization, namely participation in VCU's program (Table 2). Almost 90% of respondents participated in cooperative activities of replanting saving, agricultural production facility, sales of the FFB, savings, and loans. Several members. engaged since VCU was established in 1990, were affluent today which affected their involvement in the organization to attain an extraordinary status.

Two activities wherein members are less involved are digital participation and purchasing basic needs. VCU's readiness for its digitalization institutions becomes an issue based on the digital participation of its members.

However, only 70% of members used VCU mobile applications or WhatsApp social media. Benefits from VCU's digital participation are (1) Receiving information about FFB prices from VCU or the farmer group leader via the *Whatsapp* group; (2) Using the features of the VCU Sharia mobile application to transfer money to all state banks, top-up balance from provider and electricity, saving, and fertilizer information.

Table 2. The number of respondents by participated in the VCU

Activity	Total	%
Replanting saving	43	93
Agricultural production facility	44	96
Sale of the FFB	42	91
Savings and loans	44	96
Depositing voluntary deposits	42	91
Purchasing basic needs	32	70
The use of digitalization	32	70

Even though digitalization provided several benefits, such as simple to download, ease of business processes, extending networks, providing alternatives to fulfill needs, and less time consumed, some members with ordinary status did not participate in digitalization at VCU. They have adequate literacy about digital services from the organization. It is ubiquitous in cooperative members who are more than 40 years old. Farmers' young children usually helped their parents to engage in digitalization. In addition, many respondents of extraordinary status who were young (less than 40 years old and less than 20 years of membership at VCU) used the VCU mobile application for saving programs. They are near to the millennial generation who can be a potential digital user to support digitalization institutions at VCU through normative and cultural-cognitive pillars. Institutions pillars are interrelated and underpinned by regulative, normative, and cultural-cognitive (Scott 2013). The VCU's digitalization could be supported by the tendency of their extraordinary member schema and the group's operation standards, allowing an intensified usage of the VCU social media and mobile application. It is a practice in the middle of the formal launching of digitalization services at VCU. A similar concern is also found in organic and healthy paddy institutions, such as organizing the fertilizer and pesticide through cooperatives, farmers groups, work-labor, harvest, and post-harvest, which is not ultimately built from the cultural-cognitive pillar (Indriana et al. 2012).

The primary factor causing this slow response in digital behavior, where the normative and cultural-cognitive pillars of digitalization institutions at VCU are weak, is that the infrastructure of digital institutions, especially signal, is sometimes in poor strength. To support the readiness of VCU's digitalization, digital infrastructure is needed as it is the instrument of digital transformation (Hinings et al. 2018). With adequate robust signal, more members will be using and fulfilling their needs in plantation and household by VCU digitalization system.

The disruption of the fiber-optic installations causes a digital inequality which can be seen in rural areas than the city and in farmers and fishers groups than in other professions (Mazya 2022). Without supportive infrastructure, digitalization will become immutable. Government and non-government could deliver digital infrastructure to avoid digital inequality (Dimaggio and Hargittai, 2001) between farmers and non-farmers.

There was a correlation between VCU's participation in digitalization and member's general attitude to VCU performance (r=0.453, p=0.00). The members with a higher degree attitude correlated with their involvement in digitalization. It implied that the respondent's who have a positive attitude toward various aspects of VCU performance, such as the Cooperative Program and the benefits of participating in the VCU, will tend to participate in digitalization, using the mobile application, or joining the VCU's social media.

The attitude toward VCU could predict a respondent's cooperative behavior in an organization. The respondents had favorable general attitudes to VCU's performance (M=4.89; SD=0.53). Respondents positively assessed VCU's performance for every dimension (Table 3). Three dimensions, (1) Cooperative Program, (2) Benefit from participating in cooperative, and (3) Managerial capacity of VCU's management scored less than 5 as a moderate category. The other two dimensions obtained beyond the score of 5 were Accessibility (M=5.39; SD=0.54) and Social Relation in cooperative program services (M=5.11; SD=0.55).

Respondents positively assessed that the program at VCU was attractive and satisfied its members. The program was considered adaptive to the current situation that changed, such as the Palm oil Replanting program that respondents mostly participated in. Respondents believed that the program was able to answer the problem of palm oil aging frequently faced by respondents. This Program was regarded as the best option and even exceeded the ability of other institutions related to economic programs for respondent welfare. However, the VCU program was considered to only slightly improve the capacity of participants for the last five years. This situation was different from that the Village Cooperative had established, where it functioned as the instrument to accommodate the needs of transmigrant respondents. The VCU usually prepared various skill development programs to create plasma farmers for the state-owned plantation enterprise. VCU was also considered to reasonably provide benefits for respondents through a sense of security due to the Sharia principle applied.

Table 3. Mean of respondents' attitude towards VCU

Dimension	Min.	Max.	Mean	Std. Dev.
Cooperative program	3.00	6.00	4.92	0.58
Cooperative accessibility	4.00	6.00	5.39	0.54
Facilitation provided by cooperative management to fulfill the needs of members	3.00	6.00	4.87	0.66
Social relation in program services	3.00	6.00	5.11	0.55
Benefit from participating in the cooperative	3.00	6.00	4.90	0.67
Digital performance	2.30	6.00	4.87	0.78
Memberships financial obligation	3.00	6.00	4.78	0.62
The managerial capacity of VCU's management	3.33	6.00	4.94	0.55
Total	3.33	5.89	4.89	0.53

The two most robust positive degree dimensions in cooperative program services were accessibility (M=5.39; SD=0.54) and social relation (M=5.11; SD=0.55). Concerning accessibility, respondents considered that VCU, which had moved to other locations, was quite strategic. This positive assessment could relate to the more vigorous intensity of respondents in accessing village cooperative services. In terms of relations, it was observed that respondents experienced convenient service, friendly, and excellent quality of relations provided by VCU's management. Further, this triggered positive emotions in respondents to connect to VCU.

Even though respondents could not feel discomfort since they were not ambivalent because the overall components were favorable (Fabrigar and Wegener, 2010), the three dimensions are categorized with the lowest degree. There are digital performance (M=4.87; SD=0.78), financial obligation (M=4.78; SD=0.63), and facilitation provided by cooperative management to fulfill members' needs (M=4.87; SD=0.66). The digital performance attitude of several respondents is favorable due to ease in downloading from the play store on their smartphone, the performance of FFB price information, multi-payment, transfer, saving, and fertilizer through mobile applications and Whatsapp groups. But several members thought that VCU should be modified by adding the receiving money feature and building a tower to provide access to a better signal. A recent feature provided in the mobile application is to transfer money only and this can only be done in an area in the village with good connectivity.

Financial obligations recently seen by members was not accessible to them. Even though this is an instrument for their access to VCU's system, they did not negatively evaluate this obligation. It is in line with economic crises that come from palm oil replanting programs where they can not afford many daily needs or savings. Facilitation provided by cooperative management was believed accommodate members' needs. The management's responsiveness can be seen by providing a space for respondents to express their opinion in yearly VCU meetings. But many members did not share a similar opinion since they have different interests from the dominant VCU related to the replanting program. It causes an unstable situation in VCU where many members change their memberships to become extraordinary or tend to exit from VCU.

The general attitude toward VCUs as cooperative behavior needs to be put in its social system as a local economy unit for smallholders. This vision will pursue an impact on normative and cultural-cognitive pillars of digitalization institutions. On one side, VCU is a place for smallholders to find a profit based on palm oil plantations as their business and build a social exchange of resources between them. On the other side, historically, their social identity as early transmigrants has high cohesivity and solidarity. Their attitude to VCU performance could be affected by social identity. Data confirmed a significant correlation between general attitude toward VCUs and social identity (r=0,409; p=0.00). The more robust member’s social identification with VCU will provide them to engage a favorable attitude toward VCU.

In line with that, a social identity influenced by the perception of fairness is 17.7% (p=0.00). It means that fairness evaluation of VCU performances will construct the robustness of the social identity of respondents toward VCU. When fairness perception of VCU performance is increased by 1 degree, members' social identity will be more robust by 0,49. The Group Engagement Model covers the importance of social identity, which states that feedback from the organization, such as incentives and sanctions, will influence attitude and cooperative behavior (Tyler and Blader 2003). This study confirmed that a group is a context where the digitalization institutions in VCU are thriving and indirectly affected by fairness perception in members about VCU performance. Fair assessment by respondents, based on resources and procedures implemented at VCU, can provide them with a robust identification of VCU.

The fairness perception of respondents showed that distributive fairness was the lowest (M=4.82; SD=0.84); compared to procedural (M=5.00; SD=0.60) and interactional fairness (M=4.93; SD=0.69). The procedural fairness VCU's management, such as the replanting program and saving, is assessed as fair when implemented for their members. Interactional fairness appeared when VCU's management provided facilitative communication when respondents faced problems, for example, existing consultation related to palm oil replanting. It is consistent with earlier findings that found interactional fairness is significant in the fairness model in Indonesia (Faturochman 2002). It has shifted from collectivity issue in farmers to individuals (Riley et al. 2018). The respondents saw that the equity approach was not straightforward for them to accept in distributive fairness. This situation primarily existed when they could not access VCU's program, which will not provide them with many rewards. This result is related to a respondent's attitude about financial obligation as a VCU ordinary memberships, which is the least favorable among the other dimension of VCU's performance. Many members thought of modifying the reward system based on equality. Distributive fairness, according to Adams equity rules (1963) in Lind and Tyler (1988), is the outcome that could be based on proportional to contribution (equity), norms of equality obtained by individuals, and based on need.

Based on these findings, we can conclude that farmers' digital participation will enhance the readiness of digitalization institutions at VCU, especially through the normative and cultural-cognitive pillars. It can be drawn that members' involvement in digitalization, as a part of cooperative behavior, is correlated with their general attitude to VCU. The issue is that the general attitude to VCU correlates with social identity, while social identity is significantly predicted by fairness perception (modified to distributive, procedural, and interactional). VCU members who think fairness is practiced at the VCU will have a strong identity connection with VCU. It will indirectly stimulate a broader context in their decision-making to either participate or not in VCU digitalization as cooperative behavior through their attitude to VCU. Overall description of our findings to identify a farmer's participation in digitalization in VCU based on the *Group Engagement Model* can be seen in Fig. 1.

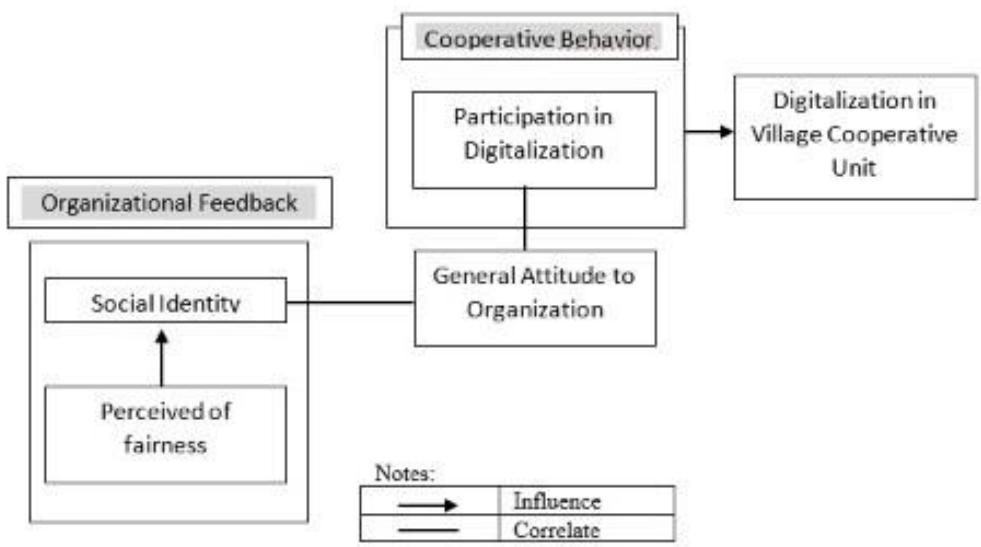

Fig. 1. Farmer's participation in digitalization at VCU

CONCLUSION AND RECOMMENDATIONS

This study constructed the role of farmers' cooperative behavior in an agricultural organization, VCU, to enhance digitalization institutions in palm-oil smallholders. The cooperative behavior perspective highlights the members' participation in support of digitalization at VCU. Around 70% of members participate in digitalization, such as using VCU mobile applications or WhatApp social media related to FFB prices from VCU and economic transactions. It identified that VCU members' general attitudes to VCU performance were significantly correlated with participation in digitalization. It instills that VCU's performance should be reorganized in a more suitable process to build a more positive attitude for members.

The *Group Engagement Model* is feasible in this study to describe how organizational feedback could influence members engagement through social identity construction. Social identity significantly correlates with members' general attitudes toward many ascects of VCU's performance. The social identity of VCU's members is impacted by their perception of fairness in VCU's performance. Concerning VCU's member's attitudes, participation, social identity, and fairness perception will generate more supportive normative and cultural-cognitive pillars in digitalization institutions at VCU.

If VCU or other stakeholders take no action to increase digital culture or digital literacies, palm oil smallholders will be eroded since they fail to gain value-added from digitalization. Government and non-government stakeholders could take part in delivering digital infrastructure to avoid digital inequality between farmers and non-farmers, including digitalization in production areas.

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PRODUCTION MANAGEMENT AND RESOURCE USE EFFICIENCY IN ORGANIC POTATO CULTIVATION IN BHUTAN: A SCENARIO FROM AN ORGANIC GASA DISTRICT

Tandin Gyeltshen[1], Supaporn Poungchompu[2*] and Panutporn Ruangchoengchum[3]
[1] Department of Agriculture, Faculty of Agriculture, Khon Kaen University, Khon Kaen, Thailand
[2*]Department of Agricultural Economics, Faculty of Agriculture, Khon Kaen University, Khon Kaen, Thailand
[3] College of Graduate Study in Management, Khon Kaen University, Khon Kaen, Thailand
*Corresponding author: psuppap@kku.ac.th

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ABSTRACT

Potatoes are one of the most important cash crops in Bhutan, both for export as well as domestic consumption. Understanding the resources used in potato production will enhance growers' efficient allocation of resources. Thus, this study aimed to analyse the economics of production and resource use efficiency of organic potato cultivation in Gasa district of Bhutan. About 90 organic potato farmers were randomly selected from the study area. A pretested questionnaire and face-to-face interviews were used to gather primary data for the 2020 cropping cycle. Descriptive statistics and Cobb-Douglas production function were used for data analysis. Farmers used 0.43 acres of land area for organic potato cultivation out of the average landholding of 3.25 acres. From the cost of production, major cost was incurred on inputs (59.19%) followed by labour (39.91%). Resource use efficiency analysis revealed that farmers were not efficient in using resources in organic potato cultivation. Cultivated area and seeds were under-utilized and therefore increasing the use of those resources could maximize productivity of potatoes. Labour, farmyard manure (FYM) and machinery were over-utilised and decreasing these would lead to better productivity. Labour, FYM, and seed constituted major parts of the cost of production hence optimum use of those resources could also enhance the profitability and resource use efficiency of organic potato cultivation. These findings will help growers in similar agricultural conditions to effectively allocate resources for potato production. The government should strengthen the existing policies to provide assistance to promote organic potato cultivation in the country.

Key words: Cobb-Douglas, cost of cultivation, economic analysis, production cost, productivity.

INTRODUCTION

Potato (*Solanum tuberosum* L.) is the most important crop in the world after wheat, rice, and maize. The historical record of potato cultivation dates back to the 17th century when the East India Company's representative George Boggle visited Bhutan (Roder et al. 2008) while the scale of production increased gradually from 1961 onwards with the onset of Five Year Development Plans (Joshi and Gurung 2009). *Desiree*, *Kufri Jyoti*, *Yusikaap*, and *Khangma Kaap* are the four potato varieties known in Bhutan of which *Desiree* is the most preferred cultivar (Joshi and Gurung 2009). As an important cash crop potato is mostly cultivated at an elevation of 2000-3500 masl (Bajgai 2018). Bhutan produced 45,500.33 metric tonnes of potato from 10,342.26 acres (Ministry of Agriculture and Forests (MoAF) 2020).

Agriculture as an important sector provides 49.9% of employment (National Statics Bureau (NSB) 2020) also contributed 19.23% to the GDP in 2020 (NSB 2021a). With the concerns of food safety and food security, the agriculture sector initiated the institution of the National Organic Programme 2006 (DoA 2006) to mark the beginning of organic farming in Bhutan. The country envisioned to go fully organic by 2020 (McCrae-Hokenson 2014) which could not be achieved. To this, the current government revamped the organic initiative through the National Organic Flagship Programme in the 12th FYP (GNHC 2019). Currently, Bhutan has 1,265 households engaged in organic cultivation in 10,095 acres of land with about 38 agricultural products certified spread across the country (Department of Agriculture (DoA) 2021). As mandated, the agriculture sector emphasizes to increase food production to ensure household food security, alleviate poverty, substitute or reduce imports through increased domestic production, generate a marketable surplus, enhance household income and employment opportunities (DoA 2019). Thus, it is necessary to assess the performance of organic agriculture in the country.

Over the years, potato cultivation has been given immense importance by the Department of Agriculture through the National Potato Program. The initiative included multi-location potato seed varieties, providing training, and seeds to the farmers (DoA 2021). Although the productivity of 4,370 kg/acre (MoAF 2020) is the lowest among south Asian countries, potato cultivation is still a profitable business (Joshi and Gurung 2009). However, Bhutan imported 4910.02 metric tonnes of potato incurring US$ 782,227 to its economy (MoAF 2019). The initiatives for import were to meet the shortage of potatoes in the country. But the source of the import and production regimes were not reliable from the food safety and security perspective. This therefore demanded improvement in the potato production system in the country, particularly for organic production methods. It is very important to understand how well producers are making plans and managing resources to maximize the returns, minimize costs, and improve overall efficiency. Several studies on potatoes such as the productivity and profitability of organic and conventional potato (Lepcha et al. 2021), weed management challenges in potato (Roder et al. 2009), marketing Bhutanese potato (Roder et al. 2007), and market performance of potato (Van Tilburg et al. 2008) were conducted in Bhutan. Besides some economic aspects of organic potato cultivation (Lepcha et al. 2021), the economics of production and resource use efficiency analysis was not covered in any of the studies.

Resource use efficiency refers to using the limited resources in a sustainable manner. Traditionally, it has been determined by calculating ratios of productivity per unit of resource (Sheriff et al. 1995). Rural communities depend on agricultural production for food and income. Natural resources such as land, water, energy, nutrients, and air are essential for agriculture and to sustain life of all living organisms. However, the overutilisation of these limited resources by human activities has called for an urgent scientific management of natural resources in agriculture to enhance use efficiency for sustainable future importance (Rani et al. 2020). The production inputs for potato cultivation such as land, labour, capital, and other inputs have significant influence on the overall yield. Thus, it is important to maximize the benefits of these inputs while minimizing waste due to overutilization or underutilization of the resources. These shortfalls can be augmented through use of optimum level of inputs by way of resource use efficiency (Verma 2007). Thus, the study aimed to analyse the economics of production and resource use efficiency of organic potato cultivation in the Gasa district. This study would help extension people broaden their advisory capacity to the farmers, policy reference directed towards helping potato farmers, and guide producers to best to allocate the scarce resources on the farm.

METHODOLOGY

Study area and data collection. Gasa is in northwestern Bhutan with an elevation ranging between 1500-4500 masl. It has a population of 4,156 with 909 households. About 29.4% its total population is engaged in agriculture (NSB 2021b). The district is further divided into four subdistricts known as

Gewogs namely Laya, Lunana, Goenkhatoed, and Goenkhamed. Gasa has been declared as the first organic areas (Duba et al. 2008) and it has over 14 years of experience in organic farming (Wangmo and Iwai 2018). Thus, two lower Gewogs that were engaged in agricultural farming were purposely selected for the current study on resource use efficiency in organic potato production. Employing the simple random sampling method, 90 households comprised of 30 households from Goenkhatoed Gewog spread across 14 villages and 60 households from Goenkhamed Gewog spread across 18 villages were interviewed for the study (Fig. 1). A structured questionnaire was used for collecting cross-sectional potato production data in February and March 2021.

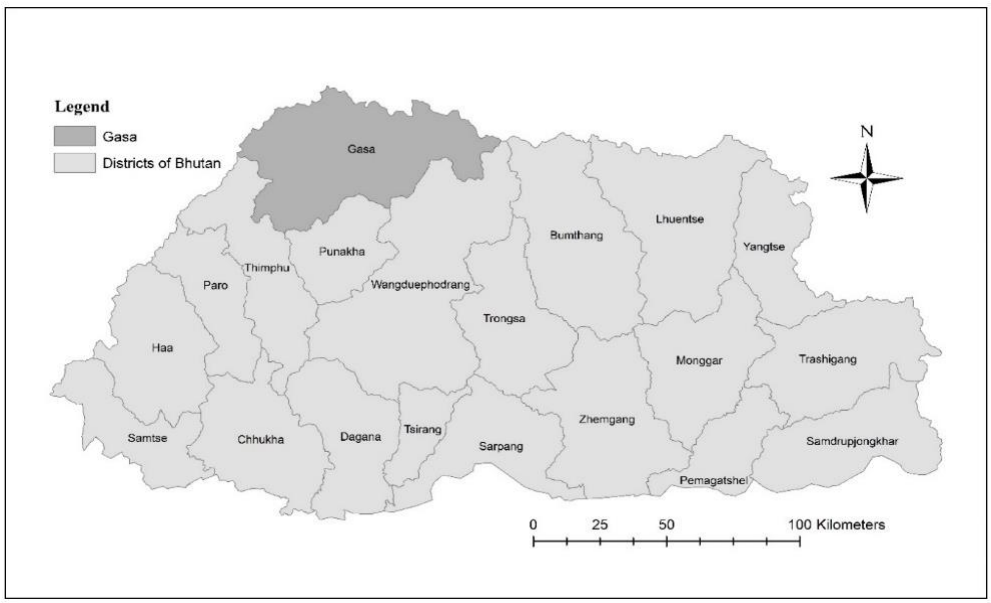

Fig. 1. Map of Bhutan with 20 districts
Source: National Statistics Bureau, 2017

Data analysis. Multiple regression analysis was employed to find the factors influencing organic potato cultivation and for evaluating the economic efficiency of resources. The linear stochastic form of the specified Cobb-Douglas function is given as:

$$Y = aX_1^{b_1}X_2^{b_2}X_3^{b_3}X_4^{b_4}X_5^{b_5}u_i \quad (1)$$

Where:
Y = Production per acre (Kg)
X_1 = Seed (Kg)
X_2 = Farmyard manure (Kg)
X_3 = Cultivated area
X_4 = Total labour (Labour hours)
X_5 = Power tiller use (Hours)
U_1 = stochastic error term
a = Intercept and b_1 to b_5 are the elasticity of coefficients.

Resource use efficiency. The estimated coefficients of significant independent variables were used to compute marginal value products (MVP). It is the marginal physical product (MPP) multiplied by the product price. The price of one unit of input is called marginal factor cost (MFC). The resource-use efficiency (r) was worked out as (Rahman and Lawal 2003):

$$r = \frac{MVP}{MFC} \quad (2)$$

where, $MVP_i = \beta_i \frac{\overline{Y}}{\overline{X}_i} * P_y$

MVP_i = Marginal value product of the i^{th} input,
β_i = Estimated co-efficient of i^{th} input,
$\overline{Y}$ = Geometric mean of the value of output,
$\overline{X}_i$ = Geometric mean of the i^{th} input, and
P_y = Price of output.

The decision rule for the efficiency analysis is if:

r = 1 resource has been used efficiently
r>1 resource is under-utilised, and increased utilisation will increase profit
r< resource is over-utilised and decreased utilisation will increase profit.

RESULTS AND DISCUSSION

Socioeconomic characteristics of farmers. Age, gender, education, family size, family labours, experience, area under cultivation, and total landholding were assessed in the study (Table 1). The age of farmers ranged from 26 to 75, with a mean of 48.8 years. This shows that majority of the farmers (76.67%) fall within the energetic age group of 20 to 60 indicating the ability to meet the physical works required for agricultural works. The female respondents constituted 77.78% followed by 22.22% of male respondents. The literacy rate of the study area was low with 72.22% of the respondents being illiterate. Majority of the households (54.44%) have the family size of 1-3 members followed by 33 households with 4-6 members, and about 7.78% households with members ranging from 7-9 and 1.11% with 10-12 members. In line with the household members, family labours are crucial for agricultural activities and relates to the dependency on hired labours. Majority of the respondents (76.67%) had 1-2 family labours followed by 21.11% of respondents had 3-4 family labours, and only two households had a family labour of 5-6 members. Farmers also had a range of years' experience in organic potato production. The minimum range of 1-5 years' experience have 24.44% of households in organic potato production. Majority (46.67%) of the farmers had experience between 6-10 years.

Majority of the organic potato farmers were female. This is evident because Goenkhatoed and Goenkhamed gewogs has more female than male population (National Statistics Bureau (NSB 2018) and also it has been observed that male population are out into off farm activities (GCF 2019). Majority of the respondents were illiterate and this is supported by NSB (2018) whereby Gasa has the lowest literacy rate according to the population and housing census 2017. The study area had two household labour on average indicating the shortage of labour-intensive organic potato cultivation (Jansen 2000) and having to rely on hired labour. Less family labour is due to migration (NSB 2018) and this agrees with the study by Karim and Muhammad (2018). Farmers were well experienced in organic potato cultivation with average of 8 years into the venture advantaged with regular trainings from the agriculture department. On average each household owned 3.25 acres, of which 2.62 acres were under cultivation. Only 0.43 acres (13.09%) of the total landholding was found to be under potato cultivation.

Table 1. Characteristics of the sampled households

Particulars		Frequency	Percent
Number of Households		90	100
Age, years	<20	0	0.0
	20-59	69	76.67
	60-69	17	18.89
	70>	4	4.44
Gender	Male	20	22.22
	Female	70	77.78
Education	Illiterate	65	72.22
	Literate	25	27.78
Family size	1 - 3	49	54.44
	4 - 6	33	36.67
	7 - 9	7	7.78
	10 - 12	1	1.11
Family labour	1 - 2	69	76.67
	3 - 4	19	21.11
	5 - 6	2	2.22
Farming experience (Years)	1 - 5	22	24.44
	6 - 10	42	46.67
	11 - 15	19	21.11
	16 - 20	5	5.56
	20>	2	2.22
Average area under potato (Acres)	0.43		
Average cultivated area (Acres)	2.62		
Average landholding (Acres)	3.25		

Economic analysis of organic potato cultivation. The average of various cost items of organic potato production is presented in Table 2. The cost covers both fixed and variable costs. The constituents of variable costs are human labour, power tiller hire charges, and materials cost such as cost of seed and farmyard manure. Rental value of the cultivated area and tax were the fixed cost incurred for organic potato cultivation in the study area.

Total inputs cost constituted the highest share (59.19%) followed by labour cost (39.91%) of the variable costs. The highest labour hour for organic potato production was for harvesting with mean 95.27 followed by weeding and earthing up with the mean of 94.45. Planting incurred 6.60% followed by land preparation with 6.22% of total costs. Total cost of production per acre of organic potato was found to be US$ 965.14. The average yield of organic potato in the study area was 1,666.67 kg per acre. The output input ratio was found to be 1.01.

The study found that majority of the cost components were from variable costs while the fixed cost was insignificant. This agrees with the findings of Kahan (2013) where small holder farmers cost are variable costs. Labour costs incurred significantly constituting about 37.12% of the total cost.

Labour was incurred more in harvesting followed by weeding and earthing, planting, land preparation, FYM application, fencing, and transportation respectively. The results of the labour-intensive nature of the organic cultivation agrees with findings of Jansen (2000), Kassali (2011), and Tashi and Wangchuk (2016). The average yield of organic potato (2,275.91 kg/acre) was much lower than the national average yield. The findings were consistent with the studies by Ierna and Parisi (2014), Lepcha et al. (2021), and Maggio et al. (2008) that reported the overall low productivity in organic production system. Despite low productivity compounded by high production cost of $ 0.58 per kg, organic potato cultivation is still profitable indicated by the output-input ratio of 1.01 indicating the profit of $ 0.01 from investment of $ 1 in organic potato cultivation. This agrees with the studies by Tashi and Wangchuk (2016) and Crowder and Reganold (2015) on the profitability of organic cultivation. Thus, increasing the land under organic potato cultivation would increase in the returns to the farmers.

Table 2. Cost and return analysis of organic potato cultivation in Gasa district, Bhutan in 2020.

Variable costs (VC)	**Value (USD/Acre)**	**Percentage**
Inputs costs		
Seeds	168.60	17.47
FYM	179.05	18.55
Machinery	97.92	10.15
Other inputs	125.76	13.03
Total	**571.33**	**59.20**
Labour costs		
Fencing	16.00	1.66
Land preparation	60.06	6.22
FYM application	48.49	5.02
Planting	63.68	6.60
Irrigation	0.07	0.01
Pest/disease management	0.59	0.06
Weeding and earthing	94.45	9.79
Harvesting	95.27	9.87
Transportation	6.62	0.69
Total	**385.23**	**39.91**
Fixed Costs (FC)		
Land tax	0.21	0.02
Land lease	8.38	0.87
Total	**8.59**	**0.89**
Total Cost (FC+VC)	**965.14**	**100.00**
Yield (Kg/Acre)	1666.67	
Return ($/Acre)	975.66	
Output-input ratio	**1.01**	

Note: 1 US$ = BTN 76.41

Farmers in the study area used extensive FYM and practised crop rotation for maintaining soil health. Similar results were obtained by crop rotation, intercropping, fertility building crops, crop residues, FYM, compost, and complete recycling methods adopted to retain soil organic matter (Heckman 2013; Watson et al. 2002). Farmers in the study area also practised pasturing animals in the fallow area, burning weeds, weed biomass, and crop residues to replenish the soil nutrient loss with removal of crops from the field which are consistent with the findings of Stark et al. (2020) and Roder et al. (2006). Organic potato farmers relied solely on rainwater knowing that potatoes require less water compared to water intensive crops like wetland rice. Most importantly the practices in the study area were in conformity with the package of organic practices (NCOA 2020). National Plant Protection Centre reported that popular insects, diseases, and animals that harm the potatoes in Bhutan are peach potato aphid, potato tuber moth, red ant, white grub, early and late blight, potato virus, and wild boars (NPPC 2018). However, only 12% of respondents reported nuisance from wild pigs on organic potato production while other pests were insignificant in Gasa district. Farmers applauded government's assistance in providing electric fencing to reduce the organic potato damage by wild animals. This finding is consistent with the national assessment electric fencing by Dorjee et al. (2021). Organic potato farmers also used mechanical, cultural, and biological methods to manage pest infestation. The mechanical methods include picking up pests as well as removing the part or whole of the infested plants by pests. The cultural method such as crop rotation, deep ploughing, proper use of fertilisers, timely or late sowing, and proper harvesting of potatoes seemed more effective to control pests. Farmers reported that these methods were easy to adopt. Similar findings were reported in the studies by Bond and Grundy (2001), Watson et al. (2002), and Wyss et al. (2005).

Distribution channels of organic potatoes. Organic potatoes from the study area were sold in the regional markets of Gasa, Punakha, Wangduephodrang, Thimphu, and Phuentsholing respectively. The channels of distribution of organic potatoes from the study area were presented in Fig. 2.

Producers refer to the organic potato farmers in the study area. Channel 1 was used by 34% of farmers to sell their products to consumers directly. About 16% traded through Channel 2, 34% through Channel 3 and 8.89% via Channel 4. Others in Channel 4 refers to the organic potatoes being sold to the institutions or other marketplaces that were adopted either through the farmers' own initiative or with the assistance of agriculture extension officers.

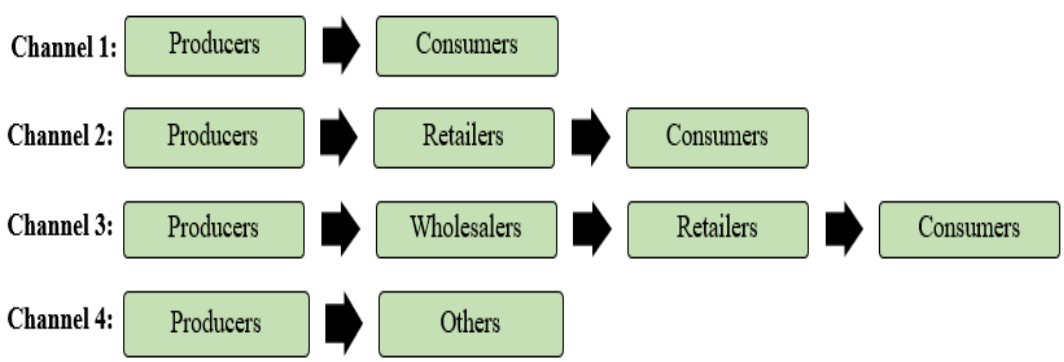

Fig. 2. Distribution channels of organic potatoes.

Resource use efficiency of organic potato cultivation. Resource use efficiency deals about how farmers' effectively use resources for agricultural production processes. The estimated coefficients and other statistics concerning the resource use employing the Cobb-Douglas production function are presented in Table 3. Five explanatory variables namely cultivated area, seeds, farmyard manure

(FYM), machinery, and labour were used in the estimation. From the analysis, seed and area cultivated were found to be underutilised as indicated by greater than unity ratio. Increasing the investment on seed and land for cultivation would lead to profit maximisation. Efficiency ratio indicated over utilisation of resources in terms of FYM (0.91), labours (0.03), and machinery (0.01) respectively.

The requirement of human labour in organic potato cultivation starts from fencing to land preparation until the harvest and storage. Potato farmers spent lot of labour hours for weeding and harvest. The most popular machine used in the study area is power tiller for land preparation in organic potato cultivation which traditionally were carried out by human labour or use of animal power, similar to findings reported by Phuntsho et al. (2022). However, the use of power tiller is less efficient due to high gradients. Power tillers were used with additional manpower to carry out the land preparation to reduce full dependency on man and animal power in organic potato cultivation. The respondents on average use 14.63 hours of power tillers per acre for land preparation in organic potato cultivation. The timing includes ploughing and rotatory by power tiller in the cultivation area. The farmers use no other machineries in other processes of organic potato cultivation; in other words, other activities such as fencing, weeding, earthling up, irrigation, and harvesting were all carried out using traditional methods.

The overutilisation of these factors would be neutralised by minimising the use of these resources or increasing the utilisation of land and seeds for better economic returns. Regarding, in the plain area, power tillers or tractors can be used to their full potential without the involvement of human labour but Bhutan being a mountainous country, the use of power tillers become less efficient. Although power tiller reduces the workload on human labour especially for land preparation in organic potato cultivation by significant time yet the dependency on human labour for other work processes in organic potato cultivation is more. That is why the ratio of efficiency results in the overutilization of both labour and machinery in organic potato cultivation.

Table 3. Efficiency of resource use in potato production

Particulars	Geometric Mean	AVP	Coefficient	MPP	MVP	MFC	r
Returns	1730.58						
Cultivated area (Acre)	0.31	5661.88	0.01	66.41	2970.62	652.00	4.56
Seeds (Kg)	304.47	5.68	0.96	5.46	244.07	30.83	7.92
FYM (Kg)	4873.21	0.36	0.11	0.04	1.81	1.98	0.91
Machinery (BTN)	4448.04	0.39	-0.30	-0.12	-5.16	462.78	-0.01
Labour (Hours)	304.05	5.69	0.01	0.05	2.17	76.89	0.03

Note: Price of output is 44.73 BTN/kg.

Land lease rate of Nu. 640 per acre per annum was adapted from the Government rate as the potato growing communities do not have land lease practise (NLCS 2009).

Resource use efficiency analysis was carried out employing the Cobb-Douglas production function on organic potato production. Similar studies on potato were carried out in Nepal (Sapkota and Bajracharya 2018) and Bangladesh (Sujan et al. 2017) while analysis on other crops were carried out using the Cobb-Douglas production function (Bapari 2016; Barmon and Islam 2017; Sapkota et al. 2020; Subedi et al. 2020; Sharma et al. 2021). The analysis revealed that farmers underutilised cultivated area and seed used for organic potato cultivation. Farmers in the study area have difficulty in

getting quality seeds due to high price and lesser supply during the plantation season. It has been reported that agriculture extension helps to supply seeds during the season, but the quantity supplied were inadequate. Thus, increase in the seed supply would enhance the efficiency of seed thereby increasing the profitability. Similar results on underutilisation of land were reported by Adebayo et al. (2020) and Tasila et al. (2019) while reported on underutilisation of seeds have been reported (Sharma et al. 2018; Subedi et al. 2020; Rana et al. 2016). As majority of the Bhutanese farmers practice self-sustaining, integrated and subsistence agricultural production system with small land holdings, farmers rear livestock to meet their household requirements where the current study area is not an exception. That is the reason for over utilisation efficiency ratio of FYM. Barmon and Islam (2017) and similar results indicated taking advantage of the FYM availability by increasing the land size for cultivation (Sapkota and Bajracharya 2018). Labour, which accounted for a substantial portion of the cost of production in the study area were also overutilized. Similar results were reported by Sapkota and Bajracharya (2018) and Khanal et al. (2020). The overutilisation of labour is due to small land size for organic potato cultivation and which involved mostly family labour. For maximisation of economic benefits from organic potato production, it is recommended to increase the area under cultivation and supply of good quality seeds.

Power tillers were the most used farm machinery ever since its distribution started in Bhutan 1984 (Thinley et al. 2011). The use of power tillers can till almost four times more area coverage than by ploughing with animal power. While many other farm machineries and tools were used in paddy and other crops, potato production relied only on power tillers mostly for land preparation in Bhutan. As in the case of Bangladesh (Sarker and Barton 2006) and other South Asian countries (Aryal et al. 2021), use of power tillers immensely benefited farmers in reduction of cost of production and agricultural productivity including potato production. However, the efficiency ratio machinery in the form of power tiller (0.01) indicated slightly over utilisation in the study area due to the inadequate supply of power tillers (Tobgay 2016), and thus, many farmers have to rely on hired power tillers from state owned enterprise and private owners. The potato farmers in the study area depended mostly on hire of power tillers from their neighbours. The farmers reported that, in certain cases, the hire charges include the actual working hours in the field and the transportation time of power tiller to and from the actual working field. Secondly, due to rugged terrain, the field sizes are small and the efficiency of power tillers are not efficient as they used to be in bigger fields (Tobgay 2006). Thirdly, the average area under potato cultivation is minimal compared to the total average landholding per household. Therefore, the farmers should increase the area under potato cultivation and use of seeds to get optimum resource efficiency from FYM, labour, and machinery usage.

CONCLUSION AND RECOMMENDATIONS

This study provides an analysis of economics of production and resource use efficiency of organic potato cultivation in Bhutan. The area under cultivation has significant influence on the overall productivity of organic potato. Farmers underutilised area for cultivation and seeds in organic potato production resulting in higher expenses on resources such as machinery, FYM, and labour. Investing more in land under cultivation and obtaining adequate seed would improve the inefficient use of FYM, labour, and machinery used in organic potato production and would lead to maximum economic benefit from the production. Due to time and resource constraints, the current study could only cover organic potato production in the study area. Further research on the other vegetables grown by the farmers in the study would give a clearer picture of the resource allocation and farming efficiency.

It is recommended that policies be directed to improve availability and accessibility of adequate high-quality seeds, either for free or subsidised, to obtain higher returns. Such actions from the government would resolve the problem of overutilization of machinery, FYM, and labour in organic potato production and enhance economic returns from production. In addition, the government should

increase subsidies in organic agriculture to encourage more farmers to adopt organic farming to enhance national food security.

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TECHNICAL EFFICIENCY OF ORGANIC RICE FARMING UNDER CONTRACT ARRANGEMENT IN PREAH VIHEAR PROVINCE, CAMBODIA

Chanmony Sok[1], Tomohiro Uchiyama[2], Nina N. Shimoguchi[2] and Rika Terano[2]*

[1] Graduate School of International Food and Agricultural Studies,
Tokyo University of Agriculture

[2]Faculty of International Agriculture and Food Studies, Tokyo University of Agriculture
1-1-1 Sakuragaoka, Setagaya-ku, Tokyo 156-8502 Japan

*Corresponding author: rt200005@nodai.ac.jp

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ABSTRACT

Organic rice contract farming is considered an important livelihood to secure farmers' income in Cambodia. Although farmers have increased their income through contract farming, their productivity remains low. This study aims to determine the factors influencing productivity and measure the technical efficiency of organic rice farming under contract arrangement in the Preah Vihear province of Cambodia. A series of field surveys were conducted in February 2021. Using the data of 50 organic rice farmers, the Stochastic Frontier Analysis model revealed that planted area, cow manure inputs, share of land harvested using combine harvester to total cultivated land, and level of comprehension and adaption of training contents significantly increased productivity. In contrast, disasters, the number of plots per ha, and unskilled laborers are the factors that led to decreased productivity. In addition, the average level of technical efficiency in the study area is 83%, indicating that the current production inputs and technology levels have the potential for productivity improvement. For further study, long-term studies on organic rice farming efficiency are recommended, specifically those that will examine factors such as level of risk-taking, the training content, and the training methods.

Key words: organic rice, productivity, stochastic frontier analysis, technical efficiency

INTRODUCTION

About 75% of the Cambodian population lives in rural areas (World Bank 2021), and they rely directly or indirectly on agriculture for their livelihood. Agriculture has reduced poverty from 47.8% in 2007 to 9.5% in 2019. However, the agriculture value-added showed slowly growth from 2013 (ADB 2021). In addition, limited farming techniques, inefficient agriculture input utilization, inefficient mechanization, and lower human capital are the challenges, resulting in low productivity and insufficient income (Lao 2019). Moreover, the continuous decline in the agricultural labor force is also a vital issue as promising additional income opportunities in the industrial and services sectors have been attracting farmers to engage in off-farm activities, especially for the past two decades (Fig. 1).

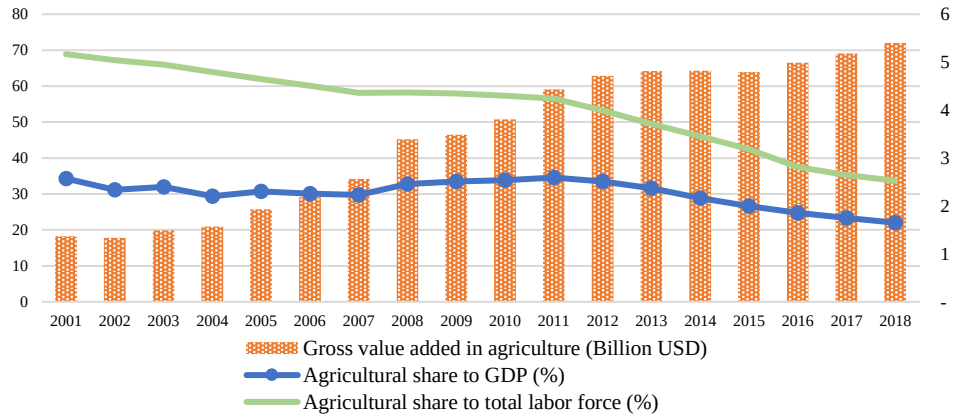

Fig. 1. Agricultural contribution to GDP and labor force, 2001-2018
Source: MAFF Cambodia, 2020

To address these challenges, the Cambodian government has been promoting two development strategies: organic and energy crop production since 2011 (Setboonsarng and Markandya 2015). Organic farming is mainly promoted through contract farming to provide appropriate training, access to proper markets, and opportunities for sustainable income (MAFF Cambodia 2011). Despite the government and private initiatives, the share of organic farming land is still limited to 0.6% (Willer et al. 2021). Moreover, only a few research studies are conducted on organic rice and contract farming. Based on the study conducted in Preah Vihear province, there was an increasing trend of small-scale farmers engaging in contract farming (Sok 2019). Some farmers even expanded their farmland due to the lucrative premium price. Contract farming entities also regularly provided technical training to farmers. However, these farmers still experienced low yields and labor shortages (Sok 2020).

Agricultural productivity and efficiency studies, including rice production efficiency studies, appear to be scarce in Cambodia, especially in organic rice production. Since there is limited research conducted on efficiency of rice production in Cambodia, it is essential to estimate the technical efficiency and inefficiency of organic rice production and clarify the factors increasing organic rice productivity.

This study attempted to determine the factors influencing the productivity of organic rice under contract farming in the Preah Vihear province, Cambodia. Specifically, this study aimed to clarify the support mechanism of organic rice contract farming, describe the characteristics of organic rice farmers under contract farming, and determine the influencing factors of the technical efficiency of organic rice farming and the distribution of technical efficiency.

MATERIALS AND METHODS

Study area. Preah Vihear province is located in the northern Cambodia about 400 km from Phnom Penh (Fig. 2). This province has the second largest arable area for agriculture production, and the largest organic granary under contract farming. It is in the plateau mountain area, which covers 14,031 km^2 with a population of 254,827 people (NIS 2019). Since the province is remote and the least populated area, about 85% of the province's population depends on agriculture. However, only 6% of the land was used for rice farming, 2.5% for other crop farming, and 0.8% for fruit farming. Hence, farmers are the main actors of this rural economy, but most farmers have low educational attainment and organic rice farming is done once a year due to rainfed farmland. Thus, adopting new technologies and improving productivity are their challenges. (Preah Vihear Provincial Department of Agriculture 2020).

Fig. 2. Map of Preah Vihear Province, Cambodia
Source: Google map, 2022

Sample selection. Preah Vihear province has a total of 6,099 organic rice farm households. During the field survey in February 2021, a combination of purposive, cluster, and random sampling methods was utilized based on previous studies (Asravor 2022; Muluneh et al. 2022; Saputra et al. 2022). The study area was then divided into seven groups (districts). Of the seven districts, three districts, namely Cheysen (1,446 HH), Rorvieng (1,191 HH), and Koulen (825 HH), were selected as the top three districts with the most organic rice contract farming households. Only 50 respondents were randomly selected from these three districts due to time constraints and Covid-19 pandemic restrictions on mobility and social contact. Face-to-face interviews were conducted with the guidance of a structured questionnaire. Since almost all farmers only had limited educational attainment, more time and effort were required to conduct the interviews.

Data analysis. In order to clarify the support mechanism of organic rice contract farming and describe the characteristics of organic rice farmers under contract farming, descriptive analysis was utilized. Support mechanism of organic rice contract farming were clarified using a combination of information collected from the field survey 2021, key-informant interview, previous literature reviews and secondary data. The field survey in 2021 collected 2020 observed data on the yield, number of farming household, agricultural cooperative in contract farming, and other information such as regulation and supports. The technical, regulation, standards support, yield, and other information from 2013 to 2019 were collected from previous literature reviews and secondary data such as the provincial department of agriculture reports.

The Stochastic Frontier Analysis (SFA) measured productivity and technical efficiencies. Typically, the concept of productivity refers to the volume of output produced with given inputs. Productivity rises when more output is produced with the same amount of input. Input and output are difficult to measure, both theoretically and empirically. Productivity measurement factors, including labor productivity and land productivity which scholars commonly apply, are referred to as partial measures of productivity. These partial measures of productivity cannot wholistically determine the inefficiency of productivity (Coelli et al. 2005). To clarify the mechanism of productivity, SFA is an efficient method applied by scholars. SFA is adopted mainly for farm management analysis with the benefit of statistical tests on the production structure and the inefficiency level (Coelli 1995b). SFA can also be used in most farming conditions and types, such as rice and vegetable farming Coelli et al.

(2005). In addition, this method also has the benefit of letting statistical tests be done on the levels of efficiency and inefficiency by figuring out why farms are not working well while separating random noise from efficiency. SFA was therefore used instead of the data envelopment analysis (DEA) method since DEA incorporates noise as part of the efficiency. In addition, the estimation methods of SFA for efficiency by using Frontier 4.1 software enable the separation of noise effects from inefficiency components and produce accurate outcomes for a single output and multiple inputs (Kebede 2001).

SFA has been used in the various fields, including agricultural research. Technical inefficiency affects rice production in a Tidal wetland in south Kalimantan, Indonesia. This study revealed that planting area, inorganic fertilizers, and pesticides were significant determinants. At the same time, the number of household members and frequency of field expansion were influential factors in the technical inefficiency of rice farming (Azis et al. 2018).
In the case of northwest Cambodia, increasing harvested land, fertilizer usage, and pesticide use significantly affected rice production, while overuse of labor in rice fields was the inefficiency factor of rice farming. Moreover, factors in the technical inefficiency model also showed that disasters (droughts, floods, and insects), household head education, family size, and cultivated area for other crops decreased the technical efficiency of household rice production. On the other hand, irrigated area, plot area, and household head gender increased technical efficiency (Kea et al. 2016).

SFA was also applied to crops (e.g., potato and rice) integrated with other crops (Nahraeni et al. 2012; Suharyanto et al. 2013). Using the Frontier 4.1 software, according to the research conducted by Nahraeni et al. (2012) on the technical efficiency of highland potato farming in Indonesia, production was highly sensitive to total land, seed, and organic fertilizer. In addition, the land slope and contour-aligned cultivation system increased efficiency, with a mean technical effectiveness of 0.84. For rice production with other crops, another research was conducted in Bali province on the technical efficiency of rice integrated crop management (ICM) (Suharyanto et al. 2013). This study used the frontier production function with the Maximum likelihood estimates method to measure the level of technical efficiency in rice with the ICM approach. As a result, the level of technical efficiency ranged from 0.71 to 0.99, with an average of 0.88. The rice yield was affected by the usage and volume of various inputs (seeds planted, active nitrogen, organic fertilizer, pesticide) and precipitation amount. The farmers' age, level of education, farm experience, and the number of owned plots per ha significantly affected their technical inefficiency.

The Stochastic frontier model was applied, which was first suggested by Aigner et al. (1977) and Meeusen and Van de Broeck (1977). The SFA model's general form is shown below:

$$Ln\ y_{it} = Ln\ f\ (x_{it}; \beta) + v_{it} - u_{it} \quad (1)$$

Where Ln represents the form of natural logarithm function, y_{it} and x_{it}denotes rice yield and inputs per ha during the time period *t*, respectively. Whereas β represents correlation coefficients; v_{it} is randoms error, assumed to be the normal distribution; u_{it} forecasts technical inefficiency, assumed to be truncated distribution; v_{it} and u_{it} are considered to be independent (Aigner et al. 1977; Ali et al. 2019; Kea et al. 2016). These technical inefficiency factors can be described in general terms as follows:

$$u_{it=\delta_0+\sum_{k=1}^{n}\delta_k z_{kit}+\omega_{kit}} \quad (2)$$

Where ω_{kit} is the stochastic noises; z_{kit} indicates external variables that are factors affecting organic rice technical efficiency; δ_0 and δ_k are estimated coefficients; if δ_k is negative, it indicates a positive correlation among affecting factor variables and technical efficiency of rice farming. Contrarily, if δ_k is positive, it shows a negative correlation between technical efficiency and determining factors. Following equations (1) and (2), the estimation SFA model parameters can use the maximum likelihood method, which estimates the likelihood function based on two variance parameters (Coelli and Battese1996；Kea et al. 2016).

$$\gamma = \frac{\sigma_u^2}{\sigma_s^2};\ \sigma_s^2 = \sigma_v^2 + \sigma_u^2 \quad (3)$$

In equation (3), gamma parameter (γ) has values ranging from zero to one ($0 \leq \gamma \leq 1$) and represents validity of the random disturbances (u_i , v_{it}). If γ is closer to zero, it indicates that the difference in actual output and the maximum possible output primarily caused by other uncontrolled random factors. This result makes the use of stochastic frontier model useless. In contrast, if γ is closer to one, it indicates that the gap is primarily caused by z_{ki} the effects of one or more external and personal characteristic variables, and it indicates that using stochastic frontier model is more relevant (Coelli and Battese 1996; Kea et al. 2016).

Application call "FRONTIER 4.1 (free download from University of Queensland, Australia)" was the package most often used to estimate the SFA model. FRONTIER 4.1 is a one-step program for the estimation of stochastic frontiers, and technical efficiency and inefficiency models (Coelli 1995a). FRONTIER 4.1 is frequently used to calculate the stochastic frontiers as mentioned in the literature review above. Moreover, it has been used in numerous rice production studies (Mayston 2015; Kea et al. 2016; Yekti et al. 2015). In this study, maximum likelihood was applied to estimate the parameters of the stochastic frontier model by using the Frontier 4.1 software.

There are 14 variables in which Y (rice yield) is a dependent variable, and independent variables are divided into two parts (Table 1). First, the efficiency included efficiency factors: planted area, amount of seeds used, amount of fertilizer used (cow manure), family labor, hired labor, exchange labor, and share of land harvested using combine harvester to total cultivated land. It should be noted that share of land harvested using combine harvester to total cultivated land is considered as one independent variable because a combined harvester can significantly reduce grain loss compared to manual harvesting methods. Combine harvester usage can save on the average of 4.9% grain losses compared to manual harvesting methods (Jones et al. 2019). Second, the inefficiency factors included age of head household, year of attended school, the number of the plot per hectare area, attended training times per year, level of comprehension and adaption of training in percentage, and percentage of cultivated land affected by disaster. Lastly, natural disasters in the study area were mostly drought, flood, and infestation of insects.

Table 1. Description and measurement of parameters of Stochastic Frontier Analysis model

Variable	Efficiency Parameters
Y	Total rice yield (ton/ha)
X_1	Total planted area (ha)
X_2	Total amount of seed used (ton/ha)
X_3	Total amount of cow manure(ton/ha)
X_4	Total family labor (man-day/ha)
X_5	Total hired labor (man-day/ha)
X_6	Total exchange labor(man-day/ha)
X_7	Share of land harvested using combine harvester to total cultivated land (%)
	Inefficiency Parameters
Z_1	Age (years)
Z_2	Education (years)
Z_3	Number of plots per ha (number)
Z_4	Frequency of training attendance per year (times)
Z_5	Level of comprehension and adaption of training (percentages)

Variable	Inefficiency Parameters
Z_6	Share of cultivated land affected by disaster (percentages)

Source: Author's compilation based on field survey and literature review, 2021

RESULTS AND DISCUSSION

Characteristics of farmer respondents. Table 2 shows that almost three out of four family members engage in farming. The average age of the farmer-respondents is 47.4 years old. Most of these respondents have had formal education for only five years, indicating that most farmers cannot read and write properly. Thus, reading comprehension of technical documents or learning to use new machineries are expected to be complex tasks for them.

The average cultivated area for organic rice per household in the study area is 4.24 ha, and total holding land is around 6.79 ha which is larger than national average total landholding of 1.3 ha (ADB 2021). It should be noted that the study area is remotely located, thus more farmlands are available for a small population. Since organic rice is only planted in single cropping as a seasonal activity, farmers also need to cultivate other crops such as cashew nuts and cassava to earn additional income. There is approximately 1.9 ha of other crop farming land per household.

Table 2. General characteristics of farmer and organic rice farming in 2020 (N=50)

Items	Mean	SD
Family member (persons)	4.02	1.30
Members engage in agriculture (persons)	2.90	1.21
Educational attainment (years)	5.60	3.18
Age (years)	47.40	12.22
Organic rice yield (tons/ ha)	1.84	0.25
Organic rice cultivated area (ha)	4.24	2.45
Other crop cultivated area (ha)	1.90	1.41
Fallow land (ha)	0.56	0.78
Total owned land (ha)	6.79	4.78

Source: Field survey, 2021

Support mechanism for organic farmers in Preah Vihear Province. Organic rice in Preah Vihear province has been supported by the Support to the Commercialization of Cambodian Rice Project in partnership with the Cambodia Organic Agriculture Association (COrAA) since 2013 (Table 3). For the first year, the project supported agriculture cooperatives to produce organic paddy in compliance with COrAA private organic standards expected to be sold directly to rice millers or exporters who had expressed interest. However, the commercialization of organic paddy was not yet satisfactory due to inefficient logistics and coordination at harvest time.

To address this logistics issue and secure the marketing channel of organic paddy, the approach was changed from a direct selling model to a contract farming approach in 2014. Changes in organic rice standards from Cambodian Organic Agriculture Association (COrAA) standards to European Organic Standard (EOS) and National Organic Program (NOP) standards were apparent, requiring external certification by an international certification body (e.g., Ecocert). Since then, contractors, government extension workers, and NGOs have worked together and provided training on organic rice standards and technical support to farmers to meet international certification requirements. Meang and

Jean-Marie (2018) found that the government or contractors only provided training, and farmers did not receive any subsidy.

Farmers commonly practice growing fragrant and non-fragrant rice in the study area because it is suitable for the wet season and land conditions. Farmers commonly start land preparation in May and do harvesting between late November and early December. Most of them followed their cultivation practice even though they received some training from extension workers, contractors, and NGOs. From the interview, even though combine harvester was used in the study area, some farmers still harvested by hand and sun-dried rice paddy in the field due to limited services, difficult land conditions, and farm location. In line with Sok's (2020) study, farmers have still faced the challenges of low yields, despite receiving technical supports and training from contractors and extension workers since 2014 (Table 2). On the other hand, contract farming enables organic rice farmers to sell harvested rice at a premium price but seems unable to improve their productivity. Although farmers have tried expanding their planted area to increase their production, it resulted in severe labor shortages. Moreover, organic rice farmers could not adapt to new machinery, and access to machinery services is very limited due to the farm location and few service providers.

Yield. The average yield of organic rice in 2020 was approximately 1.84 tons per ha (Table 4), which is still lower than average rice yield of 2.79 tons per ha (Preah Vihear Provincial Department of Agriculture 2020). Thus, farmers still seemed to underestimate their potential income.

Table 4. Mean of organic rice farming parameter per hectare of SFA model (N=50)

Variable	Mean	SD
Efficiency Parameters		
Yield (ton/ha)	1.84	0.25
Planted area (ha)	4.24	2.45
Seed (ton/ha)	0.10	0.04
Fertilizer (ton/ha)	0.09	0.14
Hire labor (pp-day/ha)	9.58	6.06
Family labor (pp-day/ha)	11.44	5.56
Exchange labor (pp-day/ha)	16.78	10.81
Share of land harvested using combine harvester to total cultivated land (%)	0.21	0.30
Inefficiency Parameters		
Age (years)	47.38	12.22
Educational attainment (years)	5.54	3.18
Number of plots (plots/ha)	39.27	8.66
Frequency of training attendance per year	5.56	1.66
Level of comprehension and adaption of training (%)	0.52	0.11
Share of cultivated land affected by disaster (%)	0.47	0.21

Source: Field survey, 2021

Table 3. Support mechanism for organic rice farmers in Preah Vihear province

Activities	2013	2014	2015	2016	2017	2018	2019	2020
Technical support	Agricultural Cooperatives (ACs) producing organic rice through **Goverment and NGOs led project**							
	Training on livestock and crop production organized by **Goverment, NGOs**							
			Training on international standards organized by **Contractors, NGOs**					
Regulatory support		Promotion and implementation of contract farming organized by **Goverment, Contractors**						
			Establishment of internal control system supported by **Goverment, Contractors, NGOs**					
				AC union establishment supported by **Goverment, Contractors, NGOs**				
Certification and standards	local OR standards by COrAA	Organic rice certification by ECOCERT with support from **Contractors, NGOs**						
						start of fair trade led by **Contrators**		
Market access	Direct purchase from buyers		Contract agreement between ACs and contractors; start of organic rice purchasing					
				Contract agreement between AC (with union support) and contractors				
Number of Agricultural Cooperatives	5	8	12	25	32	34	38	38
Number of households under contract farming	n/a	891	1,669	3,151	5,162	5,053	5,341	6,099
Yield(tons/ha)	n/a	n/a	1.96	2.15	2.07	1.81	2.01	1.84

Source: Author's compilation based on Meang et al. (2018); Provincial Department of Agriculture (2020); Sok (2020); and Field survey (2021)

Organic rice farming characteristics per hectare

Planted area. Total organic rice planted area is about 4.24 ha per household. Compared to the study of Sok (2020) that reported about 3.82 hectare per household, farmers seemed to increase cultivated land for rice farming to increase production.

Seeds. In 2020, farmers used about 100kg of seeds per ha. Since farmers conducted seed selection a season earlier for the upcoming growing season, contract companies do not conduct any rice genetic background prior to seed selection. The physical characteristics of the rice plant, specifically its thickness and heaviness of seed, are common key factors considered during the selection process. Before seeding, farmers need to separate unfit grains from the healthy seeds by soaking the seeds in water containing salt, and the floaters are separated from the sinkers.

Fertilizer. Farmers commonly use a small amount of fertilizer (cow manure) with an average of 90 kg per ha. Moreover, farmers tend to apply cow manure more after cultivating their land for more than five years to improve soil fertility. Because farmers collect cow manure from their cows, as the number of owned livestock increases, their supply of cow manure is also expected to increase, especially those farmers with matured cows.

Labor input. Most southeast Asian countries that cultivate rice rely heavily on family, exchange, and hired labor (Morooka et al. 1991). In line with field observations, this study found the same categories. Exchange labor refers to the labor inputs which farmers do not need to pay. Instead, they must return the labor input received by working in other farmers' paddy fields. Exchange and hired labors are commonly used in the study area during transplanting and harvesting. Annual exchange labor input was about 17 man-days per ha, considered the highest among labor inputs. In addition, family labor was the primary labor involved in all stages of cultivation. However, there is a shift to family labor as primary labor source when there is a lack of hired labor, especially during the high availability of off-farm activities.

Combine harvester services. During harvesting time, recently, farmers started to use combine harvester services. Most farmers use rental services because owning a combine harvester requires a high cost (Table 3). The average rental cost for combine harvester services ranges from KHR 350,000 to KHR 500,000 per ha or about USD 87 to USD 125 per ha. Currently, there are limited opportunities for farmers to lease combine harvesters depending on the availability of rental services. Some rice fields also do not have suitable field conditions to use combine harvesters. Therefore, the harvesting process still requires laborers to proceed manually.

Inefficiency parameters. The average age, family size, and education level of head household farmers are explained in Table 1. The average number of plots per ha refers to the number of plots for growing organic rice per ha. Frequency of training is defined as how many times farmers attend training per year. Level of comprehension and adaption of training are subjectively defined as how many percentages they understood and practiced after attending the training, in here, farmers defined the percentage by themselves. Finally, disasters are defined in percentages of rice cultivation areas affected by floods cause from excessive rainfall, droughts, or pests within the study period, and farmers answered in percentage by farmers themselves. About 47% of organic rice cultivation have been reported to be affected by rainfalls at the end of season. While the average rainfall in 2020 was 1,551.36 mm (Preah Vihear provincial department of Agriculture, 2020).

Technical efficiency of organic rice farming. The maximum likelihood estimation reveals that the random error variation (v_{it}) was less than 3%, and gamma (γ) was 0.970 and significance at 1% (Table 5). These results show that the variation of the composite error term came mostly from the technical efficiency (u_i).

Table 5. Parameters of stochastic frontier analysis model

Variable	Coefficient	Std. Error	t-ratio	
Constant	0.704	0.135	5.198	***
Planted area	0.042	0.021	1.967	*
Seed	-0.012	0.035	-0.354	
Organic fertilizer (cow manure)	0.040	0.002	1.704	*
Family labor	-0.006	0.031	-1.600	
Hired labor	0.015	0.008	1.846	*
Exchange labor	0.019	0.010	1.949	*
Share of combine harvester usage	0.013	0.003	1.722	*
Gamma	0.970	0.004	282.872	***
Sigma-squared	0.009	0.003	2.679	***
log likelihood function OLS	37.256			
log likelihood function MLE	58.964			
LR test of the one-sided error	43.416> chi square value 23.551			

Source: Field survey (2021)

Notes: Estimation was conducted using FRONTIER 4.1

*Significant levels are shown as follows: *** p < 0.01, ** p < 0.05, * p <0.1*

SFA revealed that most of the estimated coefficients had expected positive signs except for seed inputs and family labor. Specifically, yield increased for every increase in total planted area, input cow manure, labor hired, exchange labor used, and combine harvester in the study area. Regarding planted area, the findings suggested that the more farmers expand their cultivated land, the greater the potential for increased productivity. This finding is also consistent with previous findings that showed a positive and statistically significant correlation between the size of a rice farm and its yield (Chandio et al. 2019; Tijani 2006). More cow manure could increase organic rice yield with an elasticity of 0.04, indicating that a 1% increase in cow manure input could increase in 4% yield. Similar results from previous studies showed that a 1% increase in fertilizer input could increase rice yield by 9% and potato yield by 10% increase (Kea et al. 2016; Nahraeni et al. 2012).

In addition, hired labor and exchange labor are significant factors in increasing the yield, indicating that both seemed more skillful than family labor (Table 5). This is consistent with previous findings on rice farming efficiency that indicated an increase in hired labor positively increased rice yield in Vietnam (Khai and Yabe 2011). Combine harvesters is also a significant factor in increasing yield because these machines could reduce the harvest losses compared to manual harvesting. Despite the benefits from these agricultural machines, many farmers still use hired labor and exchange labor during transplanting and harvesting, because of the limited availability of combine harvester in the study area. Therefore, even though seed and family labor were not statistically significant with negative signs, these convey that farmers tend to use unskilled family labor and seed excessively and inefficiently.

Technical inefficiency of organic rice farming. Table 6 shows the technical inefficiency of organic rice farming. This model explains inefficiency with a negative sign on a parameter which explains the positive effect of the variables on improving technical efficiency. In contrast, a positive sign indicates inefficiency. Significant technical inefficiency factors include disaster, number of plots per ha, and training comprehension and application. A 1% increase in land area affected by disaster and the number

of plots per ha decreases technical efficiency by 25% and 5%, respectively. Farmers try to make many small plots per ha to control water but the result cause decreasing the efficiency. Therefore, if farmers could reduce plot numbers per ha and maintain the good level of water, this may increase the efficiency. This is consistent with findings by Kawasaki (2010) wherein land partition was found to reduce rice production efficiency. A study on northwest Cambodian conventional rice farming revealed that for every 1% increase in disaster-affected rice field, the rice technical efficiency decreased by 27% (Kea et al. 2016). Since the studied farms are in the plateau mountain area, the number of plots per ha is significant, conveying that there is a need to decrease the number of plots to improve technical efficiency.

Table 6. Organic rice farming technical inefficiency model parameters estimation.

Inefficiency Model	Coefficient	Std. Error	t-ratio	
Age	0.039	0.074	0.527	
Education	0.021	0.047	0.438	
Number of plots per ha	0.046	0.013	2.099	*
Frequency of training attendance per year	0.079	0.097	0.818	
Training comprehension and adaption of training	-0.438	0.120	-4.500	***
Share of cultivated area affected by disaster	0.255	0.060	2.715	***

Source: Field Survey (2021)

Notes: Estimation was conducted using FRONTIER 4.1

*Significant levels are shown as follows: *** p < 0.01, ** p < 0.05, * p <0.1*

The frequency of training attendance showed no relationship to the output. However, training comprehension and application had a negative coefficient and was a significant factor at 1%, conveying that their level of understanding and application positively impacted the technical efficiency of organic rice farming in the study area. Thus, if farmers could increase the level of comprehension and application by 1%, technical efficiency could be increased by 44%. Moreover, farmers could only understand and practice less than 50% of the training contents. From the interviews, it was confirmed that some farmers could not apply or understand well the training contents. At times, they were also not the first-hand receiver of training since their children or spouse attended the training on their behalf. From observation, farmers have difficulty adapting because some technical practices (e.g., transplanting, picking good seeds) from training require more time and labor. Therefore, the training contents and method need to be improved for better comprehension and field application and to encourage farmers to join the training.

Technical efficiency distribution of organic rice farmers. Figure 3 illustrates the technical efficiency level distribution of organic rice farming in the study area. Frontier 4.1 revealed that an individual household's production technical efficiency per hectare ranges from 52% to 99%, with a mean of 0.83, indicating that organic rice farmers attained 83% of rice production at the current production inputs and technology level. By comparing the average efficiency level of farmers (83%) to the highest efficiency of farmers (99%), with the same level of inputs, rice yield could have been increased further by 16% if farmers had been technically efficient. These results also revealed a significant gap among organic rice farmers and room for organic rice productivity improvement in the study area.

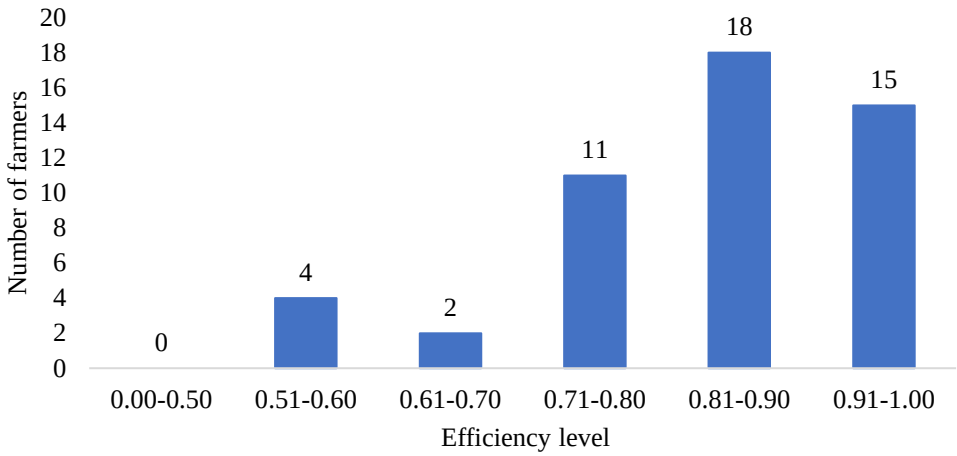

Fig. 3. Technical efficiency distribution of organic rice farmers in Preah Vihear province, Cambodia
Source: Field Survey, 2021
Note: Estimation was conducted using FRONTIER 4.1

CONCLUSION

Organic rice contract farming is considered an important livelihood to secure farmers' income in Cambodia. In Preah Vihear province, organic rice and contract farming have been promoted since 2013 and 2014, respectively. Although the income of organic rice farmers has increased through these initiatives, farmers continue to experience low yields. Thus, this study attempted to review the support mechanisms to organic rice farmers, characterize organic rice farmers under contract farming, and identify influencing factors of technical efficiency.

The review of support mechanisms confirmed that public and private institutions have been extending various support services related to certification, market access, production, and regulation since 2013. Farmers also received a premium price, which is one of their reasons for engaging in organic rice farming under contract agreement. However, farmers had low educational attainment, which led to challenges in understanding training and adapting new agricultural technologies.

SFA revealed that an increase in technical efficiency could be attributed to land expansion, increased usage of cow manure, and increased harvested land using combined harvesters. On the other hand, the share of disaster-affected land and the number of plots seemed to decrease technical efficiency. Therefore, there is a need to promote proper land preparation and minimize the number of plots. Moreover, the provincial government may consider constructing irrigation facilities to improve productivity and address flood and drought related issues. Moreover, SFA showed that understanding and application of training contents significantly and positively impacted technical efficiency, despite the frequency of attending training's insignificant impact on technical efficiency. Thus, there is a need for customized training and other support services for farmers and their family members in line with their needs and capabilities.

For further research, long-term study on organic rice farming efficiency is vital. Inclusion of factors such as level of taking risks, training contents, and training methods are suggested.

ACKNOWLEDGEMENT

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HOMOLOGY MODELING, MOLECULAR DOCKING AND MOLECULAR DYNAMICS STUDY OF TWO DIASTEREOMERS BINDING TO FALL ARMYWORM *SPODOPTERA FRUGIFERDA* ARYLALKYLAMINE N-ACYLTRANSFERASE

Edwin Alcantara*
National Institute of Molecular Biology and Biotechnology (BIOTECH),
University of the Philippines Los Baños, College, Laguna 4031, Philippines
*Corresponding author: epalcantara@up.edu.ph

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ABSTRACT

The discovery of a new mode of action insecticide is needed to augment existing tools for effective management of fall armyworm (FAW) *Spodoptera frugiferda*. This study sought to investigate the binding mechanism of two diastereomers, PubChem CID 162987453 (D1) and CID 162987454 (D2), to FAW arylalkylamine N-acyltransferase (aaNAT). Homology modeling was performed to predict the 3D structure of the FAW aaNAT receptor. A homology model with the ligands D1 and D2 was used for molecular docking. The docking results were confirmed by molecular dynamics simulation and molecular mechanics Poisson-Boltzmann Surface Area analysis. Finally, per-residue energy decomposition analysis was performed to identify specific amino acid residues involved in ligand binding. Molecular docking revealed the hydrophobic and allosteric binding sites of the two ligands. The binding was stable at 300 ns as shown by molecular dynamics simulation. The binding free energies (ΔG_{bind}) of D1 and D2 were -7.16 kcal/mol and -7.06 kcal/mol, respectively. The hydrophobic residues Ile108 and Leu112 contributed significantly to the binding interaction with D1 and D2, as revealed by per-residue energy decomposition analysis. Together, D1 and D2 are good prime candidates for development as FAW aaNAT allosteric inhibitors.

Key words: sequence alignment, allosteric binding site, inhibitor, hydrophobic, molecular recognition

INTRODUCTION

The fall armyworm (FAW), *Spodoptera frugiferda* is a major insect pest of corn in the Americas and has recently spread to Africa and Southeast Asia (Navasero et al. 2019). FAW is a polyphagous pest that attacks other economically important crops such as sorghum, rice, cotton and vegetables (Hruska 2019). The insect has acquired resistance to many synthetic insecticides (Mota-Sanchez and Wise 2019; Ayil-Gutierrez et al. 2018) including resistance to certain genetically modified (GM) corn events (Monnerat et al. 2018; Huang 2020). Diamides and spinetoram are still generally effective, but resistance to these compounds has been observed in some countries (Gutierrez-Moreno et al. 2019; Okuma et al. 2022). If the trend does not change, the number of available modes of action will be very limited in the future. In the absence of an effective control measure, FAW can spread rapidly after being introduced into an agricultural area (FAO 2020; Wang and Wu 2020).

Insect arylalkylamine N-acyltransferase (aaNAT) is a promising target for the development of new insecticides. Insect aaNAT is responsible for regulating important physiological functions such as cuticle morphology and neural signaling. Inactivation of aaNAT would impair insect survival not only by disrupting neural signaling but also by severe consequences of impaired cuticle development (O'Flynn et al. 2018). Compared to mammals, insects are particularly susceptible to aaNAT inactivation because they lack the enzyme, monoamine oxidase, which functions to inactivate arylalkylamines in mammals (Ganguly et al. 2002). Recent work that provided information on both the 3D structure and critical amino acid residues in the active site of the red flour beetle *Tribolium castaneum* aaNAT (O'Flynn et al. 2020) is an important contribution that could be applied to designing a new insecticide that targets the FAW aaNAT. At the time of this writing, no previous studies describing FAW aaNAT as an insecticidal target have been reported. To date, the IRAC has 32 mode of action classification of insecticides (Sparks et al. 2020). The classification scheme does not include aaNAT inhibitors.

The use of molecular docking and molecular dynamics simulation has been suggested to accelerate the development of new environment friendly insecticides, (Iyison et al. 2021). In fact, it is increasingly being used to study the insecticidal properties of lead compounds (Iyison et al. 2020; Du et al. 2021; Muhseen et al. 2021; Shahraki et al. 2021; Crisan et al. 2022). This has been made possible by recent advances in insect genomics (Asma and Halfon 2021) and computer technology (Buch et al. 2010; Banegas-Luna et al. 2019). The application of computational methods has also become a promising approach to elucidate insect resistance mechanisms (Banba 2021).

Diastereomers are a pair of compounds with the same chemical properties but have different physical properties (Parente et al. 2018). They are stereoisomers that cannot be superimposed and are not mirror images of one another (Nilos et al. 2009). Typically, these compounds are separated using high-performance liquid chromatography (Di Fabio et al. 2013; Kato et al. 2017; Cha et al. 2021). A recent survey of new agrochemicals used as plant protection agents showed that a significant proportion of such products are mixtures of diastereomers (Jeschke 2018). A diastereomeric natural product has recently been reported as a promising FAW insecticide (Silva et al. 2022).

To explore the potential of FAW aaNAT enzyme as a potential target for the development of new insecticides, the three-dimentional (3D) structure of the enzyme must be known. However, the experimentally determined 3D structure of FAW aaNAT is not available in the Protein Data Bank. In the absence of a 3D structure, enzyme structure prediction by homology modeling is a suitable option to enable *in silico* studies (Schmidt et al. 2014). The use of homology models of target insect enzymes has recently been a well-accepted approach to successful screening campaigns for insecticide discovery (Lin et al. 2020; Samurkas et al. 2020; Rodrigues et al. 2021).

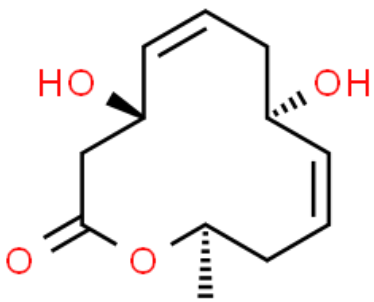
This study used homology modeling, molecular docking and molecular dynamics simulation to elucidate the binding mechanism of potential FAW aaNAT inhibitors, focusing on two diastereomers, PubChem CID 162987453 and PubChem CID 162987454 . These two compounds are closely related to the relatively rare dendrodolides for which no insecticidal activity has been reported so far (Sun et al. 2013; Oppong-Danquah 2020). Thus, studying the molecular structure to further decipher the biological function of these unique compounds provides an opportunity to discover a new mode of action insecticide, which is urgently needed to increase the options available for effective management of FAW populations. In addition, the results of this study provide theoretical insights into the molecular recognition of FAW aaNAT inhibitors.

MATERIALS AND METHODS

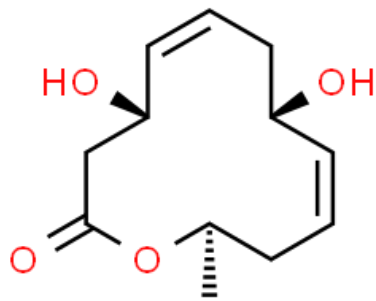
Target protein receptor. The amino acid sequence of FAW aaNAT protein, heretofore referred to as receptor was obtained from the NCBI database (NCBI 2020) and uploaded to the ModWeb server (Modbase 2020) for homology 3D structure modeling. The 3D structure of the FAW receptor has not

been determined experimentally. Then, the 3D structure of the ModWeb-generated receptor was uploaded to the PREFMD server (PREFMD 2018) for further structure refinement. PREFMD is a protein structure refinement method based on molecular dynamics (Lim and Feig 2018). The refined receptor model was uploaded to the ProBis web server to predict the ligand and its binding site (Konc and Janezic 2010). The position of the allosteric site was predicted using the AlloSite web server (Song et al. 2017). Ramachandran plot analysis of the model receptor was performed using the Molprobity web server (Chen et al. 2010).

Preparation of ligands. The 3D structures of the two diastereomers (PubChem CID 16297453 and PubChem CID 16297454) were obtained from the PubChem compound database in sdf format (Kim et al. 2019). Heretofore, each compound is referred as D1 and D2, respectively. The 2D structure and other molecular properties of these two compounds are shown in Fig. 1.



IUPAC Name: (4S,5Z,8R,9Z,12R)-4,8-dihydroxy-12-methyl-1-oxacyclododeca-5,9-dien-2-one
Mol. Wt= 226.27 g/mol
PubChem CID 162987453



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Mol. Wt= 226.27 g/mol
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Fig. 1. Chemical structure of two diastereomers PubChem CID 16297453 and PubChem CID 16297454 utilized for *in silico* studies. Image source: https://pubchem.ncbi.nlm.nih.gov/compound/

Molecular docking. Molecular docking is a computational tool often used to model the interaction between a small molecule and a protein. The docking process involves predicting the conformation and binding pose as well as the binding affinity of the ligand (Meng et al. 2011). D1 and D2 were docked separately with the model receptor in a previous step using Autodock Vina as implemented in AMDock (Valdes-Tresanco et al. 2020). Each ligand and receptor was protonated at pH 7.3 using the built-in program PDB2PQR. Search space has been set to automatic to enable blind docking. All potential binding sites for each ligand were identified using the built-in AutoLigand program. Blind docking was used to scan the entire receptor surface to identify the predicted allosteric site (Amamuddy et al. 2020). Ligand binding poses in the receptor active site were visualized with LigPlus ver. 2.2.5 (Laskowski and Swindells 2011).

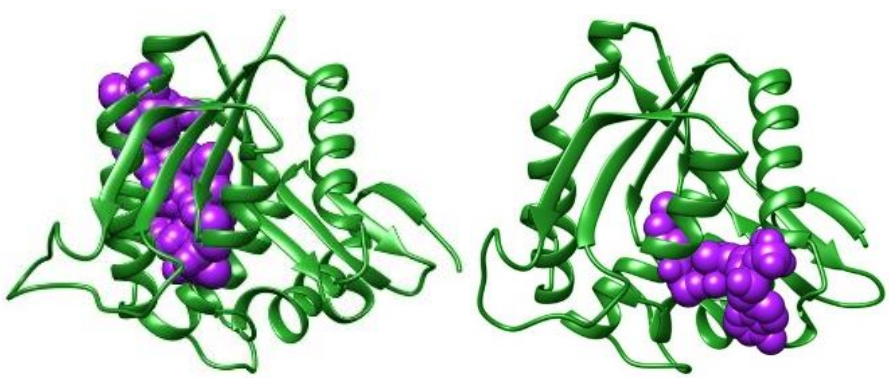
Molecular dynamics (MD). Each bound complex with the highest score from molecular docking study was selected as the starting structure for MD simulation using the software GROMACS version 2020.4 (Abraham et al. 2015). The steps performed for MDS of unbound and bound receptor-ligand complexes, such as topology preparation, system solvation, energy minimization, equilibration and production run, have been previously described (Lemkul 2019). The topology of each ligand was generated using the CGenFF server (Vanommeslaeghe et al. 2010; Yu et al. 2012). The production run for each bound complex was performed for 300 ns on a high performance computing device with 850 CPU cores. A 300 ns MDS iteration was performed for unbound and bound complexes.

Analysis of trajectory files. Trajectory files generated by MD simulations were analyzed using built-in Gromacs utilities. Before the analysis, the periodic boundary condition was removed from the system by using the gmx trjconv tool. The stability of the system was then analyzed with gms rms command. The gyration radius was calculated using gmx gyrate to determine the compactness of the system during the simulation. The number of H-bonds and the distance between receptor and ligand were calculated using gmx hbond. Data were plotted using xmgrace software version 5.1.25 (Cowan and Grosdidier 2000). 500 frames taken from the 1-300 ns MD simulation of each complex were used for both binding free energy and per-residue energy analysis by gmx_MMPBSA (Valdes-Tresanco et al. 2021).

RESULTS AND DISCUSSION

The growing problem of insecticide resistance and the increased risk of beneficial non-target insects being exposed to non-selective synthetic insecticides have prompted crop protection technologists to seek new mode of action insecticides for FAW control. An insecticide that specifically inhibits FAW aaNAT has not yet been developed (Tsugehara et al. 2013). Thus, the identification and development of compounds as FAW aaNAT inhibitors would be an important contribution to the effective management of insecticide resistance in FAW control. Therefore, this study used the available computational tools to investigate the nature of the inhibitory activity of the selected compounds at the insect target site in order to assess its potential to be developed as lead compounds for new insecticidal activity against FAW.

Homology modeling. Homology modeling was used to obtain the model receptor needed for molecular docking of the selected compounds. Homology models have been successfully used for docking analysis (Lohning et al. 2017) and have been shown to be effective when experimental protein structures are not available (Cavasotto 2011). A homology model of the receptor together with the predicted location of the allosteric site is shown in Figure 2A. The putative ligand (i.e., acetyl coenzyme A) and its binding site on the receptor are shown in Figure 2B.



(A) (B)

Fig. 2. (A) Homology model containing the predicted allosteric site (depicted as purple spheres) in fall armyworm *Spodoptera frugiferda* arylalkylamine-N-acyltransferase. The list of allosteric site residues includes the following: Phe30, Glu34, Leu36 Asn37, Leu42, Leu51, Leu52, Gln54, His55, Ser58, Leu84, Asn87, Thr88, Asp89, Ile90, Ser93, Lys96, Glu99, Ile100, Phe105, Ile108, Phe109, Leu112, Tyr113, Asn116, Leu117, Ile119, Asn120, Leu121, Phe122, Lys123, Ile130, Glu132, Ile133, Arg134, Ile135, Lys168, Thr169, Asp170, Thr172. (B) Model protein receptor with bound acetyl coenzyme A (depicted as purple spheres) in the active site. Figures were generated through UCSF Chimera software ver. 1.14 (Pettersen et al. 2004).

Acetyl coenzyme A has previously been shown to play an important role in the catalytic activity of insect aaNATs (Chu-Ya et al. 2020) . The aaNAT 3D structure of the red flour beetle *T. castaneum* (Protein Data Bank I.D. 6V3T) aaNAT was used as a template to build a 3D model of the FAW protein receptor. Based on the results of the ModWeb server (data not shown), the *T. castaneum* template has the highest amino acid sequence identity (34%) with the FAW protein receptor. Amino acid sequence identity below 30% tends to give an inaccurate estimate of the 3D structure of the protein model (Baker and Sali 2001). The Ramachandran plot is used in the final step of structure determination, such as by homology modeling, to identify unrealistic conformations in the model (Hollingsworth and Karplus 2010). The Ramachandran plot represents the distribution of torsion angles phi (Φ) and psi (Ψ) for each amino acid residue in the FAW model receptor (Fig. 3). The distribution of these torsion angles in the diagram indicates that the conformation is energetically favorable (Kleywegt and Jones 1996), because there are no steric clashes between amino acid residues in the predicted model. The plot showed that 98.1%, 100% and 0% of the total residues were in favored regions, in allowed regions and outliers, respectively. The proportion of residues in the favored region (indicated as the inner contour lines in the plot) exceeded the 90% threshold required for a model to be considered highly reliable (Laskowski 1993). The absence of outliers also indicates that no error was introduced into the model during structure refinement.

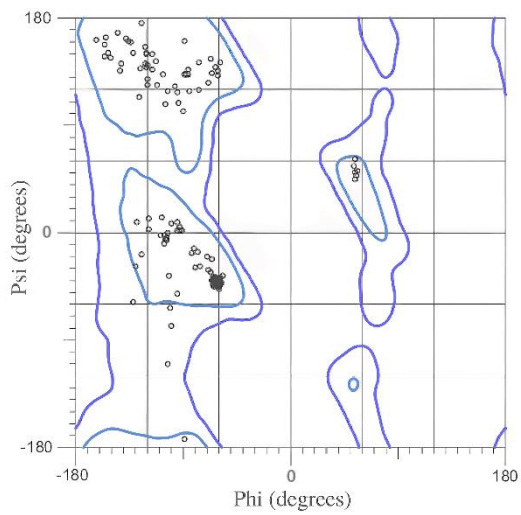


Fig. 3. Ramachandran plot of predicted homology model of fall armyworm *Spodoptera frugiferda* arylalkylamine-N-acyltransferase. Inner blue contour line indicates the favored region, outer purple contour line indicates allowed region. Open circles depict the ϕ,ψ values for each amino acid residue in the model.

Alignment of the sequence with other insect aaNATs (Fig. 4) revealed that the predicted active site of the protein receptor contains eleven highly conserved residues (Phe30, Glu34, Ile135, Leu136, Ser137, Val138, Arg143, Gly144, Gly146, Ala148 and Phe175) which aligned with insect aaNATs such as the red flour beetle, *T. castaneum* (6V3T), the yellow fever mosquito, *Aedes aegypti* (4FD6), and the fruitfly, *Drosophila melanogaster* (3TE4).

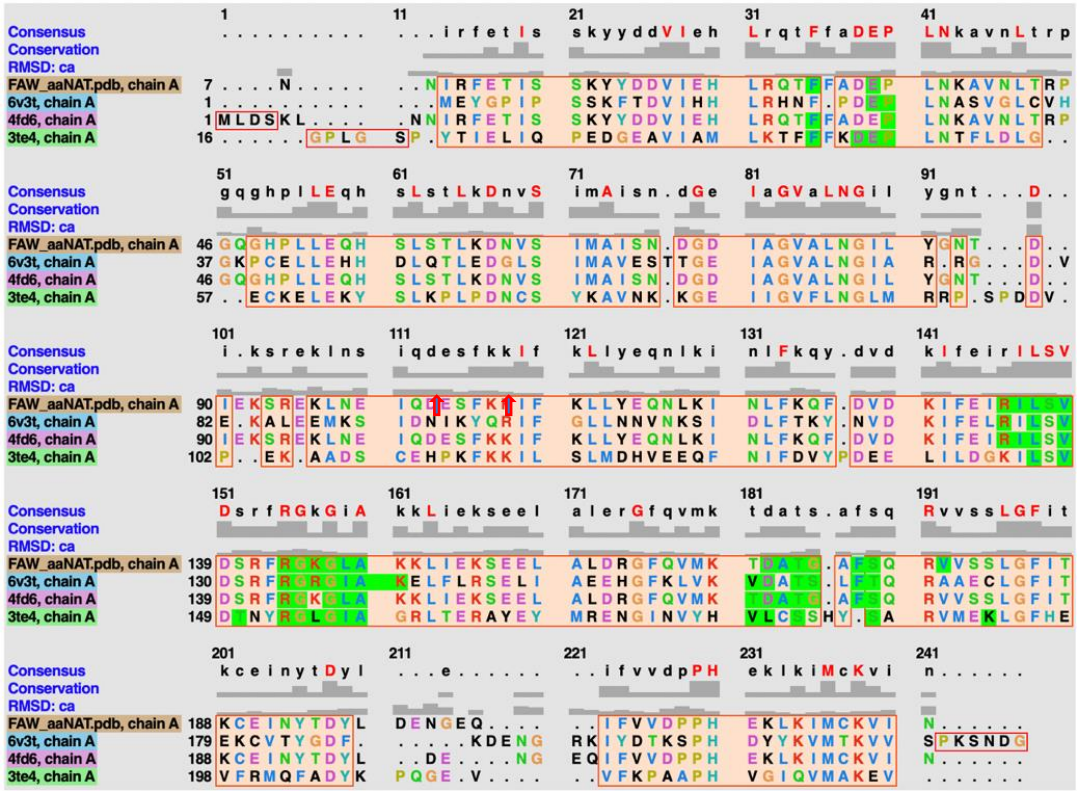


Fig. 4. Sequence alignment of fall armyworm *Spodoptera frugiferda* (FAW_aaNAT.pdb), red flour beetle *Tribolium castaneum* (6V3T), dengue mosquito *Aedes aegypti* (4FD6), and fruitfly *Drosophila melanogaster* (3TE4) arylalkyl N-acyltransferase. Residues highlighted with green background are in interaction with acetyl coenzyme A. Position of predicted hotspot residues Ile108 and Leu112 in protein receptor is indicated by red arrow. Sequence alignment was performed with UCSF Chimera software built-in sequence alignment tool (Petersen et al. 2004).

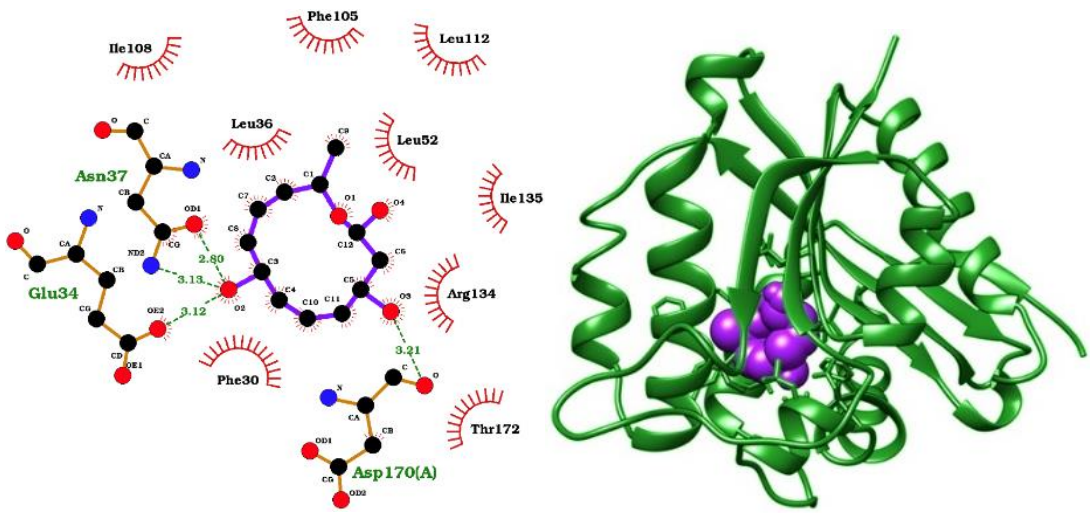
Corresponding residues of Glu34 in *D. melanogaster* aaNAT (i.e, Glu47), silkworm *Bombyx mori* (i.e., Glu27) and *T. castaneum* (i.e., Glu25) showed critical amino acid residues for catalysis (Cheng et al. 2012; Battistini et al. 2019). Complete loss of enzyme activity resulted from the Phe166Ala mutant in *T. castaneum* aaNAT (O'Flynn et al. 2020). Glu34 and Phe175 are hypothesized to have a similar important function in the active site of the protein receptor. The predicted allosteric site contained the charged amino acids Glu34, His55, Asp89, Lys96, Glu99, Lys123, Glu132, Arg134, Lys168 and Asp170 (Fig. 2A). In addition, seventeen residues were hydrophobic, consisting mainly of leucine and isoleucine.

Molecular docking. The purpose of molecular docking is to predict the conformation of a ligand in its receptor and provide an estimate of the affinity of its interaction (Guedes et al. 2014). In this study, blind docking was performed to identify potential targets of D1 and D2 in the FAW receptor. The estimated LE values indicated that both compounds had a good interaction with the binding site (Table 1).

Table 1. Molecular docking results of *Spodoptera frugiferda* arylalkylamine N-acyltransferase.

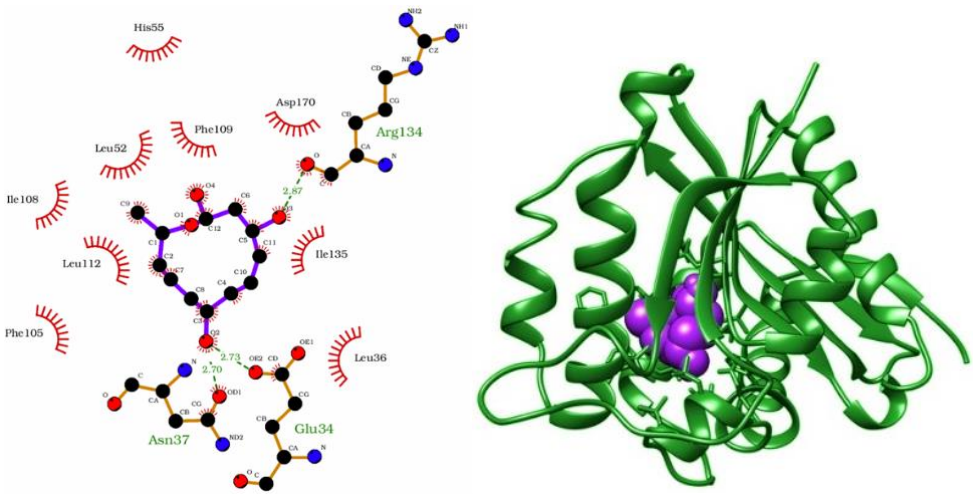
Compound code	PubChem ID	Binding affinity (kcal/mol)	K_i (μM)	Ligand efficiency (LE)
D1	CID 162987453	-7.1	6.25	-0.44
D2	CID 162987454	-7.4	3.77	-0.46

The preferential binding of D1and D2 to a common protein receptor target is shown in Figures 5A and 6A. Nine residues (Phe30, Leu36, Leu52, Phe105, Ile108, Leu112, Arg134, Ile135 and Thr172) were in hydrophobic contact with D1. On the other hand, three residues (Glu34, Asn37 and Asp170) were hydrogen bonded with D1. Similarly, nine residues (Leu36, Leu52, His55, Phe105, Phe109, Ile108, Leu112, Ile125, Ile135) were in hydrophobic contact with D2. Three residues (Glu34, Asn37, Arg134) in the binding site were hydrogen bonded with D2. In both cases of binding, the residues are located within the protein receptor (Fig. 5B and 6B).



(A) (B)

Fig. 5. Receptor-ligand interaction analysis. A) PubChem compound CID 162987453 (D1)-FAW aaNAT docked complex structure generated through LigPlus ver. 2.2.5 showing hydrogen bonds with Glu34, Asn 37 and Asp170 as green dashed lines and hydrophobic interactions as red arcs. B) PubChem compound CID 162987453 (D1)-FAW aaNAT docked complex structure generated through UCSF Chimera software ver. 1.14 (Pettersen et al. 2004).



(A) (B)

Fig. 6. Receptor-ligand interaction analysis. A) PubChem compound CID 162987454 (D2)-FAW aaNAT docked complex structure generated through LigPlus ver. 2.2.5 showing hydrogen bonds with Glu34, Asn 37 and Asp170 as green dashed lines and hydrophobic interactions as red arcs. B) PubChem compound CID 162987454 (D2)-FAW aaNAT docked complex structure generated through UCSF Chimera software ver. 1.14 (Pettersen et al. 2004).

Molecular dynamics. All-atom molecular dynamics simulation is a technique that allows understanding the structural dynamics, conformational behavior and stability of proteins and protein-ligand complexes (Hollingsworth and Dror 2018). The root mean square deviation (RMSD) was used to evaluate the overall stability of the system. The RMSD profile of the simulated systems was calculated for protein backbone and is shown in Figure 7A. It can be seen from the plot that the RMSD profile of both the unbound receptor and the bound complexes do not show significant differences. This indicates that the three systems were stable under the given simulation conditions. The RMSD of the unbound receptor (black line) began to increase continuously from 0.1nm beginning at 0 ns to 0.155 nm until 100 ns. Thereafter, the RMSD gradually decreased and stabilized at approximately 0.15 nm from 200 ns to the end of simulation. The RMSD profile of D1-FAW receptor complex (red line) remained stable at approximately 0.15 nm throughout the simulation. The RMSD profile of D2-FAW receptor complex (green line) was similar to that of the unbound receptor. From 0.125 nm at 0 ns, the RMSD increased continuously to 0.2 nm until 100 ns and then, leveled off to 0.15 nm until end of the simulation. No large variations were observed in the simulation, indicating that no significant conformational changes occurred in the three systems (Zrieq et al. 2021). Thus, the convergence shown by the three systems towards an equilibrium state indicated stability. The observed stability may indicate that D1 and D2 are potentially active protein receptor inhibitors. It was previously reported that the molecular stability of the interaction between a compound and its pharmacological target correlates with the actual inhibitory effect (Ramos et al. 2020).

The radius of gyration indicates compactness of a system. The radius of gyration (R_g) of the unbound and bound complexes is shown in Figure 7B. The average R_g value of the two bound complexes was approximately 1.73 nm. The average R_g value of the unbound receptor was approximately 1.71 nm. The slightly higher R_g value of the two bound complexes indicates that the tight binding of D1 and D2 had stabilized the receptor structure. A similar observation has been described previously (Khan et al. 2021).

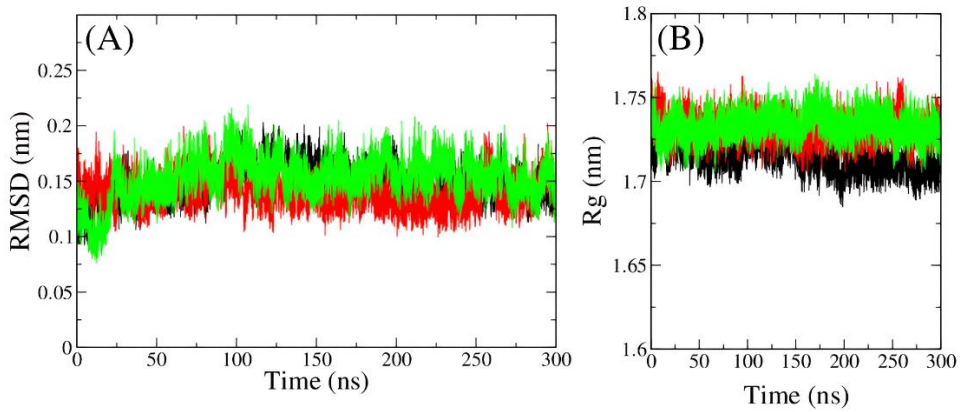


Fig. 7. A) Root mean square deviation of fall armyworm *Spodoptera frugiferda* arylalkyamine-N-acyltransferase during 300 ns MD simulation. Unbound (black), bound ligand D1 (red), bound ligand D2 (green). B) Radius of gyration of fall armyworm *Spodoptera frugiferda* arylalkyamine-N-acyltransferase during 300 ns MD simulation.

Iintermolecular H-bonds were calculated to evaluate the stability of the two bound complexes (Sakthivel et al. 2019). The number of hydrogen bonds present during the simulation between the two bound complexes varied between 1 and 5 H-bonds. The observed hydrogen bonding, which occurred frequently during the simulation, provides further evidence for stable binding of D1 and D2 to the protein receptor (Fig.8A and Fig. 8B).

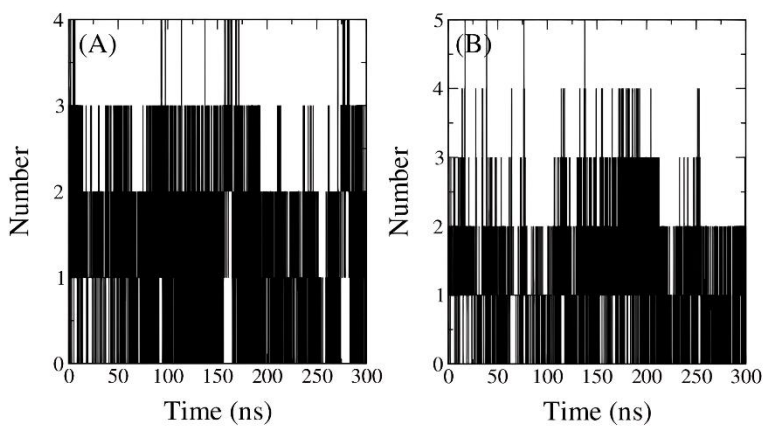


Fig. 8. Number of hydrogen bonds occurring between A) D1 (PubChem CID 162987453) and B) D2 (PubChem CID 162987453) in fall armyworm *Spodoptera frugiferda* arylalkyamine-N-acyltransferase putative allosteric site during 300 ns MD simulation.

Free energy of binding and energy decomposition analysis. The binding affinity of the two diastereomeric compounds obtained from molecular docking was investigated by evaluating the free energy of binding using the MMPBSA method. The total free energy of binding (ΔG_{bind}) components are shown in Table 2. The calculated ΔG_{bind} of both bound complexes indicated favorable binding of D1 and D2 to the protein receptor.

Table 2. Free binding energy of receptor-ligand complexes using MMPBSA method implemented in gmx_MMPBSA, where: energy components contributes to total relative binding energy (ΔG total) of the ligand including van der Waals (VDWAALS) molecular mechanics energy, electrostatic molecular mechanics energy (EEL), polar contribution to the solvation energy (EPB), non-polar contribution of repulsive solute-solvent interactions to the solvation energy (ENPOLAR), non-polar contribution of attractive solute-solvent interactions to the solvation energy (EDISPER), total gas phase (ΔG gas) molecular mechanics energy, total solvation energy (ΔG solv) (Nguyen et al. 2022) .

Complexes	**Free binding energy (kcal/mol) ± standard deviation**							
	VDWAALS	**EEL**	**EPB**	**ENPOLAR**	**EDISPER**	**ΔGGAS**	**ΔGSOLV**	**ΔGTOTAL**
FAW aaNAT-D1	-25.61±2.00	-2.89±1.31	5.35±0.89	-21.44±0.87	37.43±0.92	-28.50±2.00	21.34±1.33	-7.16±2.10
FAW aaNAT-D2	-25.67±2.08	-2.29±1.07	5.14±0.77	-21.69±0.89	37.46±0.90	-27.97±2.13	20.91±1.35	-7.06±2.23

Binding is mainly driven by ΔGGAS in both bound complexes, which consists of van der Waals (VDWAALS) and electrostatic forces (EEL). A large energy contribution from van der Waals forces is expected because the cavity of the protein receptor binding site is mostly lined with hydrophobic residues. Another study found that non-polar interactions exhibit the highest energy in the binding process (Lin et al. 2015). van der Waals forces have been shown to be determinants of the formation and stability of protein-ligand complexes (Humphris and Kortemme 2008; Bitencourt-Ferreira et al. 2019). In both complexes, the effect of electrostatic force on ΔGGAS was small but significant. The estimated total energy of solvation (ΔGSOLV) was not energetically favorable in either complex. ΔGSOLV consists of a polar contribution to the solvation energy (EPB) and a non-polar contribution to the attractive solute-solvent interaction (EDISPER). In addition, the non-polar contribution to the solvation energy (ENPOLAR) from repulsive solute-solvent interactions is favorable for both complexes. Overall, the favorable total gas phase molecular mechanics energy (ΔGGAS) counterbalanced the negative effect of ΔGSOLV, which ultimately affected the favorable ΔG_{bind} of D1 (-7.16±2.10 kcal/mol) and D2 (-7.06±2.23 kcal/mol) binding to the protein receptor. The 0.1 kcal/mol difference in ΔG_{bind} between D1 and D2 seemed negligible. A similar observation was reported previously, where two enantiomers bound to the same allosteric site had only a small difference in binding affinity (Hernandez et al. 2019).

To further elucidate the role of specific residues in ligand binding, a per-residue binding free energy decomposition analysis was performed using the program gmx_MMPBSA. Details of per residue contribution to the binding free energy of the bound complex are shown in Table 3. Most of the interacting residues of both ligands were similar and hydrophobic, consistent with the buried location of the binding site. In particular, the hotspot hydrophobic residues Ile108 and Leu112 made the most important contribution to binding of these two ligands. Ile108 contributed -1.0 kcal/mol and -1.21 kcal/mol to D1 and D2 binding, respectively. Leu112 contributed -1.10 kcal/mol and -1.19 kcal/mol to D1 and D2 binding, respectively. The hotspot residue contributes at least -1.0 kcal/mol to the binding energy of the interaction with the receptor (Humphris and Kortemme 2008). Ile108 is located at conserved sites in four insect aaNATs (Fig. 3). Therefore, it is probably related to the important regulatory function of the protein receptor. Leu112 is a non-conserved residue that may be crucial for binding site selectivity. In addition to stability provided by strong hydrophobic interactions in the binding cavity, the orientation of D1 and D2 to their bound form may result from hydrogen bonds with Asn37 and Arg134, respectively. The electrostatic interaction generated by the hydrogen bond is important because it directs the ligand to its binding form and ensures specific interactions with the complex (Talibov et al. 2021). Thus, both MD simulation and per-residue energy decomposition analysis results confirmed and clarified the docking results.

Table 3. Per-residue energy contributions to the formation of ligand-fall armyworm *Spodoptera Frugiferda* arylalkylamine N-acyltransferase complex.

Complex	**Residues (Per-Residue Energy (kcal/mol±standard deviation)**
D1-FAW aaNAT	Phe30 (-0.58±0.25), Asn37 (-0.58±0.25), Leu52 (-0.67±0.25), His55 (-0.70±0.67), **Ile108** (-1.0±0.24), Phe109 (-0.57±0.22), **Leu112** (-1.10±0.27), Arg134 (-0.81±0.48), Ile135 (-0.87±0.31)
D2-FAW aaNAT	Phe30 (-0.54±0.25), Leu36 (-0.55±0.28), Leu52 (-0.55±0.30), Phe105 (-0.61±0.27), **Ile108** (-1.21±0.30), Phe109 (-0.56±0.23), **Leu112** (-1.19±0.28), Arg134 (-0.82±0.53), Ile135 (-0.82±0.30), Asp170 (0.55±047)

Note: Hot spot residues are shown in bold.

Limitations of the model receptor, docking method (Pantsar and Poso 2018), and MD simulations (Hollingsworth and Dror 2018) do not allow firm conclusions about receptor specificity in this study based on reported results alone. However, the aforementioned simulations can be particularly useful for the design of new FAW insecticides that target a binding site different from the native ligand. For example, simulations have been shown to be able to identify allosteric sites in protein structures (Hollingsworth and Dror 2018). This study further hypothesizes that D1 and D2 bind to the allosteric site of the protein receptor, as suggested by the results of MD and per-residue energy decomposition analyses. Gene cloning and site-directed mutagenesis studies should be performed to confirm the role of Ile108 and L112 in FAW aaNAT allosteric inhibition.

CONCLUSION

The FAW aaNAT binding interaction of D1 and D2 diastereomers was successfully investigated using homology modeling, molecular docking and molecular dynamics simulations. Binding calculations and per-residue energy decomposition analysis showed that hotspot and hydrophobic residues Ile108 and Leu112 played an important role in the molecular recognition of D1 and D2 in the binding site. D2 is a better candidate for development as a lead FAW aaNAT allosteric inhibitor, based on its higher ligand efficiency.

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THE INFLUENCE OF CULTURAL VALUES ON CONSUMERS' GREEN PURCHASE INTENTION IN SOUTH KOREA

Pham Ngoc Huong Quynh[1], Duong Thi Tra My[1], To The Nguyen[1], Phan Thi Thu Hoai[2] and Nguyen Van Phuong[1,*]
[1]VNU University of Economics and Business, Vietnam National University, Hanoi, Vietnam
[2]Faculty of Marketing, Thuongmai University, Hanoi, Vietnam
*Corresponding author: vanphuong@vnu.edu.vn

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ABSTRACT

With the aim of filling in such a research gap, this research was conducted to explore factors influencing consumers' green purchase intention. The Theory of Planned Behavior was employed as a theoretical framework to analyze the influences of cultural values on consumers' intention to purchase green products. The hypotheses were tested in South Korea, a previously low-income country that has successfully graduated to a high-income, developed status in Asia. By studying a sample of 394 South Korean consumers with collected data being processed via the SmartPLS software, it is concluded that both collectivism and long-term orientation significantly influence consumers' intention. In particular, collectivism exerts both direct and indirect effects on green purchase intention, in which the mechanism underpinning the latter path is via attitude, subjective norms, and perceived behavior control. Despite not having a direct impact, long-term orientation also imposes an indirect impact on green purchase intention through the same media as collectivism.

Key words: collectivism; long-term orientation; green consumption; PLS-SEM; theory of planned behavior

INTRODUCTION

In recent decades, environmental problems have become globalized in terms of their existence and impacts, arousing great public concern (Park et al. 2013; Chen et al. 2018). In response, governments tried to develop a global culture of respect for the environment, and sustainable development (Kinnear et al. 1974; Agan 2013; Zinoubi 2020). "Sustainable consumption" or "pro-environmental behavior" is therefore increasingly gaining traction along with the rise of sustainability-conscious customers (Arli et al. 2018; Nguyen et al. 2021; Kong et al. 2014), leading to a considerable increase in green growth and green consumption (Park et al. 2013). Many concepts of consumers' environmental ethics have been proposed, in which green purchase intention is considered to be among the most popular ones in the eyes of both academics and the public (Yadav and Pathak 2017; Biswas and Roy 2015).

Discussions about green product consumption have appealed a growing number of researchers worldwide (Zinoubi 2020; Emekci 2019; Kim et al. 2021; Mazhar et al. 2022). Furthermore, consumers are more and more concerned about environmental issues and green product attributes, so green consumption-oriented marketing activities become more and more effective in promoting brand images (Laroche et al. 2001; Emekci 2019; Chen 2013). Many studies have investigated the intention to purchase green products in various countries (Yadav and Pathak 2017; Zheng and Chi 2015; Wang et al. 2020, Nguyen et al. 2021), such as India, China, and Vietnam. Most studies successfully demonstrate the effects of different factors on consumers' green intention purchase, such as: attitude, subjective

norms, and perceived behavior control; nevertheless, no study has been conducted to investigate the impact of cultural values on green intention purchase in the context of South Korea recent years.

South Korea is an exemplar of the Asian economic growth model, which has successfully transformed from a low-income into a high-income economy (World Bank 2022). Nevertheless, with a period of rapid growth comes the economic growth-environment dilemma, which urged the Korean government to reconsider its economic development model (Kim et al. 2014; Lobo and Greenland 2017). Over recent years, the Korean government has implemented many apparent solutions to encourage green consumption (OECD 2012; Lim et al. 2019), resulting in an expanded market for green products despite the rate of consumer green consumption behavior still remaining fairly low (Park et al. 2013).

Many previous studies showed that governments in developed countries, including South Korea, have actively taken action to increase consumers' cognition of eco-friendly behavior and encourage indigenous firms to adopt sustainable consumption strategies (Liu et al. 2017; Lee 2017; Leonidou et al. 2013; Lobo and Greenland 2017). In particular, as part of its pledge in the five-year green development plan, the South Korean government spends 2% of GDP promoting public green purchasing programs and implementing a waste fee system, which results in a 14% reduction in municipal waste and a 50% increase in recyclable waste in last decade (Lim et al. 2019).

The theory of planned behavior (TPB) is applied to measure consumers' behavioral intentions since this theory has the power to explain human behavior in a wide range of fields, especially in the field of sustainable consumption behavior (Nguyen et al. 2021; Yadav and Pathak 2017). However, existing studies show diverse outcomes in explaining green purchase intention (Zhuang et al. 2021), which can be partly explained by their failure to capture the diversity in cultural backgrounds of observation samples (Ko and Jin 2017; Lobo and Greenland 2017). With the aim of filling in such a research gap, this research was conducted to explore factors influencing consumers' green purchase intention. The Theory of Planned Behavior (TPB) was employed as a theoretical framework to analyze the influences of cultural values on consumers' intention to purchase green products. The results are expected to help policymakers, businesspeople, and producers promote green products consumption in order to better satisfy Korean consumers' needs. Actually, TPB is considered one of the most influential psychological theories for predicting and understanding human behavior that ties together intentions and actions (Ajzen 1985; Al-Swidi et al. 2014). The TPB framework has three main distinctive variables that affect consumers' behavioral intentions, which are the amalgamation of attitude, subjective norm, and perceived behavioral control (Zhuang et al. 2021; Lim et al. 2019). In this research paper, attitude, social norm, and perceived behavioral control, the three core components of the TBP model, were analyzed to envisage customers' environmental-friendly intentions and a hypotheses was proposed based on three core variables of TBP on green consumption. Firstly, attitude can be reflected as one of the important contributors to assessing the effectiveness of pro-environmental behavior. Regarding the purchase of sustainable products, consumers are willing to buy environmentally friendly products if they have a positive attitude toward these goods (Al Mamun et al. 2018). If customers have favorable attention to environmentally friendly products, they must have a positive outlook on green purchases (Kim and Chung 2011; Panzone et al. 2016). From the findings above, the following hypothesis is developed: H1: Attitude has a positive impact on green purchase intention. Secondly, Subjective norms refer to the social pressure that an individual feel under the execution or non-execution of a certain behavior (Ajzen 1991). In relation to environmental consumption, subjective norms may be expected as a suggestion for people to act and contribute to pro-environmental intention toward sustainable products (Biel and Thøgersen 2007; Thøgersen and Zhou 2012). When individuals distinguish their referents' environmentally friendly behavior, they tend to follow it (Kim and Chung 2011). In other words, subjective norms had a positive influence on customers' eco-product purchase intention (Ko and Jin 2017; Wang 2014). Hence, the following hypothesis was formulated: H2: Subjective norms have a positive impact on green purchase intention. Thirdly, according to Ajzen (1991), perceived behavior

control is one important variable of the TBP model, which indicates people's perception of their ability to carry out a certain behavior. In prevailing literature about green consumption intention, Bamberg (2003) states that individuals who recognize the importance of environmental issues tend to seek pro-environmental products and share green products with others. In addition, making purchase decisions is a sort of customer purchase behavior. Therefore, a person with positive perceived behavioral control can become a customer of a sustainable product (Zhuang et al. 2021; Joshi and Rahman 2015). Thus, the third hypothesis about sustainable consumption is developed: H3: PBC has a positive influence on the green purchase intention.

Furthermore, we also analyzed collectivism and long-term orientation which are cultural variables affecting green purchase intention. Collectivism refers to an individual's membership in one or more groups, such as family, peers, and society (Triandis 1995). Individuals with high collectivism, who subordinate to group benefits, are concerned with others, and usually work with group pairs, tend to take action actively on environmental products (McCarty and Shrum 2001). Besides, collectivist people acquiring a high level of sustainable attitudes, subjective norms, and perceived behavior control are more likely to show consumers' green behavior (McCarty and Shrum 2001). From the above discussion, the following hypotheses are proposed: H4: Collectivism has a direct effect on green purchase intention. H5: Collectivism has an indirect effect on green purchase intention via attitude, norms, and perceived behavior control. Finally, long-term-oriented individuals are generally described as careful money managers who seek opinions or advice from their primary referents when making decisions (Sharma 2010; Hofstede 2001). People with a long-term orientation usually create a long-range plan, respect families, regard both the past and the future, and consider prudently the impact of their long-term decisions and actions (Brigham et al. 2014; Lumpkin et al. 2010). Regarding environmental issues, long-term-oriented consumers have strong intention and attitude to defend the environment in order to enhance a better life for their families and themselves (Leonidou et al. 2013). Additionally, Lumpkin et al. (2010) asserted that there is a positive relationship between consumers' long-term orientation and pro-environmental behavior. From that, the sixth hypothesis was developed: H6: Long-term orientation has an indirect effect on green purchase intention via attitude, subjective norms, and PBC.

Based on the above hypotheses, a theoretical model (Fig. 1) was developed:

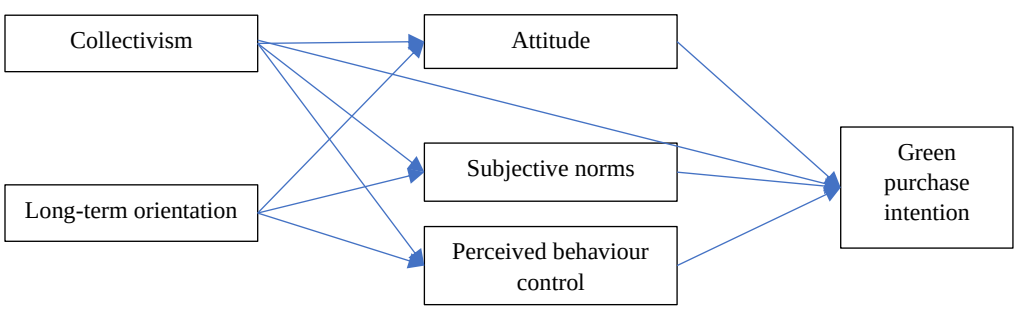

Fig. 1. Conceptual Model

METHODOLOGY

Questionnaire design. A self-administrated online questionnaire survey was created to gather data in South Korea, a developed country with the fourth largest economy in Asia (Table 1). The term of "green products" was explained at the begin of the questionnaire to the respondents. Green products in this study can be understood as products that meets one of these criteria: environmental friendliness; produced from natural ingredients or products that are nontoxic, energy and water-efficient, harmless to the environment, recyclable and biodegradable. The questionnaire consisted of six parts using core

variables based on the TBP model to examine how Korean people react to consumers' green purchase behavior (Ajzen 2006). Part 1 measures attitude towards green purchase, while part 2 assesses subjective norms and part 3 measures perceived behavior control of participants. The first three parts all include six items each. Part 4, 5, 6 respectively evaluate participants' green purchase intention, collectivism, and long-term orientation with the corresponding number of items being four, six, five. All six parts above are measured using a five-point Likert-type scale ranging from 1 to 5, on which 1 represents "strongly disagree" and 5 represents "strongly agree". To avoid misinterpretation by respondents, all questions are designed to be simple and easy to understand. Table 1 lists more specific measuring items for the constructs.

Table 1. The items used in this study and sources

Variables	Items	Explanation	Reference
Attitude towards the green purchase	ATT1	I care about the environment when purchasing.	Ajzen (2006); Yadav and Pathak (2017); Nguyen et al. (2021)
	ATT2	I understand what green purchase behavior is.	
	ATT3	I think green purchase behavior positively affects the environment.	
	ATT4	I think green purchase behavior significantly reduces natural resource exploitation.	
	ATT5	I love buying green products.	
	ATT6	If I have the opportunity, I am willing to buy green products.	
Subjective norms	SNO1	My acquaintances are very responsible for the environment.	Ajzen (2006); Dixon et al.(2015); Al Mamun et al. (2018); Zhuang et al. (2021)
	SNO2	My acquaintances think green purchase behavior is essential for the environment.	
	SNO3	My acquaintances advised me to implement green purchase behavior.	
	SNO4	My acquaintances introduce me to green products.	
	SNO5	My acquaintances support my green purchase behavior.	
	SNO6	My acquaintances' viewpoints influenced my decision to purchase green products.	
Perceived behavior control	PBC1	I believe in the quality of green products provided in the market.	Ajzen (2006); Lobo and Greenland (2017); Joshi and Rahman (2015)
	PBC2	I do not need much time to find information about green products.	
	PBC3	I can easily buy green products when I want.	
	PBC4	I have sufficient financial conditions to purchase green products.	
	PBC5	I can control my decision-making in any situation.	
	PBC6	I always carefully consider before I decide to buy a green product.	

Variables	Items	Explanation	Reference
Green purchase intention	INT1	I will consider purchasing green products.	Ajzen (2006); Han et al. (2010)
	INT2	I want to purchase green products.	
	INT3	I will purchase green products in my next shopping.	
	INT4	I would recommend other people to purchase green products.	
Collectivism	COL1	I am a sociable person when I participate in group activities.	Hofstede (2001); Laroche et al. (2001), Lee (2017)
	COL2	I think members of a group should stick together even if they are not in harmony.	
	COL3	Even if my contribution is not recognized, I still work hard for mutual goals.	
	COL4	I like to share ideas and spend time with groups.	
	COL5	Team members' happiness is important for me.	
	COL6	I always put mutual interest as a priority.	
Long-term orientation	LTO1	Financial management is essential to me.	Hofstede (2001); Sharma (2010); Brigham et al. (2014)
	LTO2	I work hard to achieve future successes.	
	LTO3	I tend to use my money wisely in the present to save for the future.	
	LTO4	Failure does not stop me from trying.	
	LTO5	I do not mind giving up today's joy for future successes.	

Data collection. The study hypotheses were tested using data on green consumption behavior collected in Seoul, Busan and Incheon in South Korea during July 2021 to March 2022. A self-completion online questionnaire using the random sampling method enabled respondents to quickly and effortlessly get access to the questionnaire and answer the questions in Korean. To ensure accuracy, this study collaborated with a team of graduate Korean students trained in survey data gathering. The students shared the questionnaire to social network and suggested their friends in three above cities sharing the questionnaire to others. As the result, a total of 394 questionnaires were collected among consumers. The questionnaire provided could be completed in 12-15 minutes and sent directly to the researcher after completion to test the study's hypotheses.

Analysis method. Structural equation modeling, a multivariate statistical analysis technique that is used to analyze structural relationships. In this study the partial least squares structural equation modelling (PLS-SEM) was utilized with the data processing software being SmartPLS 3.0, to examine the proposed hypotheses. PLS-SEM was used to "estimate complex cause-effect relationships in path models with latent variables" (Hair et al. 2019). To test hypothesis using PLS-SEM, the total sample must be more than 5 times items (Hair et al. 2022). In this analysis, we have 33 items; the total sample must be more than 165 observations, so 394 respondents is enough for analysis.

Cronbach's alpha, composite reliability (CR) and average variance extracted (AVE) were implemented to scrutinize the reliability of the measurement items. Cronbach's alpha is utilized to examine the reliability of items the questionnaire to identify the possible errors of a questionnaire, result to improve the reliability of the questionnaire. Cronbach's alpha value more than 0.7 is considered as

acceptable (Hair et al. 2022). The AVE and the CR coefficients are related to the quality of a measure. AVE is a measure of the amount of variance that is taken by a construct in relation to the amount of variance due to measurement error. To be specific, AVE is a measure to assess convergent validity. The value of AVE and CR ranges from 0 to 1, where a higher value indicates higher reliability level. AVE is more than or equal to 0.5 confirms the convergent validity (Hair et al. 2019). Furthermore, Hair et al. (2022) demonstrated that PLS-SEM use the nonparametric approach to modelling, which means that PLS-SEM makes fewer demands on the data in terms of normality distribution, and sample size.

RESULTS AND DISCUSSION

The demographic characteristics of the respondents are detailed in Table 2.

Table 2. Demographic characteristics of the respondents (N=394)

Variables	Scale	Count	Percentage
Gender	Male	193	48,98
	Female	201	51,02
Age	< 30	98	24,87
	> 30	296	75,13
Education	Under high school	21	5,33
	High school	104	26,40
	Undergraduate	217	55,08
	Post-graduate	52	13,20
Total		**394**	**100**

Smart-PLS has been used to examine the previously formed hypotheses (H1, H2, H3, H4, H5, and H6). Smart PLS also evaluates the psychological properties of the measurement model and estimates the parameters of the structural model. The reliability results of the experimental measurement models are shown in Table 3.

Table 3. Evaluation of measurement model

Items	Outer loading	Cronbach's Alpha	Composite Reliability (CR)	Average Variance Extracted (AVE)
Attitude		**0.881**	**0.910**	**0.629**
ATT1	0.756			
ATT2	0.732			
ATT3	0.819			
ATT4	0.779			
ATT5	0.859			
ATT6	0.807			
Collectivism		**0.847**	**0.891**	**0.621**
COL2	0.776			
COL3	0.772			
COL4	0.835			
COL5	0.807			
COL6	0.747			

Items	Outer loading	Cronbach's Alpha	Composite Reliability (CR)	Average Variance Extracted (AVE)
Green Purchase Intention		**0.906**	**0.935**	**0.784**
INT1	0.776			
INT2	0.920			
INT3	0.931			
INT4	0.906			
Long term orientation		**0.835**	**0.883**	**0.603**
LTO1	0.719			
LTO2	0.817			
LTO3	0.783			
LTO4	0.787			
LTO5	0.771			
Perceived behavior control		**0.782**	**0.860**	**0.605**
PBC3	0.777			
PBC4	0.823			
PBC5	0.769			
PBC6	0.740			
Subjective Norm		**0.905**	**0.927**	**0.679**
SNO1	0.727			
SNO2	0.810			
SNO3	0.890			
SNO4	0.864			
SNO5	0.831			
SNO6	0.813			

Cronbach's alpha is a measure of the reliability of each project's items, with an acceptable value ranging from 0.7 to 0.9 (Hair et al. 2022). All of our Cronbach's alpha measures qualify for the range. The results show that the measurements are robust in terms of their internally consistent confidence, as indexed by the reliability of their aggregation. The composite confidence values for all constructs ranged from 0.782 to 0.906, which exceeds the recommended cut-off value of 0.70 (Nunnally 1978). This indicates that the items used to measure all internal structures are highly consistent.

Different measurement models of reflected and formed structures were examined. This measurement is intended to ensure a representative set of all possible entries in the conceptual domain of the structure (Diamantopoulos et al. 2012), often using extracted mean variance (AVE) to evaluate the validity of the index (Fornell and Larcker 1981). Higher values generally indicate a higher level of confidence. An AVE value higher than 0.5 is considered acceptable in exploratory research (Hair et al. 2019). However, values of 0.95 and above are problematic because they indicate that the entries are redundant, thereby reducing the validity of the structure (Diamantopoulos et al. 2012). In our study, the AVE of all measures was from 0.603 to 0.784, respectively, supporting the measures.

The diagonal elements of the matrix, which represent the square root of the AVE, are always greater than the diagonal elements of their respective rows and columns (Table 4). This result supports the discriminant validity of the scales. All items load more favorably on their respective structures than

on other structures in effect. Most entries, with some exceptions, loaded above or near the 0.50 threshold (Hair et al., 2019). This suggests that all constructs are reliable and can be further analyzed to confirm the relationship between structures of dependent and independent variables.

Table 4. Discriminant validity test results

	Attitude	**Collectivism**	**Green purchase intention**	**Long term orientation**	**Perceived behavior control**	**Subjective norm**
Attitude	0.793					
Collectivism	0.555	0.788				
Green purchase Intention	0.724	0.508	0.885			
Long term orientation	0.534	0.485	0.419	0.776		
Perceived behavior control	0.504	0.465	0.541	0.512	0.778	
Subjective norm	0.549	0.500	0.468	0.415	0.536	0.824

Table 5 outlines the multi-group checking of the direct effects and indirect effects. The results of the first-order analysis in the structural model are described in Table 5 and Figure 2. The beta value of the path coefficient from attitude to green purchase intention has indicated a direct effect. The path coefficient of the attribute is 0.566, which means there is a positive relationship. The T value shows a statistically significant impact on the green purchase intention towards attitude ($p < 0.05$, $t = 10.458$). This result supports Hypothesis 1. However, the beta value of the path coefficient from subjective norm to green purchase intention is -0.005, showing that there is not a positive relationship ($p > 0.05$, $t = 0.110$). This result does not support Hypothesis 2.

Furthermore, the coefficient indicates of the relationship between perceived behavior control and green purchase intention has a beta value of 0.213, which explains the positive relationship between these two factors ($p < 0.05$, $t = 4.344$). Therefore, Hypothesis 3 is supported.

Next, the beta coefficient of collectivism for green purchase is 0.098 and the t-values indicate a statistically significant economic instability ($p < 0.05$, $t = 2.292$). Therefore, Hypothesis 4 is supported.

Besides, the beta value of the path coefficient underpinning the association between collectivism and green purchase intention is 0.277, which illustrates the indirect influence of the former on the latter. The T value shows a statistically significant impact on green purchase intention towards attitude ($p < 0.05$, $t = 8.350$). Therefore, Hypothesis 5 is supported.

Similarly, the coefficient indicates of the relationship between long-term orientation and green purchase intention has a beta value of 0.275 (with $p < 0.05$, $t = 6.571$), which also illustrates the indirect influence of the former on the latter. Therefore, Hypothesis 6 is supported.

Table 5. Path coefficient and total indirect effect of structural model

Path Coefficient	Original sample (Beta)	Sample mean (M)	Standard deviation (STDEV)	T Statistics (|O/STDEV|)	P values
Direct effects					
Attitude -> Green purchase intention	0.566	0.564	0.054	10.458	0.000
Collectivism -> Attitude	0.387	0.387	0.047	8.244	0.000
Collectivism -> Green purchase intention	0.098	0.097	0.043	2.292	0.022
Collectivism -> Perceived behavior control	0.283	0.285	0.045	6.293	0.000
Collectivism -> Subjective norm	0.390	0.390	0.049	7.980	0.000
Long term orientation -> Attitude	0.346	0.348	0.057	6.106	0.000
Long term orientation -> Perceived behavior control	0.375	0.376	0.050	7.429	0.000
Long term orientation -> Subjective norm	0.226	0.226	0.054	4.209	0.000
Perceived behavior control -> Green purchase intention	0.213	0.213	0.049	4.344	0.000
Subjective norm -> Green purchase intention	-0.005	-0.002	0.049	0.110	0.912
Total Indirect effects					
Collectivism -> Green purchase intention	0.277	0.278	0.033	8.350	0.000
Long term orientation -> Green purchase intention	0.275	0.275	0.042	6.571	0.000

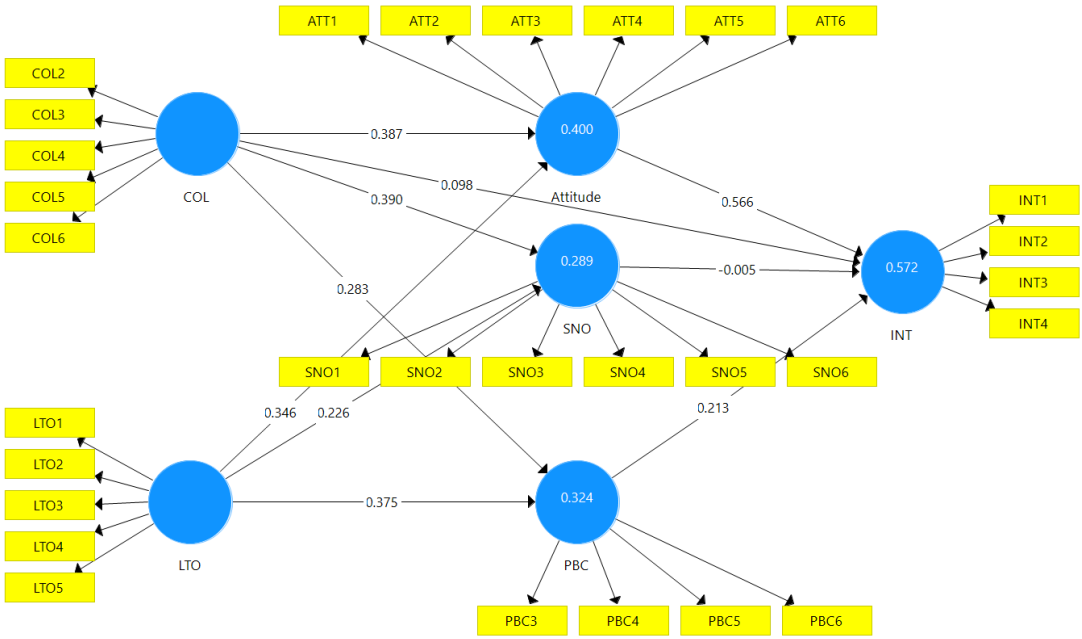

Fig. 2. Structural Model

Two factors (attitude and perceived behavioral control) significantly influenced consumers' intention to purchase green products. This result is consistent with the work of Han et al. (2010); Lee (2017); and Nguyen et al. (2021) who applied TPB to explain green consumption behavior. These results imply that in order to improve the attitude and awareness of green consumption to promote the green buying behavior of the people, it is essential that governments are able to facilitate stronger media and policy coverage of green consumption so that consumer s become familiar with the concept and tend towards consumption. Governments should open short courses on sustainable consumption, green consumption, and launch the green consumption programs through organizations and unions. In addition, local and international marketers should highlight the outstanding benefits of their green products for the environment and society in their marketing campaigns to increase product preferences among consumers. For the sake of emerging green markets, governments and stakeholders need to join hands to raise public awareness of the benefits of green products and how green labels are defined. Secondly, the result of the perceived behavioral control factor showed that people tend to trust government agencies and manufacturers. Therefore, government agencies should issue regulations on strict quality control, develop an official legal framework for green consumption, and protect the interests of consumers. Similarly, the authorities need to put in place regulations on information transparency and the handling of false advertising of green products.

Of the three individual cultural values, only subject norm value did not impact positively green purchase intention and is consistent with the results of Al Mamun et al. (2018); Zhuang et al. (2021); and Biel and Thøgersen (2007). People in South Korea are quite independent and less influenced by their families, friends, professionals, or others around them when making decisions. Therefore, using social influence such as from celebrities may not be the right way to give advice on green product consumption. Businesses should focus on changing individuals' behavior rather than affecting those around them. For example, businesses should prioritize word-of-mouth marketing while also providing incentives on price, quality, and product maintenance to better convert behavioral intentions into actual actions (Zhuang et al. 2021).

Most importantly, the findings confirm the crucial role of cultural values in the formation of green purchase intention through two aspects, collectivism and long-term orientation. In terms of collectivism, this cultural value showed, based on this study results, to have positive affect on green purchasing intentions through TPB's broadest structures with the greatest impact on subjective norms, which is consistent with the results of McCarty and Shrum (2001) and Lee (2017). Collectivist consumers sacrifice individual goals for group goals and strive to make decisions that benefit society as a whole. It suggests that governments should actively utilize people's collective attributes to increase the intention to purchase green products (Arısal and Atalar 2016; Bae and Kim 2013), and companies should also build consumer groups to enhance communication within the group as a means of exerting a more significant influence on consumer intent. Obviously, collectivism may change because of advances in economic and social development. Economic development may promote collectivism, but in a different way, economic development may erode collectivism (Ball 2001). Whatever the impact of economic advances on collectivism, trust is still essential to establish. Therefore, governments and marketers need to focus on building collective trust in individualist groups so that they can increase the intention to buy eco-friendly products. This can be done by educating consumers about the social benefits of these products. In addition, marketers should also create consumer groups to improve communication within the group to have a more meaningful influence on consumer intent. Collectivist trust can also be built through greater coverage of films and advertisements highlighting family values, traditions, and the importance of relationships with members of a family.

The long-term orientation shows that indirect effects through all of TPB's construction activities have a great influence on consumer attitudes. Therefore, marketers should reach consumers with advertisements that emphasize the long-term benefits of green products in order to garner a better customer response. Good communication of corporate sustainability values can partly increase the perceived value of sustainable consumption and build consumer confidence (Brigham et al. 2014). Governments must ensure the dissemination of credible advertising and penalize manufacturers who provide false information about their products and services. Due to economic advances, long-term orientation may change. Therefore, enterprises should identify potential customer segments for each product in each time period, thereby proposing an appropriate strategy. Initially, businesses can focus on products that serve high-income customers. After green consumption becomes a popular trend, affecting other customer groups, businesses can begin to expand their products to serve lower segments.

CONCLUSION

In this study, cultural value is assessed on the basis of collectivism, long-term orientation, and subjective norm. Both collectivism and long-term orientation significantly influence consumers' intention. In particular, collectivism exerts both direct and indirect effects on green purchase intention, in which the mechanism underpinning the latter path is via attitude, subjective norms, and perceived behavior control. Despite not having a direct impact, long-term orientation also imposes an indirect impact on green purchase intention through the same media as collectivism.

It is recommended that policymakers, business people, and producers promote the consumption of green products in order to better satisfy consumers' needs and get the highest business efficiency, perhaps by strengthening communication and promotion to Korean consumers to enhance awareness of green products, and to preserve cultural values associated with encouraging green consumption. It would be interesting for future research to widen the sample and include other cultural groups in South Korea. Furthermore, this study was conducted based solely on the Theory of Planned Behavior Framework using PLS-SEM. In the future, researchers can use other theoretical models to test the hypotheses made in this study or other novel hypotheses.

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PATHOGENICITY OF *Pythium deliense* ISOLATED FROM THE RHIZOSPHERE SOIL OF ORANGE IN VIETNAM

Tran Van Quang[1], Valentin Valentinovich Vvedensky[1], Han Viet-Cuong[2] and Ha Viet Cuong[3]*
[1] Agrarian Technological Institute, Peoples' Friendship University of Russia,
Moscow, 117198, Russia
[2]Center for Crop and Disease Management (CCDM), School of Molecular and Life Sciences, Curtin University, Bentley, Perth, WA 6102, Australia
[3] Department of Plant Pathology, Vietnam National University of Agriculture,
Hanoi, 131000, Vietnam
*Corresponding author: hvcuongnh@vnua.edu.vn

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ABSTRACT

Orange (*Citrus sinensis*), a major crop in Vietnam, has been suffering from a disease with symptoms of leaf yellowing, gummosis, and root rot. This study identified the associated microorganism of the disease and evaluated its pathogenicity to citrus species. In 2021, two *Pythium* isolates, assigned as VN-Oo16 and VN-Oo29, were isolated from the rhizosphere soil of symptomatic orange plants in Tuyen Quang province in northern Vietnam, and were identified as *Pythium deliense* based on morphological and molecular characteristics. The two isolates formed numerous inflated, lobulate, and toruloid sporangia, smooth oogonia, and aplerotic, thick-walled oospores. In the phylogenetic trees based on the Internal Transcribed Spacers (ITS) and cytochrome c oxidase subunit 1 (*cox*1) sequences, these two isolates and other GenBank *P. deliense* isolates formed a distinct species cluster. Pathogenicity assays of *P. deliense* (VN-Oo29 isolate) were conducted by inoculation of mycelium plugs or zoospore suspension on the detached fruits, stems of grafted seedlings, and roots of seedlings grown from seeds. In pomelo and orange, *P. deliense* produced typical brown rot on wounded fruits and gummosis on wounded stems. *P. deliense* was not able to infect the roots of pomelo, orange, and lime seedlings. To our knowledge, this is the first report of *P. deliense* in association with citrus in the world as well as in Vietnam.

Key words: citrus, internal transcribed spacer, cytochrome oxidase subunit I, gummosis, brown rot

INTRODUCTION

Species of the genus *Pythium* are currently classified in the family *Pythiaceae*, the order *Peronosporales*, the phylum *Oomycota* of the kingdom *Stramenopila* (*Chromista*) (Mostowfizadeh-Ghalamfarsa and Salmaninezhad 2020). *Pythium* and *Phytophthora* that is another member of the family *Pythiaceae* share many morphological and reproductive characteristics in common. However, one asexual feature that differentiates the two genera is the formation and release of zoospores. In *Pythium*, the differentiation of zoospores occurs within a vesicle that connects to sporangium through a short tube, and mature zoospores are released upon the rupture of the vesicle. In contrast, in

Phytophthora, zoospores are differentiated within the sporangium and released through the sporangial apex (Ho 2018).

In 2022, *Pythium* contains 376 species (www.mycobank.org), however, many of them are either synonymous or invalidated. Historically, in the 1980s, less than 90 species of *Pythium* were recognized, mostly based on morphological characters as described in the well-known comprehensive monograph written by Van der Plaats-Niterink (1981) that contains the identification key to 85 species. Since applying molecular approaches that are based on some housekeeping genes such as the Internal transcribed spacer (ITS) region of rDNA, β-tubulin, cytochrome c oxidase subunit 1 (*cox*1) and 2 (*cox*2), the identification and classification of *Pythium* species have become more accurate (Levesque and De Cock 2004; Robideau et al. 2011; Villa et al. 2006), and up to now, 157 species of *Pythium* are validated at the molecular level (Jayawardena et al. 2020). It is worth noting that several *Pythium* species, for example, clade K members (Levesque and De Cock 2004), that have intermediate characteristics between *Pythium* and *Phytophthora*, morphologically and phylogenetically, have been reassigned as the new genus *Phytopythium* (Bala 2010).

Pythium species are soil and water inhabitants and have worldwide distribution. They can live saprophytically but in certain conditions, they can become very pathogenic and cause rot of fruits, roots, or stems, pre- or post-emergence damping-off of seeds and seedlings of a wide range of plants (Ho 2018; Rai et al. 2020; Van der Plaats-Niterink 1981). Like other oomycetes, the major inoculum of *Pythium* is zoospores. Recently, many aspects of the interaction between *Pythium* and plants have been clarified at the molecular level, which could explain the necrotrophic lifestyle of *Pythium* (Judelson and Ah-Fong 2019).

Citrus, particularly orange (*Citrus sinensis*) and pomelo (*C. grandis*), is a major crop in Vietnam. In 2020, the area and production of citrus in Vietnam were 140,841 ha and 1,983,299 tonnes, respectively (FAO 2022). However, one of the major constraints to citrus production is a disease with the symptoms of leaf yellowing, gummosis, and root rot, resulting in plant death and a significant yield loss (Dang et al. 2004). Various species of *Phytophthora* and *Pythium* have been shown to be associated with the disease worldwide and in Vietnam (Dang et al. 2004; Maseko and Coutinho 2002; Puglisi et al. 2017). During an investigation to identify the causal agents involved in the disease in orange orchards grown in northern Vietnam, we isolated several *Phytophthora* and *Pythium* species from the rhizosphere soils and roots of orange trees that suffered from disease symptoms. Hence, this study aimed to identify a *Pythium* species isolated from the rhizosphere soil of the diseased orange in Vietnam and to elucidate its pathogenicity to various citrus species.

MATERIALS AND METHODS

Isolation of *Pythium*. Rhizosphere soils were collected in diseased orange plants showing symptoms of leaf yellowing, stem gummosis, and root rot in orchards at Ham Yen (22°04'48.0"N, 105°02'07.5"E) and Yen Son (21°53'40.8"N 105°08'11.7"E) in Tuyen Quang province in northern Vietnam in 2021. Disease incidence in these two orchards is approximately 20 and 25%, respectively. *Pythium* was isolated using a baiting method. Approximately 20 grams of soil per sample were suspended in 100 ml tap water in a plastic cup, and 5 rose petals were then floated on the surface to bait zoospores in the soil sample. After 3 days, Pythium isolates were recovered by placing the surface-sterilized symptomatic petal tissues onto water agar (WA) medium supplemented with streptomycin sulfate (100 $mg.L^{-1}$). Pythium isolates were then subcultured by transferring the hyphal tips to potato dextrose agar (PDA) or V8 agar media.

Morphological studies. Two *Pythium* isolates, assigned as VN-Oo16 (from Ham Yen) and VN-Oo29 (from Yen Son), were isolated and used for morphological studies. The assessment of the growth rates of these isolates was conducted on both PDA and V8 in 90-mm Petri dishes incubated in an incubator

set at 28°C. Hyphal growth was recorded every day until the dishes were completely covered by mycelium. The morphological investigation was based on agar plugs which were cut from the edge of actively growing colonies on PDA. The agar plugs were initially immersed in V8 liquid medium in a 60-mm Petri dish and incubated for 24 hours. V8 liquid was replaced with sterile distilled water and 5-6 young rice leaf fragments were floated on the surface to stimulate the reproduction (Vafa et al. 2021). The dish was incubated for 24 hours for observation of sporangia and zoospores, and further 48 hours for observation of sexual structures. All steps were conducted at room temperature in light. Morphological identification of isolates was achieved using taxonomic keys for *Pythium* (Ho 2011; Van der Plaats-Niterink 1981).

Molecular characterization. The two above isolates, VN-Oo16 and VN-Oo29, were used for molecular characterization.

DNA extraction. DNAs were extracted using a NaOH method which was originally adopted for plant tissue (Wang et al. 1993). A few milligrams of mycelium mat from seven-day-old PDA culture were transferred into a 0.5 ml Eppendorf tube containing 5 µl of 0.5 M NaOH and homogenized using a pipette tip. The homogenate was diluted 20 times with 100 mM Tris pH 8.0. The extract was used immediately or stored at –20 °C until use.

Polymerase chain reaction (PCR). Two widely accepted DNA barcodes for oomycetes, ITS and *cox*1 (Robideau et al. 2011), were selected for molecular identification. The ITS region was amplified using ITS4 (5'TCCTCCGCTTATTGATATGC3') and ITS5 (5'GGAAGAAAAGTCGTAACAAGG3') primers (White et al. 1990). The *cox*1 was amplified using OomCoxILevup (5'TCAWCWMGATGGCTTTTTTCAAC3') and Fm85mod (5'RRHWACKTGACTDATRATACCAAA3') primers (Robideau et al. 2011). PCRs were performed in a final reaction volume of 25 µl using MyTaq HS Mix (Meridian Bioscience). Amplification was carried out using a thermocycler programmed as follows: 1) preliminarily denaturation step at 94°C for 5 min, 2) 35 cycles of denaturation at 94°C for 15 s, annealing at 55°C for 15 s, and extension at 72°C for 1 min, and 3) a final extension step at 72°C for 5 min.

Sequencing. The PCR products were purified from agarose gel using an Expin™ Gel SV Kit (GeneAll Biotechnology), estimated for the DNA concentration using agarose gel electrophoresis. Purified amplicons were directedly sequenced using the PCR primers at the Institute of Biotechnology in Hanoi, Vietnam.

Sequence analyses. The sequences were initially compared to GenBank ITS and *cox*1 sequences using the BLAST program available at the National Centre for Biotechnology Information (http://blast.ncbi.nlm.nih.gov/Blast.cgi). Two data sets for each *Pythium* gene containing all GenBank isolates of Clade A, the ex-type isolates of the Clades B1, B2, C, D, E1, E2, G, H, I, and J (Levesque and De Cock 2004; Robideau et al. 2011) were used for phylogenetic analyses. The ex-type isolate CBS 124518 of *Phytopythium sindhum* (Bala 2010) was used as the out-group. The sequences were aligned using an online MAFFT program (Katoh and Standley 2013) available at https://mafft.cbrc.jp/alignment/server/. MAFFT alignment of ITS and *cox*1 was performed with the L-INS-i and default algorithms, respectively. The phylogenetic analyses were conducted by MEGA11 (Tamura et al. 2021). The maximum likelihood (ML) trees were constructed by using the best nucleotide substitution model (TN93+G) for ITS and (GTR + G) for *cox*1. All indels were kept which resulted in a total of 1262 and 679 positions in the final ITS and *cox*1 datasets, respectively. Supports for nodes were obtained using 1000 bootstrap replicates. Trees were rooted to the out-group.

Pathogenicity test. The representative isolate VN-Oo29 grown on V8 medium was used for all inoculation experiments. Each inoculation was replicated on three fruits or plants. All inoculated and control fruits or plants were kept at room temperature. The infection was evaluated by the appearance of the symptom and confirmed by re-isolation of *Pythium* from lesions. Three pathogenicity assays were performed.

In detached fruit inoculation, mature fruits of pomelo (*C. grandis* cv. Doan Hung and cv. Soi Ha) at 3 days after harvesting, and orange (*C. sinensis* cv. Vinh) purchased from the supermarket were surface sterilized using 70% ethanol and airdried. The fruits were wounded by making a shallow hole (5 mm in diameter and 0.5 mm in depth) at the peduncle or rind. The wounded and non-wounded fruits were inoculated by placing a 5-mm mycelium plug or dropping 200 µl of $1x10^6$ zoospore ml^{-1} in the peduncle or rind. The inoculated sites were wrapped with sticky tape and fruits were incubated in food bags to maintain moisture. For the control, V8 agar plugs or water were used.

In stem inoculation, the grafted young plants of pomelo (*C. grandis* cv. Soi Ha) and orange (*C. sinensis* cv. Vinh) with a base stem of 1.5 - 2 cm were used for the experiment. The bark (1 cm wide x 2 cm length) in the middle stem was removed. A 3-mm mycelium plug was placed centrally in the wound, with the mycelium facing inward. The wound was then covered with the bark piece and wrapped by a saran membrane. The inoculation on non-wounded plants was also performed in a similar manner. For the control, V8 agar plugs were used.

In root inoculation, seeds of pomelo (*C. grandis* cv. Doan Hung, cv. Soi Ha and cv. Duong), orange (*C. sinensis* cv. Vinh, cv. Sanh, cv. Duong and cv Duong Canh) and lime (*C. aurantifolia* cv. Tu Quy) were sowed on sterile sand in plastic pots (7 x 9 cm) for three months. At this time, seedlings had 3-4 (for orange and lime), and 5-6 (for pomelo) true leaves. The nutrient solution was supplied every week. Two methods of root inoculation were performed. In the first method, the pots were flooded several times with water to remove extra nutrient salts that may affect zoospores before inoculation. The mycelium plugs were submerged in water for the production of zoospores. Five plugs and 100 ml of a zoospore suspension (10^4 zoospores ml^{-1}) were applied to the base region of seedlings. To stimulate the further formation of zoospores, the water saturation stature of sand was maintained for two days after inoculation. In the second method, the seedlings were gently removed from the sand, and the roots were washed with water. The seedlings were hung on 300 ml water in a plastic cup using clip support. Then, the mycelium plugs and zoospore suspension were applied to the cup as for the first method. To verify the activity of zoospores, three rose petals were also floated on the water of each cup. The infection was evaluated by the lesion formation on the roots until one month after inoculation.

RESULTS AND DISCUSSION

Identification of *Pythium* isolates. Two *Pythium* isolates, VN-Oo16 and VN-Oo29, were isolated from the rhizosphere soils of orange plants showing symptoms of yellowing, gummosis, and root rot. These two isolates showed identical morphological characteristics. They grew fast and completely colonized PDA or V8 agar plates after 4 days. Colonies on PDA formed cottony aerial mycelia and have a chrysanthemum pattern with radial mycelium. On V8 agar, the aerial mycelia were thicker and had less distinct chrysanthemum patterns compared with those on PDA (Fig. 1 a, b). They did not reproduce on agar media. On the agar plugs immersed in water, they formed numerous sporangia that were inflated, lobulate, and toruloid, forming swollen side branches sometimes in clusters, mostly terminal, occasionally intercalary (Fig. 1 c). Both isolates had smooth globose oogonia, 18.9-24.6 (average 21.3) µm in diameter, mostly terminal, with oogonial stalks bending towards the monoclinous antheridia (Fig. 1 d). Their oospores were aplerotic, 18.1-22.2 (average 19.5) µm in diameter, and had a thick wall with thicknes of 1.7-2.8 (average 2.4) µm (Fig. 1 e, f).

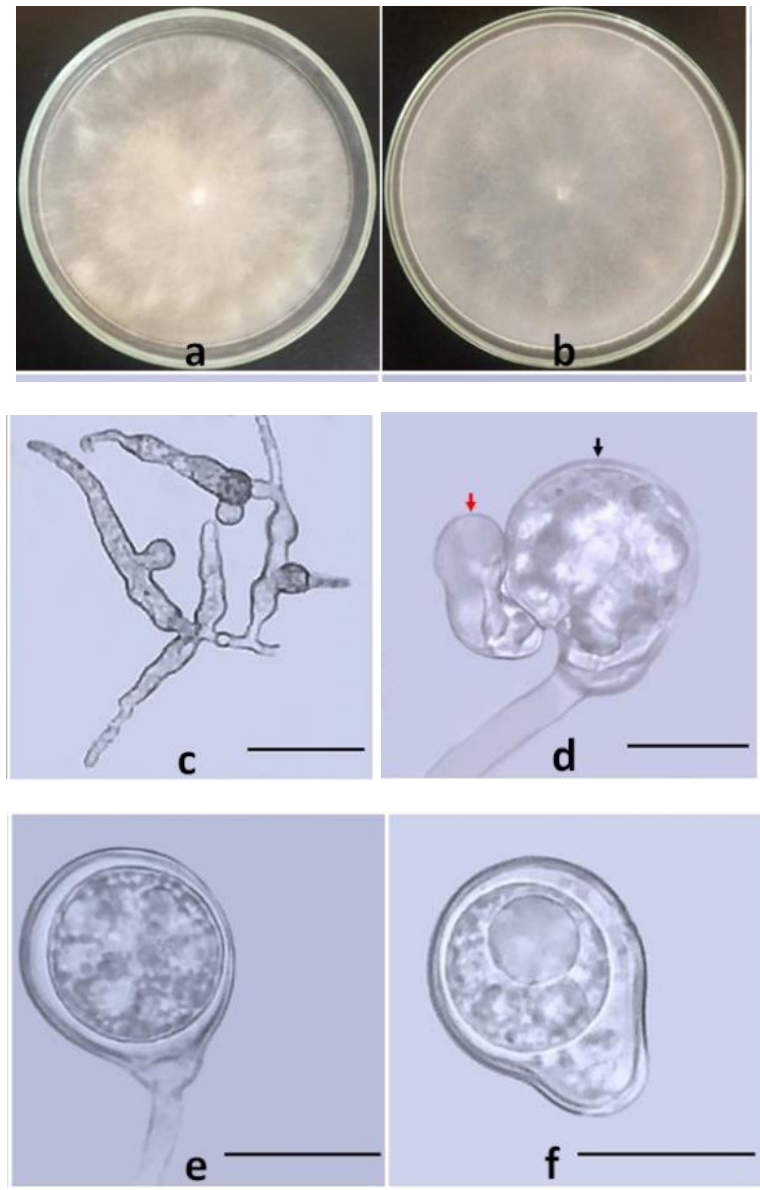

Fig. 1. Cultural and morphological characteristics of *P. deliense* – isolate VN-Oo29. Colonies on potato dextrose agar (a) and V8 media (b) after 5 days. Sporangia (c); an antheridium (red arrow) mating an oogonium (black arrow) (d); an oospore (e and f). Scale bars: 50 µm (c) and 15 µm (d, e, f).

The ITS and *cox*1 genes of the two *Pythium* isolates were successfully amplified and sequenced. The sequences were deposited in GenBank under accession numbers ON573330 (ITS, VN-Oo16), ON573331 (ITS, VN-Oo29), ON563235 (*cox*1, VN-Oo16), and ON563236 (*cox*1, VN-Oo29). The ITS and *cox*1 sequences of the two isolates were initially searched for the homologous sequences in GeneBank. The Blast searches evidenced that both *Pythium* isolates from Vietnam were most closely related to *P. deliense* isolates. In the ML phylogenetic ITS and *cox*1 trees, both *Pythium* isolates from Vietnam and other GenBank *P. deliense* isolates formed a distinct species cluster that was well bootstrap supported (99% for both markers). In the two trees, this *P. deliense* species cluster also grouped well and consistently with the *P. aphanidermatum* species cluster within the clade A of *Pythium* (Fig. 2).

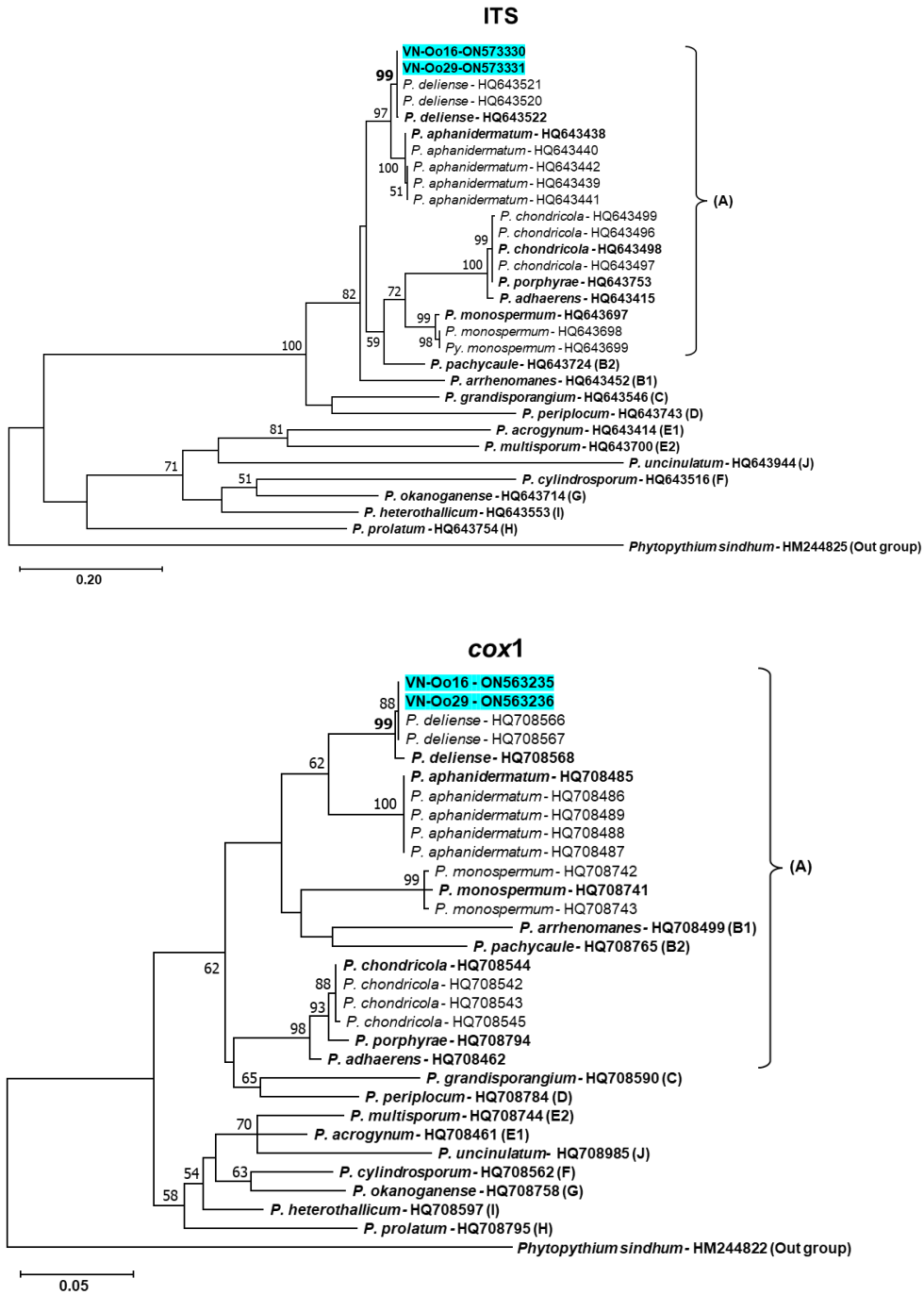

Fig. 2. Maximum likelihood trees of *Pythium* isolates based on the ITS and *cox*1 sequences. The isolates from Vietnam are shown in bold and shaded. The ex-type isolates of *Pythium* species representative for the clades A, B1, B2, C, D, E1, E2, G, H, I, and J (Levesque & De Cock, 2004; Robideau et al., 2011) are shown in bold and parentheses. The numbers at nodes represent percentage bootstrap support values calculated from 1000 replicates. Only bootstrap support values greater than 50% are shown. The scale bars indicate the number of substitutions per site.

Based on morphological and molecular analyses, the two *Pythium* isolates were identified as *P. deliense* species and as such, this species was detected for the first time in Vietnam. *P. deliense* and other 5 species, *P. adhaerens, P. porphyrae, P. chondricola, P. monospermum* and *P. aphanidermatum* are members of clade A of *Pythium* (Levesque and De Cock 2004; Robideau et al. 2011). In this clade, *P. deliense* and *P. aphanidermatum* are very morphologically similar having inflated sporangia, fast growth, and monoclinous antheridia, whereas the remaining members have filamentous non-inflated sporangia, slow growth, and diclinous antheridia (Levesque and De Cock 2004). *P. deliense* was first isolated and described from the tobacco plant in Indonesia in 1933 and then reported to cause diseases in a wide range of dicot and monocot plants in warmer regions of the world (Van der Plaats-Niterink 1981).

Pathogenicity assay. In the pomelo and orange fruit inoculation experiment, only wounded fruits inoculated with either mycelium plug or zoospore suspension developed the symptom (Table 1). The lesion appeared 2 days after inoculation and enlarged gradually to form the typical fruit brown rot symptom (Fig. 3). *Pythium* isolates recovered from the infected tissues of the inoculated fruits had morphological features identical to those of the VN-Oo29 isolate. The fruit assay in this study suggested *P. deliense* appear to be virulent to fruits of pomelo and orange producing typical fruit brown rot; on the contrary, *P. irregulare, P. aphanidermatum, P. paroecandrum* and *P. ultimum* produced small brown necrotic lesions on the wounded rind surface of citrus fruit (Maseko and Coutinho 2002).

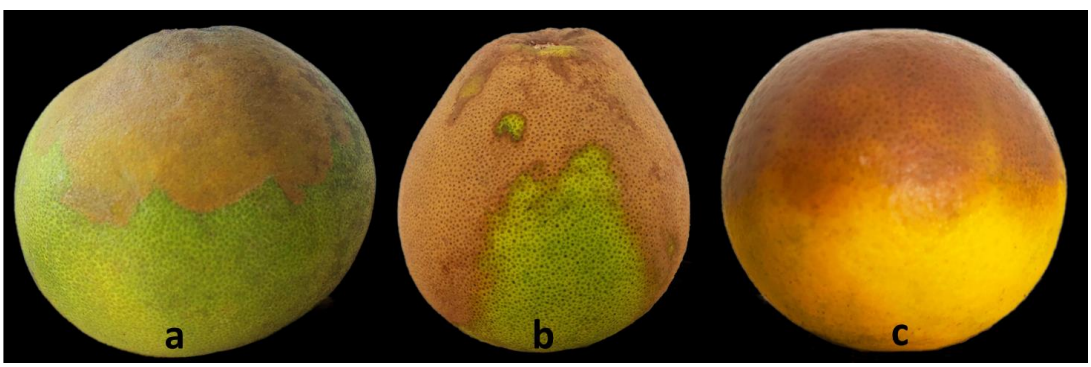

Fig. 3. Fruit rot of pomelo cv. Soi Ha (a), pomelo cv. Doan Hung (b) and orange cv. Vinh (c) inoculated with mycelium plug of *P. deliense* (isolate VN-Oo29) at wounded peduncle. Symptom was pictured at 7 days after inoculation.

It is widely known that fruit brown rot of citrus is caused by multiple species of Phytophthora when conditions are cool and wet (Graham and Feichtenberger 2015; Naqvi 2003). To our knowledge, this is the first report of *P. deliense* associated with brown rot of citrus fruit. Further, our fruit assay also indicated that *P. deliense* was not able to infect intact fruit rind or non-injured peduncle. The formation of a cuticle layer on the rind and lignification of cells adjacent to the abscission zone of the peduncle during ripening (Merelo et al. 2017; Wang et al. 2016) could prevent citrus fruits from direct penetration of *Pythium*.

In stem inoculation, the infection occurred on both pomelo and orange seedlings resulting in the gummosis and browning of the necrotic area at the inoculated sites (Table 1, Fig. 4). The stem assay in this study indicated that *P. deliense* is pathogenic to the wounded stem of pomelo and orange, inducing the formation of gummosis and vascular rot. *Pythium* isolates recovered from the infected tissues of the inoculated stem had morphological features identical to those of the VN-Oo29 isolate.

Once again, stem infection of *P. deliense* was different from that of other *Pythium* species. Maseko and Coutinho (2002) when inoculating 16 isolates of *P. irregulare, P. aphanidermatum, P. paroecandrum, P. vexans, P. rostratum, P. ultimum,* and *Pythium* group G & F concluded that only isolates of *P. irregulare, P. ultimum,* and *P. paroecandrum* were weakly pathogenic producing superficial lesions without gum exudation on the wounded stems of citrus rootstocks.

Fig. 4. Stem rot of pomelo cv. Soi Ha (a) and orange cv. Vinh (b) inoculated with mycelium plug of *P. deliense* (isolate VN-Oo29) at wounded site. Symptom was pictured at 14 days after inoculation.

In root inoculation, no difference in root systems between the inoculated and un-inoculated (control) seedlings of all cultivars was observed in both methods, suggesting this species could not infect the root (Table 1). Recent pathogenicity tests demonstrated *P. deliense* could infect underground parts of numerous plants causing pod rot in peanuts (Parkunan et al. 2014), root rot in melons (Cara et al. 2008), crown and root rot in walnut (Ghaderi and Banihashemi 2011) and periwinkle (Intaparn et al. 2019), and soft rot of ginger (Vafa et al. 2021). However, the root assays on seven citrus cultivars in this study indicated that *P. deliense* is unable to infect the root of citrus seedlings. Infection of roots from the inoculum involves pre- and post-penetration. The pre-penetration sequence of oomycetes zoospores includes zoospore movement, encystment, cyst adhesion, and germination (Deacon and Donaldson 1993) that involves multiple homing responses, including chemotaxis, electrotaxis, host-triggered encystment, and germ tube tropism (Judelson and Ah-Fong 2019). Among compounds mediating homing responses of oomycetes zoospore, Ca^{2+} in the environment was proved to play a vital role (Addepalli and Fujita 2002; Deacon and Donaldson 1993). In our root inoculation experiments, the absence of Ca^{2+} during inoculation might be a factor that affected the pre-penetration of the zoospore. Furthermore, *Pythium* is widely known to be necrotrophic oomycetes, which feed on nutrients from lysed cells and most members of this group are opportunistic root pathogens with broad host ranges (Judelson and Ah-Fong 2019). So, another possibility of no infection of *P. deliense* to the citrus root was the incompatible interaction between zoospore and citrus roots under the test condition. More work needs to be done to clarify the interaction between *P. deliense* and citrus. Likewise, it is necessary to undertake studies on management strategies against this pathogen.

Table 1. Pathogenicity test of *P. deliense* (isolate VN-Oo29) on different parts of citrus plants

Plant	Cultivar	Detached fruit[1]			Root of seedling grown from seed		Stem of grafted young plant	
		Non-wounded, mycelium plug	Wounded, mycelium plug	Wounded, zoospore suspension	Grown in sand	Hung on water	Non-wounded, mycelium plug	Wounded, mycelium plug
Pommelo (*C. grandis*)	cv. Doan Hung	-[2]	+[3]	+	-	-	nt	nt
	cv. Soi Ha	-	+	+	nt	-	-	+
	cv. Duong	nt[4]	nt	nt	nt	-	nt	nt
Orange (*C. sinensis*)	cv. Vinh	-	+	+	-	-	-	+
	cv. Sanh	nt	nt	nt	nt	-	nt	nt
	cv. Duong Canh	nt	nt	nt	nt	-	nt	nt
Lime (*C. aurantiifolia*)	cv. Tu Qui	nt	nt	nt	-	-	nt	nt

[1] Wounded and non-wounded were performed on both peduncle and rind;
[2] -: No symptoms
[3] +: With symptoms
[4] nt: not tested

CONCLUSION

This is the first report of *P. deliense* isolated from the rhizosphere soil of orange plants with the symptoms of leaf yellowing, stem gummosis, and root rot in Vietnam. *P. deliense* is pathogenic to the wounded stem and fruit of pomelo and orange. *P. deliense* was not able to infect the roots of pomelo, orange, and lime seedlings under the test condition, implying the complicated interaction between the *P. deliense* zoospores and citrus roots.

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TECHNOLOGY CHANGE IN DRY SEASON VEGETABLE PRODUCTION: A COMPARISON OF TWO VILLAGES WITH AND WITHOUT A FARMER GROUP IN KHON KAEN PROVINCE, NORTHEAST THAILAND

John S. Caldwell[1] and Arunee Promkhambut[2]
[1]Agricultural Extension Research Society of Japan,
Akihabara Shinko Dai-ichi Seimei Bldg. 8F, Ueno 3-1-2,Taito-ku, Tokyo 110-0005 Japan
[2] Department of Agricultural Extension and Development,
Faculty of Agriculture, Khon Kaen University, Khon Kaen 40002 Thailand
Corresponding author: caldweljoverseas@gmail.com

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ABSTRACT

The authors reconstructed farmers' technology change over 10 years, assessed the effect of a farmer group, and identified characteristics contributing to technology change in 2017. Farmers in two villages in Khon Kaen Province, Northeast Thailand, one with a farmers' group (SS) and the other without (NSH), described technology changes in a narration format. Reasons for and benefits of changes were converted to quantitative values and analyzed statistically. The villages differed in types and numbers of technologies. SS farmers described more technologies for fertilization and pest management, while NSH farmers indicated more technologies for water management and trellising. Most pest management changes in SS involved organic pest control and cultural practices, while most in NSH involved chemical pesticides. Changes based on farmers' own ideas and interaction were 45% more numerous in SS, and SS farmers had nearly double the number of sources of information. Working off-farm increased technology changes in SS. These results suggest that farmer groups can increase farmer-to-farmer learning in using new technologies. Methods developed in this research can enable researchers to gain insights into farmer thinking using qualitative approaches and assess differences among farmers statistically.

Key words: data conversion, farmer interaction, innovation, mixed methods research (MMR), narration, pest management, water management

INTRODUCTION

During the past 20 years, emphasis has been placed on diversification of agriculture in Northeast Thailand (Isan). Vegetables are an important component of diversification. New production techniques including soil-improving organic fertilizers and alternatives to chemical pesticides have been introduced by NGOs and the agricultural extension service. Consumer awareness of the risks of excessive pesticide use on vegetables has also increased (Nguyen, 2018). The development of the East-West corridor and the elimination of tariffs on agricultural products among ASEAN countries offer new potential markets for vegetable production in Northeast Thailand (Srisathit 2017).

However, the process of change in production techniques and the reasons why farmers make changes have not been well-documented at the village level. There are many sources of information on new production techniques available to farmers. An important source is agricultural extension agents in rural districts in Thailand. Other actors providing information at the village level include projects of universities and research organizations, Royal Projects, and various NGOs. Moreover, with the spread

of the internet and higher rural education levels, farmers can access information on their own. Considerable numbers of Isan people go to work for several years in other provinces or countries and then return. Some work in agriculture, and thereby learn new techniques and get new ideas that they bring back and apply on their farms (Grandstaff et al. 2008).

Recognition of the importance of farmers as innovators and researchers has increased in the past decades, beginning with observations by researchers carrying out on-farm research with farmers (Lightfoot 1987). Experiences in Latin America and Southeast Asia have supported the role of farmers' organizations involved in generating innovations (Ashby et al. 2000; Horne et al. 2002). Agricultural extension theory has moved away from technology transfer to emphasis on networks among multiple actors jointly producing innovations, termed Agricultural Knowledge and Innovation Systems, or AKIS (European Commission 2012).

In 2015, the Japan Agricultural Extension Research Society held a symposium on AKIS (Yokoyama 2015). This symposium included a presentation by an innovating farmer (Yokota 2015). Stimulated by this symposium, Caldwell and Ueda (2015, 2016, 2017) carried out research in 2015-2017 to develop new methods for reconstructing farmers' processes of technology use, change, and innovation; assessing the relative importance of farmers' own ideas and interaction compared with outside sources of information; and elucidating the benefits of different technologies for farmers.

Parallel with the above research in Japan, Caldwell and Promkhambut (2017) began research on technology change in Khon Kaen Province, Northeast Thailand. Several districts producing dry season vegetables were selected for a reconnaissance survey of vegetable production in March 2016. Based on the results of the survey, two villages were selected, one in SS District and the other in NSH District, for research on technology change. The first village was selected because it had a functioning farmers' organization. The second village did not have a farmers' organization, but had similar conditions of good water availability in the dry season.

This research sought to describe technologies and innovations over the past 10 years in two villages, compare technologies with and without the presence of a farmer group and identify factors contributing to the number of changes in technologies and revenue.

MATERIALS AND METHODS

Approach and assumptions. This research, conducted in 2017, combined qualitative and quantitative data collection, transformation of qualitative data into quantitative measures, and descriptive and inferential analysis of results. These methods developed in Japan and adapted for use in this research in Thailand seek to bridge the continuing gap in the research approaches of agricultural scientists working in the hypothetico-deductive tradition and anthropologists and other social scientists using inductive approaches. The two approaches are based on different conceptions of how one understands the world (Tashakkori et al. 2021). The hypothetic-deductive tradition is positivist in its assumptions and quantitative in its observation and analysis methods. While it recognizes that many phenomena in the biological and social realms are probabilistic rather than deterministic, it assumes that these phenomena are real and can be measured objectively. The inductive / constructivist tradition is qualitative in its observation and analysis methods. It assumes that the observer cannot anticipate all causes of phenomena in advance, so prior hypotheses may not be relevant, and places emphasis on elucidating the knowledge and perceptions of the people involved. It also assumes that knowledge of the world is constructed differently by each person, depending on their place in society and their personal and collective experiences (Kriterion 2015).

The combination of methods reflects the tradition of Farming Systems Research and Extension (FSRE), which began to combine qualitative and quantitative methods in multidisciplinary team-based

work in applied technology development and extension in the late 1970s, and the experiences of the senior author in carrying out FSRE and similar research and extension activities in several countries since the early 1980s. In the terminology of current Mixed Methods Research (MMR), FSRE created a sequential mixed methods design (Tashakkori et al. 2021; Creswell 2022) for applied technology development, using qualitative methods for diagnosis and design of on-farm technology trials, and both quantitative and qualitative methods for assessment of technology performance in the trials.

Farming systems research and extension (FSRE) began in the late 1970s as a pragmatic response to the difficulties of Green Revolution improved technologies for rice and wheat production (Caldwell 1994; Collinson 2000). Agricultural scientists had come to recognize that farmers did not simply adopt all new technologies created by agricultural researchers, but rejected some and adapted many others depending on their conditions. The usefulness of technologies for farmers depended not only on the effects of the technologies on crops, but also on how farmers perceived the technologies and how they used them in their on-going production under varying conditions with multiple objectives of both household consumption and market sale. In response, FSRE developed team-based methods of qualitative assessment of farmer knowledge and perceptions of existing, desired, and proposed technologies, the conditions of their use, and their differing benefits and problems. Qualitative methods were seen as more effective in revealing the real experiences and perceptions of farmers than questionnaires with a pre-determined format based on the hypotheses of researchers. This knowledge became the basis for prioritization of technology development needs, followed by design, implementation, and evaluation of applied technology trials with farmers on their fields. Evaluation combined agronomic and economic quantitative data collection and analysis with farmer group-based assessment.

Nevertheless, the differing assumptions and methods of agricultural scientists and social scientists continued to create difficulties. Agricultural researchers often found the graphs, diagrams, and calendars of farmer activities to be inadequate, simply reflecting in visual, descriptive form the practices of particular farmers. Agricultural researchers were likely to consider that understanding individual farmers' experiences and perceptions was not sufficient for assessing the potential use of technologies and identifying how these might be modified to improve their usability. Extrapolation of those experiences and perceptions to a larger target area was needed.

This research sought to combine the strength of qualitative approaches in understanding farmers' experiences and perspectives, with statistical approaches for assessing the likelihood that differences among farmers were not due simply to random variation among the group of farmers. The idea of "Mixed Methods" from Tashakkori and Teddlie (2009) was used as the starting point for creating a method to link qualitative narrative data collection and quantitative data analysis. In effect a mixed methods conversion design (Tashakkori et al. 2021) was created, although this was unfamiliar terminology when work was done in 2015-2017. It was hoped that these results could provide a starting point for broader extrapolation, future technology trial design, and wider extension of improved technologies.

Data collection. Seven farmers were selected in each of two villages. In SS district, the farmers were all members of the farmers' group in the village. In NSH district, the farmers were selected in consultation with the head of the village.

Two different methods were used for data collection from the selected farmers, depending on the nature of the information sought:

(1) A large matrix recording sheet was used to record information from each farmer's narration of 10 years of change in types of vegetables and their production practices, impetuses and sources of information for each change, and benefits of each change. Columns in the matrix represented types

of vegetables and their production practices. Rows represented different points in time and types of information about each change asked through probing questions when information did not emerge in the farmer's narration. This information was recorded on post-it tags placed in the appropriate cells in the matrix. Both farmers and researchers could see how vegetable production had changed over time, based on the principle of visual sharing of data by researchers and farmers (Chambers 1993).

Fig. 1. Narration matrix

(2) Farmer personal characteristics (age, gender, education, presence or absence of off-farm income and its relative importance, farmer household labor), and types of crops and area and income from each crop were gathered using a questionnaire.

The above narration and matrix recording method was based on the method developed for technology change in "mountain potato" yam (*Dioscorea opposita*) production in Akita, Japan (Caldwell and Ueda 2015, 2016), and adapted to the research objectives and agricultural conditions of Northeast Thailand. The surveys were conducted over seven days in January 2017.

Data entry, transformation, and analysis. The information recorded on each post-it was entered into an Excel file with the same structure as the narration recording matrix. Each crop had three stages of technology use: 1)crop establishment, 2)water management, 3) pest management and other technologies. Three additional columns were inserted to the right of each technology stage column: 1) technology, 2) impetus / sources of information, 3) benefits. Information was divided vertically into four time periods: original technology more than 10 years ago; technology 10 years ago; initial innovations within the last 10 years; second innovations after an initial innovation within the last 10 years. Two files were made for classification and quantification of the technologies. The first quantification file consisted of 25 types of impetuses and sources of information that led the farmer to create or adapt a given technology. The second quantification file consisted of 20 types of benefits of using the technology.

For a given technology, its impetus and/or information sources were placed in the corresponding impetus or information source category. The value 1 was placed in the cell corresponding to that category, if there was only one impetus or source. Each cell received a weighted value w calculated as the inverse of the number of impetuses or sources t, w = (1 / t), if there was more than one impetus or source. The same method for quantification of each benefit was applied based on categories of the benefits. A technology change file was used to classify types of technology changes. Crop establishment and management were divided into six sub-stages: land preparation, crop and variety selection, fertilization, planting, trellising, and other. Crops were combined into five groups: yard long bean, other fruiting vegetables (pepper, various eggplants, corn), leafy crops (including heading cruciferous

crops), and other crops. Changes in technologies for each production sub-stage and crop group were mapped out over the four time periods described above, and frequencies of different change patterns enumerated.

Differences in the resulting quantitative measures of types of impetus / information sources, benefits, and farm management between the two villages were assessed using Student's t. Differences in numbers of farmers in each village in different categories of impetus / information sources, benefits, and personal characteristics were assessed using the Chi-square test. Stepwise regression was used to test for contributions of farmer characteristics and three groupings of types of sources of information (farmer, research and extension, and private sector) contributing to numbers of changes in technologies and farm and vegetable revenue. All statistical analysis was done using Statistix10 (Analytical Software 2008).

RESULTS AND DISCUSSION

Farmer profile. Farmers in the two villages were similar in age, education, years farming, years off-farm experience, and household size (Table 1). All households surveyed had income only or primarily from farming. Half or more of the households in both villages had no off-farm work. Occasional off-farm work was more important in SS than in NSH. The most important difference between the districts was participation in agriculturally-related training. Over the past 10 years, farmers in SS had participated 4-5 times per year. Farmers in NSH had participated on the average only 1 time in 3 years.

Farm management. Farmers in NSH had nearly 3 times more land in rice, and overall 26% more land in agriculture compared to SS. However, land in vegetables in SS was nearly twice that of NSH. Farmers in SS had less revenue from rice than farmers in NSH, but 9 times more revenue from vegetables than NSH farmers (Table 1).

Table 1. Characteristics of farmers in two districts, Khon Kaen Province, Northeast Thailand, 2017.

Parameter	unit	SS [a]	NSH [a]	Probability [b]
Farmer characteristics [c]				
Age	year	51	50	0.79 NS
Education	year	5.4	4.0	NT
Farming experience	year	29.2	31.9	0.69 NS
Off-farm experience	year	3.2	2.9	0.90 NS
Household size	persons	4.7	4.0	0.49 NS
Training	times	45	3	0.03 *
Income sources [d]				
Only farming	%	33%	57%	0.39 NS
Farming > non-farm	%	67%	42%	
Non-farm > farming	%	0%	0%	NT
Frequency of off-farm work [d]				
None	%	50%	71%	0.43 NS
Regular or occasional	%	50%	28%	

[a] Means of 6 farmers (SS) or 7 farmers (NSH)
[b] Probability of differences, + trend at $p<0.10$, * significant at $p < 0.05$, or
** highly significant at $p < 0.01$;
NT, no statistical test .
[c] Differences between villages tested by unpaired t-test
[d] Differences between categories and villages tested by chi-square test.

Vegetables and associated crops. SS farmers planted a greater variety of vegetables and associated crops, 18 types, compared with 11 types in NSH. Both villages planted yard long bean, many kinds of leafy and heading vegetables, and several kinds of fruiting vegetables. Cruciferous crops predominated among the leafy and heading vegetables. Only five kinds of crops (yard long bean, eggplant, pepper, Chinese flowering cabbage, and coriander) were common to both villages. SS farmers also planted several kinds of fruits and herbs in the same areas where vegetables were planted (Table 2).

Table 2. Farmer crop areas and annual household agricultural revenue in two districts, Khon Kaen Province, Northeast Thailand, 2017

Crop types	Farmers		Parameter values [a]		
	SS	NSH	SS	NSH	Probability [b]
	n	n	Area (ha)		
Rice	6	7	1.33	4.40	0.01*
Other agronomic crops	2	1	3.24	1.60	NT
Other crops	2	0	0.20	0	NT
Total	5	7	4.77	6.00	0.02*
Vegetables (sum)	6	7	0.42	0.23	0.31 NS
				Revenue (bahts)	
Rice	5	7	1,120	3,200	0.18 NS
Other agronomic crops	2	1	6,000	300	NT
Other crops	2	0	1,500	0	NT
Total crops	5	7	8,620	3,500	0.82 NS
Vegetables (sum)	6	7	18,191	2,426	0.04*

[a] Means of farmers responding at each location for each parameter

[b] Probability of differences, + trend at p<0.10, * significant at p < 0.05 or ** highly significant at p < 0.01; NT, no test, due to small sample size or absence of variation in one or both villages.

Technologies and technology changes. Farmers described a total of 417 individual technologies, or an average of 30 technologies / farmer (Table 3). Farmers in SS described nearly twice as many more technologies than farmers in NSH. These technologies were divided into original technologies used 10 or more years ago, and technology changes made during the past 10 years. In both locations, more than half of both original and changed technologies described by farmers involved soil and planting. There were no differences between the two districts in relative proportions of different types of original technologies. However, farmers in SS described proportionally more changes (53%) than farmers in NSH (44%), and there was a highly significant difference in types of changes between the two villages. Changes in water management were more important in NSH than in SS, whereas more changes in pest management and other technologies were made in SS.

Table 3. Individual technologies used by farmers in vegetable production 10 or more years ago and technology changes made during the past 10 years in two districts, Khon Kaen Province, Northeast Thailand, 2017.

Technology categories	SS		NHS		All		
	No.	%	No.	%	Signif. [a]	No.	%
Individual technologies	271		146			417	
Original technologies [b]							
Soil and planting [c]	72	56%	48	59%		120	57%
Water management [c]	27	21%	17	21%	NS	44	21%
Pest mgt. and other [c]	29	23%	17	21%		46	22%

Technology categories	SS		NHS		All		
	No.	%	No.	%	Signif. [a]	No.	%
Technology changes [e]							
Soil and planting [c]	84	59%	35	55%		119	57%
Water management [c]	11	8%	17	27%	<0.01**	28	14%
Pest mgt. and other [c]	48	34%	12	19%		60	29%
Totals [d]							
Original technologies [b]	128	47%	82	56%	0.08+	210	50%
Technology changes [e]	143	53%	64	44%		207	50%

[a] Probability of differences in technologies tested by chi-square test,
** highly significant at $p < 0.01$, + trend at $p<0.10$, or * NS not significant at $p\geq0.10$.
[b] Technologies used 10 or more years ago
[c] Percentage of original technologies or technology changes
[d] Percentage of all individual technologies
[e] Technologies changed in the past 10 years

The 417 individual technologies were grouped into 83 technology types. Each type grouped several individual technologies described using similar words by different farmers. Approximately one third of the technology types were common to both villages, while two thirds were specific to one or the other village.

There were significant differences between the two villages in numbers and types of technology changes. In SS, nearly half of all technology changes involved leafy vegetables, while in NSH, more than half involved yard long bean. Differences among vegetables and types of technology changes were significant in NSH but not in SS, where pest management changes were most important for leafy vegetables. In NSH, pest management changes were most important for yard long bean (Table 4).

Farmers did not make technology changes in the same way or sequence (Table 5). Changes in fertilization involved all types of fertilizers: chemical, organic, and mixed. Water management changes tended to move from hand and hose to systems using pumps, and in some cases then changing from furrow to trickle or sprinkler irrigation, especially in NSH. Some farmers changed water sources from ponds to underground, while others improved ponds. Adding ponds was more important in SS than in NSH. There were significantly more pest management changes involving organic pest control products, cultural practices, or IPM techniques in SS, while most changes in pest management in NSH involved changes in types of chemical pesticides.

Table 4. Types of technology changes of different vegetable types in two districts, Khon Kaen Province, 2017

Technology change	Vegetable type				
	Yard long Bean	Other fruiting	Leafy	Other	All
			SS		
Crop and variety	3	4	11	10	28
Fertilization	5	5	11	1	22
Trellising	5	0	0	0	5
Water management	0	0	4	1	5
Pest management	2	5	15	4	26
All changes	15	14	41	16	86
Probability of differences [a]					

Technology change	Vegetable type				
	Yard long Bean	Other fruiting	Leafy	Other	All
All crops, SS [b]			0.12 NS		
			NSH		
Crop and variety	3	6	2	1	12
Fertilization	4	1	3	0	8
Trellising	3	0	0	0	3
Water management	4	1	2	0	7
Pest management	10	1	3	0	14
All changes	24	9	10	1	44
Probability of differences [a]					
All crops, NSH[c]			0.03*		
SS vs. NSH, all changes [d]			<0.01**		

[a] Probability of differences in technologies tested by chi-square, ** highly significant at $p < 0.01$, * significant at $p<0.05$, or * NS not significant at $p\geq0.05$.
[b] Probability of differences between vegetables x technology changes within SS; trellising and water management not included due to small numbers.
[c] Probability of differences between vegetables x technology changes within NS; other vegetables and fertilization, trellising and water management not included due to small numbers.
[d] Probability of differences between districts and vegetable types.

Table 5. Sequences of technology changes in two districts, Khon Kaen Province, 2017

Technology Groups	Technology change	SS		NSH	
		Changes	Types	Changes	Types
Crop and variety	variety	13	28	7	12
	add or change crop	15		5	
Fertilization	chemical→ organic	1	22	2	8
	change within chemical	9		2	
	change within mixed	5		3	
	change within organic	7		1	
Trellising	none→ string	0	5	1	3
	stakes→ wire and string	0		1	
	change pole type	0		1	
	net, other	5		0	
Water management	hand→ hose	0	5	1	7
	hose→ pump→ trickle	0		2	
	water source→ trickle	0		1	
	hose→ pump, source	1		1	
	pump: petrol→ electric	1		1	
	bucket→ pump→ sprinkler	0		1	
	ponds	3		0	
Pest management	change to or add organic	4	26	1	14
	cultural practices	8		3	
	IPM	3		0	
	change within chemical	8		10	
	mixed, other	3		0	

Technology Groups	Technology change	SS		NSH	
		Changes	Types	Changes	Types
Total	all technology changes		86		44
Probability of differences [a]					
X^2 test, districts x all technology groups [b]			0.28 NS		
X^2 test, districts x non-chemical vs. chemical pest management [c]				0.03*	

[a] Probability of differences tested by Chi-square, * significant at $p < 0.05$, or NS not significant.
[b] Trellising not included due to small numbers.
[c] Non-chemical pest management is the sum of change to or add organic, cultural practices, and IPM vs. change within chemical; mixed not included due to small numbers.

Impetuses and sources of information of technologies. Comparison of impetus and information sources between the two villages showed important differences between the two districts (Table 6).

Impetuses based on farmers' own ideas and interaction were 45% more numerous in SS than in NSH. Farmers' own ideas and farmers' own trials were more common in SS, while farmers' reasons for adaptive use of technology and talking among themselves were more important in NSH.

Farmers in SS obtained significantly more information from public extension and research sources, approximately 7 times more than in NSH. These included training programs and university visits that were not present in NSH. Farmers in both villages obtained similar amounts of information from the private sector and other outside sources. Information from input stores and companies was the most important private sector source. Farmers in SS also obtained considerably more information from printed publications and the internet, and from markets. In contrast, more farmers in NSH had off-farm experience that contributed information. These included farmers who had worked in Israel and Taiwan.

Overall, farmers in SS had significantly more types of sources of information, and nearly double the number of sources of information. The first measure, types of sources, indicates more diverse sources of information. The second measure, number of sources, reflects the greater number of technologies in SS. While not all individual impetus and source type comparisons were statistically significant, they provide insight into reasons why differences in total types and numbers of sources of information were significant.

Table 6. Impetus and sources of information of technologies used in vegetable production in two districts, Khon Kaen Province, Northeast Thailand, 2017

Impetuses and sources	SS [a]	NSH [a]	Probability [b]
Farmers' ideas and interaction			
Farmer's own idea	3.14	1.50	0.28
Farmers' reason for adaptive use	0.07	0.19	0.33
Family	0.71	0.00	No test
Farmers talking	0.14	1.64	0.12
Farmer organization	0.36	0.00	No test
Observing another farmer	0.83	0.68	0.69
Farmer's own trial	1.19	0.44	0.27
Farmers' ideas and interaction sum	6.45	4.45	0.22
Research and extension			
Extension information	1.50	0.55	0.23
Training program	0.68	0.00	No test

Impetuses and sources	SS [a]	NSH [a]	Probability [b]
University visit	0.94	0.00	No test
On-farm collaborative trial	0.00	0.00	No test
Research station information	0.00	0.00	No test
Other organization	0.39	0.00	No test
Research and extension sum	3.51	0.55	0.01 **
Private sector and other sources			
Store or company	2.07	2.34	0.78
Printed publication or internet	0.57	0.08	0.03 *
Private collaborative trial	0.00	0.00	No test
Market	1.39	0.57	0.16
Infrastructure	0.00	0.14	No test
Overseas experience	0.00	0.00	No test
Off-farm work experience	0.00	0.57	No test
Private sector and others sum	4.03	3.71	0.81
Number of types of sources	7.71	5.57	0.03 *
Sum of sources	14.00	8.71	0.003 **
Independence quotient[c]	0.44	0.52	0.52

[a] Mans of 7 farmers / location.
[b] Probability of differences, * significant at $p < 0.05$ or ** highly significant at $p < 0.01$; no test, one or both averages zero.
[c] Proportion of all sources comprised by farmers' ideas and interaction.

Benefits of technologies. Farmers in SS and NSH differed in benefits obtained from technologies (Table 7). Farmers in SS cited twice as many benefits as did NSH farmers. This partially reflected the 38% greater number of impetuses and sources of information in SS seen in Table 6. It also reflected more benefits per technology in SS.

Crop production and environmental benefits comprised 63% of the benefits of technologies in SS, while those benefits comprised 45% of the benefits of technologies in NSH. Economic benefits comprised only 29% of the benefits in SS, but 46% of benefits in NSH. Crop production and environmental benefits were thus relatively more important in SS, and conversely the economic quotient (proportion of all benefits) was greater in NSH. Social and individual benefits comprised less than 10% of the benefits cited in both villages.

Within the crop production and environmental benefits group, benefits for insect and disease management were the most important type of benefit in SS, cited 5 times more than in NSH. Soil fertility and fertilization benefits were second in importance in SS, while cited only occasionally in NSH. Water management benefits were also significantly more important in SS. Crop growth benefits were the most important type of benefit within NSH.

Economic benefits were cited 32% more often in SS than in NSH, but this difference was not significant. Marketability was cited 78% more often in SS. This trend might become significant if a larger number of farmers were compared in each village.

Table 7. Benefits of technologies used in vegetable production in two districts, Khon Kaen Province, Northeast Thailand, 2017

Impetuses and sources	SS [a]	NSH [a]	Probability b
Crop production and environmental benefits			
Soil fertility and fertilization	2.50	0.29	0.05 +
Water management	1.21	0.21	0.04 *
Environment	0.57	0.43	0.70
Compatibility with other technologies	0.14	0.00	No test
Weed management	1.14	0.07	0.14
Insect and disease management	5.03	1.07	0.003**
Crop growth	2.21	1.60	0.52
Product quality	0.37	0.79	0.31
Subtotal, production and envir. benefits	13.18	4.45	0.03*
Economic benefits			
Yield	1.80	1.07	0.37
Crop planted area	0.14	0.26	0.51
Production costs	0.50	0.62	0.74
Returns	0.43	0.57	0.60
Marketability	1.65	0.93	0.08+
Labor quantity, efficiency	0.58	0.33	0.41
Compatibility with other crops	0.07	0.00	No test
Ease of input obtainability	0.71	0.69	0.96
Input quality	0.21	0.14	0.74
Subtotal, economic benefits	6.11	4.62	0.20
Social and individual benefits			
Recommendation of other farmers	0.00	0.14	0.78
Recommendation by extension, etc.	0.00	0.14	No test
Farmer organization	0.17	0.00	No test
Easy, fast, etc.	1.10	0.71	0.36
Policy	0.00	0.07	No test
Others (health, home use)	0.36	0.00	No test
Subtotal, social and individual benefits	1.64	0.93	0.27
Number of types of benefits	11.57	8.71	0.09 +
Sum of benefits	20.93	10.00	0.02 *
Benefits / source	1.5	1.1	No test
Economic quotient [c]	0.32	0.45	0.099+

[a] Means of 7 farmers / location

[b] Probability of differences, + trend at p<0.10, * significant at p < 0.05 or
** highly significant at p < 0.01;
no test, one or both averages zero

[c] Proportion of all benefits comprised by economic benefits

Factors contributing to changes in technologies and revenue. Table 8 summarizes the results of stepwise regression analysis of factors hypothesized to affect the number of changes in technologies and revenue. Effects of several farmer characteristics were seen on all four dependent variables overall (both villages combined) and in SS, but only on total revenue in NSH.

Overall, greater age increased both the number of changes in technologies due to farmer ideas and interaction and the total number of changes in technologies. More years working off-farm increased

both farmer-initiated changes and the total number of changes in SS but not in NSH.

Overall, more years in farming increased farm revenue. Greater farm area increased revenue in SS, while more years working off-farm increased revenue in NSH. Family size and training increased vegetable revenue. In SS, greater farm area increased vegetable revenue, but none of the hypothesized variables contributed to vegetable revenue in NSH.

Table 8. Results of stepwise regression of effects of selected farmer characteristics on numbers of technology changes and revenue in two districts, Khon Kaen Province, Northeast Thailand, 2017

Dependent variables	**Independent variables [a] in model, significance [b], and model R^2**		
	All	**SS**	**NSH**
Changes due to farmer ideas and interaction	Mean 5.5 Age * +0.3 Years farming * +0.18 72%	Mean 6.5 Years off-farm * 0.6 84%	Mean 4.5 All variables NS No model
Total number of changes	Mean 11.4 Age * +0.07 48%	Mean 14.0 Years off-farm * 0.1 84%	Mean 8.7 All variables NS No model
Farm Revenue (baht)	Mean 66,154 Yrs farming** +6,124 60%	Mean 60.833 Total area* +1,860 89%	Mean 70,714 Yrs off-farm* +18,164 72%
Vegetable revenue (baht)	Mean 242,551 Family size* +70,991 Training** +1,421 67%	Mean 454,778 Farm area -7,311 97%	Mean 60,643 All variables NS No model

[a] Farmer and farm characteristics hypothesized as contributing to all dependent variables: age, number of years of education, number of years farming, number of years engaged in off-farm work, total farm area, number of times participated in training.
Impetuses and information sources hypothesized as contributing to revenue:
weighted number of technology changes due to farmer ideas and interaction, information from extension or research, or information from the private sector, and
total number of technology changes,

[b] Significance of dependent variables remaining the final model:
NS non-significant at $p \geq 0.05$, * significant at $p < 0.05$, or ** highly significant at $p < 0.01$;
+ indicates positive effect, - indicates negative effect.

Origins of technology changes. The above results show that farmers in the village with a farmers' organization, SS, are using a greater variety of technologies and have made more technology changes than the farmers in the village without a farmers' organization, NSH. Moreover, farmers in SS made more changes based on their own ideas and interaction than in NSH. At the same time, they also accessed outside information more than farmers in NSH.

In the oral interviews, farmers in SS described how an early training program by public extension and universities starting from 1994 had helped them organize themselves into a group and work together to produce for the market. As farmer 6 stated, "Middlemen set the price on the day of sale. There was no assurance of price. Training gave us knowledge on how to form a group. As the group became stronger, it became able to market on its own." The above quantitative analysis shows that SS farmers became more active innovators, taking information from public and private sources, combining it with their own ideas (such as making sticky traps from local materials), testing innovations in their own trials, and making more technology changes in their production. For example, farmer 5 stated that he changed

the variety of *kana* (Chinese kale, a popular leafy vegetable) "because he observed that another farmer in the group found this variety sold well." Farmer 7 stated that she changed her watering method "based on observing the vegetables and following a friend in the group." In contrast, farmers in NSH had plentiful informal interaction, but overall made fewer technology changes than in SS. These comparisons suggest that the farmers' group had a stimulating effect on technology change and innovation.

Analysis of farmer perceptions of benefits suggests that the technologies used in SS are successfully addressing more types of environmental and production problems than in NSH. SS farmers are also deriving marketability benefits more often from technologies.

Value, types, and origins of farmer organizations. These results overall support the value of farmer organizations in stimulating technology change and innovation. Extension agents, university and research institute researchers carrying out field-based, farmer-oriented applied research, and private sector actors working with farmers can support the formation and functioning of farmer organizations with the expectation that it will provide environmental, agronomic, and economic benefits to farmers. The effect of farmer organizations in increasing the use of organic and reduced chemical technologies can also result in better quality agricultural products for consumers.

In this case, the farmer group in SS consisted of 10 farmers in one village, all producing vegetables. The group formed naturally among relatives. The leader is a respected person in their kinship network and was formerly head of the village. The group has a market stall in the city where they sell products together. This selling platform is therefore one of the locations ("ba") for sharing knowledge among members. The word "ba" means "place" in ordinary Japanese usage, but Yokoyama (2015) proposed using this word as a term to refer to the location of interaction among actors involved in agricultural extension and development. Caldwell (2021) has explained "ba" as a "theater of action" for agricultural extension and development activities, both spontaneous and of external origin, and introduced this term in international agricultural extension training conducted by the Japan International Cooperation Agency (JICA).

Shigetomi (2006) divided community organizations into four types, based on field work in both Northeast Thailand and the Philippines. Organizations were classified based on their function (addressing a specific objective vs. mediating social relations) and origin (spontaneous within the village vs. from outside the village). This farmer group begin from a kinship network, but had a specific objective, to sell products together. Moreover, the farmers sought out assistance from both extension and the regional agricultural university. So in effect, they evolved from Type III (spontaneous mediating social relations) to Type I (spontaneous with a specific objective), seeking out and taking advantage of information from outside sources, similar to Type II. The authors term this a hybrid "collaboration" type. The Agricultural Extension Research Society of Japan has made the word "collaboration" the central focus of an expanded understanding of agricultural extension (Sato et al. 2020), wherein agricultural extension workers facilitate and support spontaneous development effects of farmers and communities. The word in Japanese is a combination of the characters for "cooperating" and "working."

More information on the formation of farmer groups is needed to identify the conditions for successful formation and operation of farmer groups. This information should include what stimulated formation of the group, characteristics of the location of the group (including water access, soils, and access to markets), characteristics of members of the groups (type of farming, kinship and social relationships, market access), and frequency of both formal meetings and informal interaction.

One example of the impetus to group formation can be seen in the response quoted above of farmer 6 in SS who stated in his narration that, "Training gave us knowledge on how to form a group. Because we had the problem of middlemen fixing prices." This indicates that farmers wanted to form a group

to gain the power to set their own prices. In other words, economics was the impetus to group formation.

Effects of technology changes. In contrast with results in Akita (Caldwell and Ueda 2017), greater numbers of technology changes due to farmers' own ideas and initiatives did not show a linear effect on farm or vegetable revenue. Years farming and family size had more effect on revenue than technology changes. In Akita, a major innovation stimulated other technology changes for a single crop that was the flagship brand crop of the area of study. On the other hand, in Northeast Thailand, small technology changes were spread across many types of vegetables, so the effect on overall farm and vegetable revenue of each change was likely to be small. Young farmers without other family members to help with intensive vegetable production and marketing could benefit from support in targeting production to markets to obtain meaningful economic benefits from making technology changes.

The sample size for modelling of 12 in this study was approximately half only the sample size of 23 obtained in Akita. With a larger sample size, a significant effect on farm and vegetable revenue of technology changes due to farmers' own ideas and initiatives might have been found in this study in Northeast Thailand. A larger sample size also allows for more independent variables to enter into models (Hair et al. 2010), such as in Akita, where three factors (potato planted area positively, total planted area and years of off-farm experience negatively) were found to contribute to the number of technology changes (Caldwell and Ueda 2017). Models with several variables can provide more insights for designing support programs targeting farmers most likely to obtain economic benefits from technology change.

Factors affecting technology changes. The role of off-farm employment in stimulating agricultural technology change for vegetables is in accordance with off-farm employment's positive effect on rice technology change (Grandstaff et al. 2008). Future research could trace the types of information brought back from overseas and the interactions of returning farmers with other farmers.

In a study of constraints to organic production in the same area but with different farmers, insufficient understanding of the principles of organic production and lack of training in organic production were the two greatest constraints of vegetable farmers in that area, followed by lack of organic pesticides, difficulties with organic soil management, and lack of cooperation among farmers (Mondal et al. 2014). The results presented here suggest that farmer organization can help address technical difficulties through sharing of knowledge among member farmers.

A key issue to consider in future research is the question of the effects of market type, size, and access on innovation. Farmers in SS had access to an urban market with more demand for reduced pesticide and organic vegetables, while demand in the smaller market in NSH was almost exclusively for conventionally-produced vegetables. These results suggested marketability to be a factor in technology use and change in SS, and marketability may be an underlying motivational factor in the difference between the two villages. Analysis of farmers' motivation through self-determination analysis can help farmers understand better the importance of market access, and thereby increase motivation for market-oriented technology change and innovation. This approach has been successfully applied with small-scale farmers in Kenya (Sayanagi et al. 2016).

Potential for future uses of MMR in agricultural extension and development research. The methods used in this study linked qualitative narration as a source of information about technology change and quantitative analysis to assess to what extent there were differences between the two villages, as a first step towards extrapolation. From the perspective of Mixed Methods Research (MMR), research questions 2 and 3 required integration using the conversion design the authors created to address these two questions. Mixed Methods Research (MMR) is defined by Tashakkori et al. (2021, p. 401) as "research that collects/analyzes QUAN [quantitative] and QUAL [qualitative] data and integrates the findings and makes meta-inferences based on both types of findings." This research meets the standard of the first part of this definition, but it did not set out to conduct a separate qualitative

analysis to make meta-inferences from both qualitative and quantitative types of analysis. It does, however, meet the older, broader definition of Johnson et al. (2007) quoted by Tashakkori et al. (2021, p. 47): "Mixed methods research is the type of research in which a researcher or a team of researchers combines elements of qualitative and quantitative research approaches (e.g., use of qualitative and quantitative viewpoints, data collection, analysis, inference techniques) for the purpose of breadth of understanding or corroboration."

To add an explicitly qualitative analysis for making meta-inferences in a convergent design (Creswell 2022; Creswell and Plano Clark 2022) would require a preparatory study to determine which qualitative analysis method would be most useful for the objectives of agricultural extension and development, and for identifying key farmer responses for qualitative analysis and inclusion in joint displays. The life pathways method of Aquino (2021) could be a useful method to determine how spontaneous farmer groups form. A range of qualitative methods and their uses with examples are presented Corbin and Strauss (2015), which could be used as a reference in considering the use of MMR to address research questions in agricultural extension and development.

The data conversion process, in which each key element of the narration is read one-by-one and assessed for quantification, provides context for interpreting the quantitative analysis that follows in a conversion design, but it is very time consuming. Discussion with several MMR researchers at the 2018 Symposium of the Japan Mixed Methods Research Society did not lead to a conclusion as to how computer technology might be used to speed up the conversion process without losing the qualitative understanding gained by the researcher's reading of each response for qualitative analysis and interpretation.

MMR uses joint displays as one method (Creswell 2022; Creswell and Plano Clark 2022; Fetters et al. 2013) for presenting the results of both types of analysis for meta-inference. This should be considered at the beginning of planning research that aims for meta-inference from both QUAL and QUAN analyses. The overall time requirements for the different steps in MMR need to be considered carefully and planned for in advance, as explained in more detail in Creswell and Plano Clark (2022).

The original responses of the farmers were used to identify examples of key results revealed by the quantitative analysis. This was like re-entering a forest and looking at all the individual trees one-by-one. The individual trees provide context, but one cannot see a whole forest from examination of each tree. Quantification is a tool that enables one to reduce this complexity and see the overall contours and characteristics of the forest of farmer technology change revealed in the qualitative narration by individual farmers. This is a benefit of the combination of quantitative and qualitative methods.

The integration of formal MMR methods into agricultural research on farmer technology change would need to address the above issues. This could be a topic for a graduate student interested in using qualitative and quantitative methods for understanding farmer technology change, and thereby developing better methods of farmer-centered and farmer-led collaboration in agricultural extension and development.

CONCLUSION

Overall, farmers in this study made on the average 30 technology changes over the 10 years that they described technology changes in their narrations. These technology changes differed both in content and in number between the village with a farmer group, SS, and the village without a farmer group, NSH.

The mixed methods research (MMR) conversion design developed for this study provided quantitative measures of frequency of key words in the narrations. Statistical comparisons of the

villages showed that the village with a farmer group, SS, made twice as many technology changes overall, with proportionally more technology changes in pest management. More pest management changes in SS involved organic pest control, cultural practices, and IPM. The farmers in SS were then able to gain access to a market for organic and reduced chemical vegetables.. These changes reflected more training and other support programs, greater use of public extension and research information, and greater farmer interaction in SS. Increased training had a positive impact on vegetable revenue in both villages considered together.

These results overall indicate that farmer groups can have a positive effect on technology change. In order to stimulate and support farmer group formation, more research is needed to identify the conditions that lead to the successful formation and operation of farmer groups. Life path history of farmers both belonging to and not belonging to a farmer group could reveal these conditions. This research should include identification of information brought back from off-farm work experience and patterns of sharing that information among farmers. More research on types of markets and farmer motivation in seeking new market access is also needed. The key factors identified through such qualitative research could then be tested using a similar MMR conversion model with a larger number of farmers to increase the power of statistical testing for extrapolation. Results from both qualitative and quantitative analysis could then form the basis for guidelines for agricultural extension and other collaborators in rural development to use in developing farmer-centered and farmer-led collaboration in agricultural extension and development.

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OPTIMAL SUBMERGED CULTURE CONDITIONS AND BIOACTIVITIES OF MYCELIA OF WILD MUSHROOMS, *Cyathus striatus (*HUDSON) WILDENOW, AND *Xylaria hongkongensis* (A.M.C. TANG, R.Y.C. LAM and M.W.K. LEUNG)

Janice S. Aguilar[1, 2]*, Rich Milton R. Dulay[1], Sofronio P. Kalaw[1]and Renato G. Reyes[1]
[1]Center for Tropical Mushroom Research and Development, College of Science,
Central Luzon State University, Science City of Muñoz, Nueva Ecija, 3120, Philippines
[2]Science Education Institute, Department of Science and Technology,
Bicutan, Taguig City, Philippines
*Corresponding author: janiceaguilar919@clsu.edu.ph

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ABSTRACT

The Philippines is a rich source of mushrooms with promising nutritional and pharmacological potentials. To date, a number of Philippine wild mushrooms have been investigated for their growth requirements and bioactivity profile. However, several mushrooms remain to be undiscovered and unutilized. Hence, the mycelial biomass production of *Cyathus striatus*, and *Xylaria hongkongensis* in submerged conditions was evaluated and the bioactivities of their ethanolic extracts were assessed. Potato sucrose broth at pH 6-8 favored the mycelial biomass production of both mushrooms, while coconut water and rice bran broth were also favorable for *X. hongkongensis*. The maximum yield of *C. striatus* mycelia was obtained when incubated at 28°C, lighted, and agitated submerged fermentation. *X. hongkongensis* recorded higher mycelial biomass yield at 28°C, dark, and agitated conditions. Mushroom extracts exhibited antibacterial activity against *Staphylococcus aureus*, but not against *Escherichia coli*. Both extracts showed toxic effects in brine shrimp with LC_{50} values of 134.90 µg/ml (*C. striatus*) and 141.25 µg/ml (*X. hongkongensis*). In zebrafish assay, 1000 µg/ml or higher concentrations of extracts showed 100% mortality of embryos after 48 hours of exposure, and no hatchability, indicating embryotoxicity and cytotoxicity. Extract-treated embryos also showed different morphological abnormalities, indicating teratogenic properties. The maximum yield of mycelial biomass of the two mushrooms can be obtained in optimized culture conditions, which can be sources of bioactive compounds with pharmacological properties.

Key words: mushrooms, antibacterial, cytotoxic, liquid media, mycelia

INTRODUCTION

Mushrooms are saprotrophic organisms that demonstrate remarkable nutritional and medicinal potential. They are highly considered one of the most valued crops due to the number of benefits they can offer to humanity. Edible fungi such as the group of oysters, enoki, shiitake, straw, and button mushrooms contain ample amounts of protein, vitamins, minerals, and carbohydrates (Nhi and Hung 2012; Eguchi et al. 2015). Apart from their nutritional attributes, mushrooms contain agents that can treat or prevent the emergence of serious health problems such as hypertension, cancer, diabetes, and diseases associated with microbial infection (Chaturvedi et al. 2018). Considering these numerous

capabilities of mushrooms, attention must be given to every species of mushroom to broaden the resources of excellent bioactive compounds.

Most of the wild mushrooms are observed when there is enough humidity in the environment. One of the wild mushrooms is *Cyathus striatus*, which is commonly known as a bird's nest (Zhao et al. 2008). It has tiny fruiting bodies with egg-like structures inside the nest-like fruiting body. This mushroom is usually observed in clusters attached to decaying wood or soil. It is capable of enhancing nerve growth factors and has anti-neuro-inflammatory activity since it contains neuroprotective agents (Bai et al. 2015; Yin et al. 2019). It has incredible anticancer potential as its low extract concentration inhibits cell proliferation within a short period of time (Sharvit et al. 2012). *Xylaria hongkongensis* is another species of mushroom with unique morphological characteristics wherein its pointed structure resembles a hardened coral. Mushrooms under the genus *Xylaria* contain a wide range of biologically active compounds with antioxidant, antimicrobial, and anti-cancer potentials (Changi 2015; Adnan et al. 2018). However, there is very little information regarding the optimized condition for the successful propagation of these mushrooms. Understanding the growth preferences will therefore lead to the establishment of efficient production technology.

Solid-state fermentation is the most common technology for the cultivation of mushroom fruiting bodies in the Philippines. However, this technique might not be suitable for all species of mushrooms. Some mushrooms take a longer incubation period in order to produce fruits. Submerged cultivation which involves the use of liquid media is a reliable technique that can be used to produce mycelial biomass. The fruiting body and mycelia can be good sources of bioactive compounds and can exhibit biological properties. Therefore, liquid cultivation could be used as an alternative technology that can provide a sufficient amount of mycelia.

Mycelia, as the vegetative part of mushrooms, require nutrients and appropriate environmental conditions such as temperature, agitation, and illumination. Some species of mushrooms can produce high biomass yield in a wide range of environmental conditions, others can tolerate extreme surroundings while other species have a very specific requirement. These factors clearly determine successful mycelial development, which varies depending on the species.

Many attempts have been made to culture the fruiting bodies of *C. striatus* and *X. hongkongensis*. However, the Center for Tropical Mushroom Research and Development at Central Luzon State University was not successful in culturing the fruiting bodies of these mushrooms. Since it is of utmost importance to establish the position of *C. striatus* and *X. hongkongensis* in the mushroom industry, submerged cultivation was utilized as the cultivation technique to mass produce the mycelial biomass for the screening of their biological properties. The optimal culture conditions for the maximum production of mycelia of the two mushrooms were established in this study.

MATERIALS AND METHODS

This study was conducted at the Center for Tropical Mushroom Research and Development (CTMRD) Central Luzon State University, Science City of Muñoz, Nueva Ecija, Philippines, from January to March 2022.

Source of mycelial discs. Pure cultures of *C. striatus* and *X. hongkongensis* were obtained from the culture collection of the CTMRD. To prepare the source of inoculant, mycelial blocks from the pure culture were aseptically inoculated into potato dextrose agar plates and incubated for 7 days. Mycelial discs were prepared using a cork borer,

Evaluation of culture media and pH. The optimum liquid culture media for the mycelial biomass production of *C. striatus* and *X. hongkongensis* was determined using four indigenous liquid media namely corn grit broth (CGB), potato sucrose broth (PSB), rice bran broth (RBB), and coconut water

(CW) (Dulay et al. 2022). Each medium was prepared by boiling rice bran, corn grits, and potato in water separately with the ratio of 50g rice bran: 1L water, 50g corn grits: 1L water, and 250g diced potato: 1L water. After boiling, 10g of table sugar was added to the decoction. Prior to sterilization, the prepared broth was adjusted to pH 6.5. Thirty ml of the previously prepared liquid culture media was transferred into clean bottles and covered with plastic. The bottles were sterilized in an autoclave (KT-3065A, ALP Co., Ltd., Japan) for 30 minutes at 15 psi and 121°C. The bottles were allowed to cool after sterilization and a mycelial disc was aseptically inoculated into each bottled medium. All bottles were incubated at room temperature to allow mycelial growth. Mycelial biomasses were harvested after seven days of incubation, air-dried for three days, and weighed using an analytical balance.

PSB was then used to determine the optimum pH requirement of the mushrooms. Varying pH levels of PSB were prepared (4, 5, 6, 7, 8) using 1 M NaOH or 1 M HCl and adjusted using a pH meter (ST20, Ohaus, USA). Then, 30 ml of the medium was placed in clean bottles covered with plastic, and secured with a rubber band. The mycelia were harvested after seven days of incubation, air-dried, weighed, and recorded.

Effects of physical factors. After determining the optimum pH for the mycelial biomass production of the two mushrooms, the physical factors: temperature, illumination, and agitation were evaluated. To determine the optimum temperature, the culture bottles containing PSB adjusted to pH 7 were inoculated with mycelial discs and incubated at three temperature conditions; refrigerated (8°C), air-conditioned (21°C), and room temperature (28°C). After determining the temperature preference of the mushrooms, the influence of light was assessed by incubating the culture bottles under lighted and dark conditions. The culture bottles were covered with black paper for the dark condition while the other culture bottles were exposed to artificial light (137 lux). The culture bottles were incubated in agitated (100 rpm) and static conditions with the optimum illumination and temperature conditions to determine the influence of agitation on the mycelial biomass production of the mushrooms. Harvesting of mycelial biomasses was done after seven days followed by air-drying and weighing. The optimum temperature, illumination, and agitation were determined based on the mycelial biomass yield after seven days of incubation.

Mass production of mycelia. This was carried out by inoculating mycelia discs into culture bottles containing 30 ml of PSB at pH 7. The bottles were incubated under the optimum temperature, illumination, and agitation conditions. Fifty replicates of mycelial cultures for each mushroom were prepared. The mycelial biomasses were harvested and air-dried, after seven days of incubation.

Ethanolic extraction. The bioactive compounds of the mycelia were extracted following the method of Dulay et al. (2021) with some modifications. The dried mycelia were powdered using a blender, and then soaked in 95% ethanol for 48 hours. Filtration was done using Whatman filter No.2 and concentrated to dryness using a rotary evaporator (RV 10 D S99, IKA Germany).

Antibacterial assay. Two bacterial pathogens namely: *Staphylococcus aureus* (UPLB BIOTECH 1582) and *Escherichia coli* (UPLB BIOTECH 1634) in disc-diffusion method were used. Microbial cultures were prepared in a 9 ml nutrient broth medium and the turbidity was compared to 0.5 McFarland standard. Assay plates were swabbed with the bacterial inoculum and a 6-mm sterile paper disc previously soaked in mycelial extracts was placed on the surface of the medium. Streptomycin sulfate was used as the positive control and ethanol as the negative control. The zone of inhibition was measured using a Vernier caliper after 24 hours of incubation.

Brine shrimp lethality assay. The cytotoxicity of the extract was assessed using brine shrimp (*Artemia salina*). Larvae were prepared by hatching the eggs in artificial seawater (25 g salt in 1 L dH_2O). Varying concentrations of the extract were prepared (1, 10, 100, 1000, and 10,000 µg/ml) in vials, then ten larvae were placed in vials containing the treatments. Brine solution was used as control. The

number of dead nauplii was recorded after 24 hours and the percentage mortality was calculated. Lethal concentration (LC_{50}) was computed using Probit analysis and interpreted using the following: LC_{50} values > 1000 µg/ml (non-toxic), ≥ 500 ≤ 1000 µg/ml (weak toxic), and < 500 µg/ml (toxic) (Bastos et al. 2009).

Toxicity and teratogenicity assay. The method of toxicity and teratogenicity using zebrafish embryos used by Dulay et al. (2012) was followed. Ten ml of each treatment concentration of the extract was prepared using embryo water as a diluent (1 µg/ml, 10 µg/ml, 100 µg/ml, 1000 µg/ml, and 10000 µg/ml) and control (embryo water) and placed into each well of the 12-well ELISA plate. Three embryos at the segmentation phase were transferred into each well containing the different treatments. The plate was incubated at 26°C ± 1°C. The teratogenic effect was examined using a dissecting microscope after 48 hours of incubation. Morphological endpoint evaluation of zebrafish was based on the parameters established by Nagel (2002): Lethal (coagulation, tail not detached, no somite, and no heart-beat): teratogenic (malformation of head, tail, and heart, scoliosis, deformity of yolk, and growth retardation): and normal. Percentage hatchability and mortality were determined. A test is classified as valid if 100% of the embryos in the control group show normal conditions.

Statistical analysis. All treatments were laid out in a Complete Randomized Design (CRD). Data were analyzed using Analysis of Variance (ANOVA) and compared using Tukey's HSD at 5% level of significance. Treatment means in illumination and shaking conditions were compared using paired T-tests. The two mushrooms were not compared since they are of different species.

RESULTS AND DISCUSSION

Effect of liquid culture media and pH. The successful and luxurious growth of mushroom mycelia is dependent primarily on the nutritional composition of the substrate. The mean mycelial biomass yield of *C. striatus* and *X. hongkongensis* after seven days of incubation in the different liquid culture media is presented in Table 1. The highest mycelial biomass yields were recorded significantly in PSB with 73.60 mg/30 ml (*C. striatus*) and 136.33 mg/30 ml (*X. hongkongensis*). Aside from PSB, CW and RBB were also found favorable for the mycelial growth of *X. hongkongensis*. In contrast, CGB registered the lowest biomass yield in both mushrooms.

Table 1. Mycelial biomass yield of *C. striatus* and X. *hongkongensis* grown in liquid culture media after seven days of incubation.

Culture media	Biomass yield (mg/30 ml)	
	C. striatus	***X. hongkongensis***
Potato sucrose broth	73.60±13.98[a]	136.33±12.61[a]
Corn grit broth	34.55±3.35[b]	62.93±5.51[b]
Coconut water	41.90±8.19[b]	132.20±22.00[a]
Rice bran broth	29.60±35.80[b]	132.73±9.15[a]

In each column, means with the same superscript are not significantly different from each other at 5% level of significance. Potato sucrose broth served as the control medium.

The suitability of PSB could be attributed to the nutritional component of potato that favors the efficient development of their mycelia. Potato contains high amounts of carbohydrates, proteins, vitamins, minerals, fructose, sucrose, and folic acid (Burlingame et al. 2009). Carbon and nitrogen are the primary nutrients needed by mushrooms, and the presence of other nutrients like potassium, phosphorus, manganese, iron, selenium, magnesium, copper, and molybdenum have positive effects on their growth (Chang and Miles, 2004). Mushrooms prefer the type of carbon, sugar, and other nutrients

that are present in potato sucrose broth than the other media used allowing their mycelia to grow luxuriantly. *Xylaria hypoxylon* and other *Xylaria* species also prefer potato dextrose agar (Ramesh et al. 2014; Ahmed and Jahan 2017) Meanwhile, *Xylaria nigripes* produced optimum biomass yield in media with fructose and yeast extract supplemented with magnesium sulfate heptahydrate, nitrogen, and some minerals (Chen et al. 2014). However, there were no published reports regarding the mycelial biomass production of *C. striatus* to compare with the results of this study. Other species of mushrooms that prefer potato as the carbon source include *Pleurotus floridanus* (Khan, 2017) and *V. volvacea* (Kalaw et al. 2016).

The mycelial biomass yield of the two mushrooms is influenced by pH (Table 2). Potato sucrose broth at pH 7 registered the highest yield of mycelial biomass with a mean value of 136.37 mg/30 ml and 140.43 mg/30 ml for *C. striatus* and *X. hongkongensis,* respectively. However, those biomass yields were found statistically comparable with those at pH 6 and 8 which also produced high yields. pH levels lower than pH 6 produced a low biomass yield of both mushrooms. The response of *X. hongkongensis* to illumination coincides with the findings for *Cyclocybe cylindracea, Pleurotus djamor,* and *Pleurotus salmoneostramineus,* which did not show sensitivity to light (Landingin et al. 2020; Jacob et al. 2015). The response of *X. hongkongensis* to illumination coincides with the findings for *Cyclocybe cylindracea, Pleurotus djamor,* and *Pleurotus salmoneostramineus,* which did not show sensitivity to light (Landingin et al. 2020; Jacob et al. 2015).

Table 2. Mycelial biomass yield of *C. striatus* and *X. hongkongensis* grown in PSB with varying pH levels after seven days of incubation.

pH	Biomass yield (mg/30 ml)	
	C. striatus	***X. hongkongensis***
4	90.70±4.61^{b}	50.77±8.24^{b}
5	99.10±26.10^{b}	86.37±16.27^{b}
6	102.70±8.60^{ab}	130.00±2.00^{a}
7	136.37±4.13^{a}	140.43±12.11^{a}
8	135.00±20.70^{a}	133.97±8.24^{a}

In each column, means with the same superscript are not significantly different from each other at 5% level of significance.

The differences in the yield among varying pH levels could be explained by the direct effect of the hydrogen ions in the media. pH affects the entry of sodium ions and necessary molecules present in the media to the individual cell (Elisashvili 2012). It can limit the absorption capacity of the cell membrane leading to poor mycelial growth since the necessary nutrients are not provided (Deacon 2006). These results are in consistent with earlier findings where pH 6-7 was found to be the optimum pH for most wild mushrooms collected in Central Luzon namely: *G. lucidum, L. tigrinus*, and *Coprinopsis cinerea* (Kalaw et al. 2016), Likewise, the mycelia of *Ganoderma applanatum* showed excellent growth in pH 6 – 9 (Jo et al. 2009). Previous reports on the pH preferences of mushrooms under the genus *Xylaria* obtained different results. These differences in pH preferences of mushrooms though under the same genus are possibly correlated to the location and type of substrate from where they are collected. *X. nigripes* in China preferred pH 5 (Chen et al. 2014): *X. hypoxylon* collected in Bangladesh was favored at pH 6 (Ahmed and Jahan 2017) while another strain of *Xylaria* species from India showed the highest biomass yield at pH 5.5 (Ramesh et al. 2014).

Effect of physical factors. The most suitable media with the best pH were used to evaluate the influence of physical factors such as temperature, illumination, and agitation on the mycelial biomass production of the two mushrooms. To efficiently exploit mushroom biomass, it is necessary to understand the relationship between these environmental factors and biomass production. Three different temperature conditions including 8°C, 21°C, and 28°C were used in this study in order to understand which of these conditions can support the better growth of the two mushrooms. Table 3 presents the mean biomass yield after 7 days of incubation to different physical factors. Cultures incubated at 28°C significantly recorded the highest mycelial biomass yield of both mushrooms, indication that the two mushrooms are tropical species. However, lower yield was obtained at 21 °C and no biomass growth was observed at 8 °C. Other species under the genus *Xylaria* showed optimum biomass production under 28°C -30°C such as *Xylaria* sp. (Ramesh et al. 2014) and *X. hypoxylon* (Ahmed and Jahan, 2017).

Table 3. Mycelial biomass yield of *C. striatus* and *X. hongkongensis* grown in PSB at pH 7 as affected by temperature, illumination, and agitation after seven days of incubation.

Physical factors	**Biomass yield (mg/30 ml)**	
	C. striatus	***X. hongkongensis***
Temperature		
8°C	0.00±0.00^{c}	0.00±0.00^{c}
21°C	106.63±11.65^{b}	145.90±44.64^{b}
28°C	205.80±19.00^{a}	183.80±1.20^{a}
Illumination		
Lighted	283.62±14.70^{a}	201.39±51.72^{b}
Dark	234.50±22.50^{b}	249.40±37.20^{a}
Agitation		
Agitated	407.62±65.91^{a}	266.11±12.91^{a}
Static	199.51±7.87^{b}	236.40±9.33^{b}

In each column, means with the same superscript are not significantly different from each other at 5% level of significance.

Illumination is crucial to the physiological processes of all organisms including mushrooms, some species rely on the availability of light for successful mycelial proliferation (Cheng et al. 2012). The culture bottles of *C. striatus* incubated at 28 °C under lighted conditions produced a higher yield (283.6 mg/30ml) compared to mycelial cultures incubated in the dark. The presence of light has a positive impact on the biomass production of *C. striatus*. Illumination helps greatly in regulating the fermentation process which in turn enhanced the mycelial biomass production of fungi (Cheng et al. 2012). This study supports the findings of other related studies (Smania et al. 1997; Kalaw et al. 2016). However, dark conditions favored biomass production of *X. hongkongensis* (249.4 mg/30ml). The response of *X. hongkongensis* to illumination coincides with the findings for *Cyclocybe cylindracea, Pleurotus djamor,* and *Pleurotus salmoneostramineus*, which did not show sensitivity to light (Landingin et al. 2020; Jacob et al. 2015).

Agitation (100 rpm) improved the yield of both mushrooms which produced 407.6 mg/30 ml (*C. striatus*) and 266. 1 mg/ 30 ml (*X. hongkongensis*). The mycelial biomass of *C. striatus* and *X. hongkongensis* in their optimized submerged culture conditions are shown in Fig. 1.

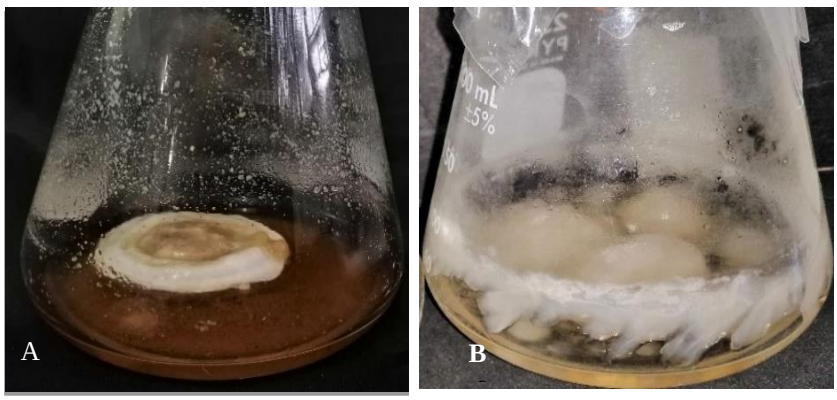

Fig. 1. Mycelial biomass of *C. striatus* (A) and *X. hongkongensis* (B) in an optimized submerged culture condition after seven days of incubation.

A significant increase in the biomass yield in agitated condition could be attributed to shaking of the media which resulted in the efficient distribution of nutrients and oxygen to the entire network of mycelia (Cui et al. 1997; Peng et al. 2000). There is a positive correlation between biomass growth and energy dissipation in liquid fermentation (Kelly et al. 2004). Similarly, the agitation has a positive effect on *Xylaria nigripes* which produced a high biomass yield in a shaker at 100 rpm (Chen et al. 2014). A considerable increase in biomass production, glucose consumption, and lipid biosynthesis was observed in different mushroom species including *P. ostreatus*, *G. lucidum*, *Auricularia auricula*, and *Lentinus edodes* in shaking conditions (Diamantopoulou et al. 2012).

Antibacterial activity of mycelial extracts. The ethanolic extracts of *C. striatus* and *X. hongkongensis* mycelia were assessed for their antibacterial potential against Gram-negative, E. *coli*, and Gram-positive *S. aureus* bacteria. The mean diameter zones of inhibitions after 24 hours of incubation are shown in Table 4. The two extracts showed inhibitory activity against *S. aureus* with a mean of 11.93 mm diameter zone of inhibition for *X. hongkongensis* and 11.27 mm diameter zone of inhibition for *C. striatus* while no inhibitory activity was observed against *E. coli* (Fig. 2).

Table 4. Antibacterial activities of *C. striatus* and *X. hongkongensis* mycelial extract against *E. coli* and *S. aureus.*

Treatments	Zone of inhibition (mm)	
	E. coli	***S. aureus***
C. striatus extract	6.00±0.00^{b}	11.27±0.55^{b}
Streptomycin sulfate	32.33±0.45^{a}	33.13±0.15^{a}
Ethanol	6.00±0.00^{b}	6.00±0.00^{c}
X. hongkongensis extract	6.00±0.10^{b}	11.93±0.15^{b}
Streptomycin sulfate	32.33±0.45^{a}	33.13±0.15^{a}
Ethanol	6.00±0.00^{b}	6.00±0.00^{c}

In each column, means with the same superscript are not significantly different from each other at 5% level of significance. Streptomycin sulfate served as positive control and ethanol served as negative control.

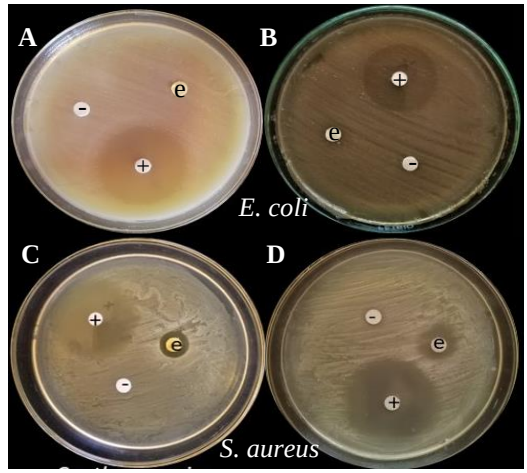

Fig. 2. Antibacterial assay plates showing the zone of inhibitions exhibited by *C. striatus* (A, C) and *X. hongkongensis* (B, D) mycelial extract against *E. coli* and *S. aureus.*

C. striatus and *X. hongkongensis* are capable of suppressing the growth of pathogenic bacteria. Previous studies demonstrated that mushrooms naturally produce some novel secondary metabolites with known antibacterial properties such as triterpenes, alkaloids, and anthrones (Chudzik et al. 2015). Related studies on *Xylaria* species revealed the antibacterial potential of these mushrooms. For example, *X. papulis* demonstrated an intermediate effect against *S. aureus* and *E. coli* (De Leon et al. 2020). Another *Xylaria* extract inhibited the growth of *Pseudomonas aeruginosa* and *S. aureus* (Ramesh et al. 2014). Ten *Xylaria* isolates inhibited the growth of *S. aureus,* Bacillus *subtilis, Pseudomonas aeruginosa,* and *Escherichia coli* (Orachaipunlap et al. 2015). Other mushrooms under the genus *Cyathus* including *C. intermedius, C. colensoi,* and *C. pallidus* possess antibacterial activity against *E. coli*, *Mycobacterium tuberculosis, Staphylococcus albus, Streptococcus hemolyticus α, Streptococcus hemolyticus β* and *Streptococcus pneumonia* (Liu and Zhan 2004; Shakouri et al. 2014).

Cytotoxic activity of mycelial extracts. Mushrooms are good resources for bioactive compounds with promising anti-cancer activity. Brine shrimp lethality assay, a preliminary step to a more advanced study of cell toxicity, can be used to reveal anticancer activity. Both extracts were toxic to brine shrimp larvae with LC_{50} values of 134.90 µg/ml for *C. striatus* and 141.25 µg/mm for *X. hongkongensis*. Although the mushroom extracts demonstrated toxicity, their efficacy is incomparable to cyclophosphamide, an anticancer drug, which is cytotoxic at a concentration of 16.30 µg/ml (Moshi et al. 2010). Both extracts were more toxic than *X. hypoxylon* and *X. papulis* extracts (Ahmed and Jahan 2017; De Leon et al. 2020). In addition to this, *Cyathus subglobisporus* and *C. striatus* showed great potential in inhibiting the proliferation of cancer cells (Nitthithanasilp et al. 2018; Sharvit et al. 2017). *Xylaria curta* and other *Xylaria* species also exert antitumor activities against lung cancer cells (Orachaipunlap et al. 2015; Ramesh et al. 2015). The cytotoxic activities of these mushrooms can be linked to the secondary metabolites they contain such as terpenoids, steroids, flavonoids, and phenolic compounds (Ramesh et al. 2015). Indolic compounds, striatins C and cyathins from *C. striatus* demonstrated strong activity against human breast and pancreatic cancer cells (Sharvit et al. 2021; Fares et al. 2022). Although indole derivatives exhibited strong cell growth inhibition ability, these can be used in anticancer drug development since they are not toxic to normal cells (Fares et al. 2010). *X. allantoidea* was found to contain several secondary metabolites such as allantoside which demonstrated antiproliferative activity (McCloskey et al. 2017). However, the compounds responsible for the cytotoxic activity of *C. striatus* and *X. hongkongensis* used in this study are still unknown. Further studies need to be conducted in order to investigate the mycochemical constituents and their mechanisms of action.

Teratogenic and toxic effects of *C. striatus* and *X. hongkongensis* extract. The use of zebrafish embryos is an efficient way to assess the teratogenic activity of certain compounds in humans. The morphological abnormalities can be readily observed due to the transparency and visibility of the internal structures; aside from that, its genetic composition is almost similar to the human genome (Howe et al. 2013). Some compounds can negatively affect the processes during embryonic development which in turn alters the morphological characteristics of the organism and is obviously shown as it matures (Elefant et al. 2020).

In this study, the hatching rate was found to be dependent on the concentration of the extracts (Table 5). Control embryos hatched normally while a lower hatching rate was observed in groups directly exposed to the different concentrations of the extracts after 72 hours. The highest number of embryos hatched at 1 µg/ml, with 88.91 % (*C. striatus*) and 77.85 % (*X. hongkongensis*) being not significantly different from the control group. However, embryos exposed to 10 µg/ml concentration of the extracts showed 44.4% and 33.3% hatching rates. A much lower number of embryos hatched at 100 µg/ml extract concentration (22.2 % for *C. striatus* and 11.1 % for *X. hongkongensis*) however, no embryos hatched at 1000 and 10,000 µg/ml suggesting that the extracts have a dose-dependent effect on the hatchability of zebrafish embryos.

Table 5. Hatchability rate of zebrafish embryos at different concentrations of the mushroom extracts after 72 hours.

Mushroom Extract (µg/ml)	% Hatchability	
	C. striatus	***X. hongkongensis***
Embryo water	100.00±0.00^{a}	100.00±0.00^{a}
1	88.91±19.27^{a}	77.85±38.59^{ab}
10	44.41±19.28^{b}	33.34±0.00^{bc}
100	22.28±19.23^{bc}	11.17±19.27^{c}
1,000	0.00±0.00^{c}	0.00±0.00^{c}
10,000	0.00±0.00^{c}	0.00±0.00^{c}

In each column, means with the same superscript are not significantly different from each other at 5% level of significance. Embryo water served as the control.

Growth retardation was observed among the embryos as a result of direct exposure to the mushroom extracts (Fig. 3B-E). It is possible that the mushroom extracts inhibit enzymes responsible for hatching (David and Pancharatna 2009). Aside from growth retardation, embryos with bent tail were observed in 100 µg/ml concentration of *C. striatus* extract (Fig. 4F). These morphological abnormalities are considered teratogenic effects of the extracts (Nagel, 2002). Tail malformations (perverted tail, hook-like tail, twisted tail) wavy somite, pericardial edema, and head deformation are the other reported morphological abnormalities caused by other mushroom extracts (Dulay et al. 2012; Dulay et al. 2014; De Castro et al. 2016; Sogan et al. 2018). Natural teratogenic compounds from mushrooms trigger irregular circulation of blood, limiting the cells with the necessary nutrients it needs to function properly, similar to known teratogens like hydroxyurea, retinoic acid, and valproic acid. It also affects the expression of genes responsible for the production of glucose, triglycerides, and cholesterol (Miao et al. 2022). Thus, these results demonstrated that exposure of zebrafish embryos to *C. striatus* and *X. hongkongensis* mycelia extracts can lead to undesirable morphological conditions.

Fig. 3. Teratogenic and toxic effects of *C. striatus*. and *X. hongkongensis* extracts on zebrafish embryos after 72 hours. Normal hatched embryo (A), growth retardation at 1 µg/ml to 1000 µg/ml extract concentration (B-E), Bent tail at 100 µg/ml extract concentration of *C. striatus* (F), coagulated embryo (G, H)

Embryo mortality (100 %) was observed at 10, 000 µg/ml concentration of both extracts after 12 hours (Table 6). Increasing mortality was observed in 1000 µg/ml as a result of prolonged exposure to the extracts. However, dead embryo was not observed in 1 to 100 µg/ml concentrations of both extracts in all observation periods except for 100 µg/ml concentration of *X. hongkongensis* which caused 11.11 % mortality after 48 hours. These results indicate that high concentrations and prolonged exposure of zebrafish embryos to *C. striatus* and *X. hongkongensis* extracts have lethal or toxic effects (Nagel et al, 2002).

Table 6. Mortality of zebrafish embryos exposed to the different concentrations of mushroom extracts after 12, 24, and 48 hours.

Mushroom Extract (µg/ml)	**% Mortality**		
	12H	**24H**	**48H**
C. striatus			
Embryo water	0.00±0.00^{b}	0.00±0.00^{b}	0.00±0.00^{b}
1	0.00±0.00^{b}	0.00±0.00^{b}	0.00±0.00^{b}
10	0.00±0.00^{b}	0.00±0.00^{b}	0.00±0.00^{b}
100	0.00±0.00^{b}	0.00±0.00^{b}	0.00±0.00^{b}
1000	11.11±19.20^{b}	88.90±19.20^{a}	100.00±0.00^{a}
10000	100.00±0.00^{a}	100.00±0.00^{a}	100.00±0.00^{a}
X. hongkongensis			
Embryo water	0.00±0.00^{c}	0.00±0.00^{b}	0.00±0.00^{b}
1	0.00±0.00^{c}	0.00±0.00^{b}	0.00±0.00^{b}
10	0.00±0.00^{c}	0.00±0.00^{b}	0.00±0.00^{b}
100	0.00±0.00^{c}	0.00±0.00^{b}	11.11±19.20^{b}
1,000	33.33±0.00^{b}	88.99±19.20^{a}	100.00±0.00^{a}
10,000	100.00±0.00^{a}	100.00±0.00^{a}	100.00±0.00^{a}

In each column, means with the same superscript are not significantly different from each other at 5% level of significance. Embryo water served as the negative control

The introduction of various chemicals to the embryonic system can cause obstruction in blood flow leading to the malfunctioning of the different organs (Wang and Zhong 2020). Coagulation observed at high concentrations and longer exposure time could be due to the activity of toxic compounds present in the extracts. Some active compounds require a longer time of accumulation in the cell before taking effect (Abdel-Tawab 2021). Results obtained in this study validate the findings for *X. papulis* ethanol extract which caused heart problems in zebrafish embryos (De Leon et al. 2020). These mushrooms are natural sources of triterpenes, flavonoids, and alkaloids, all of which have cytotoxic properties (Mendoza et al. 2020). Given these findings on the cytotoxic ability of *C. striatus* and *X. hongkongensis*, further testing against various cancer cell lines to determine their antiproliferative property needs to be done. Furthermore, the identification of the active constituents responsible for this biological activity should be elucidated.

CONCLUSION

The productivity of mushrooms in submerged fermentation varies depending on the species. In this study, both species prefer potato sucrose broth with pH 6-8 as their optimum nutrient source. *X. hongkongensis* can be effectively grown in PSB, CW, and RBB. Incubation at room temperature and agitating conditions increased mycelial biomass production. The presence of light can improve the mycelial growth of *C. striatus* while it has no direct effect on the growth of *X. hongkongensis*. Both mushrooms have antibacterial properties against *S. aureus*. The ethanol extracts of the mushroom mycelia have potential cytotoxic and teratogenic properties. Therefore, these mushrooms can be used as sources of novel compounds for the development of effective antibacterial and cytotoxic drugs.

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IMPLEMENTATION OF ARTIFICIAL INTELLIGENCE AND BLOCKCHAIN IN AGRICULTURAL SUPPLY CHAIN MANAGEMENT

Yandra Arkeman[1], Nizmah J Hidayah[2], Aries Suharso[3], Faza Adhzima[3] and Try Kusuma[3]
[1]Department of Agricultural Industrial Technology, Faculty of Agricultural Technology, IPB University, Bogor Indonesia
[2]Doctoral Student, Agro-Industrial Engineering Study Program, Postgraduate School, IPB University, Bogor, Indonesia
[3]Doctoral Student, Department of Computer Science, IPB University, Bogor, Indonesia
Correspomding author: yandra@apps.ipb.ac.id,aniznizmah@apps.ipb.ac.id

ABSTRACT

Today's agricultural sector cannot avoid the need for advanced digital technology, such as robots, artificial intelligence, and the Internet of Things to deal with various agricultural problems that can disrupt national stability. Agro-industrial supply chain management ensures that agricultural products reach consumers in quantity, quality, and safety required. The supply chain includes from farm to consumer, to table, from farmer to processing producer, and distribution to the consumer, as well as other long chains that cover from upstream to downstream. Some of the problems that need to find solutions include a lack of information on raw materials needed in processing, transparency of product sources, information on production processes, information on logistics distribution to consumers, and lack of traceability and traceability (products, quality, documents, costs, halal status, and value chain). Digital transformation along the supply chain must be carried out immediately through: (1) implementation and improvement of traceability systems using blockchain technology, and (2) blockchain integration with smart and rapid tests or Internet of Things (IoT) using Artificial Intelligence (AI) to enhance blockchain intelligence. Smart and rapid tests are needed to detect and identify the substance levels of a product, while the IoT architecture applied along the supply chain connects various supply chain actors to share data. Machine learning and cloud computing are used for data processing and communication networks for information transfer. Blockchain and AI-based digital systems allow consumers to track transaction history and product status in just a few seconds. Blockchain integration with AI will bring about a more reliable system called a smart blockchain.

Key words: agricultural supply chain management, artificial intelligence, blockchain.

INTRODUCTION

The development of human civilization in the 21st century is marked by the Industrial Revolution 4.0 where there has been rapid progress in digital technology in various fields, combining automation technology with cyber technology "cyber-physical system". Innovations such as internet-connected robots, AI (Artificial Intelligence), cloud computing, microchips, machine learning, deep learning, cloud analytics, blockchain, nanotechnology, quantum computers, biotechnology, 3D printing, and autonomous vehicles.

The Industrial Revolution 4.0 not only has tremendous potential in overhauling the industry but also changing various aspects of human life. The concept of the industrial revolution 4.0 brings together physical industrial resources with the development of digital technology. In the agricultural sector, which is the main sector providing food for the lives of many people, digital transformation is a necessity in overcoming upstream and downstream challenges. In agricultural management, combining digital technology with potential local wisdom resources will renovate the agro-industry to become an efficient upstream-to-downstream production value chain business model. The successful impact of

agricultural development with advanced technology 4.0 has an impact on increasing income and availability of staple food for the community.

In precision agriculture, the use of the right resources and the application of digitalization technology to agricultural production systems is an important part of agricultural development in Indonesia. The application of Artificial intelligence (AI) and blockchain technology in the agricultural industry helps address the availability of world food needs with innovative and efficient production systems. Blockchain technology combined with AI enables tracking, information tracking, and transparency in the food supply chain so that data can be tracked in real-time.

Artificial Intelligence and Blockchain: State of the Art

A. Artificial intelligence (AI)

*Artificial intelligence (AI)*is the part of computer science concerned with the design of computer systems that mimic human intelligence. The system takes input data, processes it, and produces an output which that can perform many complex tasks even more difficult for humans with very large data capacities.AI itself is the forerunner of today's intelligent machine development by imitating human abilities. Including machine learning and deep learning which are sub-fields of AI. The following are types of artificial intelligence:

- Adaptive learning and systems
  The ability to adapt behavior and develop general rules based on experience (Adamu and Awwalu 2019).
- computer vision
  The ability to analyze perceived scenes by relating them to internal models that represent "knowledge of the world" (Karn 2021).
- Robots and sensors
  A combination of most or all of the above abilities with the ability to move over terrain and manipulate objects (Chen et al. 2021).
- Understanding language
  The ability to "understand" and respond to natural language (Mah et al. 2022).
- Problem-solving/expert system
  The ability to formulate problems in appropriate representations, plan solutions, and know when and how to get new information (Rao 1999).
- *Speech recognition*
  Users can communicate with the computer using voice. An example is the voice search feature provided by Google (Smadi et al. 2015).

The benefits of AI include automated decision-making, recurring tasks, and reducing human errors. AI fuels the development of technologies like big data, the internet of things (IoT), and robotics, AI Tools *Neural Networks, Fuzzy Logic, Expert Systems, Genetic Algorithms, Cellular Automata*, and others

B. *Cloud Computing, Big data, and Machine learning.*

Cloud computing is a combination of the use of computer technology in data storage and management, and internet-based (cloud) development, where users are given access rights (login) to use the cloud. Big data or data with large volumes in real-time provides information that can increase the productivity and efficiency of using the data (Behari et al. 2016; El-Seoud et al. 2017).

Machine Learning is a scientific field regarding computer algorithms that are used to automatically improve the performance of computer programs based on data. The way it works is to collect, process, and compare data (from small to large) to look for patterns and analyze the differences. Data processing with data ingestion, data mining, data mapping, and data science.

In the agricultural sector, AI, cloud computing and big data, and machine learning are used predictive properties of algorithms for smart farming. Wind speed prediction is needed to increase the amount of energy generated. Power demand and price forecasting accuracy are considered as one the most important research problems in electrical engineering today and in the future. The predictive nature of various algorithms makes them the best instrument for meeting energy and power engineering challenges (Arumugam et al. 2022). The data can be used and drawn as predictions about rainfall patterns, water cycles, and fertilizer requirements and is used by farmers to make smarter decisions, such as what crops to plant for better profits and when to harvest. This ultimately increases the volume of agricultural output and financial returns.

C. Blockchains

Blockchains can be interpreted as a distributed ledger that tracks every activity in the blockchain from the transactions of every entity involved. The application of blockchain technology can provide benefits for all supply chain actors, from small producers, processors, and distributors to consumers(Zheng et al. 2017; Usman et al. 2021). Blockchain technology helps improve supply chain efficiency by providing a tracking system for all events that occur in the supply chain and product quality. The principles used by blockchain are:

1. Blockchain is a peer-to-peer data storage technology in the form of a distributed system.
2. Every peer or authority who has a real identity can make transactions and verify the information directly with other parties, without going through a third party.
3. *Blockchains* has a consensus data verification system and a named transaction record security system *cryptography*
4. *Blockchains* that we use in Indonesia can be connected to blockchain networks in other countries.

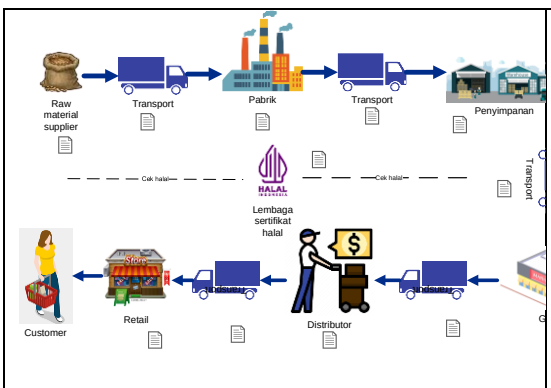

Fig. 1. Conventional supply chain business model

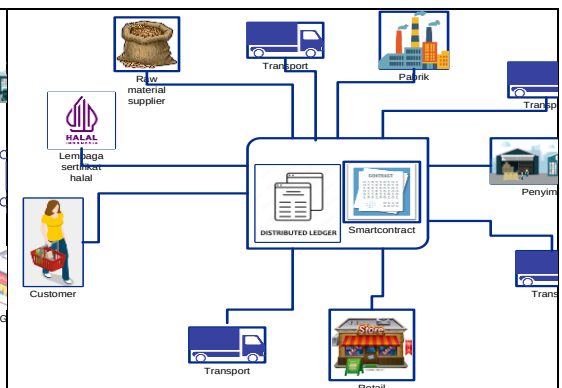

Fig. 2. Blockchain business model

Differences between conventional supply chain business models and blockchain business models:

Conventional supply chain management business model

- The length of the value chain in marketing (Gosier, distributor, and retail)
- Value chain information from procurement to consumers is not well documented because the system is still centralized
- a centralized system will be vulnerable to falsification of information on every activity in the value chain
- The mix between halal and non-halal materials, and equipment is very large and cannot/difficult to trace
- Halal traceability will be difficult because of the falsification, which results in delays in the halal audit process

Blockchain supply chain business model

- *Blockchains* making the system integrated so that the value chain in marketing can be shorter, namely only located at the retailer
- Value chain information from procurement to consumers using a distributed system
- Where the information is Trusted, Transparent, and Traceable (3T), it helps make it easier for auditors to track product halalness in all activities in the value chain.

Blockchain reliability has 3T characteristics(Iansiti and Lakhani 2017), that is :

- All transaction records (records or BLOCK) before being stored must be validated by all members (peers) visible to all entities involved (Transparent)
- The current record is linked to the previous record and the next record (such as a chain or CHAIN) and is locked with a highly secure cryptographic system (such as a password) so that the record is irreversible. (trusted)
- Because they are well structured (block & chain), transaction records are easy to trace both backward and forwards (tracing, tracking, and traceable)

METHODOLOGY

System design. The general method used in research systems consists of several stages, namely problem-solving, architectural design, program implementation, and evaluation (Fig. 3).

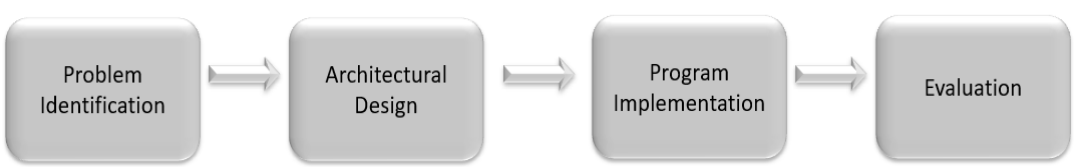

Fig. 3. The general method of system design development

Developing a system design begins with identifying backgrounds and problems, Requirement analysis provided comprehensive information to develop a system design of AI and blockchain. the stages of system design development are problem identification, architectural design, program implementation, and evaluation. (Fig. 4).

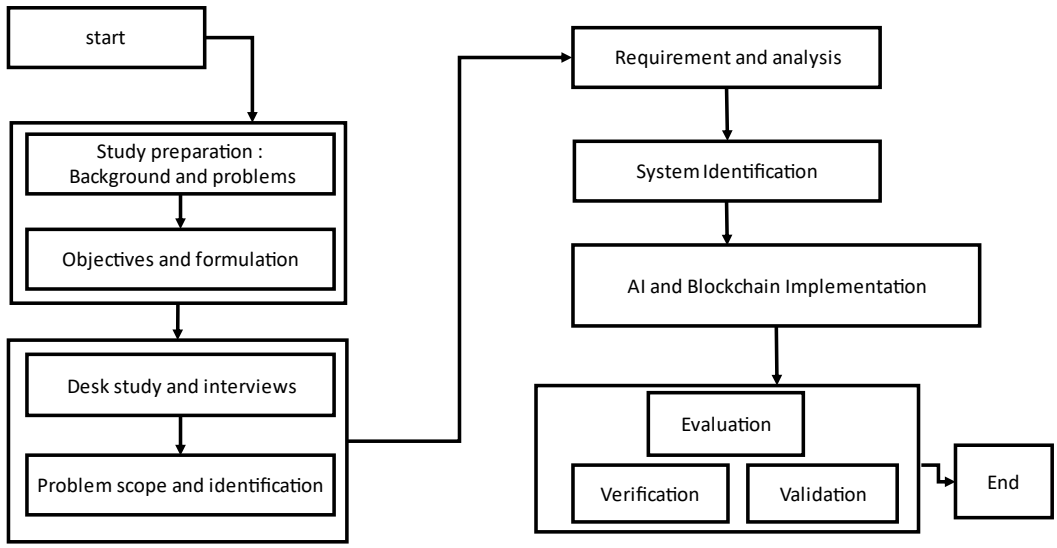

Fig. 4. System design implementation flowchart

Artificial intelligence focuses on image, text and voice-based applications, leading to the groundbreaking development of self-driving cars, speech recognition algorithms and recommendation systems. alternative graphic-based machine learning systems that deal with three-dimensional spaces, which are more structured and combinatorial than images, text, or sound. In particular, function-based learning to produce conceptual designs. use of neural networks to evaluate existing designs encoded as graphs, extract significant building blocks as subgraphs, and combine them into new compositions. exploring the application of generative adversarial networks to come up with completely new and unique designs (As et al. 2018).

Designed the system architecture into two main components, i.e., hardware and service. The hardware-oriented design included IoT devices, servers, and smartphones. In contrast, the service-oriented design included client, system management, and cloud services (Surasak et al. 2019). Blockchain system development needs to be preceded by a system requirements analysis to determine the components needed in the blockchain system. Requirements analysis also helps with the coding process. Two Unified Modeling Languages (UML) diagrams, namely use case diagrams and sequence diagrams, are used to analyze system requirements. The results of their study show that the blockchain system requires two inputs, namely (1) the structure of the supply chain and the activities that occur within it, and (2) four stakeholders (farmers, collectors, agro-industry, and exporters) (Iswari et al. 2019).

Implementation of artificial intelligence (Ai) and blockchain in agricultural supply chain management

The concept of agriculture in the future will not only increase agricultural production (push strategy) to be sent to the market (domestic and export) regardless of whether the market needs these commodities or not, but must be built with a pull strategy. Agro-industry must be established a lot to boost the agricultural sector. With this approach, agricultural products will be accommodated by the industry to be used as materials with higher added value required by the market. The problem of

overproduction of agricultural commodities will never occur because regardless of the amount of these agricultural commodities, the industry will absorb them at reasonable prices and farmers will be more prosperous (Arkeman 2021). The strategic role of agro-industry is to connect the agricultural sector on the upstream side, and the industrial sector on the downstream side. To accelerate upstream and downstream connectivity, agricultural digitization is the right step so that farmers and producers, and industry can expand market share both domestically and abroad. Digital technology can increase farmers' technical knowledge, enabling more efficient calculations of the use of fertilizers, seeds, or other agricultural inputs; and improve farmer decision-making through information on weather, crop management, market conditions, or livestock data (World Bank Group 2020).

Currently, the world's population reaches 7.9 billion and the World Bank estimates this number will continue to grow to reach 9.7 billion in 2050 and 11.2 billion in 2100. Requires an increase in agricultural and food production by 70% to meet the needs, so the intervention of computational tools and forecasting strategies of artificial intelligence and machine learning as the integration of a predictive multidisciplinary approach to improve the food and agriculture sector (Ben Ayed and Hanana 2021).

To catch up with demand, consumer needs must be met with safe supply, advanced digital technology is part of the agro-industrial system by transforming raw materials into agricultural products that are ready to be used and have added value, both from an economic aspect, social and environmental.AI is applied to increase productivity and efficiency, as well as to address labor shortages and environmental sustainability concerns (Lakshmi and Corbett 2020). The problems in the supply chain are the long flow of supply chain information, lack of transparency, less extensive marketing reach, and documentation activities that are not good enough so blockchain-based digitization using the System Development Life Cycle (SDLC) method and the Unified Modeling Language results in a more systematic, documented and effective flow of supply chain information (Iswari et al. 2020).

AI influences production process factors, information sharing, and supply chain integration (Supply Chain Integrity), and AI positively influences Supply Chain Risk Management, which can mitigate supply chain risks by increasing visibility, risk, sourcing, and distribution capabilities. five critical areas of AI usage; (i) transparency, (ii) ensuring, last-mile delivery, (iii) offering solutions to upstream and downstream supply chain stakeholders, (iv) minimizing the impact of disruptions, and (v) facilitating dynamic procurement strategies (Modgil et al. 2021). The concept of AI is used to observe the quality of food and agricultural products with techniques that are fast and do not spoil the product. The expert system approach, artificial neural network (artificial neural network), and fuzzy logic (AI consisting of an expert system, artificial neural network (ANN), and fuzzy logic) determine the quality of food to produce high and optimal results, the best modeling and effective in accurate time monitoring technique (real-time) (Mohd Ali et al. 2021).

The development of smart technology in the potato agro-industry sector shows the success of optimal and adaptive system design, the use of the Internet of Things (IoT) in remote sensing is carried out to predict the amount of crop production and production capacity (Yusianto *et al.* 2020).

Artificial neural networks using the Hadoop framework predict efficient agricultural yields using metaheuristics, exploring ANN models with multi-temporal satellite data systems in areas that have the potential to produce corn and soybeans.(Saranya and Nagarajan 2020). An example of the convergence of precision agriculture is where farmers respond in real time to changes in plant growth with nanotechnology and artificial intelligence (AI) controlling the nutrient cycle and crop productivity (Zhang et al. 2021).

The advantages of blockchain can identify existing stakeholders in the supply chain in each ecosystem from upstream to downstream so that it can transform an inflexible and uncertain supply chain system into an efficient performance supply chain with transparency and trust between

stakeholders so that integration, collaboration, and accuracy of accurate data information (Ekawati et al. 2021).

A new method was developed using an internal camera on a camera-based smart device for estimating lycopene content and grading tomatoes by classifying color values. This method detects quickly and does not harm the fruit. Traditional methods for determining the lycopene content in tomatoes usually rely on destructive sampling followed by chemical analysis, which is not only expensive but also time-consuming (Ye et al. 2018).

Growing applications of precision agriculture include driverless tractors for tillage, drones for the monitoring of fields and plantations, and even a combination of bioinformatics and genetic algorithms for searching for superior seeds. The harvest and post-harvest processes are automated using fast and non-destructive quality testing methods. The agricultural product collection system can determine the shortest vehicle route with ant colony optimization techniques. Blockchain and big data technologies increase the transparency of goods and monetary flows along the value chain of agricultural products(Arkeman 2021).

Robotics and artificial intelligence technology, an intelligent packaging system, an adaptive inventory system with a non-linear model for perishable agro-industrial products, an intelligent vision system for product sorting, non-destructive quality testing for final product quality assessment, intelligent bioreactors, intelligent agro-logistics systems using blockchain technology and agent-based modeling techniques to study changing consumer preferences and many others(Arkeman 2021).

Image-based meat purity detection methods that can be operated on Android device, so the proposed computationally method is Convolutional Neural Network (CNN). The method can do the learning process independently with object extraction and classification. While the other capabilities that can handle image deformation such as rotation and scale. Molecular Technology can be used as an accurate alternative solution to authenticate/ensure DNA content. Molecular technology with a rapid test method for identifying contaminants in meat and processed food is the Polymerase Chain Reaction (PCR) technique. Both conventional PCR, Multiplex PCR, and the latest is Real Time PCR. This technique is an analytical technique used to detect DNA in one type of living thing and is less precise when used to detect commercial food products consisting of a mixture of several types of meat (Yulianti et al. 2021; Purwantoro et al. 2022).

Digital transformation along the supply chain must be carried out immediately through: (1) implementation and improvement of traceability systems using blockchain technology, and (2) integration of blockchain with smart and rapid tests or Internet of Things (IoT) using Artificial Intelligence (AI) to increase intelligence blockchains. Smart and rapid tests are needed to detect and identify the substance levels of a product, while the IoT architecture applied along the supply chain connects various supply chain actors to share data. Machine learning and cloud computing are used for data processing and communication networks for information transfer (Dwivedi et al. 2021).

Case Study AI And Conceptual Design with Blockchain

Clustering types of diseases of rice plants based on leaf image, results of clustering types of diseases of rice plants are optimized using fuzzy c-means (FCM) and genetic algorithm (GA).

Occasionally, rice plant diseases can threaten growth and even cause crop failure. The damage to leaves can be identified and identified by the application of clustering image processing techniques to identify the types of diseases present in rice leaves. Machine learning for identifying rice diseases is the fuzzy C-means (FCM) approach. The advantage of the FCM method is that it can reach convergent cluster centers and has an unsupervised nature. The disadvantage of this method is that

local optima occur frequently and are sensitive to the initial cluster centers, so changing the initial clusters can strongly affect the results (Chaghari et al. 2018).

Many researchers have proposed and implemented optimization algorithms to address the shortcomings of this method. One of the proposed optimization algorithms is the Genetic Algorithm (GA). GA method can be used as an alternative method that can be applied to the optimization process to avoid local optimum(Mas`udia and Wardoyo 2013). Image processing and computer vision have the potential and play a very important role in technology in the field of agriculture (Kurniawati et al., 2009). Computer vision can be used to analyze all kinds of data, not just images from cameras (Nixon and Aguado 2012).

Recognition using the artificial sense of human vision and limited knowledge of rice disease types would be time-consuming, laborious, and inefficient. Therefore, machine learning methods are helpful for help to make predictions on large amounts of data and a learning algorithm (GA) is proposed. The GA-FCM optimization evaluation results show that the addition of cluster groupings achieves the best cluster centroids more precisely compared to FCM, and the proximity of the optimized model using GA-FCM shows a larger range of cluster centers. Additional variation patterns for distances and cluster-centers

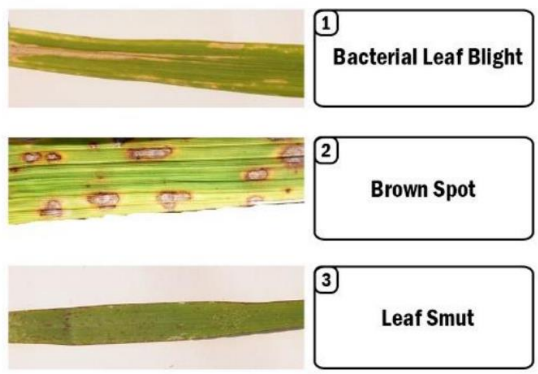

Fig. 5. Image of rice leaf on rice leaf diseases

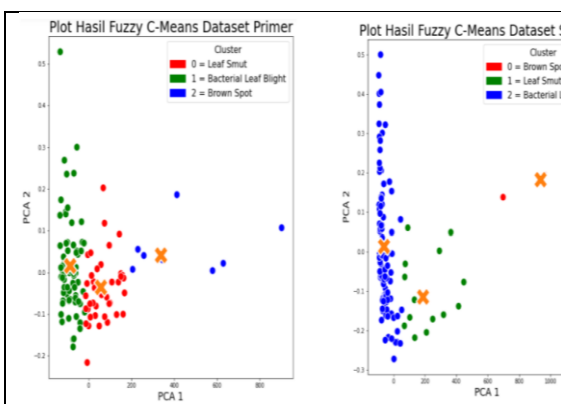

Fig. 6. Identification of the type of disease (FCM)

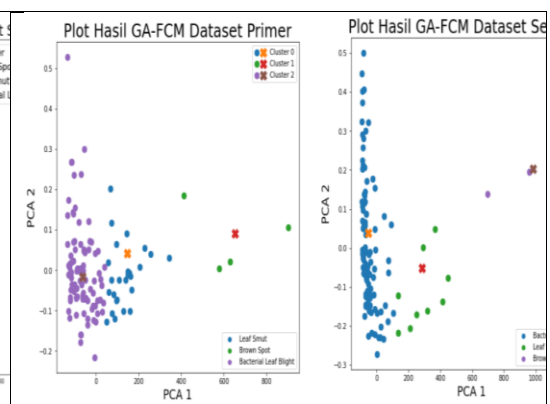

Fig. 7. Identification of the type of disease (GA-FCM)

Random forest prediction model for drought sugarcane land using satellite image data

One of the productivity problems of rainfed sugarcane farming is prolonged drought, lack of rainfall, and lack of water supply in the soil during the vegetative growth phase.(Reyes-González et al. 2018).In addition, high temperatures during the ripening phase can reduce the conversion of sucrose to fructose and glucose(Mishra and Singh 2010). Climate change can also cause diseases and pests (Li and Yang 2015).

It is necessary to monitor drought conditions to schedule proper irrigation based on the response of plants to drought at different stages of vegetation (Mahan et al. 2012). Measuring crop response to drought is difficult and complex Similarly detecting and integrating crop water deficits is still complex based on single crop responses (Hull et al. 2018).

Grouping of four methods for monitoring plant response to drought (Ihuoma and Madramootoo 2017), that is:

(1) groundwater measurement;
(2) Groundwater balanced approach;
(3) Plant-based approach;
(4) Remote sensing method.
the best remote sensing approach is based on the vegetation spectral index obtained from Unmanned Aircraft Vehicle (UAV) hyperspectral sensors, aircraft, and satellite imagery in space. Considering that sensor rental costs are relatively inexpensive, the determination of indicators of leaf moisture status and plant stomata conductance is high. Non-destructive, non-labor intensive, and suitable for automation. So remote sensing methods are widely adopted as irrigation scheduling decisions.

Classification and prediction of sugar cane fields that experience drought have quite complex challenges, this is due to the need to build sufficient resolution land cover maps under conditions of rare satellite visits and lack of cloud-free data. This greatly affects the quality of classification and prediction.

The developed machine learning model is capable of predicting the drought level of sugarcane fields in the PTPN X Kediri region, East Java Indonesia, which can then be developed into an early warning system as an effort to support precision agriculture 4.0 for sugar cane farmers and sugar mills to schedule patterns irrigation in rainfed sugarcane fields. The research objectives are:

- Overcoming the lack of cloud-free satellite image observation data by combining some Landsat-8, Sentinel-2, and MODIS satellite imagery data for the 2017 - 2020 period

- Develop a good workflow for processing satellite image data before the classification process (Pre-Process) by applying the Pan-Sharpening image sharpening stage and Watershed image segmentation patterns and determining satellite image features based on Gray Level Co-occurrence Matrices (GLCM) •contrast, •energy, •homogeneity, •entropy

- Designing a prediction model for drought in sugarcane fields using the Ensemble Learning RF concept mapped crop drought with hyperspectral index through machine learning classification method for persistent cloud areas with high temporal dynamics of land cover types. To overcome the main problems in monitoring satellite imagery-based remote sensing, image processing methods along with machine learning can be adopted in classifying land use land cover (LULC) to the phonology of certain plants (sugarcane).

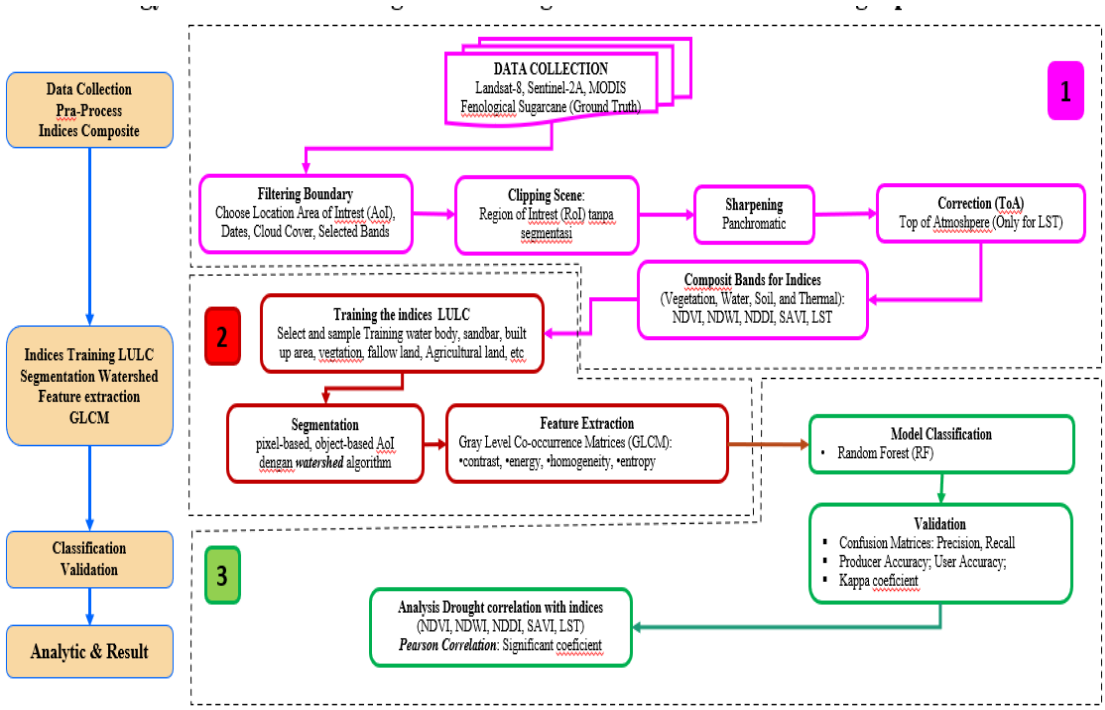

Fig. 8. Classification of sugarcane drought based on remote sensing optical data

Fig. 9. RGB Composite, NDVI, LST

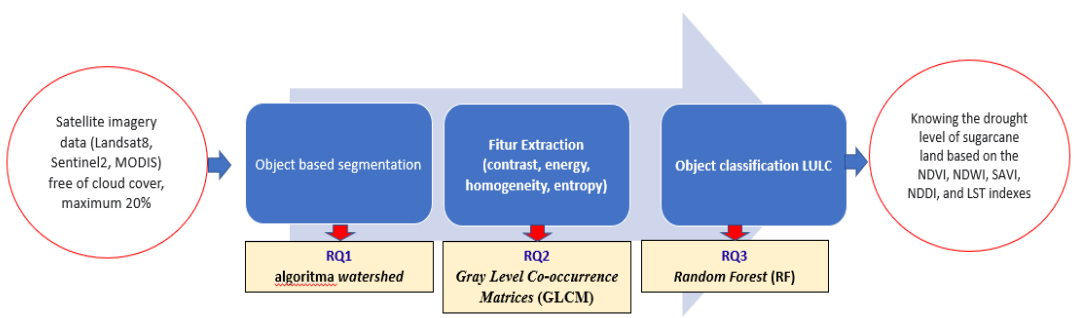

Fig.10. The workflow of the sugarcane field drought prediction model using remote sensing methods

Multi-drone coordination for precision farming using k-means clustering algorithm, ant colony optimization, genetic algorithm & 3-opt

Environmental issues regarding the use of pesticides for plants and their effects will potentially pollute the environment which can experience movement and degradation(Scholtz and Bidleman 2007; Liu et al. 2015).

Reducing the negative impact of environmental pollution using pesticides, applying pesticides to plants according to the dosage or dosage. Effective fertilizer or pesticide spraying drone operating system on a very wide agricultural land based on plant health level data. Drones with camera payloads can be used for plant health monitoring(Neupane and Baysal-Gurel 2021; Hafeez et al. 2022). The multispectral camera capability installed on the UAV produces data that is more analytical and effective compared to satellite data(Bollas et al. 2021).

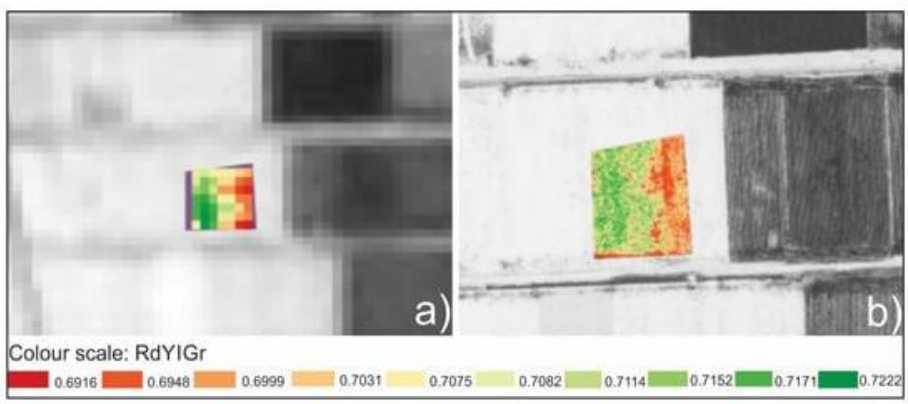

Fig. 11. Comparison of image data (a) Sentinel-2 data and (b) UAV multispectral data at a scale of 1:1000
(Bollas et al. 2021)

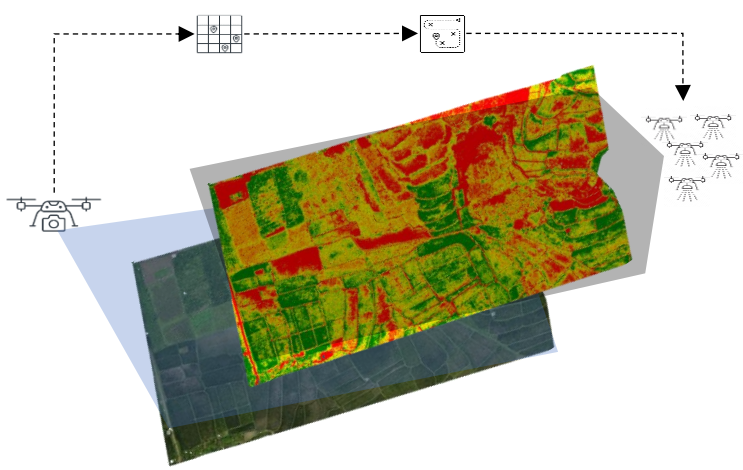

Fig. 12. Illustration of the operating system concept to be implemented

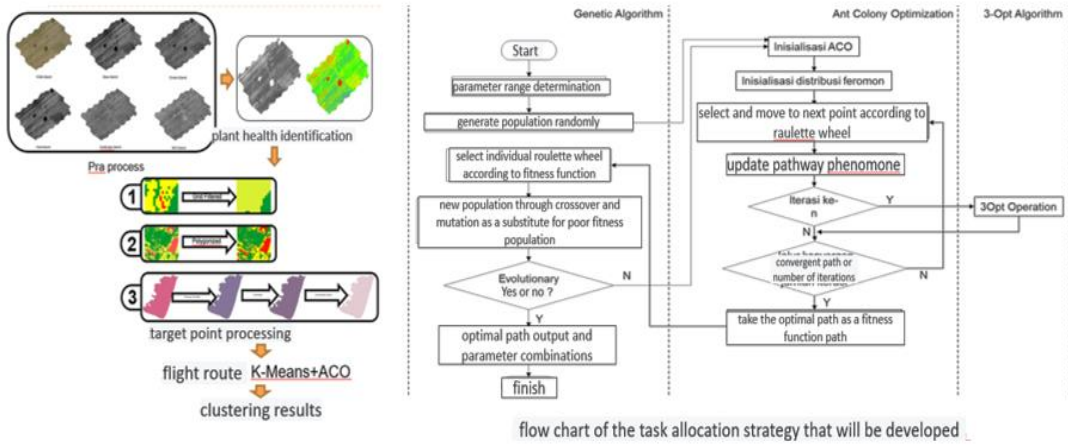

Fig. 13. Research stages

1. K-Means Clustering is used to group spraying target points into several groups, depending on the size of the area and the number of drones available
2. Genetic Algorithm to update the parameters contained in Ant Colony Optimization
3. Ant Colony Optimization functions to allocate drones to each area group (the result of the K-Means Clustering process)
4. 3-Opt is used to optimize the results of Ant Colony Optimization (local optimization) calculations

This method is used to create a point database with plant health level parameters, as well as to produce a task allocation strategy for several drones which are expected to be able to design flight routes effectively.

The application of AI when connected to the blockchain as a traceability application and data integration will produce applications with the highest intelligence in solving problems in agriculture. Presently, Supply chain Management (SCM) has been also growing in Agriculture and Healthcare. Blockchain offers excellent capability of supply chain management in a transparent and distributed manner. The IoT, AI can be integrated with the Blockchain to expand the performance of the real-world application. Integration of IoT, AI and Blockchain fulfill the dynamic requirement of supply chain stakeholders in Agriculture (Singh and Singh 2020; Dwivedi et al. 2021).

Combining artificial intelligence and blockchain technology, decentralized AI applications and algorithms are built with access to identical visions of a trusted and shared data platform to store knowledge, record and make decisions. This platform is useful when keeping reliable records of all AI algorithms before, during and after the learning and decision making process (RegPac 2022)

CONCLUSION

With digitalization in the agro-industry, it is very easy for the government and stakeholders to ensure product availability, both for domestic and foreign consumers. With the development of blockchain and AI technology, it can innovate super smart tools for agroindustry. Integration of machine learning, AI, and blockchain supported with information systems, creating a quality monitoring system to deliver real-time information and quality predictions. The system increases the ability of supply chain actors in making related decisions. The integration of blockchain technology and AI in the development of intelligent systems and applications in supply chain management introduces

new solutions for food safety and transparency, trust, and traceability. The use of blockchain technology and AI can improve quality monitoring, and halal status and control production effectively and efficiently using smart devices. Further research is needed to combine AI with blockchain into a smart application or platform to overcome problems on the agro-industrial supply chain management.

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SHAPING A BETTER PRIMARY INDUSTRY THROUGH SMART TECHNOLOGIES

Ramadhona Saville*, Katsumori Hatanaka and Nina N. Shimoguchi
Department of Agribusiness Management, Tokyo University of Agriculture,
1-1-1 Sakuragaoka, Setagaya-ku, Tokyo 156-8502 Japan
*Corresponding author: sr203424@nodai.ac.jp

ABSTRACT

This paper presents the development of smart technologies application for shaping a better primary industry (agriculture and fishery) with several study cases in Asia, namely Japan and Indonesia. In the next decades, increasing the productivity of small-scale crop, livestock, fishery and forestry production systems will be key to achieving global food security. Smart technologies in the primary industry certainly help farmers and fishermen to monitor many aspects of the production part which can lead to better decision making, management, efficiency and eventually productivity. In order to shape a better primary industry, we applied smart technologies combined with data science for the agriculture and fishery sector in several case studies. Specifically, (1) application of a low-cost sensor network and data science to analyze the variables influencing fruit tomato sweetness in a Japanese greenhouse farm; (2) fish finder and data science utilization to evaluate catch amount and fish kind classification within set-net in Japan; (3) application of smart technologies for better mariculture in Indonesia. In light of the evidence, smart technologies can enhance the primary industry.

Key words: Smart technologies, Data science, Primary industry,

INTRODUCTION

The United Nation estimated that the world population will reach 8.5 billion people in 2030, 9.7 billion people in 2050 and will eventually reach 11 billion people in 2100 (UN 2019). The estimated growth of the population means a big challenge to provide food for the people in the future. Several reports have strategized how to provide and distribute food for the world in the future 2050 (FAO 2009; Searchinger et al. 2019) and 2100 (IARFR PAS 2021). At the same time, as the world economy rises compared to several last decades, people tend to increase the consumption of higher quality foods and more resource-intensive, animal-based foods (Sans and Combris 2015; Ranganathan et al. 2018; Andreoli et al. 2021). On the other hand, cutting greenhouse emissions from every aspect including agriculture and fishery as the main source of food is needed to ensure sustainability (Garnett 2011). Therefore, a strategy is crucial to fulfilling the want and need for food production in the future sustainably and effectively.

Primary industry is generally defined as an industry that involves in extraction and production of raw materials and natural resources, including forestry, mining, agriculture and fishery. Primary industry is very essential for our basic needs, as it included agriculture and fishery, the major producer of food for humans and feed for animals. Agriculture itself is broadly defined in Oxford English Dictionary (1971) as "The science and art of cultivating the soil, including the allied pursuits of gathering in the crops and rearing livestock (sic); tillage, husbandry, farming (in the widest sense)." While fishery is defined as "A part of the sea or a river where fish are caught in large quantities and also a place where fish are bred (kept in order to produce young) as a business (Oxford English Dictionary 1971)." As the definition stated above, the fishery can be divided into wild or capture and culture (Edwards and Demaine 1998; Lam 2013). Several institutions and previous studies considered fishery as agriculture in a broader sense (Scannes 2018; USDA 2020). Several institutions such as the

United States Department of Labor (2020) and the United States Bureau of Labor Statistics (2022) suggested that agriculture and fishery belong to the same category, food-related producers.

It simply means, straining agriculture and fishery is essential for not only present but also future sustainable, effective and efficient food production. In the last decade the term "smart agriculture" and "smart fishery" are widely used in both academic and mass media (Aqeel and Shaikh 2009; Ray 2017; Rose and Chilvers 2018; Honarmand-Ebrahimi 2021). This study defines smart agriculture as the combination of agriculture and the application of smart technologies. Similarly, the smart fishery is the combination of fishery and the application of smart technologies. The "smart" in smart technologies was initially an acronym of "Self-Monitoring, Analysis, and Reporting Technology" and was used in computer hardware in the early 1990s (IBM 2019). Yet, nowadays smart technologies are defined as innovative or new technologies that can integrate computing and telecommunication technology into other technologies that did not previously have such capabilities, such as the Internet of Things (IoT), Artificial Intelligence (AI), robot, remote sensing and so on (Kozlova 2021). It is necessary to investigate the application of smart technologies in the actual site in the agriculture and fishery sector. This study aims to enrich the discussion of shaping a better primary industry through smart technologies' application in the actual site, namely in several study cases of our research projects in the agriculture and fishery sector as the major food producer. Specifically, (1) application of a low-cost sensor network and data science to analyze the variables influencing fruit tomato sweetness in a Japanese greenhouse farm; (2) fish finder and data science utilization to evaluate catch amount and fish kind classification within set-net in Japan; (3) application of smart technologies for better mariculture in Indonesia.

Application of smart technologies for greenhouse fruit tomato. This section discusses the application of smart technologies, namely a sensor network, farming record, cloud system, database and data science for investigating factors that affect the sweetness degree of tomato (Saville et al. 2020). Tomato is one of the most consumed food vegetables in Japan, with a value of 224 billion JPY (approximately 2 billion USD) in 2020 even though it is categorized as fruit (MAFF 2022). Several previous studies showed that Japanese consumers seek quality (high sweetness degree) over quantity when purchasing tomatoes, they are even willing to pay a decent price for the high-quality tomato (Higashide et al. 2012; Amano and Hatanaka 2019). Japanese farmers are reportedly aware of the demand of Japanese consumers and put effort to provide high quality (Amano 2020). Several farmers screen every single tomato using a refractometer to sort tomato grade based on sweetness degree before shipping it to the market. One of the most popular tomato types is the fruit tomato, which is generally known as a type of tomato with a sweetness degree of more than 7 (Yabe et al. 2009; MAFF 2022). According to Amano and Hatanaka (2019), the price of tomato with unmeasured sweetness degree per 250g was 86 JPY, the price of sweetness degree between 7.0 to 8.4 was 500 JPY, 8.5 to 9.9 was 600 and for sweetness degree more than 10.0 was 700 JPY. No wonder why tomato farmers would like to produce sustainable high-quality fruit tomatoes. While some farmers with tens of years of expertise or large industrialized farms can produce sustainable high-quality fruit tomato, small-scale farmers still find it difficult to do so. Farmers would like to know what factors affect the sweetness degree of tomatoes. Therefore, in this section, a study was conducted to investigate the application of a sensor network and data science for determining factors affecting the sweetness degree of tomatoes in actual site operated by fruit tomato farmers (Saville et al. 2020).

This study was conducted in a greenhouse hydroponic tomato farm in Nara Prefecture, Western Japan. The data gathering was conducted from June 2017 to January 2020. This study used two types of data, namely, greenhouse microclimate environment and production data. The microclimate data was automatically acquired from the sensor network (Netatmo) installed inside the greenhouse (Fig. 1), while production data is manually input by the farmer. The data transference scheme in this study is shown in Fig. 2. The sensor network automatically recorded temperature, humidity, air pressure and CO_2 and send the data to the Netatmo cloud server. On the other hand, farmer recorded fertilizer input (NO_3 and Ca), pH, electrical conductivity (EC), water stress, cropping calendar,

the daily amount of harvest and average daily sweetness degree into Google Drive. These two data are then combined in one database server in order to be analyzed. The analysis result is shared with farmer in order to support the decision making of the farmer.

Fig. 1. Sensor network used in fruit tomato greenhouse.

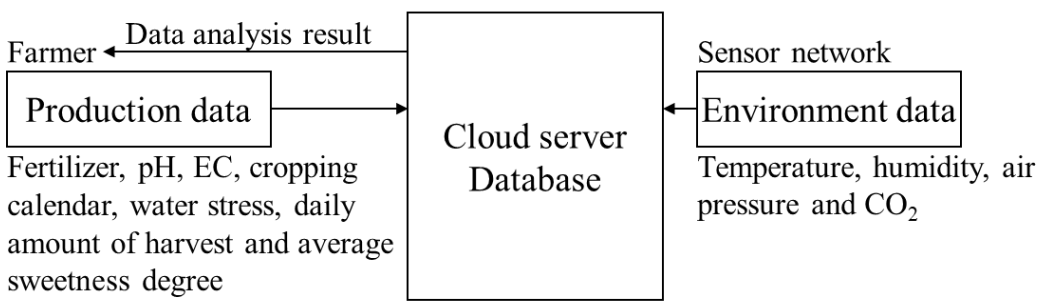

Fig. 2. Data transference scheme used in this section.

EC record through production cycles resemble an S-shaped curve as shown in Fig. 3. The first day of the production cycle is transplanting tomatoes from nursery. After several days, the tomato plant starts growing through the phase of growing time until it produces a flower and eventually tomato. Then, the tomato plant will reach maturity and farmer will start harvesting until the end of the production cycle. The cultivation time during the monitoring period of this research project range between 85 days to 150 days. With this in mind, this study extracted several independent variables from EC record, namely, growing time, growing time slope derivative, average, maximum, minimum and standard deviation of EC value both in growing time and cultivation time.

The gathered data were analyzed by using multiple regression analysis (MRA) in order to determine the factors affecting sweetness degree. Independent variable selection is needed to avoid multicollinearity before conducting MRA (Farrar and Glauber 1967). One of the ways is to check the correlation of independent variables. This study used the extracted EC variables and exclude fertilizer (both Ca and NO_3) as well as pH due to the high correlation between them. The independent variables. This study also considered maximum, minimum, average, cumulative as well as standard deviation of temperature and humidity as independent variables. Besides, this study also considered cumulative of

CO_2 and water stress. The dependent variable was the sweetness degree of fruit tomato. Next, a pre-processing standardization using z-score was conducted in order to make the variables at the same level of value. Subsequently, MRA was conducted several times to get the best model possible in this study. The MRA was evaluated using AIC and R-squared values (Akaike 1974). When AIC was comparatively high and R-value was comparatively small, this study omitted the independent variable with high P-value as well as changed the combination.

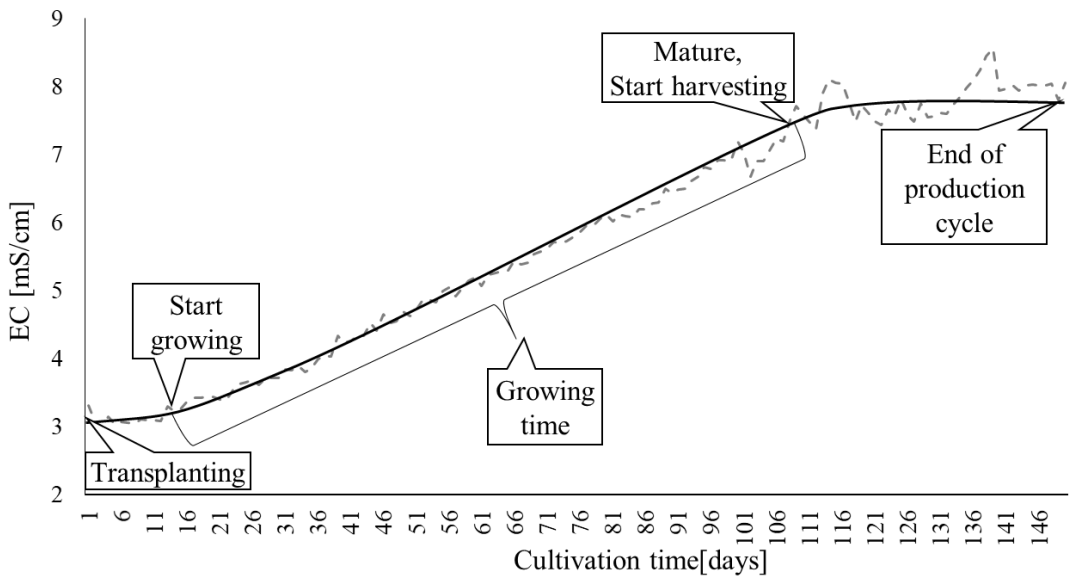

Fig. 3. EC record in one production cycle of tomato fruit.

After conducting several MRA, the best result was gained when the R-value was 0.91 and AIC was 13.11. The summary of the best MRA model is shown in table 1. The most significant independent for sweetness degree was growing time as indicated by the smallest P-value. This study then investigated the relationship between growing time and sweetness degree. The relationship between growing time and sweetness degree is shown in Fig. 4. The longer the growing time, the sweeter the fruit tomato will be. Yet, the sweetness degree of a fruit tomato will have its peak because the tomato will rot at a certain degree. Due to that reason, this study decided to use a three-degree polynomial curve as a trendline. In light of the evidence, smart technologies are useful to investigate factors affecting the quality of fruit tomato. The result was then shared with farmer as a decision support system for decision-making in production.

Table 1. MRA summary of sweetness degree of fruit tomato production

Variables	Estimate	Std. Error	T-value	P-value	α
Intercept	7.5329	0.07553	9.739	< 2e-16	
Growing time	0.61848	0.12402	4.987	0.00041	***
Cultivation time	0.65096	0.18156	3.585	0.00428	**
CO_2 cumulative	-0.3109	0.1653	-1.881	0.08674	.
Water stress cumulative	-0.0805	0.08979	-0.897	0.38913	

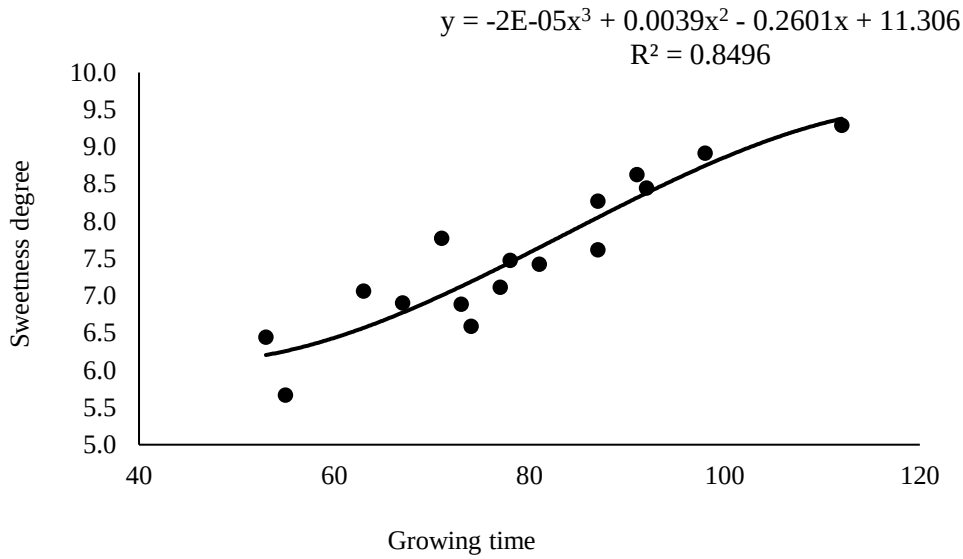

Fig. 4. The relationship between growing time and sweetness degree of fruit tomato.

Application of smart technologies for set-net fishing. This section discusses the application of smart technologies, namely ubiquitous buoy (Wada et al. 2008), catch fishing records, cloud system, database and data science for predicting fish catch in set-net (Saville et al. 2015). Set-net is one of the most popular fishing methods in Japan and across Asia. The fact that fishermen do not know the catch quantity in advance and will only be aware of the harvest situation once they arrive at the set-net region is one of the challenges with set-net fishing. When the amount of fish trapped in the set-net is particularly large and the fishermen are not ready for such a significant number of catches, they must load the fish and travel to the port, then return to the set-net to load the rest. The fishermen must do more effort, lose time and pay more for labor and gasoline. It is necessary to create a more effective and efficient set-net fishery monitoring system in order to address the issue. Thus, fishermen need a real-time monitoring system of fish trapped in set-net.

This study was conducted in set-net sites in Hokkaido, Toyama, Shizuoka and Mie Prefecture, Japan. The data gathering was conducted from June 2013 to July 2015. This study used two types of data, namely, ultrasonic wave monitoring data and catch data. The ultrasonic wave monitoring data was automatically acquired from a ubiquitous buoy installed on the final trap of the set-net, while production data is manually input by the fishermen (Fig. 5). All of the data was compiled in one cloud database. Next, the data was analyzed and visualized in website, therefore, the fishermen can monitor the fish trapped in the set-net in real-time. The ubiquitous buoy in this study is a modification of a fish finder that can transmit data (ultrasonic wave ping data) to the cloud in real-time. The ubiquitous buoy was installed in the final trap of the set-net because the fish most probably cannot get out of the final trap and the trapped fish is the catch of the fishermen. Fish finder is generally known for the ability to detect based on swim bladder. The intensity of ping data is highly dependent on the amount of fish below the water. In Fig. 6, body of the water is colored blue, when the buoy detects fish, the monitor indicates in green, yellow and red brownish color. The more the number of fish, the higher the ping value is with the darker color.

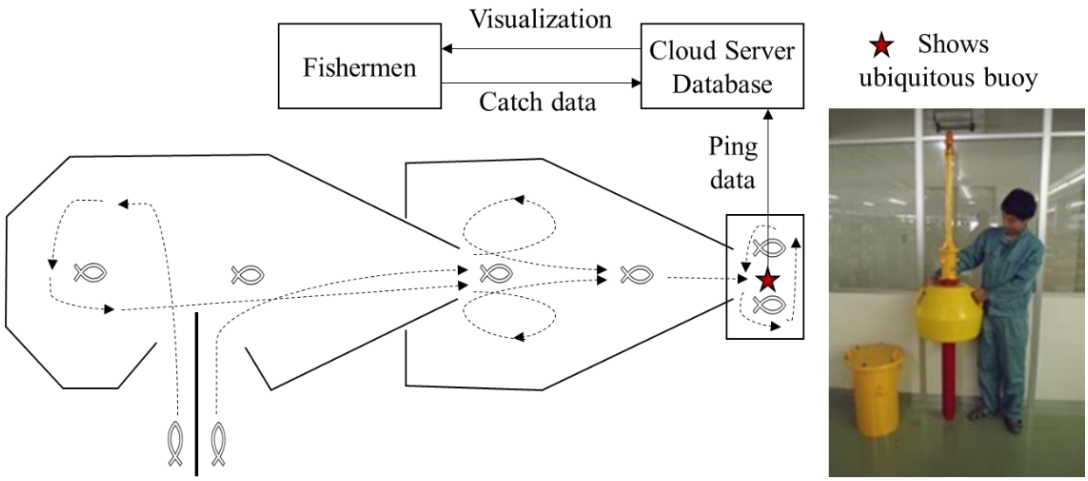

Fig. 5. Sketch of set-net and data transference.

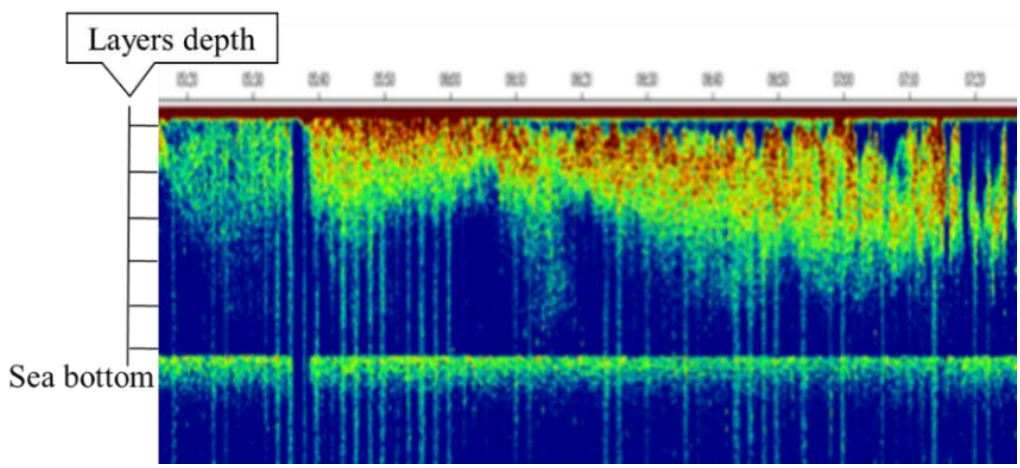

Fig. 6. Screenshot of ping data from ubiquitous buoy.

This study used MRA in order to predict the amount of fish trapped in a set-net. The independent variables were ping data. Yet, this study divided the water into 5 depth levels because fish usually move in different level depth at different time. In particular, the independent variables were the maximum, minimum, and average of ping values of each layer and sea bottom. The dependent variable was the catch amount. However, the amount of catch varies greatly, ranging from tens of kilograms to several tons. To minimize this variability, the study applied the Box-Cox Transform pre-processing, which scales the data values to fall at the same level. After the preprocessing, the data is used for MRA. As also discussed in the previous section, MRA was conducted several times to get the best model possible in this study. Next, the estimation results are converted into the same unit of catch by reverse transformation.

The best result in this study was obtained by using the maximum value of each layer and the minimum value of the sea bottom with the R-value was 0.97. The comparison of catch estimation and the real catch is shown in Fig. 7. Relative Absolute Error (RAE) is employed in this study to quantify the discrepancies between the algorithmic catch estimation and the real catch. The RAE of catch

estimation in Toyama was 22% compared to the real catch record. The fishermen stated, besides fish catch estimation, real-time monitoring to show the situation in the set-net was already useful for fishermen. The fishermen can check the set-net condition anytime anywhere as long as they have an internet connection. Finally, it is important to note that smart technologies are useful for supporting set-net fishermen.

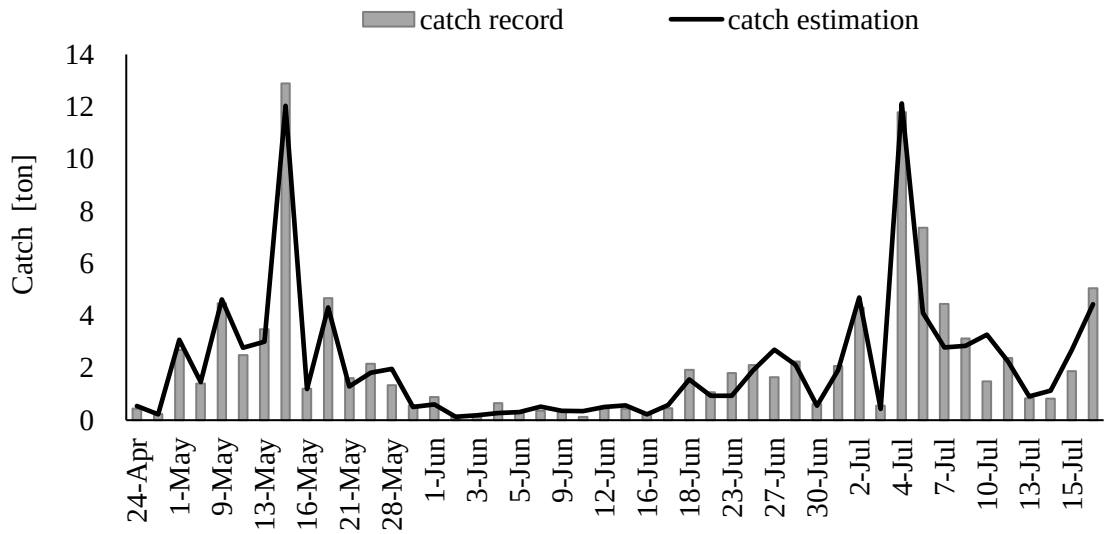

Fig. 7. Comparison of estimated catch amount and real catch data in Toyama site.

Application of smart technologies for Indonesian mariculture. This section discusses the application of smart technologies to face issues in Indonesian mariculture. Indonesia's unique geography, with over 17,000 islands and 81,000 km of coastline, provides an ideal environment for the development of the fishery sector, including both capture fishery and aquaculture. In 2020, Indonesia's production of capture fishery amounted to 7 million tons, while the production of aquaculture reached over 14 million tons, according to FAO (2022). The most commonly farmed species in Indonesia include shrimp, pelagic fish and seaweed as reported by the Indonesian Ministry of Marine Affairs and Fisheries (MMAF 2022). The MMAF has been actively promoting aquaculture activities among coastal communities, with a particular emphasis on mariculture, which involves the cultivation of aquatic animals and plants in marine environments (MMAF 2021).

Indonesia is one of the largest producers of aquaculture in the world. Indonesian aquaculture produced a massive 14.8 million metric tons in 2020, second to China (World Bank 2023). Yet, many issues occur in Indonesian mariculture. For example, grouper fish mass mortality in mariculture sites, Harmful algal blooms (HABs) caused some people to stop running aquaculture or investigate the suitability index for seaweed aquaculture. Indonesia is one of the largest exporters of grouper globally, yet sustainable production and day-to-day operation in mariculture site is affected by mass mortality. Therefore, a mortality monitoring system, as well as prediction, is necessary for grouper fish farmers' decision-making. On the other hand, HABs that periodically occur cause negative effects on marine biotas including fish, including mariculture sites. Hence, the investigation of the trigger of periodic HABs is necessary in order to prevent the occurrence in the future. Factors affecting seaweed production need to be clarified for better production and day-to-day operation planning. A seaweed suitability index in a form of a visualization map is important to clarify factors affecting seaweed production.

Gondol, Northwestern Bali Island is one of the largest grouper mariculture centers in Indonesia. However, Gondol also has the same problem as grouper mariculture elsewhere, high mortality rates. In general mortality rate of grouper in mariculture is 50%. When the number of groupers today is 10,000 but tomorrow 100 die, day-to-day operations, for example, the feed that must be prepared and given to the fish is also different for today and tomorrow. That's why a system is needed to monitor the number of groupers in the mariculture site, including a prediction system for the number of fishes. Data transference to monitor and predict fish mortality is shown in Fig. 8. The water quality sensor network (Wada et al. 2019) on grouper mariculture site automatically transmits temperature, current, salinity, conductivity, chlorophyll, turbidity and DO (dissolved oxygen) once in 30 minutes. While fish farmer input daily fish mortality records to the same cloud server database. Those data were then combined to develop the fish mortality prediction using the Random Forest algorithm (Saville et al. 2022). The result is shared to fish farmer on the website.

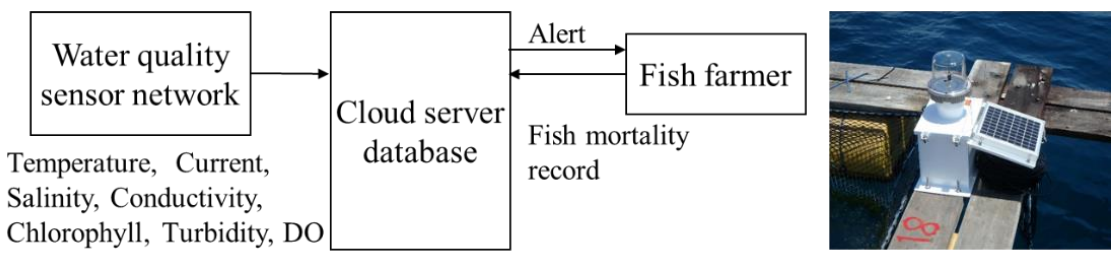

Fig. 8. Data transference scheme used in this section. The water quality sensor network is pictured in the right side.

Periodic HABs in Lampung Bay, Indonesia have caused a huge economic loss for the area surrounding the Bay. The HABs occurred every year, especially during the rainy season since its massive outbreak in 2012. The occurrence of HABs affected mariculture, in 2010 150 mariculture sites existed in the Bay, but, in 2018 only 26 survived (Saville et al. 2019). Therefore, an investigation of the trigger effect of HABs in the Bay is crucial. This study combined spatial analysis and *in situ* water quality monitoring in order to investigate the trigger of periodic HABs (Saville et al. 2022). The spatial analysis was conducted to investigate land use surrounding the Bay. While *in situ* quality monitoring was conducted to measure the nutrient upstream and downstream of the watershed.

The data used for spatial analysis was the 2018 high-resolution satellite (WorldView-3) and the 2011 Digital Elevation Model (DEM) obtained from NASA. Besides, a five-day extensive land use ground truth survey was also conducted in February 2020. The spatial analysis was divided to land use classification to map the land use and hydrologic characteristics analysis to map the watershed and water flow toward Lampung Bay. The spatial analysis was conducted using ArcGIS. Meanwhile, for *in situ* water quality, this study monitored pH, EC (electric conductivity), NO_2 and NH_3 in four sampling stations (two upstream and two downstream) from February 2020 to March 2021. NO_2 and NH_3 were monitored to represent Nitrogen (N) in the water, as N is known as one of the triggers of HABs. N is also generally known as one of the main compositions of fertilizer as this study suspects fertilizer run-off to be one of the triggers of HABs. The samplings were conducted after rain to test the run-off hypothesis. The analysis results are shown in Fig. 9. This study found that agricultural land in the target area was 1,200 ha with numerous sloppy hills and water streams toward the sea. The water stream passes through residential and urban areas. Moreover, the concentration of nutrients downstream was statistically significantly higher than upstream. The results indicated that fertilizer run-off might be one of the triggers for HABs occurrence. Besides, anthropogenic activities from residential and urban areas might also be one of the triggers.

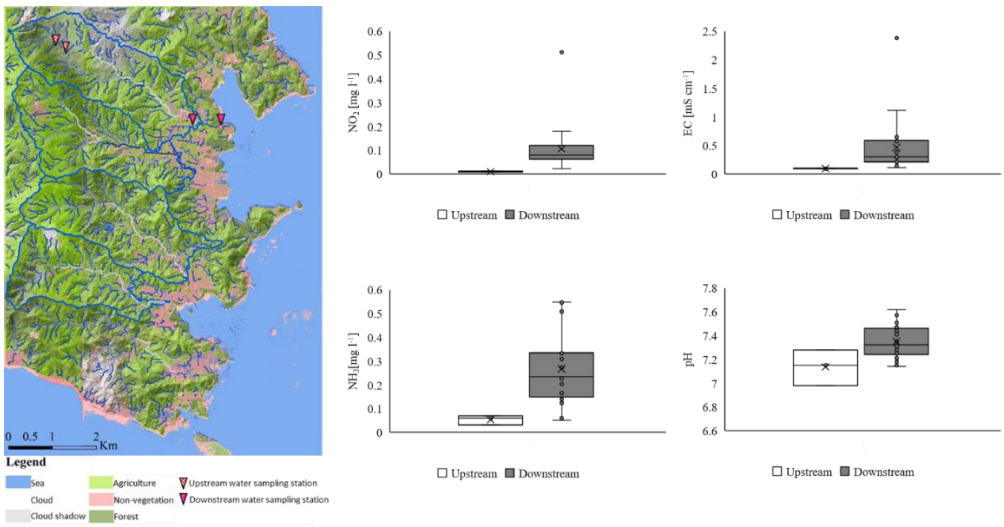

Fig. 9. Map of the spatial analysis result on the left. Boxplot of nutrients in upstream and downstream sampling stations.

Indonesia ranks second in the world in seaweed production at 10 million tons (FAO 2022). Seaweed farming has been a focus of the Indonesian government since 2016 (MMAF 2020). Needless to say, seaweed farming is an important source of income for Indonesian coastal communities. One of the pilot areas for seaweed farming promoted by the Indonesian MMAF is in Seriwe Bay, West Nusa Tenggara Province. However, according to the government of West Nusa Tenggara Province (2018), seaweed production in Seriwe Bay decreased from 14,000 tons in 2012 to 7,000 tons in 2017. Factors affecting seaweed production in Seriwe Bay are necessary to be examined. In order to understand the variables influencing seaweed production, it is necessary to visualize the seaweed suitability index on a map.

To realize the development of the seaweed suitability index, the same water quality sensor network as in Gondol was installed in the Bay. Meanwhile, seaweed farmers input their production data into a cloud server database. However, because there is only one sensor network, it cannot be used for the entire bay. Therefore, this study used the Landsat8 satellite image data series from January 2019 to December 2020 to obtain the sea surface temperature of the whole Bay. The temperature data from the sensor network was used for calibration of sea surface temperature obtained from the satellite. Other than that, this study also used the data of water depth and water circulation flow rate obtained from bathymetry in September 2018. The area of the bay was divided into several rectangular grids size 500×500m. The suitability index was formulated using MRA. The dependent variable was seaweed yield. The result of the suitability index is shown in Fig. 10, where this study divided the index into two seasons in Indonesia, rainy and dry. The P-value of Sea surface temperature and water depth were less than 5%, while, the water circulation flow rate was less than 0.1%. The results indicated that all of the independent variables were significant. Yet, the R-value was 0.13, which means, there is still a remaining unknown independent of 87% for the seaweed suitability index.

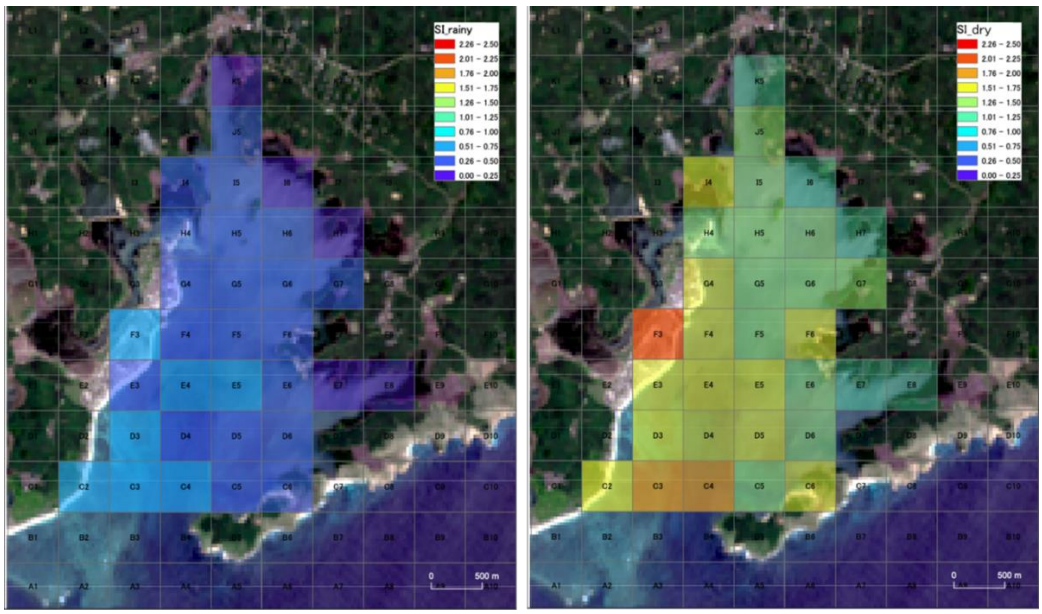

Fig. 10. Map of suitability index of seaweed production for rainy and dry season in target area.

CONCLUSION

This study discussed the application of smart technologies to enhance the agriculture and fishery sector as the primary industry with three study case research projects. The application of sensor networks, cloud database server and data science enable to examine factors affecting sustainable production, provide visualization for both agricultural as well as farmers and ultimately can enable farmers to make informed decisions about resource allocation for day-to-day operations. It should be evident that the research projects are pilot projects and should be continued to gain more stakeholder engagement in this industry. Because if more and more stakeholders apply smart technologies, most probably it will advance the primary industry as a whole, which in turn will be able to produce food more effectively, efficiently and sustainably.

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EXTENDED ABSTRACTS

2022 ISSAAS VIRTUAL NATIONAL SCIENTIFIC CONGRESS

Mainstreaming Climate Change Resilience: Strategies and Management in Agricultural and Food Systems in the New Normal

13-14 OCTOBER 2022

INTERNATIONAL SOCIETY FOR SOUTHEAST ASIAN AGRICULTURAL SCIENCES (ISSAAS) PHILIPPINES

AND

THE AURORA STATE COLLEGE OF TECHNOLOGY

RESPONSE OF GARLIC "ILOCOS WHITE" TO ORGANIC MANURES AND INORGANIC FERTILIZER UNDER CONTROLLED CONDITION IN LEYTE, PHILIPPINES

Nicolasa N. Florentino and Ryan L. Paguibo
Visayas State University, Villaba, Leyte, Philippines
Corresponding author: nnflorentino@vsu.edu.ph

INTRODUCTION

Garlic (*Allium sativum*) is one of the most highly valued vegetables due to its culinary and pharmacological properties. It is one of the ancient allium crops acclaimed by numerous researchers used in treating various diseases (Londhe et al., 2011, Petrovska & Cekovska, 2010)) and among the top most spice in the list of every household. In the Philippines, this crop is grown widely in Ilocos region, provinces of Nueva Ecija, Pangasinan, and Mindoro – all in Luzon area. Therefore, consumers from other regions largely depend garlic, from these areas and imports, at higher cost. Leyte province, situated at the eastern part of the Philippines, is about 1000 km east of Luzon. In this province products like rice (lowland), coconut, rootcrops and fruit vegetables like eggplant, ampalaya, cucumber squash, pepper are produced year-round. To have new crop, an adaptability trial of garlic was conducted (under protective structure) from January to May 2022 in Villaba, Leyte, Philippines. The study primarily aimed to evaluate adaptability performance in the area and specifically to assess the growth and yield responses to the combined farm manure and inorganic fertilization scheme. Due to high N requirement of garlic for foliage and bulb development (Santos et al., 2017), inorganic fertilizer was combined with farm manures in this preliminary investigation.

MATERIALS AND METHODS

An area of 30 m2 (4m x 7.5m) under the greenhouse was manually cleaned, pulverized and divided into 12 subplots each plot measured 1m x 1.5 m with 0.5 m alleyway in between. The study was laid in Randomized Complete Block Design replicated three times with four treatments: T_1 – chicken manure (20 t ha^{-1}+ Inorganic), T_2 – Vermicast (10 t ha^{-1}+ Inorganic), T_3 - Chicken Manure + Vermicast + Inorganic), and T_4 (control)– Inorganic fertilizer (High N 30-10-10). Farm manure was incorporated in the designated plots one month before planting. Garlic var. NSIC 2017 Gr 01 'Ilocos White', with reported yield of 5,550 kg/ha was used. One hundred outer cloves were dibbled (January 10, 2022) up to its length at 10 cm apart in each plot previously applied with specified animal manure. Thereafter, plots were covered with 3-inch thick dried rice straw and watered up to field capacity. Following the recommended dosage, inorganic fertilizer was applied at 14, 28 and 42 days after planting. Watering and weeding were done as needed. Twenty sample plants per plot/replication were randomly selected to obtain data on plant height, number of leaves, bulb weight, number of cloves per bulb and weight of 10 outer cloves. Harvesting was done 110 days after planting and data were analyzed using Statistical Tool for Agricultural Research (STAR) software version 2.0.

RESULTS AND DISCUSSION

Results revealed that the different treatment combinations significantly affected only the number of cloves per bulb but not on the percent survivability, plant height, number of leaves, bulb weight, number of outer cloves, weight of 10 outer cloves and final yield of garlic. Irrespective of treatment combinations, average survivability was 67.5 %, plant height of 61.27cm and 7 leaves at 60 days after planting. Plots with combined vermicast, chicken manure and inorganic (T_3) produced highest number of cloves per bulb (22) and statistically the same ($p>0.05$) with plots applied with chicken manure + inorganic fertilizer (T_1) and vermicast + inorganic (T_2), both with 21 cloves per bulb.

Plots applied with inorganic fertilizer alone (T_4) significantly ($p>0.05$) produced the least number of cloves per bulb (19). Though highest yield was recorded in T_3 (6,430 kg/ha), this was not significantly different from the yields of other treatment plots which was 6,040, 5,650 and 5,560 kg/ha; respectively for T_2, T_1 and T_4, implying chicken and vermicast as good sources of organic materials and partner of inorganic fertilizer for garlic production. More surviving plants, heavier bulb weight and a greater number of cloves produced per bulb contributed to the higher yield of T_3 plots.

CONCLUSION

Though conducted under controlled condition, one location and growing period in Leyte, yield results are comparable to that reported by the NSIC (2017), obviously showing the potentiality of garlic as new crop for Leyte farmers. In addition, the combined effect of incorporating chicken dung and vermicast and inorganic fertilizer material in the growing medium favors adaptation, complete differentiation of garlic cloves and higher yields. A similar study will be conducted under farmer's field condition in different municipalities of Leyte to further assess its productivity under the prevailing agro-climatic conditions.

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INDIGENOUS RICE (*Oryza sativa*) FARMING USING URINE AS ALTERNATIVE SOURCE OF FERTILIZER

Jonnie F. Huervana
West Visayas State University-Calinog Campus, Simsiman Calinog, Iloilo, Philippines.
Corresponding author: jonnie.huervana@wvsu.edu.ph

INTRODUCTION.

Indigenous rice farming was practiced in the upland part of Panay Island and using commercial fertilizer is very expensive. Farmers are searching for alternative sources of cheap fertilizer for rice production. The use of human urine with a compost mixture is the potential solution to this problem (AdeOluwa 2012) and a good source of nitrogen fertilizer substitute (Ranasinghe, et al 2016). Urine and feces are complete fertilizers, rich in NPK and organic matter (Johnson 2004). Urine contributes a major proportion of NPK (Hoglund 2001). Human urine was applied as fertilizer in cabbage and tomatoes without posing any microbial or chemical risk (Pradhan, 2007,2009), however, according to (Club 2010), Sustainable Sanitation Practices should be followed, and removal of pharmaceutical residues is essential (Pronk 2009). Human urine is rich in plant nutrients, but limited information on crop production (Shrestha 2013). The Philippine rice is considered drought resistant with water consumption of 50% less compared to traditional lowland rice varieties (Lampaya 2003), and producing rice with less water is essential for food security (Tuong 2003). Traditional rice (Ulikan) planted in Kalinga Province at 500-2000 above sea level got an average yield of 2.2 to 3.8 tons/ha (Mountain Partnership, 2021). Rice is the staple food for over half of the world's population (Muthayya 2014), and about 3 billion consumed > 100 kg/ person per year (Van Nguyen 2006), and increasing rice production is needed to feed the growing population (Giri 2000). Studies were conducted on vegetables utilizing human urine as a source of fertilizer, but limited information on rice production, hence this investigation is undertaken. This study was conducted to determine the growth and yield performance of indigenous rice in Panay Island using human urine as an alternative source of fertilizer.

MATERIALS & METHODS

The 4 indigenous rice seeds (Malido, Palawan, Kutsiyam, and Sulig) were laid out in Randomized Complete Block Design (RCBD), distributed randomly in treatments: A – Malido, B-Palawan, C – Kutsiyam, and D – Sulig. Urine was taken from BSA male students of WVSU- Calinog and stored for 30 days period before using it as the source of liquid fertilizer. The urine application was done once a week, 5 liters of urine/ square meter for 140 days period of the experiment, conducted at West Visayas State University-Calinog on August 1, 2019, to December 19, 2019. The 10 plant samples/ subplot at harvest includes; the plant height at harvest (cm), the number of tillers, the weight of 1000- grains (g), and rice yield (kg/ha) at harvest were the sources of data for analysis. Analysis of Variance (ANOVA) was used in evaluating and analyzing the data for all the treatments using the Statistical Tool in Agriculture Research (STAR) software.

RESULTS AND DISCUSSION

The application of human urine at the rate of 5 liters/m^2 significantly influenced the height of indigenous rice (tallest- Malido, 150.2 cm) and weight of 1000 grains, (heaviest- Malido 29.60 g) with comparable results in the number of tillers/hill, and the yield of 4 indigenous rice varieties after the experiments. The highest yield of 3,348 kilograms/ha harvested during the trials was within the range of the average yield of Ulikan rice of Kalinga province with an average yield of 2.2 to 3.8 tons per hectare (Mountain Partnership 2021). Indigenous rice produces productively with the application of

human urine used as liquid fertilizer with a 1:1 ratio with water, likely the same result of the study of tomato and cabbage (Pradhan 2007).

CONCLUSIONS

Indigenous rice farming using human urine as an alternative source of fertilizer was effective in the productivity and yield performance of rice. The application at the rate of 5 liter/m^2 was a sufficient amount to supply the NPK requirements of rice for a good harvest.

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GENETIC DIVERSITY AND CHARACTERIZATION OF RHIZOBIA WITH CLIMATE CHANGE MITIGATION POTENTIAL ISOLATED FROM SOYBEAN IN THE PHILIPPINES

Maria Luisa T. Mason[1, 3], Baby Lyn T. De Guzman[2]
[1]Ramon Magsaysay-Center for Agricultural Resources and Environment Studies (RM-CARES), Central Luzon State University.
[2]Department of Crop Science, College of Agriculture, Central Luzon State University.
[3]Department of Soil Science, College of Agriculture, Central Luzon State University.
Corresponding author: mltmason@clsu.edu.ph

ABSTRACT

The global share of agriculture activities to greenhouse gases emissions has increased significantly over the years, especially the N_2O gas. In this study, some indigenous strains of rhizobia with potential ability to minimize N_2O emission to the atmosphere were isolated. Through the Polymerase Chain Reaction (PCR) and sequence analysis of the 16S rRNA gene, internal transcribed spacer (ITS) region, and the rpo*B* housekeeping gene, the 31 representative isolates from the initial 405 isolates were genetically identified wherein all the isolates were classified as bradyrhizobia namely: *B. japonicum*, *B. diazoefficiens*, *B. elkanii* and *Bradyrhizobium* sp. The microcosm experiment indicated that the *B. diazoefficiens* species showed the most significant contribution to soybean inoculation. This study was able to identify some potential microbial inoculant for soybean production and potential strains for climate change mitigation strategy.

Keywords: *B. diazoefficiens*, food security, Polymerase Chain Reaction, symbiotic efficiency

INTRODUCTION

Soybean (*Glycine max* [L.] Merill) is an important food legume in the world and can form symbiosis with rhizobia which allows the plant to use the fixed N by the rhizobia whereas the rhizobia receives "food" from the plant in the form of carbon substrates from photosynthesis. Nitrous oxide is reportedly recognized as the most important ozone-depleting substance in the stratosphere and is closely related with the biogeochemical cycling of N, application of N fertilizers, and microbial activity in the soil. This current study aimed on isolating strains that may possess this particular *nos*Z^+ gene that could also be used as inoculant to increase the production of soybean in the country.

METHODOLOGY

Eleven soil samples were collected from eleven locations in the Philippines which represented the Luzon, Visayas, and Mindanao and these were used to isolate soybean-nodulating rhizobia by using PSB-SY2 as the trap crop. The procedure for growing the soybean plant in growth chamber is reported (Mason et al. 2017, 2018, 2021). After collecting the nodules at 28 days after sowing, the nodules were used to obtained bacterial colony which were isolated, purified, and the DNA was extracted as described (Hiraishi et al. 1995; Minami et al. 2009). The, PCR amplification and sequence analysis of the 16S rRNA gene, ITS region, and rpoB gene were conducted.

RESULTS AND DISCUSSION

Genetic diversity of rhizobia. It can be noted in Figures 1A and 1B that rhizobia isolated on 11 locations in the Philippines is genetically varied. However, it can also be noticed that some isolates

such as NE2-37, BO-52, GI-4, SO-1, LT-3, NE1-65, and NE2-3 do not belong to a particular cluster within the three species.

Symbiotic ability of the representative isolates. The table below shows the amount of N fixed and the symbiotic efficiency (SE) of each representative isolate on soybean. In comparison with the control, all the isolates were able to increase the amount of N on the shoots and this was supported by the computed SE as reported by the methods of Risal et al. (2010).

Isolate	N fixed (mg/plant)	Symbiotic Efficiency	Isolate	N fixed (mg/plant)	Symbiotic Efficiency
IS-2	1.48	5.81	LT-36	0.81	4.55
GI-4	0.63	2.86	BO-4	-1.31	-5.49
GI-8	1.01	4.09	BO-15	1.14	4.55
BA-24	1.50	8.94	BO-52	1.43	7.70
BA-41	1.10	5.92	NR-1	1.28	6.41
BA-42	0.84	3.20	NR-2	1.72	6.65
NE1-6	1.02	4.55	NR-40	0.91	5.62
NE1-19	1.33	6.67	NR-48	1.40	5.68
NE1-34	1.15	6.87	NR-60	2.37	14.31
NE1-65	0.81	3.84	SK-1	1.30	6.75
NE2-1	0.81	4.35	SK-2	1.96	9.07
NE2-3	1.97	8.09	SK-5	1.21	4.85
NE2-37	3.10	9.91	SK-12	2.64	8.57
NE2-66	0.89	3.82	SC-3	1.85	6.74
SO-1	2.23	9.32	SC-49	1.40	5.44
LT-3	0.53	3.05	CONTROL		

CONCLUSION

Potential novel isolates were identified as inoculants for soybean with climate change mitigation ability.

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AN INNOVATION, CONSTRUCTION AND PERFORMANCE EVALUATION OF A ROTARY DRUM DRYER

Agustin L. Rote
College of Agriculture and Forestry, Negros Oriental State University,
Bayawan-Sta. Catalina Campus,
City of Bayawan, Negros Oriental, Philippines 6221
Corresponding author: roteagustin@gmail.com

INTRODUCTION

The advent of the Republic Acts No. 8435, No. 8559, No. 10915 and No. 11203, has stirred Farm mechanization in support of agricultural production of paddy (unhusked rice grains) with the ultimate objective of lowering production cost through efficient utilization of farm machineries.

Rice (*Oryza sativa L.*) production has indeed increased with all the support programs and subsidies by the Philippines Department of Agriculture. But efficient production needed to be synchronous, thus harvest was almost at the same time. This requires Bulk post- harvest facilities for rice drying operation to maintain, improve longevity, shelf life, storability and subsequent post production processing. Often paddy drying is dependent on sun drying which is usually undertaken in concrete paved spaces as basketball courts, and national highways. This coincides usually the time when there are typhoons and thus, drying is hampered and extended time renders our harvest to be spoiled. Thus we need mechanical dryers independent of weather condition without side effects on quality of grains for its subsequent post production processing. Current drying facility using the flat bed batch type drying has some disadvantages such as un-even drying of the material and inclusion of smoke from the heating material used, with its chemical interaction on the dried product. A rotary drum dryer using the principle of conduction drying wherein the heat produced by combustion was used without its by-products. Studies by **(**Acasio and Belonio. 1983**)** and (Belonio and Stickney. 1985) using the Rotary Drum Dryer made with the IRRI – International Rice Research Institute, using a rice hull gasifier as the source of heat. At that time the cost of the dryer system was very expensive, while the operation needed to be properly controlled since excessive heat made pop rice as one of its consequence. The operation used a rice hull furnace as its source of heat and a connector chute from the furnace to the drying cylinder. This is to minimize and control heat to the drying cylinder. After the drying cylinder, a grain cooler was made to receive the heated grains from the drying cylinder. Result of the performance evaluation showed that the dryer system was able to dry the wet paddy from 20 to 27 per cent initial moisture content at a rate of 5 to 6 per cent. Rice hull consumption of the operation is 14 to 16 kg per hour, with the computed thermal efficiency is from 12 to 19 per cent. Milling and head rice recoveries of the rotary-dried paddy in a commercial mill were higher compared with sundried paddy.

MATERIALS AND METHODS

A rotary drum grain dryer was innovated, constructed and evaluated in its performance for paddy drying. The innovation of the dryer system consisted of: 1.) the rice hull furnace, the source of heat of high temperature drying was situated directly below the, 2.) drying cylinder, where the paddy grains were heated for improved heat transfer and, 3.) grain cooler which receives the heated grains, where grains were further cooled to effect the temperature reduction, and /or added low temperature drying. Data of sample paddy rice grains on moisture content and temperature was made using the Shega III grain moisture meter and the standard mercurial type maximum thermometer respectively. Data on different portions of the dryer was taken namely at the inlet hopper, before the drying cylinder,

after the drying cylinder, and after the grain cooler. Retention time of the paddy in the dryer system was recorded using a standard wristwatch.

Rotary-dried paddy rice was evaluated for viability and milling recovery. Viability data was taken by sampling the portion of dried paddy. The total grain paddy rice (TG) was individually counted and recorded. These were tested for viability using the standard seed viability (germination) testing method. Percent Viability (%V) was computed as %V = SG/ TG x 100

Milling recovery was made using a rubber roll rice mill. Initial (TG) total grain mass (kg) was taken before milling and the (TM) total milled rice (kg) was taken after milling, using a platform scale. Per cent milling recovery (% MR) was computed as %MR = TM/TG x 100

RESULTS AND DISCUSSION

Results of the performance evaluation showed the dryer system can dry rice paddy by reducing moisture content of the paddy from initial moisture of 16% mc w.b. reduced to 14.5% mc w.b. after passing the high temperature drying at the drying cylinder and reduced to 10.5% mc w.b. after passing the grain cooler, thus a low temperature drying effect was observed. Therefore, there was moisture reduction in both high-temperature and low-temperature drying. The resulting grain temperatures during the drying operation starts at 30 ^{o}C at the initial receiving hopper. The grains were heated to 55 ^{o}C after passing the drying cylinder for high temperature drying and a reduced temperature to 33 ^{o}C after passing the grain cooler. The paddy can be contained in bags due to grain temperature being optimum for short term safe storage.

Retention time of the paddy grains in the drying cylinder was from 1 to 2 minutes. Viability test of paddy grains had 88 % germination, while result of total milling recovery was 64 %. These data showed that rotary-dried Paddy rice can be used both for commercial milling and as well as seed purposes. The rotary-dried paddy grains as observed were not affected by the products of combustion as smoke, compared to other types of mechanical dryer systems.

CONCLUSION

The dryer system can effect paddy rice drying both in the high-temperature conduction drying with the drying cylinder and in the low-temperature drying with the grain cooler. It can be stored due to its low temperature and reduced moisture content. The rotary dried paddy has no after -effect of the combustion products. The rotary dried paddy can be used for seeds purposes due to its high germination viability result. The rotary dried paddy can be used for commercial milling purposes due to its acceptable milling recovery. Since the current innovation of the rotary drum dryer system is in its prototype, the materials and techniques/ technology skills used in the innovation are considered crude or locally-available, this can be upgraded. The drying machine as a System needed further development, in its capacity and size, with refinement in its operating sections for more efficient operation, making innovation for commercial feasibility.

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YIELD AND QUALITY ATTRIBUTES OF COFFEE BERRIES AS INFLUENCED BY ETHEPHON APPLICATION AT DIFFERENT STAGES OF MATURITY

Jonah Gumban-Zante
Aurora State College of Technology
Corresponding author: jonahgz@yahoo.com

INTRODUCTION

Quality is the key importance in the specialty coffee trade. The paramount consideration is to picked coffee beans when fully ripe to attain the desired quality. Harvest of coffee is a complex and intricate process. Selecting only red, ripe fruits during harvest increased cost of production. Synchronized ripening of berries can help in reducing harvesting frequency thus reduce cost. Now a days, growth regulator is popularly used to effect marked change. One of these is ethylene which is known as the ripening hormone. A carrier of ethylene is ethephon which can promote ripening, flowering and abscission.

The study focuses on the utilization of ethephon to induce uniform ripening of coffee berries. Generally, the study aimed to determine the yield and quality attributes of coffee berries as influenced by ethephon application at different stages of maturity. Specifically it seek to find the ripening of coffee berries in response to different concentrations of ethephon and determine the quality attributes of ripened coffee berries as influence by ethephon application at different stages of maturity.

MATERIALS AND METHODS

Concentrations of ethephon applied to coffee berries 6 months after flower initiation (AFI) were 100 ppm, 500 ppm, 1000 ppm, 1500 ppm, and 1920 ppm. Study 1 was set up following RCBD with six treatments and six replications. The concentration that promoted faster and uniform ripening was used in Study 2. The solution was applied in berries of varying stages of maturity as follows: 5 months old, 6 months old and 7 months old. The untreated berries (8 months old) served as the control. RCBD with 4 treatments and 3 replications was used. Chemical constituents of naturally ripened and ethephon induced coffee berries through sensory evaluation and chemical analysis.

RESULTS AND DISCUSSION

The more marked influence of ethephon was observed in days to ripening. Ethephon is an effective ripening agent especially, if used from 1500 to 1920 ppm. Ripening is faster and more uniform. However, it must be used only for six-month-old or older berries since Ethephon inhibits growth and development of coffee berries. Five-month-old treated berries failed to attain the desired quality of beans after Ethephon treatment. From 21.00 days to ripening of the control berries, the ripening period significantly decreased with the application of Ethephon. The shortest period was in T_4 which ripened at 8.00 days after induction. Younger berries had longer ripening period although still significantly shorter than the control.

Ethephon treatment can also reduce the number of develop berries as well as size and weight, thus, lower production of berries. Significantly faster and uniform ripening was attained with the use of 1920 ppm Ethephon. The process of ripening in the said treatment lasted only for 12 days compared to 40 days of the control or untreated berries. The difference between the two treatments was 28 days, which is highly significant.

Ethephon had no influence on the number of harvested berries but fresh weight of treated berries significantly decreased with increasing concentration. The degree of growth suppression since Ethephon contains ethylene which is a growth inhibitor. In Study 2, the number of acceptable berries was significantly greater in the control (T_1) than T_2 and T_3 but comparable with T_4. However, the number of coffee berries was significantly lower in Ethephon-treated tree than the control. Fresh weight was likewise affected by Ethephon. However, this was only observed when Ethephon was applied to T_2 and T_3. Weight of berries in these treatments was significantly lower than T_4 and the control (T_1). T_2 berries were not able to attain the desired quality. Only T_4 and the control (T_1) were found to be fully developed in terms of yield characteristics, such as fresh weight and size.

Sensory evaluation disclosed that they have the same cupping quality as the control or untreated berries. The chemical analysis of the berries in all treatments were statistically the same except for total carbohydrates and fats. Chemical analysis of green beans also showed similar caffeine content in berries from six-month-old to eight months old (control).

CONCLUSIONS

Results showed that 1920 ppm induced berries promote faster and uniform ripening. It took only 11 days for all berries in the lateral branch to ripen as compared to 40 days of the control. Number of harvested and percent rejected beans were not affected by Ethephon. On the other hand, number and fresh weight of berries significantly decrease with decreasing age of berries upon Ethephon application. The more dramatic effect of Ethephon was on ripening. It took only 8 days to complete the ripening process when Ethephon was applied to 7 months old berries after flower initiation as to compared to 21 days of the untreated berries (control). Ripening of other treated berries (5 and 6 months old) was also significantly earlier than the control with mean of 11 and 14 days after Ethephon application. Diameter of beans significantly decrease with decreasing age of berries while percent rejected beans significantly decreased with age upon application of Ethephon.

Cupping quality of coffee berries (aroma, acidity, flavor, body and aftertaste) was the same for 6 months old, 7 months old and the control berries. The same was true for the chemical constituents (caffeine, food energy, ash and crude protein). Total fat of 6 months old was significantly higher while total carbohydrates was significantly lower than the older berries.

VULNERABILITY ASSESSMENT TO CLIMATE CHANGE OF CAPTURE FISHERIES AND AQUACULTURE IN NORTHERN AURORA, PHILIPPINES

Ramos, Glaizel P , Garcia, Jhon Paul, Daylo, Opalyn, Lorilla, Robilyn, Maglayon. Harvey, **Travallo, Elvie and Mark Joseph R Rafael**
Aurora State College of Technology, Casiguran Campus,
Department of Agriculture and Aquatic Sciences, Casiguran, Aurora, Philippines

INTRODUCTION

Climate change is one of the most challenging conflicts that the world faces, specifically the Philippines. This study aims to assess the vulnerability to climate change in the two coastal municipalities in Aurora, Philippines: Casiguran and Dinalungan, particularly in aquaculture and capture fisheries, identify their top commodity, gear used in capture fisheries, propose adaptive strategies and policy for coastal management.

MATERIALS AND METHODS

Municipality of Casiguran and Dinalungan were selected as study areas with nineteen (19) coastal barangays with 30 respondents each barangay and aquaculture has fouthteen (14) barangays with a total of 75 aqua farmers as respondents. Site selection was conducted in coordination with Municipal Agriculture Office of Casiguran and Dinalungan. The modified Fisheries Vulnerability tool was used to gather information on exposure, sensitivity and adaptive capacity. Using the questionaires, interviews were conducted for each barangay within 3 months of the duration period. The data was analyzed using the modified fisheries vulnerability assessment rubrics and computed the results using Fish Vool Excel System (Aguila et al. 2021)

RESULTS AND DISCUSSION

Oreochromis niloticus and *Chanos chanos*, are only two commodities for aquaculture and five in capture fisheries namely, *Nemipterus hexodon*, *Katsuwonus Pelamis*, *Auxis thazard*, *Thunnus albacares*, and *Gaza minuta*. Fishermen used handline, longline, gill net, speargun, trawl line, fish corral, floater, and bottom set longline. The findings revealed that the two municipality is vulnerable to climate change because the potential impact is high and the adaptive capacity is medium. As a result, vulnerability assessment is an effective tool for determining a community's vulnerability to natural hazards. The findings are consistent with the findings of reported effects of climate change which are already evident, causing a high level of vulnerability in districts and cities with long coastlines based on interviews in Vietnam (Do Tra My Tran et al. 2020). Furthermore, when exposure becomes severe, the absolute level of vulnerability increases (Kim et al. 2019).

Building levees around the pond complex, installing run pipes, vertical net must be performed, constructing a fish shade, and building fish pens that can withstand the effects of climate change are some adaptive strategies to combat climate change. The two municipalities create regulations and marine protected areas to ensure the sustainability of fishery resources. Generally, the study provided knowledge and understanding of climate change for future management and conservation of fisheries in municipalities.

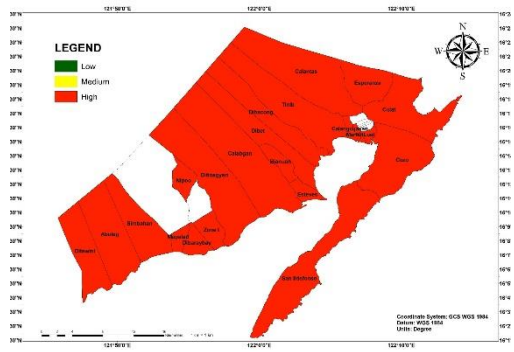

Fig. 1. Climate change vulnerability map of capture and aquaculture fisheries in Casiguran and Dinalungan, Aurora

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FORAGING BEHAVIOR OF POLLINATORS ASSOCIATED WITH FLORAL TRAITS OF TOMATO (*Solanum lycopersicum* L.)

Rodelina C. Deyto
Institute of Biological Sciences, College of Arts and Sciences,
University of the Philippines Los Baños, Philippines
Corresponding author: rcdeyto@up.edu.ph

One of the most important plant-animal interactions for reproduction of plants is pollination. Pollinators can improve the quality, shelf life and commercial value of agricultural products. About 35% of global food production and 45% of global nutrient supply for humans depends on animal pollination (Roubik 1995; McGregor 1976). Pollinator visits are known to increase the quantity and quality of crops by moving outcross pollen among individuals and increasing the total amount of pollen deposited on flower stigmas (NRC 2007). Tomato (*Solanum lycopersicum* L.) is the second most consumed and widely grown non-starchy vegetable in the world after potato. They are extensively utilized in Middle Eastern, Mediterranean, Spanish and Italian cuisines as the main ingredient in pizzas, spaghetti and pastas (Padmanabhan et al. 2016). The type of pollination depends on the floral types. In case of tomato, nectar is absent and the presence of anthers with poricidal dehiscence limit the number of potential pollinator visitors. The pollen grains are released from small pores at the apex of the anther through a vibration mechanism, resulting to a specialized pollination system known as buzz pollination. Solitary bees and many native bees like the bumblebees were observed to either sonicate or buzz the flowers for pollen. Although tomato is self-compatible and self-pollinated, they require insect pollination to increase fruit set and seed set. Since tomato is one of the major economic crops not just in the Philippines but worldwide, it is necessary to ensure that the amount of its yield is maintained and improved. In order to maximize the yield and effectively produce quality fruits of tomato, it is imperative to understand its floral biology and identify its significant pollinators. In addition, this knowledge will guide growers on how to properly implement pest control techniques to conserve the pollinators and produce greater yield. This study was conducted to determine the foraging behavior of pollinators associated with buzz pollination in tomato. Specifically, the study will establish the floral biology (days to flowering, anthesis, time of anther dehiscence, pollen viability, and stigmatic receptivity) of tomato; and identify its pollinators and their foraging behavior. The field trial was conducted at the Organic Agriculture Demo Area, University of the Philippines Los Baños, Laguna, College of Agriculture and Food Science (CAFS). Laboratory analyses were done at the Palynology Laboratory at the Institute of Biological Sciences.

The flowers of tomato are herkogamous, solanoid with poridical anther and nectar-free, leaving only the pollen as reward to flower visitors and pollinators. The flowers are non-terminal and short lived (three days). Five to twelve flowers are clustered in a helicoid cyme inflorescence. The anthesis of tomato occurred during morning hours, 0600 to1200 h with peak at 0900 to 010 h. The pollen grains were simple and numerous with high viability (81 $\pm$ 17.4 %). Receptivity of stigma synchronized with pollen viability. The stigma was most receptive at 0700 h with 56 $\pm$ 10.13 % germinated pollen. Tomato is a partially protandrous plant. The floral structure (solanoid form) of tomato serves as selective pressure for a specialized pollination mechanism known as buzz pollination that deter ineffective floral visitors (Buchmann 1983; Sun and Rychtar 2015). The presence of poricidal anthers is the plants' strategy to increase pollen export efficiency and to reduce pollen loss during transport (Harder and Barclay, 1994). Asynchronous flowering, dichogamy and herkogamy in tomato plants are adaptive strategies to promote outcrossing and for reproductive assurance. The floral visitors of tomato were very limited and consisted mainly of insects (Anthophorid, Xylocopa or carpenter bee, Halictid, and beetle). Among these species, carpenter bee (*Xylocopa* spp.) was the most numerous and the most frequent visitor throughout the flowering period. The foraging activity of carpenter bee started as early as 0630 h. Because of their weight and larger size compared to flowers of tomato, the flowers bent down as carpenter bees landed on flowers. When foraging, carpenter bees directly landed and

grabbed the anther tubes. The body was curved on top of the anthers and stigma, hanging like a bat with fully flexed wings such that the dorsal sides of its wings were touching. They performed a vibrating movement, and a buzzing sound can be heard when they were gathering pollen. Carpenter bees have significantly shorter handling time when foraging on flowers, spending 1-4 sec (ave 2 $\pm$ 0.68 sec) per visit. They visited 4-21 (ave 11 $\pm$ 8) individual flowers before flying away. Different foraging behavior was observed on anthophorid bees while foraging on flowers of tomato. During pollen collection on tomato flowers, the body of anthophorid bees was rounded atop of the anther column wherein the whole ventral side of the body was in contact with the anther column. Subsequently, the bees wiggled its body while tapping and shaking the anther column with the posterior end of its abdomen. Then alternately, they performed grooming, transferring pollen from the ventral side of its body using the midlegs to the pollen basket on its hindlegs. The foraging activity of anthophorid bees lasted for 5-25 sec (ave 14 $\pm$ 6.63 sec). They visited 3-7 (ave 4 $\pm$ 2.31) flowers and then flew away. The buzzing behavior was also observed on halictid bees when gathering pollen from tomato flowers. They spent 4-22 sec (ave 13 $\pm$ 8.99 sec) during pollen collection and visited 5-6 (ave 5 $\pm$ 0.58) flowers before moving away. The average number of floral visitation per foraging trip of carpenter bees during anthesis was 11 $\pm$ 7.59, which was significantly higher than that of halictid (5 $\pm$ 0.58) and anthophorid bees (4 $\pm$ 2.31).

The solanoid floral morphology, particularly the presence of poricidal anthers of tomato need natural or artificial vibrations to release pollen through their apical pores. In open areas, shaking by wind is usually sufficient to trigger pollen release, promoting self-pollination. However, the fruit set and seed set of tomato are significantly enhanced by insect pollination, as shown in previous studies. Although tomato is autogamous and self-compatible, it exhibits features that are attractive to insect pollinators, such as the yellow corolla and intense yellow anthers that reflect ultraviolet light, and the high amount of pollen. The foraging activity of pollinators was synchronized with anthesis. During anthesis, pollen presentation is greatest and very important for pollination. Carpenter bee and Anthophorid bee were considered as effective pollinators of tomato, since they are capable of buzzing and removing pollen from the poricidal anthers and function as pollen vectors, and they visited the flowers during anthesis regularly. Since carpenter bees and Anthophorid bees do not form large nests, they are expected to contribute most to crop pollination when honeybees are ineffective. Nest site augmentation in their natural habitat and in agro-ecosystem is needed to enhance the reproduction of wild bee populations. Halictid bee was not an effective pollinator of tomato due to its low number of visits. The small body size of halictid bee did not allow it to press the anther thecae, making it inefficient pollinator. This findings highlights the importance of conserving this natural wild bee fauna in and around crop fields even during the off-season of crops. They may contribute to pollinate the flowers as they were found sonicating or buzzing the flowers. It is essential to understand the attributes of native bees such as nesting, social behavior or not, foraging behavior (flight distance, type of food resource) and pollination (buzz pollination and other behaviors) considering their relevance in nature and for food production. The protection of pollinators is a core step in ensuring food security and ecosystem integrity for the future.

EFFECTS OF DILUTED SEAWATER AND TABLE SALT ON THE POSTHARVEST QUALITIES OF TOMATO *(Solanum lycopersicum* L.*)*

Celades JP[a*], Gonzaga ZC[a], Obeda WL[b] and Datan MB[b]
College of Agriculture and Natural Resources, Bohol Island State University, Bilar-Campus
Department of Horticulture, Visayas State University, Visca, Baybay City,
Leyte, 6521-A Philippines.
*Corresponding author: jennifer.celades@bisu.edu.ph

ABSTRACT

Tomato is a major component of daily meals in many countries and constitutes an important source lycopene and β-carotene. Recent consumer interest in tomatoes has focused on improvements of flavour quality. The experiment was conducted in the Department of Horticulture Postharvest Laboratory, Visayas State University, Visca, Baybay City, Leyte to evaluate the effects of diluted sea water and table salt with different concentrations on improving fruit quality of tomato. Tomatoes were placed in the Department of Horticulture Postharvest Laboratory. All harvested fruits per treatment were placed in separate plastic trays and initial weight were measured. Data gathering was done at 3 days interval and during termination of the study. Fruits were evaluated according to the sources of salt solution and different concentrations imposed on the standing crop. The physical and chemical characteristics of tomatoes were gathered which include visual quality rating, cumulative weight loss, firmness, postharvest life, color changes, pH, total soluble solids (TSS) and titratable acidity (TA). The experiment was laid out in a Complete Randomized Design and analyzed using Statistical Tool for Agricultural Research (STAR).

The result of the experiment showed that salt sources and the different concentrations had no significant effects on visual qualities and cumulative weight loss of tomato fruits stored under ambient conditions. Nevertheless, a significant interaction was observed on salt sources and concentration on color changes of tomato. Earlier change in color was noticed on table salts solution. Total soluble solids were significantly higher in fruits harvested from plants applied with 5 % concentration. Postharvest life on the other hand, was extended under sea water treatment at 2.5 % concentration. Furthermore, sea water treatment of 5 % enhanced the citric acid content of tomato fruits whereas diluted table salt treatments had comparable effects on citric acid content and the different salt concentrations which were significantly higher than control.

Based on the results obtained, application of salt solution from two sources and three concentrations did not significantly influence the visual quality rating (VQR) and cumulative weight loss of tomato fruits during storage. Color changes in tomato were slowed down in fruits harvested from sea water treated plants as compared to table salt treated plants. Higher concentrations of salt solutions increased the total soluble solids but not on total acidity. Fruits from sea water treated plants 2.5 % had longer postharvest life. Citric acid content on the other hand, was improved as the salt concentration applied to plants was increased. The use of sea water with 2.5 % concentration is recommended in improving the fruit quality of harvested tomato fruits.

Keywords: VQR, TSS, TA, Citric acid, cumulative weight loss

THE FFECTS OF TUNA FISH VISCERA PROTEIN HYDROLYSATE LIQUID FERTILIZER ON THE GROWTH AND YIELD RESPONSE OF LETTUCE (*Lactuca Sativa*) 'LOLLO BIONDA EVELYN' AND 'BLACK ROSE' VARIETY

Arvin Jonathan L. Flores, **Vince S. Clemente**, **Lee Andrew S. Villarez**
Occidental Mindoro State College, College of Agriculture,
Murtha, San Jose, Occidental Mindoro 2100 Philippines
Corresponding author: arvinlumata1991@gmail.com/arvin_flores@omsc.ph.education

ABSTRACT

Conducted in a field experiment following the layout in Split-Plot for Randomized Complete Block Design (RCBD), the effects of tuna fish viscera protein hydrolysate on lettuce variety were investigated on the growth and yield of lettuce (*Lactuca sativa)*.Various data gathered were analyzed using ANOVA for treatment effects and LSD for mean comparisons. In data processing, the Statistical Tool for Agricultural Research (STAR) software was used. Results revealed that the application of fish protein hydrolysate had increased the growth performance of green and red lettuce in terms of height. The same was observed in number of leaves, but not statistically significant. However, it had no significant effect on fresh weights. There was no evidence of an interaction effect, but the application rate of 15 ml L^{-1} fish protein hydrolysate resulted in taller plants, more leaves, and more fresh weights. Hence, using of fish protein hydrolysate at the said rate was suggested. Similar studies with more lettuce varieties and other test crops, as well as higher application rates of fish protein hydrolysate, were also recommended.

Key words: *Lactuca sativa,* Fish protein hydrolysate, viscera, tuna, growth, and yield, growth performance

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EFFICACY OF VERMICOMPOST TEA AND GUANO TEA MIXTURE ON THE GROWTH AND YIELD OF TOMATO (*Lycopersicum esculentum*) +SWEET PEPPER (*Capsicum annuum*) CROPPING SYSTEM

Arleen S. Upod and Ryan Mark A. Ambong
College of Agriculture-Murtha Campus
Occidental Mindoro State College
Corresponding author: fritzarleen23@gmail.com/rambong@omsc.ph.education

ABSTRACT

Alternative materials are being utilized to deal with the harmful effects of chemical fertilizer. Organic-based fertilizers using agricultural wastes are being continuously developed by researchers with the primary objective of reducing, if not totally eliminating the use of chemical fertilizer, and enriching the soils to lessen environmental pollution. This study was conducted with the aim of determining the effect of vermitea and guano tea on the growth and yield of intercropped tomato and pepper. It further aims at producing tomato-pepper in a better but cheaper way through the use of organic agricultural practices. The results reveal that various amount of vermicompost tea and guano tea and cropping system significantly affect the growth and yield of tomato and pepper. However, it is also revealed that there was no significant interaction effect on growth response but there is a significant interaction effect on the yield responses between varying levels of treatment fertilizers and cropping system.

Keywords: vermicompost, intercropping, fertilizer, farming system

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PROPERTIES AND FERTILITY STATUS OF SOILS UNDER COCONUT IN MT. PANGASUGAN

Christine Mae C. Atup[1] and Victor B. Asio[2]
[1]College of Agriculture and Natural Resource, Bohol Island State University, Zamora Bilar Bohol, Philippines. Email: christinemae.atup@bisu.edu.ph
[2]Visayas State University (VSU), Philippines
Department of Soil Science, Visayas State University, Visca, Baybay City, Leyte, 6521-A Philippines.
Corresponding author: vbasio@vsu.edu.ph

ABSTRACT

The yield of coconut is influenced by the properties and fertility status of the soil. However limited information is available on the soil constraints affecting coconut. This study was conducted to evaluate the properties and fertility status of soils in coconut plantation in Mt. Pangasugan. Three sites was evaluated and sampled. To be able to compare the soils under the coconut with the nearby forest soils, a fourth site (a secondary forest) was also sampled. All four sites were within a 200 m distance and were presumed to have similar pedogenesis. A soil profile was dug in each site and the composite surface soil samples were collected from each site.

Results revealed that the soils are generally clay loam, acidic, with moderate organic matter content, low total N content, and sufficient amounts of available phosphorus, and exchangeable bases (Ca, Mg, K, Na) contents of soils were moderate. The result also revealed that the soils under coconut were more compact than the forest soils. Based on the results, application of fertilizer particularly the containing N and cover cropping with leguminous crops may improve coconut performance. Also improve the physical condition of the soil.

Key words: Fertility, soil profile, sub-surface soil, elevation

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