Journal of
Zoological and Botanical Gardens

MDPI

Article

Tree Species Composition and Diversity in a Secondary Forest along the Sierra Madre Mountain Range in Central Luzon, Philippines: Implications for the Conservation of Endemic, Native, and Threatened Plants

Christian Ofalla Llait [1,2]

[1] Department of Forestry, College of Agriculture and Forestry, Tarlac Agricultural University, Camiling 2306, Tarlac, Philippines; llaitchristian1@gmail.com; Tel.: +63-936-7091-152
[2] School of Graduate Studies, Aurora State College of Technology, Baler 3200, Aurora, Philippines

Abstract: The Sierra Madre Mountain Range (SMMR) is the backbone of the Luzon Islands that contains a high concentration of highly important ecological resources distributed among the 68 protected areas therewith. The present study aimed to assess the composition and diversity of tree species in a secondary forest within the SMMR. A 2.25 km transect with 10 900-m^2 plots were established to record tree species with a diameter at breast height of at least 10 cm. The findings revealed 148 individuals of trees from 38 morphospecies, 28 genera, and 20 families. Importance values unveiled the Aurora endemic *Macaranga stonei* Whitmore as the most important species in terms of the relative values of its abundance, frequency, and dominance. The area was also found to be home to 33 natives, 12 endemics, five IUCN threatened species, and nine Philippine threatened trees. Furthermore, the study site was also found to have considerably high diversity, with a Shannon–Weiner Index value of 3.269 and a relatively even distribution of individuals among species, as supported by the Simpson's Evenness index value of 0.9453. Significant correlational relationships were also found among species richness, Shannon–Weiner index, and Simpson's Evenness index, with correlation coefficients ranging from 0.881 to 0.934, with all significant at $p < 0.001$. Lastly, the study was able to produce a distribution map, which is necessary for implementing targeted conservation strategies. These findings provided valuable implications for future research and implementation of targeted and participatory biodiversity conservation and protection strategies.

Keywords: biodiversity; biodiversity hotspot; correlation analysis; distribution maps; Shannon–Weiner index; Simpson's Evenness index

Citation: Llait, C.O. Tree Species Composition and Diversity in a Secondary Forest along the Sierra Madre Mountain Range in Central Luzon, Philippines: Implications for the Conservation of Endemic, Native, and Threatened Plants. *J. Zool. Bot. Gard.* **2024**, *5*, 51–65. https://doi.org/10.3390/jzbg5010004

Academic Editors: László Bakacsy and Ágnes Szepesi

Received: 30 October 2023
Revised: 17 December 2023
Accepted: 3 January 2024
Published: 9 January 2024

1. Introduction

The Philippines, an archipelagic country known for its rich biodiversity, stands proudly as one of the 18 megadiverse nations on Earth [1]. This designation signifies that it harbors over two-thirds of the world's biodiversity resources, which play a crucial role in supporting human well-being and survival while maintaining ecosystem stability [2,3]. Biodiversity generously provides us with essential resources like food, water, raw materials, and clean air. Moreover, it diligently regulates climate and protects us from natural disasters [4]. Ecologically speaking, biodiversity enables vital processes such as pollination, nutrient cycling, water filtration, and soil stabilization and erosion control—all working together to create balanced ecosystems and desirable environmental conditions [5–7].

Unfortunately, biodiversity has long been facing numerous threats that jeopardize its existence and the critical services it provides. Climate change, along with many undesirable human activities such as deforestation, habitat destruction, land use change, and overexploitation, primarily drive biodiversity loss globally [8]. Due to these, scientists were able to identify biodiversity hotspots that contain very high rates of endemism and

drastic loss of vegetation and habitat that threatens various key biodiversity species [9]. At present, there are already 36 biodiversity hotspots, including the Philippines [10]. This signifies the need for immediate planning and implementation of strategies to prevent total biodiversity loss.

In the Philippines, various conservation and rehabilitation efforts are continuously implemented. The establishment and monitoring of protected zones under the National Integrated Protected Areas Systems (NIPAS) Act is considered one of the most important tools in conserving the country's key biodiversity resources, as recommended by the Convention on Biological Diversity [11]. Other conservation and rehabilitation programs, such as the National Greening program under Executive Order No. 26 [12], community-based forest management under Executive Order No. 263 [13], and sustainable ecotourism [14], among others, are recognized as greatly contributing to biodiversity conservation while educating people about its values and services.

However, there were critical issues in some rehabilitation and conservation programs. One of these is the unsuitable choice of plant species to rehabilitate a degraded or disturbed area. Several efforts in the past used exotic and invasive species such as *Gmelina arborea* Roxb. And *Swietenia macrophylla* King [15] in many greening activities. Some used native species, but there was a lack of pre-assessment of the site–species relationships thus introducing the natives to inappropriate habitats and hindering their successful growth and survival [16]. This is where the importance of plant inventory and assessments comes in. The data and findings yielded by these studies provide essential information on the population structure, composition, and ecology of an area and its resources that are beneficial in recovery planning, such as biodiversity conservation and habitat rehabilitation [17].

This current study aims to contribute to the conservation of Philippine biodiversity by assessing tree diversity in the municipality of San Luis in the province of Aurora. The province is a part of the Sierra Madre Mountain Range, the longest mountain range in the country, which is considered a highly important area in terms of valuable ecological resources distributed among its 68 protected areas [18,19]. Furthermore, there are very few studies about the plant composition and diversity in the province, which only cover the tree species in the municipalities of Baler [20] and Dipaculao [21], as well as the diversity of ferns in the municipalities of Maria Aurora [22] and Baler [23]. Hence, this study will pioneer the assessment of plants in the municipality of San Luis, which is beneficial in identifying the area's key biodiversity resources, such as the endemic, native, and threatened species, which is a crucial step in biodiversity conservation. Specifically, the study aimed to determine the tree species composition, including ecological classifications (i.e., indigeneity, endemism, and conservation status), calculate the importance values and diversity indices and explore the underlying relationships among diversity parameters and ecological variables (i.e., elevation).

2. Materials and Methods

2.1. Study Site

The study was conducted in April 2023 in Barangay L. Pimentel in the municipality of San Luis, province of Aurora, situated approximately 15°41′2.94″ N and 121°30′1.23″ E (Figure 1). The barangay is composed of residential, agricultural, and mountainous forest lands. Specifically, the survey was carried out in mountainous forest lands, which is a portion of the Sierra Madre Mountain Range, the backbone of Luzon Island, that serves as a protector and barrier from typhoons coming from the Pacific Ocean [24]. The survey area had a moderately steep topography, with elevations ranging from 273 to 581 masl. Climate-wise, the municipality has average monthly temperatures ranging from 26 °C to 30 °C (high temperature) and 22 °C to 25 °C (low temperature), and average monthly rainfall ranging from 118.3 mm (March: average of 7 rainfall days) to 416.9 mm (October: average of 17 rainfall days) in 2023. In the past 10 years, the average annual temperatures have usually ranged from 26 °C to 28 °C, while rainfall has been 100.23 mm to 624.86 mm. During the study period, the area had an average temperature of 28 °C during daytime and

24 °C during nighttime and there were 8 rainy days, with precipitation of around 300 mm during the month of April [25].

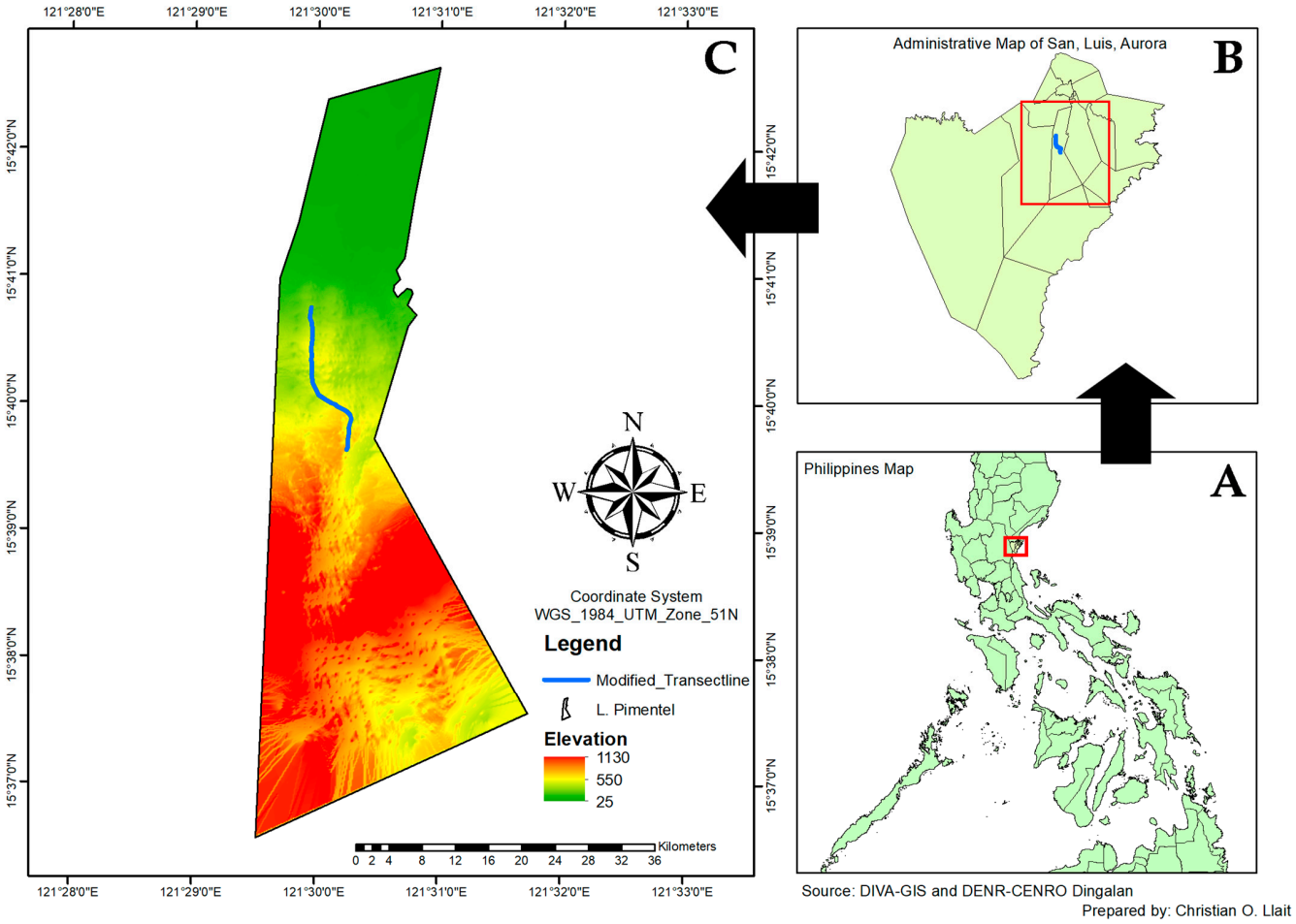


Figure 1. Location map of the study site in San Luis, Aurora: (**A**) study site pointed in the Philippine map, (**B**) location of the site pointed in the map of San Luis, Aurora, (**C**) elevation map of Barangay L. Pimentel showing the location of modified transect.

2.2. Survey and Mapping of Tree Species

The inventory of tree species was carried out along a 2.25 km transect line with 10 30 by 30 m quadrats established at every 250 m point (Figure 2). The transect line was established following the trail while the quadrats were positioned alternately at the left and right of the transect line, with an approximate distance of 5 m away from the trail. The total coverage of all the quadrats was 9000 m^2. The use of transect in conducting this plant inventory was used to ensure that the quadrats were evenly distributed throughout the forest stand [26].

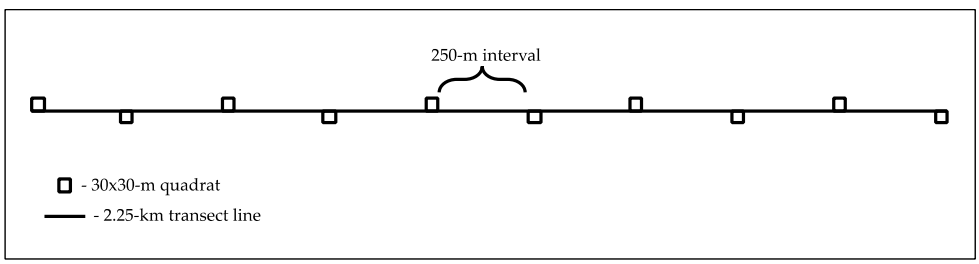


Figure 2. Arrangement of quadrats along the transect line.

After the establishment of the transect and quadrats, the plant survey was carried out. Trees with a diameter at breast height (DBH) of at least 10 cm were included in the study, following the DBH cut-off of many tree species inventories undertaken in the Philippines [27,28]. Plant identities were determined in the field using morphological characteristics. For individuals that were not identified in the field, photos were taken for further verification. References and databases such as Co's Digital Flora of the Philippines [29] and Revised Lexicon of Philippine Trees [30] were used to verify plant identities. Finally, the accepted scientific names of plants were determined using the Plants of the World Online database of the Kew Royal Botanic Gardens [31]. Significant ecological statuses (i.e., indigeneity, endemism, and conservation status) of all species were also assessed. Indigeneity and endemism were obtained from the Co's Digital Flora of the Philippines [29]. Meanwhile, conservation statuses were determined using the IUCN Red List of Threatened Species [32] for the global scale and DAO 2017-11 or the Updated National Checklist of Threatened Plants and their Categories [33] for the national scale.

Mapping was also carried out to visually present the location of each individual tree, which will also serve as the basis for the future implementation of targeted biodiversity conservation and management measures. Initially, the Locus map (a mobile outdoor navigation application) was used to record the location of the transect line and quadrats. Then, the geographic coordinates of each tree were recorded and encoded in Microsoft Excel. Geographic coordinates in decimal degree format were then converted into the Universal Transverse Mercator (UTM) format using the ArcGeek Coordinate Conversion Tool [34] before feeding it to ArcGIS software (v. 10.4). After that, the locations of all trees were plotted on the map. Lastly, final editing was undertaken to produce the final copy of the map in .jpeg format.

2.3. Data Analysis and Interpretation

2.3.1. Species Richness, Abundance, and Importance Values

Species richness, abundance, and importance values were either counted or calculated to discover the species composition in the area. Species abundance refers to the number of individuals of a species in an area [35], while species richness is the number of species or taxa present [36]. Hence, the number of species and its individuals were counted to determine the species richness and abundance. Lastly, importance values (IVs) serve as an index to measure how dominant a certain species is in a forest area through the relative values of its abundance, frequency, and dominance [37]. Thus, IVs were computed using the following equations [38]:

$$Density = \frac{number\ of\ individuals\ of\ a\ \ species}{total\ area\ sampled} \quad (1)$$

$$Relative\ Density = \frac{density\ of\ a\ species}{total\ density\ of\ all\ species} \quad (2)$$

$$Frequency = \frac{number\ of\ plots\ in\ which\ a\ species\ occur}{total\ number\ of\ plots\ sampled} \quad (3)$$

$$Relative\ Frequency = \frac{frequency\ of\ a\ species}{total\ frequency\ of\ all\ species} \quad (4)$$

$$Basal\ Area = 0.7854\ \left(DBH\ of\ a\ species^2\right) \quad (5)$$

$$Dominance = \frac{basal\ area\ of\ a\ species}{total\ area\ sampled} \quad (6)$$

$$Relative\ Dominance = \frac{dominance\ of\ a\ species}{total\ dominance\ of\ all\ species} \quad (7)$$

$$Importance\ Value = Relative\ Density + Relative\ Frequency + Relative\ Dominance \quad (8)$$

2.3.2. Diversity Indices

Biological diversity can be quantified using mathematical functions known as the diversity indices [39]. In this study, the widely accepted Shannon–Weiner (H') and Simpson's Evenness (E) were employed as the species diversity indices and computed through Paleontological Statistics (PAST v 3.18) software. The choice of these indices aligns with the standards set by previous biodiversity studies undertaken in the country and uses the Fernando Biodiversity Scale, which has been widely adopted in diverse ecological investigations in the Philippines to effectively facilitate the interpretation of computed values [40,41] (Table 1).

Table 1. Fernando Biodiversity Scale.

Interpretation	Shannon–Weiner	Simpson's Evenness
Very high	3.5 and above	0.75–100
High	3.0–3.49	0.5–0.74
Moderate	2.5–2.99	0.25–0.49
Low	2.0–2.49	0.15–0.24
Very Low	1.9 and below	0.05–0.14

2.3.3. Correlation Analysis

Exploring intricate relationships among key variables is essential in deeply understanding the dynamics of forest ecosystems. Therefore, Pearson correlation analysis was used to explore the underlying relationship (i.e., monotonic association) among important variables, namely, elevation, species richness, abundance, Shannon–Weiner, and Simpson's Evenness. This was computed at a significance level of $p < 0.05$ through JASP v. 0.16.1, an open-source statistical software package. The results were interpreted using the computed correlation coefficient values (r-values) and their associated p-values, as well as the conventional approach in interpreting r-values, contextualized as a direct or inverse relationship [42] (Table 2).

Table 2. Conventional approach in interpreting correlation coefficient [42].

Absolute Value of r	Interpretation
0–0.09	Negligible correlation
0.10–0.39	Weak correlation
0.40–0.69	Moderate correlation
0.70–0.89	Strong correlation
0.90–1.0	Very strong correlation

3. Results and Discussion

3.1. Tree Species Composition

The study recorded a total of 148 individuals of 38 morphospecies of trees from 20 families and 28 genera. In terms of the families, Dipterocarpaceae and Moraceae were the most speciose with seven and five species, respectively. The most abundant families were Euphorbiaceae, Dipterocarpaceae, and Moraceae, with 29, 28, and 21 individuals, respectively. These families are abundant in the Philippines, especially in tropical lowland evergreen forests that are dominated by dipterocarps [43]. Sadly, dipterocarps are among the most threatened plant species in the Philippines and in Southeast Asia due to deforestation, and their timbers have been massively exported in the past [44,45]. Species-wise, *Macaranga stonei* Whitmore was the most abundant, followed by *Parashorea malaanonan* (Blanco) Merr., with 24 and 9 individuals, respectively. Given that the study plots covered 9000 m^2, which is 9/10 of a hectare, it is estimated that these species, *M. stonei* and *P. malaanonan*, had 26 and 10 individuals in a hectare of the study area, respectively.

The importance values computation also revealed significant findings in terms of the species composition. Eleven (11) species had individual IVs of more than 10 (Figure 3). In

total, these 11 species contributed 55.50% of the total IV of all species in the area. Among them, *M. stonei* had the highest IV of 30.35, which is equivalent to 10.11% of the total IV of all the species recorded, followed by *Parashorea malaanonan* (Blanco) Merr, with 21.63 (7.21%). *M. stonei*'s high IV was related to its high abundance of 24, its occurrence in six plots out of all ten plots, and a total basal area of 136.73 m^2. *M. stonei* is Aurora province-endemic and a critically endangered plant species belonging to the family Euphorbiaceae [29,32]. This keystone species lacks focus in terms of research, thus dictating the need to study this species more and include it as one of the top priorities for conservation due to it being a species restricted to the province of Aurora.

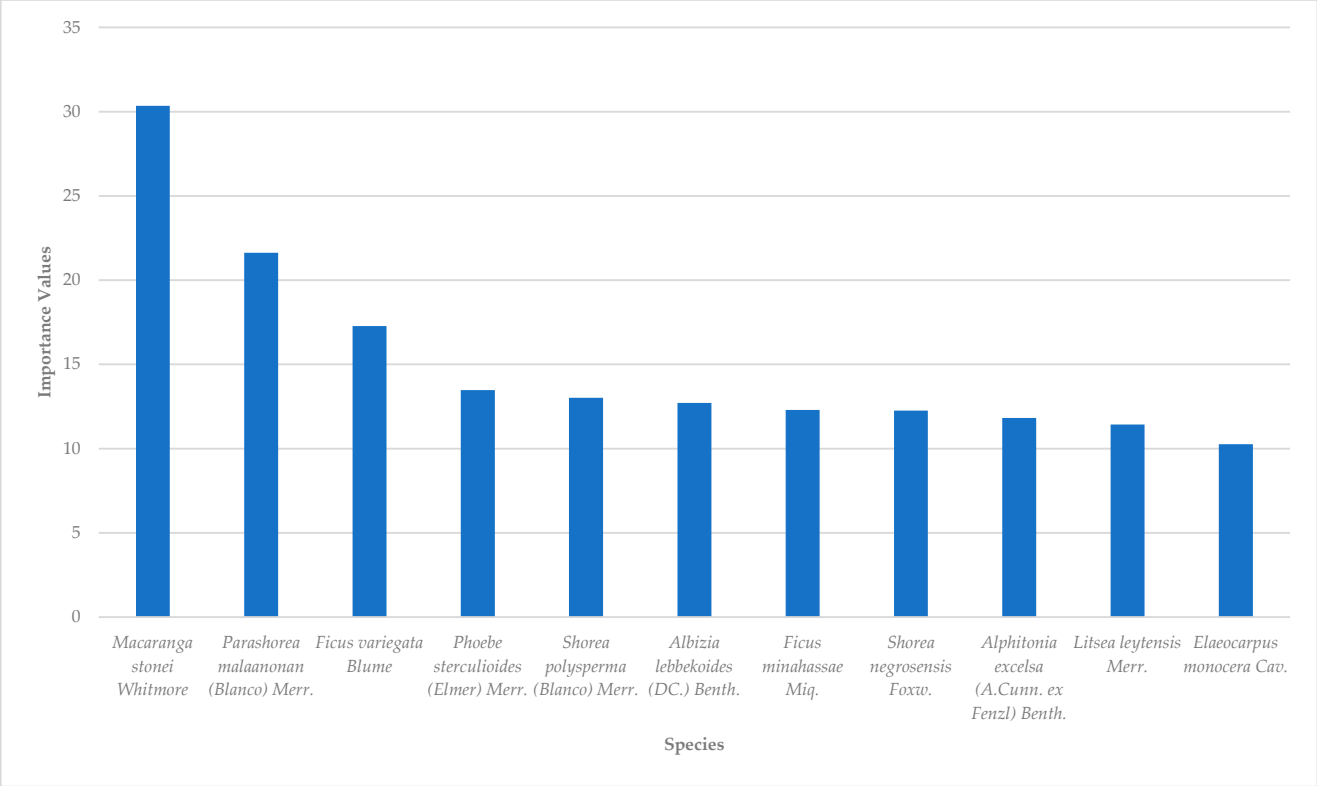


Figure 3. Top eleven species with highest importance values (IVs).

The surveyed forest was also found to be home to ecologically important species, namely, native, endemic, and threatened species (Table 3). Out of the 38 species found, 33 (86.84%) were natives, while five were exotics (with one invasive *Gmelina arborea* Roxb. ex Sm.). The native species were composed of 12 endemics, five IUCN threatened species, and 9 Philippine nationally threatened species. Specifically, there were one critically endangered, two endangered, and two vulnerable species found in the IUCN. Furthermore, there were two endangered, six vulnerable, and one other threatened species found in DAO 2017-11 or the Philippine Red List. The most notable among the Philippine endemic species were the IUCN critically endangered *M. stonei* and the IUCN vulnerable and DAO 2017-11 endangered *Hopea acuminata* Merr, and the IUCN endangered and DAO 2017-11 vulnerable Philippine national tree *Pterocarpus indicus* Willd. The presence of critically important plants in the area dictates the need for immediate action to conserve, protect, and even spread their population. It is emphasized that these species, particularly the endemics, have higher probabilities of extinction because of their narrow and restricted habitat than widespread species [46]. The native and endemic plant species also provide suitable habitats and enough food sources for native and endemic fauna species [47]. In fact, we were able to witness a couple of the Philippine endemic Luzon Rufous Hornbill (*Buceros hydrocorax* Linnaeus) during the survey. However, the presence of invasive species like the *G. arborea*

adds pressure to the survival and propagation of the native and endemic flora and fauna species due to the aggressive nature of most invasive plants [48]. Actual representative photos of some critically important plant species in the area and an image of *B. hydrocorax* individuals are shown in Figure 4.

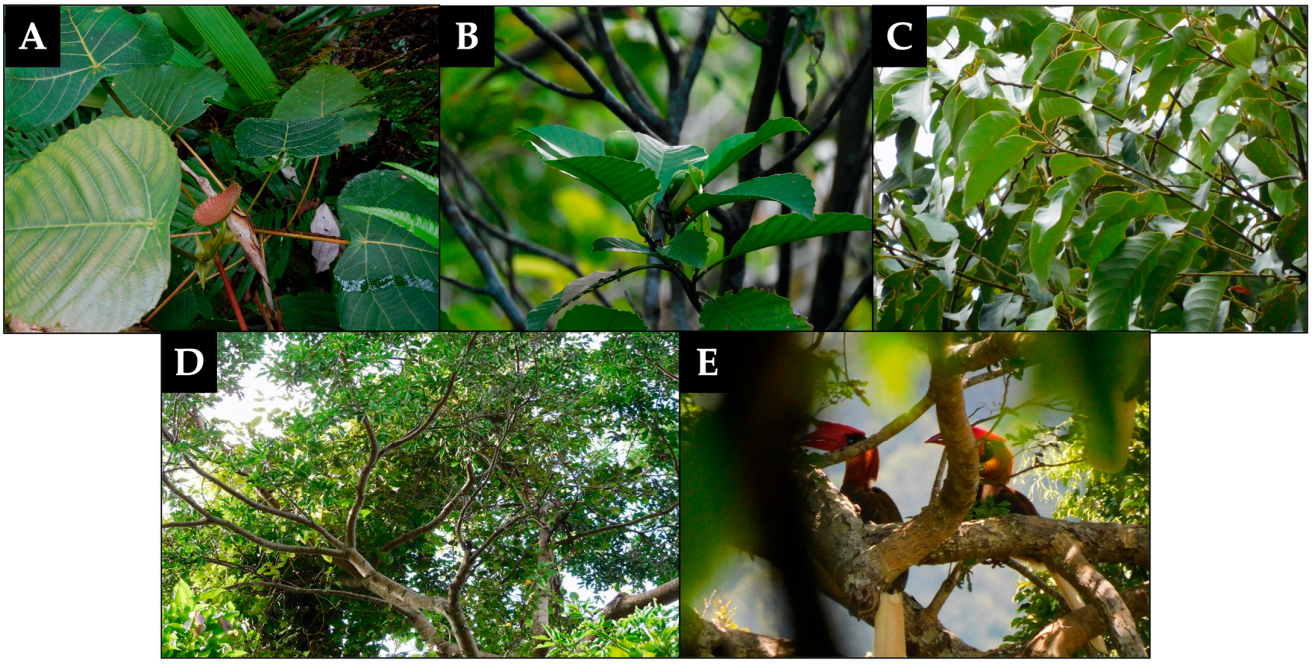


Figure 4. Representative photos of critically important species in the area. (**A**) *Macaranga stonei* Whitmore (endemic, IUCN critically endangered), (**B**) *Dillenia philippinensis* Rolfe (endemic, IUCN near threatened), (**C**) *Shorea polysperma* (Blanco) Merr. (endemic, DAO 2017-11 vulnerable), (**D**) *Shorea contorta* Vidal (endemic, DAO 2017-11 vulnerable), (**E**) a couple of *Buceros hydrocorax* Linnaeus (endemic, IUCN vulnerable, Philippine Red List endangered).

Table 3. Taxonomic list of native species recorded with their corresponding endemism and conservation statuses.

Family	Species	Endemism [1]	Conservation Status [2]	
			IUCN Red List	DAO 2017-11
Anacardiaceae	*Koordersiodendron pinnatum* (Blanco) Merr.	NE	ND	OTS
Brownlowiaceae	*Diplodiscus paniculatus* Turcz.	PE	LC	ND
Cannabaceae	*Celtis philippensis* Blanco	NE	LC	ND
Dilleniaceae	*Dillenia philippinensis* Rolfe	PE	NT	ND
Dilleniaceae	*Tetracera scandens* (Linn.) Merr.	NE	ND	ND
Dipterocarpaceae	*Dipterocarpus grandiflorus* (Blanco)	NE	EN	VU
Dipterocarpaceae	*Hopea acuminata* Merr.	PE	VU	EN
Dipterocarpaceae	*Parashorea malaanonan* (Blanco) Merr.	NE	LC	ND
Dipterocarpaceae	*Shorea contorta* Vidal	PE	LC	VU
Dipterocarpaceae	*Shorea negrosensis* Foxw.	PE	LC	VU
Dipterocarpaceae	*Shorea polysperma* (Blanco) Merr.	PE	LC	VU
Dipterocarpaceae	*Shorea squamata* (Turcz.) Benth. & Hook.	PE	LC	ND
Elaeocarpaceae	*Elaeocarpus cumingii* Turcz.	NE	LC	ND
Elaeocarpaceae	*Elaeocarpus monocera* Cav.	PE	ND	ND
Euphorbiaceae	*Macaranga grandifolia* (Blanco) Merr.	NE	VU	ND
Euphorbiaceae	*Macaranga stonei* Whitmore	PE	CR	ND
Euphorbiaceae	*Macaranga tanarius* (L.) Muell. Arg.	NE	LC	ND
Euphorbiaceae	*Mallotus paniculatus* (Lam.) Müll. Arg.	NE	LC	ND

Table 3. *Cont.*

Family	Species	Endemism [1]	Conservation Status [2]	
			IUCN Red List	DAO 2017-11
Fabaceae	*Albizia lebbekoides* (DC.) Benth.	NE	LC	ND
Fabaceae	*Pterocarpus indicus* Willd.	NE	EN	VU
Hypericaceae	*Cratoxylum sumatranum* Blume	NE	LC	ND
Lauraceae	*Litsea leytensis* Merr.	PE	NT	EN
Lauraceae	*Phoebe sterculioides* (Elmer) Merr.	PE	LC	ND
Meliaceae	*Aglaia luzoniensis* (Vidal) Merr. & Rolfe	NE	NT	ND
Moraceae	*Artocarpus blancoi* (Elmer) Merr.	PE	LC	ND
Moraceae	*Ficus minahassae* Miq.	NE	LC	ND
Moraceae	*Ficus nota* (Blanco) Merr.	NE	LC	ND
Moraceae	*Ficus variegata* Blume	NE	LC	ND
Myrtaceae	*Syzygium nitidum* Benth.	NE	ND	VU
Myrtaceae	*Syzygium tripinnatum* (Blanco) Merr.	NE	ND	ND
Rhamnaceae	*Alphitonia excelsa* (A.Cunn. ex Fenzl) Benth.	NE	LC	ND
Sterculiaceae	*Sterculia ceramica* R.Br.	NE	ND	ND
Urticaceae	*Leucosyke capitellata* (Poir.) Wedd.	NE	LC	ND

[1] Endemism classifications: PE—Philippine endemic; NE—Not endemic. [2] Conservation status classifications: CR—Critically endangered; EN—Endangered; Vu—Vulnerable; OTS—Other Threatened Species; NT—Near threatened; LC—Least concern; ND—No data.

3.2. Tree Species Diversity

The diversity indices of the secondary forest in San Luis are presented in Figure 5. The Shannon–Weiner index values per quadrat ranged from 1.626 to 2.3384 and were interpreted as very low to low based on the Fernando Biodiversity Scale. In terms of Simpson's Evenness, the values ranged from 0.8182 to 0.9619, which were interpreted as very high. Quadrat 2 had the highest diversity ($H' = 2.384$ and $E = 0.9619$). Overall, the study area had a high Shannon–Weiner index ($H' = 3.269$) and a very high Simpson's Evenness index ($E = 0.9453$), which means that the trees in the area were relatively diverse and had a considerably even distribution of individuals among species. In most ecological studies in the Philippines, H' values generally range from 1.5 to 3.5, wherein higher values dictate higher species diversity [49]. The overall H' value of the present study falls within this range and was interpreted as high, which can possibly be attributed to the variety of native and endemic species that still thrive therewith. This is comparable with some studies undertaken in the Philippines, such as in a lowland forest in Agusan del Sur ($H' = 3.32$, $E = 0.52$) [50], in a secondary forest in Benguet ($H' = 2.40$) [49], and in a secondary forest in Pampanga ($H' = 2.2807$, $E = 0.8549$) [51], which were all categorized as having low to moderate diversity based on the Shannon–Weiner index. Similarly, these study sites were either under the management of upland communities or near their residential or agricultural sites. In contrast, the values are lower than the studies in a private mountainous forest in Baler, Aurora ($H' = 4.096$; $E = 0.9735$) [18], in the Quezon Protected Landscape ($H' = 3.90$, $E = 0.81$) [52], and in the Mt. Makiling Forest Reserve ($H' = 3.50$, $E = 0.91$) [53]. The common characteristics that possibly caused these high values were their classifications as private property, with strict monitoring and considerably high protection for the site in the first study and being classified as protected areas under the law of the second and third study sites, relating to the monitoring and protection activities of the government.

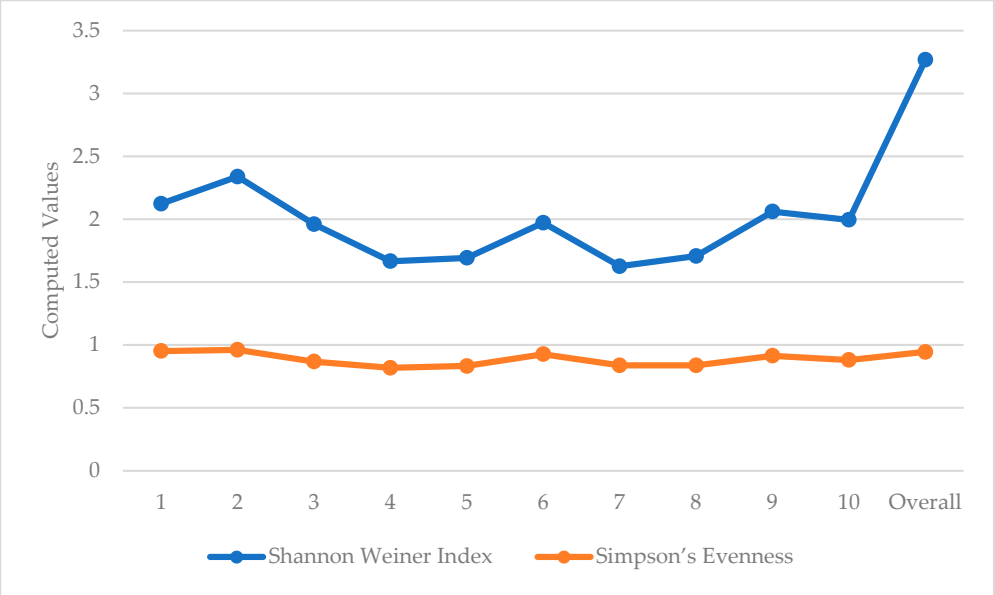


Figure 5. Computed diversity indices per quadrat and for the entire survey area.

3.3. Correlation among Parameters

This study also tested the correlations among elevation, species richness, abundance, Shannon–Weiner, and Simpson's Evenness values. As a result, significant correlational relationships were only observed for the following: (a) Species Richness and Shannon–Weiner (r = 0.881, $p < 0.001$); (b) Species Richness and Simpson's Evenness (r = 0.885, $p < 0.001$); and (c) Simpson's Evenness and Shannon–Weiner (r = 0.934, $p < 0.001$) (Figure 6). Based on the r-values, there was a strong positive correlation between species richness and Shannon–Weiner index as well as between species richness and Simpson's Evenness, as supported by a very high significance value of p that is less than 0.001. This relationship suggests that as species richness increases, the values of the Shannon–Weiner and Simpson's Evenness indices also tend to increase. Thus, this observation indicates that having a greater variety of species can lead to a higher diversity, as measured by using the mentioned indices. Furthermore, there was a very strong positive correlation found between the Simpson's Evenness index and Shannon–Weiner index based on the obtained r-value, which is backed up by a very high statistical significance with $p < 0.001$. This indicates that as the value of Shannon–Weiner index increases, the value of Simpson's Evenness also tends to increase. The findings are corroborated by the study of DeJong, which also found a very strong correlation among species richness, Shannon–Weiner index, and Simpson's Evenness index, with correlation coefficients of more than 0.96 [54]. However, no significant correlational relationships were found between the following: (a) elevation and other variables, and (b) abundance and other variables. A similar finding was found in a study at a mountain range in Southern Mindano, suggesting that elevation did not greatly affect biodiversity parameters such as the diversity indices [55]. In essence, these results are beneficial in understanding the dynamics of an ecosystem, which can be the foundation for implementing management and rehabilitation strategies in different areas within the study site with the goal of improving biodiversity.

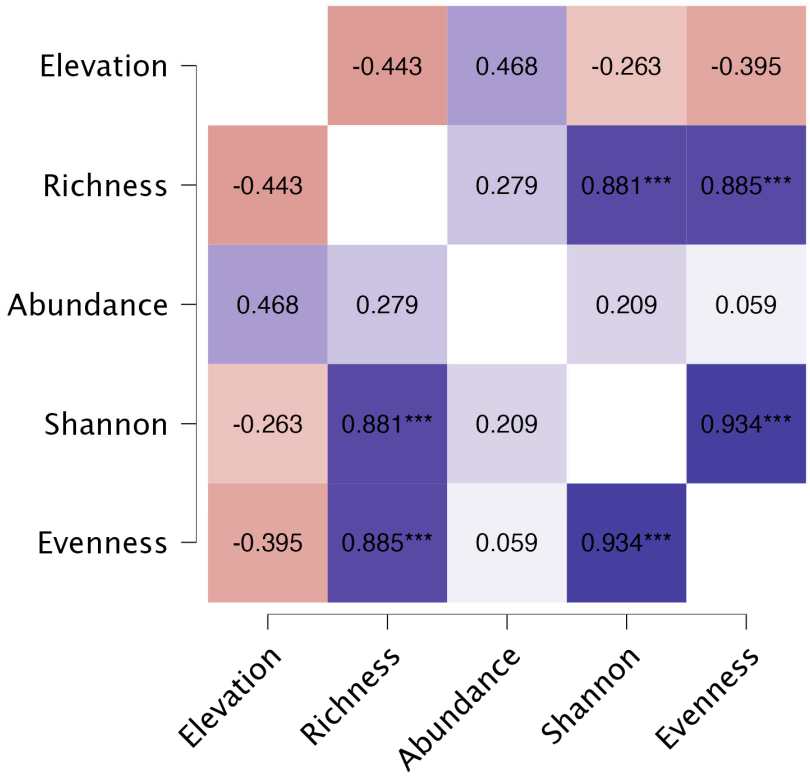


Figure 6. Heatmap of Pearson's *r* correlation among elevation and biodiversity parameters (*** $p < 0.001$).

3.4. Spatial Distribution of Trees

Figure 7 shows the spatial distribution of trees across the sampling plots in the secondary forest of San Luis in Aurora, Philippines. This map shows the position of the transect line and the approximate location of each individual tree, represented by colored dots (legend placed on the right-hand side), based on the recorded coordinates. As observed in the map, the plots were zoomed in to show the locations of the trees more clearly. We can also see in the background of the zoomed image of the plots the actual image of the forest cover in the area, as reflected in the base map used. Mapping the spatial distribution of trees is a crucial element in devising strategies for the sustainable management and conservation of natural resources [56]. For instance, locating the trees can help us identify areas with possible sources of mother trees of the targeted species that we aim to propagate [57]. For example, if we are looking for a source of planting materials for a high-priority species such as *M. stonei*, which is a very important species in the area due to the fact that it is an endemic and critically endangered species, we can refer to the map and see that it can be seen in plots 3, 5, 7, 8, 9, and 10. Furthermore, distribution maps can visually present areas needing attention and immediate measures, such as in the case of our study, the presence of invasive *G. arborea* that poses a threat to the native biodiversity. Knowing the location of its recorded individuals (present in plots 2 and 3) will allow the forest managers to perform targeted measures in managing specific portions of the area where invasion issues arise [48]. Lastly, we can identify micro-biodiversity hotspots among the sampling plots in the study area by determining the number of critically important species [58].

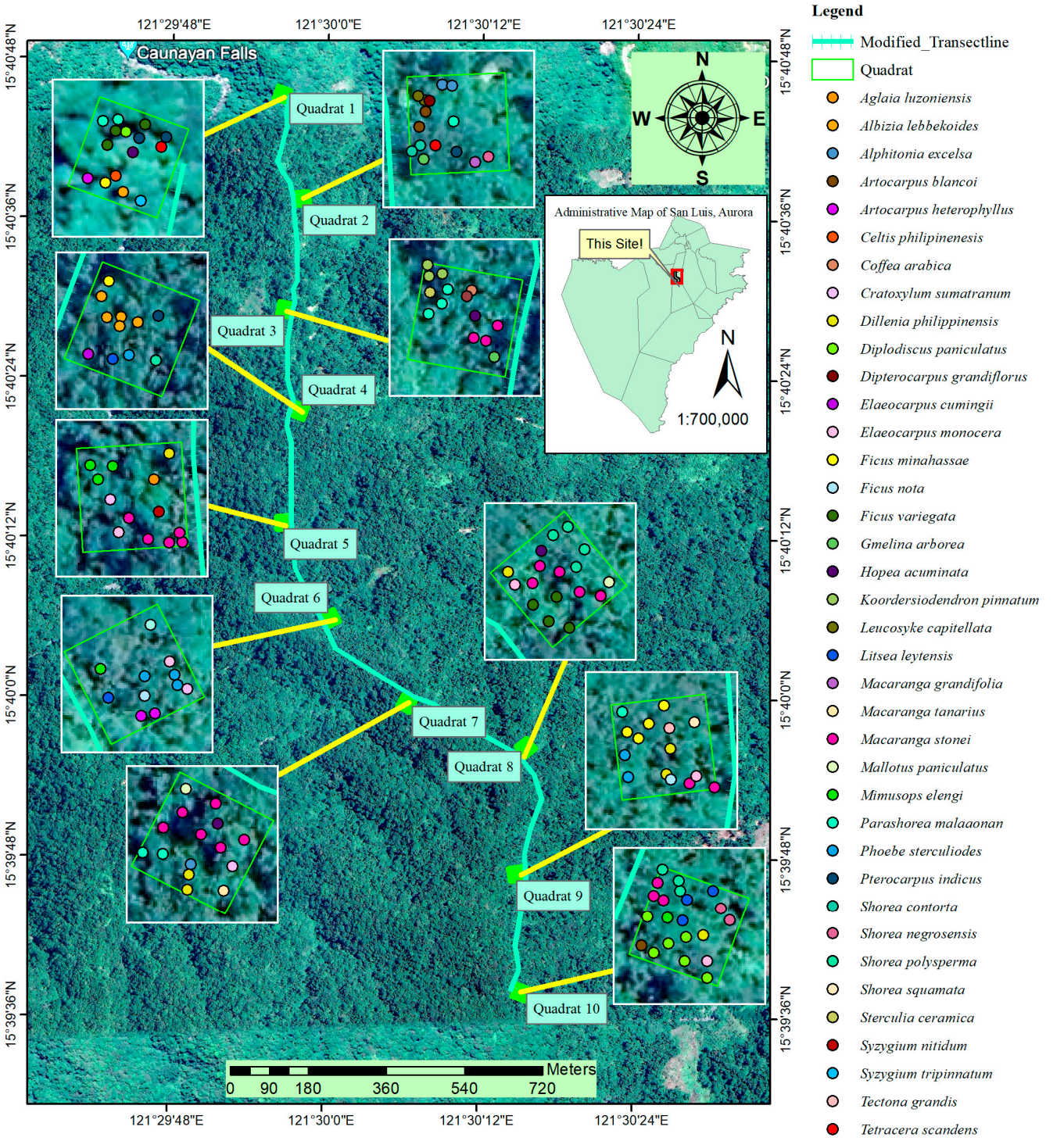


Figure 7. Distribution map of all trees across sampling plots in the secondary forests of San Luis, Aurora.

3.5. Research Limitations

This study provides valuable insights into the composition and diversity of tree species in the San Luis area of the SMMR. However, certain limitations were acknowledged. The research scope was limited to a short duration, and only a specific portion of the secondary forests of San Luis, Aurora, were covered by the transect, where variations in topography, exposure, and elevations, as well as forest dynamics, were not fully explored. Thus, these issues limit the generalizability of the findings in terms of larger ecosystems and dictate

the need for long-term monitoring and the eventual establishment of a protected area. Furthermore, tree species were the only life forms included in the study, opening the door for a more comprehensive assessment of other biodiversity components, such as understorey and ground vegetation, wildlife, and soil characteristics, which were beyond the scope of this study. These limitations are crucial in interpreting the results to guide future research directions and in planning a holistic and more effective biodiversity management and conservation.

4. Conclusions and Implications

This study yielded valuable findings and insights regarding the species composition and diversity of a secondary forest in San Luis, Aurora. Overall, the area had a relatively high diversity and significant conservation, as signified by the recorded 148 individuals of 38 morphospecies belonging to 20 families and 28 genera, with 33 natives, 12 endemics, five IUCN threatened, and nine Philippine threatened species. Furthermore, diversity was found to be high in terms of the Shannon–Weiner index ($H' = 3.269$) and very high in terms of the Simpson's Evenness index ($E = 0.9453$). Significant correlational relationships were also found among species richness, Shannon–Weiner index, and Simpson's Evenness index. Lastly, individual trees were mapped to serve as a guide for targeted conservation measures. These findings are critical in the following applications for the conservation of native, endemic, and threatened species:

1. The presence of many native, endemic, and threatened species underscores the immediate need to prioritize the conservation of these species through the aid of the map produced in locating the micro-biodiversity hotspots in the area. Furthermore, many endemic species lack scientific studies, highlighting the need to conduct focused studies to explore the ecology and distribution of these critically important species. Furthermore, this can serve as a basis for the Department of Environment and Natural Resources to include the forest as one of the high conservation priorities or to expand protected areas to cover the area surveyed.
2. The relatively high diversity values and even distribution of plants calculated for the area somehow indicate a relatively healthy ecosystem. Thus, this underscores the need for intensified law enforcement to protect the remaining forests that serve as habitats for native and endemic wildlife, such as *Buceros hydrocorax* Linnaeus.
3. The presence of introduced and invasive species such as *Gmelina arborea* Roxb. poses a very significant threat to local native biodiversity. Targeted and participatory invasive species management is needed to control and eventually eradicate the impact of invasive plants in the ecosystem.
4. All the implications and conservation strategies discussed above will need the participation of locals and other stakeholders due to the fact that the area is adjacent to residential communities. Thus, information and educational campaigns, as well as a participatory approach in implementing conservation strategies, are ideal tools to ensure more effective biodiversity conservation and protection.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Data Availability Statement: Dataset available on request from the authors.

Acknowledgments: The author would like to thank the following individual for their great contributions to this work: Diomedes Llait, Diadema Llait, Cherie Win L. Sanchez, and John Paul Llait for financial and moral guidance. The author would also like to extend gratitude to the faculty of the Department of Forestry, Tarlac Agricultural University, Christian Arvin M. Arce and Mark Anthony L. Bagsit for their assistance when conducting the research. The author also greatly appreciated the Local Government Unit of Barangay L. Pimentel, San Luis, Aurora for allowing the research to be conducted in their vicinity. Finally, a sincere thanks is given to the faculty of the School of Graduate Studies of Aurora State College of Technology, especially Mary Jane Aragon-Marigmen, RB Gallego,

Michelle Resueño, Jay Amon, Maria Cristina Cañada, and Afed Daiwey, for their untiring support and advise.

Conflicts of Interest: The author declares no conflicts of interest.

References

1. Vidallon, S.L.; Arriola, A.H. A preliminary checklist of Rubiaceae in Mt. Mariveles, Bataan, Philippines. *Biodiversity* **2023**, *24*, 1–10. [CrossRef]
2. Davids, S.; Mavumengwana, Z. Stocktaking SA's natural treasures. *Veld Flora* **2020**, *106*, 34–37.
3. Li, F.; Altermatt, F.; Yang, J.; An, S.; Li, A.; Zhang, X. Human activities' fingerprint on multitrophic biodiversity and ecosystem functions across a major river catchment in China. *Glob. Change Biol.* **2020**, *26*, 6867–6879. [CrossRef] [PubMed]
4. Ansari, N.A.; Agus, C.; Nunoo, E.K. Foundations of 'SDG15–LIFE on Land': Earth, Forests and Biodiversity. In *SDG15–Life on Land: Towards Effective Biodiversity Management*; Emerald Publishing Limited: Bingley, UK, 2021; pp. 7–48.
5. Sahu, D.; Sahu, J.K.; Kumar, V.; Gupta, P. Role of Floriculture in Promoting Biodiversity and Enhancing Ecosystems: A Comprehensive Review. *Int. J. Environ. Clim. Change* **2023**, *13*, 2077–2084. [CrossRef]
6. Keybondori, S.; Abdi, E.; Deljouei, A.; Lázaro-Lobo, A.; Ervin, G.N.; Shakeri, Z.; Etemad, V.; Borz, S.A. Effect of forest roadside on vegetation characteristics in the Hyrcanian temperate forest. *Eur. J. For. Res.* **2023**, *142*, 455–473. [CrossRef]
7. Sefidi, K.; Copenheaver, C.A.; Sadeghi, S.M.M. Anthropogenic pressures decrease structural complexity in Caucasian forests of Iran. *Écoscience* **2022**, *29*, 199–209. [CrossRef]
8. Caro, T.; Rowe, Z.; Berger, J.; Wholey, P.; Dobson, A. An inconvenient misconception: Climate change is not the principal driver of biodiversity loss. *Conserv. Lett.* **2022**, *15*, e12868. [CrossRef]
9. Jepson, P.; Canney, S. Biodiversity hotspots: Hot for what? *Glob. Ecol. Biogeogr.* **2001**, *10*, 225–227. [CrossRef]
10. Cunningham, C.; Beazley, K.F. Changes in human population density and protected areas in terrestrial global biodiversity hotspots, 1995–2015. *Land* **2018**, *7*, 136. [CrossRef]
11. Hind, E.J.; Hiponia, M.C.; Gray, T.S. From community-based to centralised national management—A wrong turning for the governance of the marine protected area in Apo Island, Philippines? *Mar. Policy* **2010**, *34*, 54–62. [CrossRef]
12. Gregorio, N.; Herbohn, J.; Harrison, S.; Pasa, A.; Ferraren, A. Regulating the quality of seedlings for forest restoration: Lessons from the National Greening Program in the Philippines. *Small-Scale For.* **2017**, *16*, 83–102. [CrossRef]
13. Lasco, R.D.; Pulhin, J.M. Environmental impacts of community-based forest management in the Philippines. *Int. J. Environ. Sustain. Dev.* **2006**, *5*, 46–56. [CrossRef]
14. Catibog-Sinha, C. Biodiversity conservation and sustainable tourism: Philippine initiatives. *J. Herit. Tour.* **2010**, *5*, 297–309. [CrossRef]
15. Mangaoang, E.O.; Pasa, A.E. Preferred native tree species for smallholder forestry in Leyte. *Ann. Trop. Res.* **2003**, *25*, 25–30.
16. Navarrete, I.A.; Peque, D.P.; Macabuhay, M.D. Soil information as a reforestation decision-making tool and its implication for forest management in the Philippines. In *Environmental Resources Use and Challenges in Contemporary Southeast Asia: Tropical Ecosystems in Transition*; Springer: Berlin/Heidelberg, Germany, 2018; pp. 97–116.
17. Appiah, M. Tree population inventory, diversity and degradation analysis of a tropical dry deciduous forest in Afram Plains, Ghana. *For. Ecol. Manag.* **2013**, *295*, 145–154. [CrossRef]
18. Coracero, E.E.; Malabrigo, P., Jr. Carbon storage potential of the tree species along the ultramafic forest in Sitio Dicasalarin, Barangay Zabali, Baler, Aurora, Philippines. *AIMS Environ. Sci.* **2020**, *7*, 589–601. [CrossRef]
19. Forest Foundation Philippines. Sierra Madre Mountain Range: Backbone of Luzon. 2022. Available online: https://www.forestfoundation.ph/wp-content/uploads/2022/04/Sierra-Madre-Mountain-Range_Landscape-Profile.pdf (accessed on 2 July 2023).
20. Coracero, E.E.; Malabrigo, P.L., Jr. Diversity Assessment of Tree Species in Sitio Dicasalarin, Barangay Zabali, Baler, Aurora, Philippines. *Open J. Ecol.* **2020**, *10*, 717–728. [CrossRef]
21. Bambalan, J.M.; Palapal, I.K.S.; Guleng, R.V.; Coracero, E.E.; Gallego, R.J.; Suniega, M.J.S. Tree diversity and carbon stock in North Poblacion and South Poblacion (Dipaculao, Aurora, Philippines). *Theor. Appl. Ecol.* **2022**, *2*, 198–208. [CrossRef]
22. Balberona, A.N.; Noveno, J.J.; Angeles, M.G.B.; Santos, R.I.; Cachin, E.J.D.J.; Cruz, K.G.J. Ethnomedicinal plants utilized by the ilongot-eǵongot community of Bayanihan, Maria Aurora, Aurora, Philippines. *Int. J. Agric. Technol.* **2018**, *14*, 145–159.
23. Barrogo, K.N.; Delos Santos, M.P.; Montes, A.A.T.; Quiben, A.D.; Rotaquio, E.L., Jr.; Valete, E.J.P. Fern and fern allies as non-timber forest product in Baler, Aurora, Philippines. *Int. J. Agric. Technol.* **2021**, *17*, 423–432.
24. Coracero, E.E.; Malabrigo, P.J.L.; Bambalan, J.M.; Palapal, I.K.S.; Guleng, R.V.; Gallego, R.J.; Suniega, M.J.A. Diversity, Species Composition, and Carbon Stock Assessment of Trees in Aurora, Philippines: Variations between Preserved and Developed Ecosystems. *Environ. Sci. Proc.* **2022**, *22*, 29.
25. World Weather Online. San Luis Annual Weather Averages. 2023. Available online: https://www.worldweatheronline.com/san-luis-weather-averages/aurora/ph.aspx (accessed on 5 July 2023).
26. USDA. *Multiparty Monitoring and Assessment Guidelines for Community Based Forest Restoration in Southwestern Ponderosa Pine Forests*; US Department of Agriculture, Forest Service, Southwestern Region: Washington, DC, USA, 2003; 94p.

27. Coritico, F.P.; Lagunday, N.E.; Galindon, J.M.M.; Tandang, D.N.; Amoroso, V.B. Diversity of trees and structure of forest habitat types in Mt. Tago Range, Mindanao, Philippines. *Philipp. J. Syst. Biol.* **2020**, *14*, 1–11.
28. Malabrigo, P.; Tobias, A.; Eduarte, G.; Terbio, L.; Hernandez, J.; Umali, A.G. Tree diversity and stand structure of a 2-hectare Permanent Biodiversity Monitoring Area (PBMA) in Mts. Iglit-Baco National Park, Mindoro Island, Philippines. *Ecosyst. Dev. J.* **2022**, *12*, 83–94.
29. Pelser, P.B.; Barcelona, J.F.; Nickrent, D.L. Co's Digital Flora of the Philippines. (2011 Onwards). Available online: http://www.Philippineplants.org (accessed on 1 May 2023).
30. Rojo, J.P. *Revised Lexicon of Philippine Trees*; Forest Products Research and Development Institute, Department of Science and Technology: Laguna, Philippines, 1999.
31. POWO. Plants of the World Online. 2023. Available online: https://powo.science.kew.org/ (accessed on 2 July 2023).
32. IUCN. The IUCN Red List of Threatened Species. 2023. Available online: https://www.iucnredlist.org (accessed on 2 July 2023).
33. DENR. *DAO 2017-11*; Department of Environment and Natural Resources: Quezon City, Philippines, 2017.
34. Fox, S.; Stefánsson, H.; Peternell, M.; Zlotskiy, E.; Ásbjörnsson, E.J.; Sturkell, E.; Wanner, P.; Konrad-Schmolke, M. Physical characteristics of microplastic particles and potential for global atmospheric transport: A meta-analysis. *Environ. Pollut.* **2023**, *342*, 122938. [CrossRef] [PubMed]
35. He, F.; Gaston, K.J. Estimating species abundance from occurrence. *Am. Nat.* **2000**, *156*, 553–559. [CrossRef]
36. Chao, A.; Chiu, C.H. Species richness: Estimation and comparison. *Wiley StatsRef Stat. Ref. Online* **2016**, *1*, 26.
37. Ismail, M.H.; Zaki, P.H.; Fuad, M.F.A.; Jemali, N.J.N. Analysis of importance value index of unlogged and logged peat swamp forest in Nenasi Forest Reserve, Peninsular Malaysia. *Int. J. Bonorowo Wetl.* **2017**, *7*, 74–78. [CrossRef]
38. Hernandez, J.; Umali, A.G.; Malabrigo, P. Floristic diversity assessment of Caramoan National Park, Camarines Sur, Philippines. *Ecosyst. Dev. J.* **2021**, *11*, 73–81.
39. Daly, A.J.; Baetens, J.M.; De Baets, B. Ecological diversity: Measuring the unmeasurable. *Mathematics* **2018**, *6*, 119. [CrossRef]
40. Pampolina, N.M.; Coracero, E.E.; Eco, K.O.; Tingson, K.N. Floristic Composition, Diversity, and Ecology for Conservation of Lower Agno Watershed Forest Reserve, Mountain Province, Philippines. *Asian J. Biodivers.* **2022**, *13*, 1–23.
41. De Villa, K.R.; Lagat, R.D. Species Diversity and Habitat Association of Ferns and Lycophytes in Mts. Palay-Palay Mataas na Gulod Protected Landscape. In *Plant Diversity in Biocultural Landscapes*; Springer Nature Singapore: Singapore, 2023; pp. 135–161.
42. Schober, P.; Boer, C.; Schwarte, L.A. Correlation coefficients: Appropriate use and interpretation. *Anesth. Analg.* **2018**, *126*, 1763–1768. [CrossRef] [PubMed]
43. Salvaña, F.R.P.; Lopez, C.K.C.; Mangaoang, C.C.; Bretaña, B.L.P. Diversity and community structure of trees in two forest types in Mt. Apo Natural Park (MANP), Philippines. *Biodivers. J. Biol. Divers.* **2019**, *20*, 1794–1801. [CrossRef]
44. Langenberger, G. Habitat distribution of dipterocarp species in the Leyte Cordillera: An indicator for species—Site suitability in local reforestation programs. *Ann. For. Sci.* **2006**, *63*, 149–156. [CrossRef]
45. Pang, S.E.; De Alban, J.D.T.; Webb, E.L. Effects of climate change and land cover on the distributions of a critical tree family in the Philippines. *Sci. Rep.* **2021**, *11*, 276. [CrossRef] [PubMed]
46. Volis, S.; Belolipov, I.V.; Asatulloev, T.; Turgunov, M. Role of endemism and other factors in determining the introduction success of rare and threatened species in Tashkent Botanical Garden. *J. Zool. Bot. Gard.* **2023**, *4*, 325–334. [CrossRef]
47. Manchester, S.J.; Bullock, J.M. The impacts of non-native species on UK biodiversity and the effectiveness of control. *J. Appl. Ecol.* **2000**, *37*, 845–864. [CrossRef]
48. Coracero, E.E. Distribution and Management of the Invasive Swietenia macrophylla King (Meliaceae) at the Foot of a Protected Area in Luzon Island, Philippines. *J. Zool. Bot. Gard.* **2023**, *4*, 637–647. [CrossRef]
49. Batani, R.S.; Basbas, A.V., Jr.; Loncio, R.S.; Napaldet, J.T. Floral diversity in a secondary forest managed by indigenous community: The case of Mt. Kili-kili in Benguet, Cordillera Central Range, Northern Philippines. *Biodiversity* **2023**, *24*, 212–230. [CrossRef]
50. Lleno, J.V.; Ligalig, R.J.; Sarmiento, R.T.; Along, A.A. Tree diversity, composition, and stand structure of lowland tropical forest in Prosperidad, Agusan del Sur, Philippines. *J. Surv. Fish. Sci.* **2023**, *10*, 4810–4830.
51. Mancera, J.P.; Ragragio, E.M.; Su, G.L.S.; Rubite, R.R. Plant community structure of a secondary forest at Barangay Camias, Porac, Pampanga, The Philippines. *Philipp. J. Sci.* **2013**, *142*, 135–143.
52. Carig, E.T.; Manuel, R.P. Tree Diversity and Timber Resources Assessment in Secondary Forests of Quirino Forest Landscape Project, Philippines. *Asian J. Biodivers.* **2021**, *12*, 36–54. [CrossRef]
53. Castillo, M.L.; Castillo, L.A.; Canceran, M.S.; Gonzalvo, K.J.P.; Barua, L.D.; Alegre, A.C.; Barredo-Parducho, V.O.; Gestiada, E.; Breva, R.; Bantayan, N.C. Distribution, diversity and spatial analysis of tree species in a long-term ecological research plot in Molawin-Dampalit Watershed, Mount Makiling Forest Reserve. *Asian J. Biodivers.* **2018**, *9*, 12–36. [CrossRef]
54. DeJong, T.M. A comparison of three diversity indices based on their components of richness and evenness. *Oikos* **1975**, *26*, 222–227. [CrossRef]
55. Gevaña, D.; Pollisco, J.P.; Pampolina, N.; Kim, D.; Im, S. Plant diversity and aboveground carbon stock along altitudinal gradients in Quezon Mountain Range in Southern Mindanao, Philippines. *J. Environ. Sci. Manag.* **2013**, *16*, 20–28. [CrossRef]
56. Pu, R. Mapping tree species using advanced remote sensing technologies: A state-of-the-art review and perspective. *J. Remote Sens.* **2021**, *2021*, 9812624. [CrossRef]

57. Engay-Gutierrez, K.; Dimailig, E.; Yacon, J. Plus and Mother Trees in Mt. Banahaw de Lucban, Quezon, Philippines. *J. Environ. Sci. Manag.* **2022**, *25*, 33–48. [CrossRef]
58. Harris, G.M.; Jenkins, C.N.; Pimm, S.L. Refining biodiversity conservation priorities. *Conserv. Biol.* **2005**, *19*, 1957–1968. [CrossRef]

Development and Assessment of Outdated Computers: A Technology Waste for Alternative using Parallel Clustering

Jeffrey John R. Yasay

Department of Computer Studies, College of Engineering and Technology
Tarlac Agricultural University, Camiling Tarlac, Philippines

jryasay@tau.edu.ph

Abstract. Technology is constantly evolving to the point that computers that are purchased then are inevitably outmoded in terms of speed and their ability to process new applications. The study aims to provide procedure and measurement in viewing the process of the parallel clustered computers via graphical representation. The idea of the development procedure has been conceptualized by the author to elevate obsolete computer for alternative use. Likert scale was used (experts and users) in assessing the system. It was found out that the development has a promising result as evident in the assessment of experts on the system's reliability (availability and stability) and the users' assessment of the system's accessibility (ease of use and flexibility). It is also noted that obsolete computers have alternative disposal technique of e-wastes. With this, the development of clustering (using the interconnectivity of a master node and slave nodes) that is reliable, accessible and with a minimal cost was conceptualized as an alternative for managing e-waste and addressing the demand of new technology in the public sectors.

Keywords: Parallel clustering, processing power, alternatives & e-waste.

1 Introduction

Nowadays, technology has become an essential part of our lives. New technology has paved the way for smartphones, faster and more powerful computers, more compact televisions and so much more. Technology has made our lives simpler, quicker, safer and more enjoyable.

Technology has truly revolutionized the way we live and the way we work. It has provided opportunities for productivity and development. It has made working more effective and efficient in general as companies continue to invest in cutting-

edge technologies.

With all the promising outcomes of technology, companies have embraced it and enjoy all the profits it could give. It has played a crucial role in companies that technology is no longer seen as cost but more of an investment. At present, various companies and industries have strategically advanced their technologies to cope with the ever-changing world.

However, as technology progresses, there were also setbacks created by them. So much of the wastes from various industries come from the technologies that are utilized in their gateways. E-wastes, or the electronic products nearing the end of their "useful life" such as computers, televisions, copiers, and fax machines are some of the challenges in the fast-paced technology development.

E-waste, also known as "a wide and growing range of electronic devices ranging from large household appliances such as refrigerators, air conditioning, cell phones, personal stereos and consumer electronics to computers that have been discarded by their users" [1], has a major effect as technology progresses. Technology has developed and progressed so fast. Rapid application development has become challenging for developers to adapt, although some are searching for alternatives that will potentially help urbanized communities develop those technology.

As we live in a world that is geographically complex and unpredictable, new business forces are generated by the rush of mega-trends, including dramatic shifts in globalization and advances in technology. For any organization to survive and prosper in such an environment, innovation is imperative.

However, innovation is no longer just for creating value to benefit individuals, organizations, or societies. Innovation's overall goal can be far more far-reaching, helping to build a smart world where people can achieve the highest possible quality of life [2].

Over the past decade, technical advances have accelerated the exponential use of multimedia tools by learners of all ages. These global trends also include the constant progression of the e-learning assessment. Evaluation is the practice of clarifying what needs to be done and relating it to what needs to be done, in order to promote the evaluation of performance and how it should be achieved [3]. In terms of speed and their ability to process new applications, computers which are then bought are ultimately outdated. When this happens, outdated computers are considered to be redundant. This also happens in sectors where computation plays a crucial role in development and achievement. As necessity dictates, there is a need to find a way in which these devices, considered redundant and worthless, can be useful in constructing computers that can meet the demands of whatever

endeavours.

A cluster consists of a series of interconnected stand-alone computers operating together as a single consolidated computing resource and is a type of parallel or distributed computer system [4]. Clustering is commonly used in a network to reduce the energy consumption and thus increase the network longevity [5]. In other terms, cluster is a series of separate and inexpensive computers, used together to provide a solution as a supercomputer.

Cluster computing provides a single general approach for designing and implementing high-performance parallel systems independent of individual hardware manufacturers and their product preferences [6]. A typical application of cluster parallel computing is to load and disperse the demand for processes by the master node to the slave nodes. The information is transmitted from the source to its respective cluster head and then to the base station in order for the selected head to bear all of the information that needs to be transmitted and route it to the intended target [7]. A commodity cluster is an array of entirely autonomous computer systems that are interconnected by an off-the-shelf networking network of commodity interconnections [8] and play a major role in redefining the supercomputing concept. As a result, high-performance high-throughput, and high-availability computing has arisen as parallel and distributed standard platforms.

With this, the development of clustering (using the interconnectivity of a master node and slave nodes) that is reliable, accessible and with a minimal cost was conceptualized as an alternative for managing e-waste in the public.

2 Build and Architecture

2.1 The parallel clustered uniform set-up

After the selection of obsolete system attachments on peripherals, cluster computers must be built.

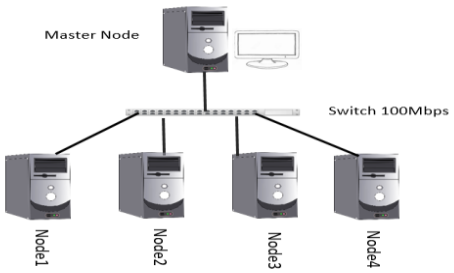


Fig. 1. Indicates the cluster clustering connectivity. The development was based on computer architecture clustered in parallel.

2.2 Production Instruments

The design of the clustered computers was based on the hardware and software needed to meet the demand of cluster computers are (a) personal computers consist of the same basic components: a CPU, memory, circuit board, storage, and input/output devices [9] (b)fast ethernet switch [10] (c)straight cable (T568A – T5668A) [11] and (d) Ubuntu ABC GNU/Linux [12].

2.2 Setup Clustering

Homogeneous computing is used to interconnect identical processor cores or units to create a high-performance device in order to use a homogeneous parallel clustering mechanism [13]. The nodes 1-4 and the master node all come in the same "Boot to Network" BIOS (basic input output system) configuration connected via T568A using Cat-5E UTP cable.

2.3 Installation (Software)

The next move is to install the program after the computers have been assembled. ABC GNU Linux (Ubuntu 9.04) [10] was used with the default kernel as a basis. Upon the installation of ABC GNU Linux (Ubuntu 9.04), gathered the information about the hardware specifications.

2.4 Specification and checking of device

Step 1: Upon determining the master node and slave node this will be the basis of hetorogenousity of the system as the specification be Processor: Intel Celeron M CPU with a CPU Speed: 2266 MHz

Step 2: Setting up of ABC GNU Linux kernel ISOLINUX3.63 Debian to the master node. Boot from the CD-ROM then choose an install mode, press enter then

follow the directions on the screen. The default language of the distro is Spanish. Changing to your preference language is necessary. After which select use entire disk to partition the hard disk, then create username and password and lastly install ABC GNU (Ubuntu 9.04)

Step 3: Setting up the slave nodes, first enter the configuration or setup of CMOS, choose halt on ALL ERROR, and finally set-up to boot from the network.

Step 4: This procedure will check the master node via Command Line Interface (CLI), master@master-desktop:~$ cat clusterhosts 192.168.0.1. Upon checking proceed to connectivity check this will test the network connectivity of Master Node, Node1, Node 2, Node 3, and Node 4, master@master-desktop:~$ cat clusterhosts 192.168.0.1 192.168.0.13 192.168.0.3 192.168.0.10 192.168.0.8.

3 Monitoring

3.1 Cluster interpretation of GANGLIA monitoring tool (GUI) [14]

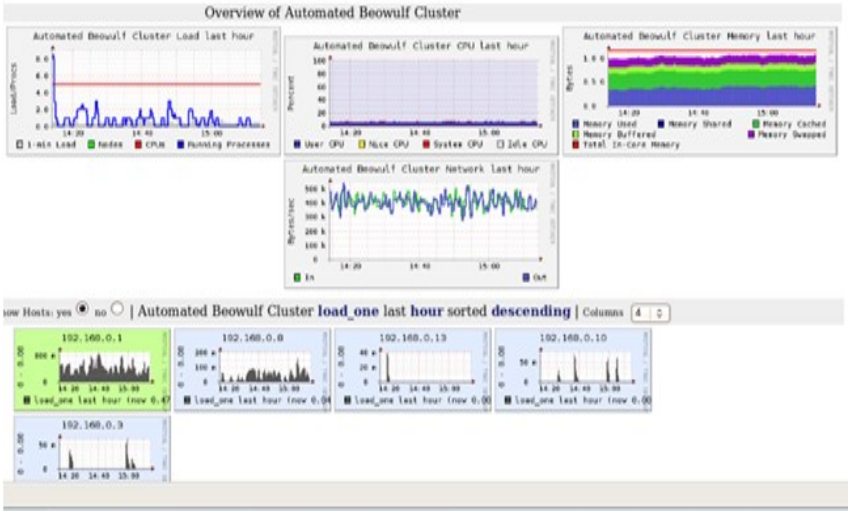


Fig 2. Overview of Automated Beowulf Cluster using Ganglia

Fig 2. Shows the device view of the cluster. A series of small graphs display the master node, and processes are used for nodes 1-4. It also indicates that the master node and nodes 1-4 work with various processes.

3.2 Performance differences of machine loaded

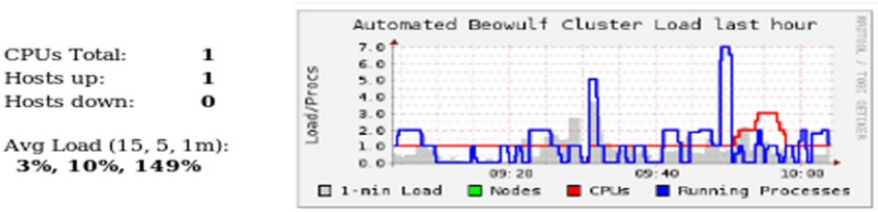


Fig 3. Performnace of Total hosts (1 CPU)

Fig 3. Displays performance representation from 1 host. It showed that the average capacity of a single CPU was 3%, 10% and 149%, showing that it is hard for a single host to process.

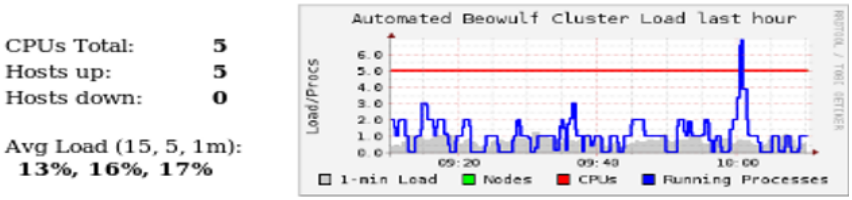


Fig 4. Performnace of Total hosts (5 CPU)

Fig 4. Shows the performance of 5 host computer. It indicates that the average load of performance is 13% , 16% and 17% which reveal that a multiple hosts process smoothly.

3.3 Network flow by graph (Master Node and Node 1)

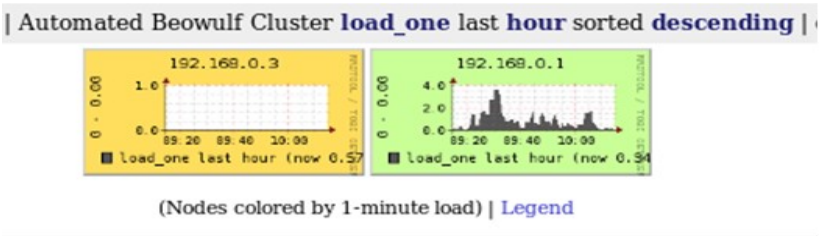


Fig 5. Master Node and Node 1

Fig 3. Reveals the master node and node 1. It ensures that the Master Node process and Node 1 process are distinct from one another. This also shows how process efficiency and relation identification are calculated.

3.4 Network movement process by graphs (Master Node and Node 1-4)

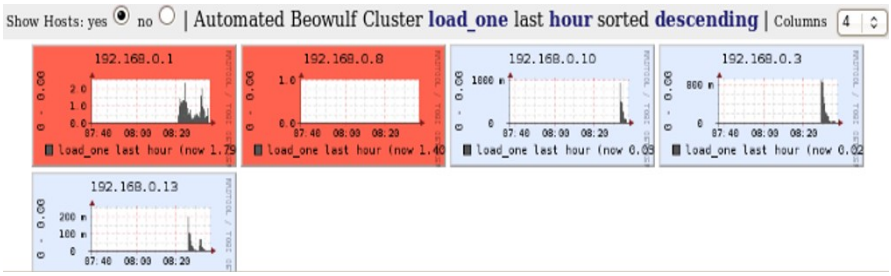


Fig 6. Process Identification of Nodes

Fig 6. Shows that the use of the CPU is 100%, it also shows that the Master Node and Node 1 used their processing power in the process distribution. It also reveals that different nodes have distinct processes.

3.5 Network movement process by graphs in Shutting down of Nodes

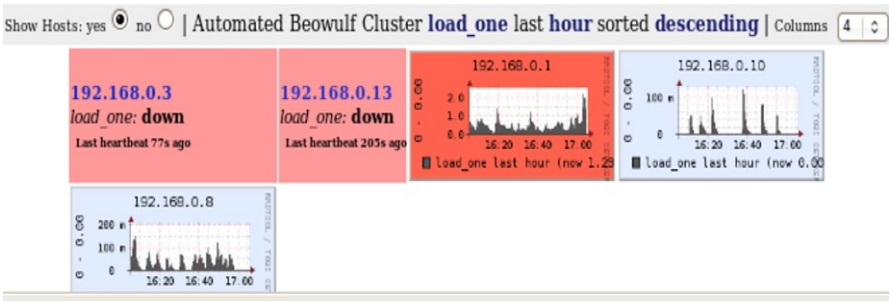


Fig 7. Node Process in Shutting Down

Fig 7. Indicates the Nodes have been successfully shut down. In the image and graph, the master node and the remaining nodes used that homogeneous parallel clustering processes are established and used.

4 Evaluation and Results

Two approaches are applied to test the homogeneous parallel clustering of alternatives for success acceptance and creation: by IT experts and by the users. The IT experts assessed the system as to Reliability with system availability and system stability [15] while the users rated the system as to accessibility with ease of use and flexibility of the system [16]. The questionnaire was based on the

Likert scale suggested by ISO 9126 [17] and used to analyze the results from scales 4.01-5.0 as excellent, 3.01-4.0 as very good, 2.01-3.0 as good, 1.01-2.0 as fair and 0-1.0 as poor with the following informative equivalents.

4.1 IT Experts

Table 1. Assessment of the System by IT Experts

Assessment Criteria	Mean	Descriptive Rating
Realiability (Composite Mean: 4.08)		
System Availability	4.50	Excellent
System Stability	3.67	Very Good

Table 1 shows the results of the evaluation based on the reliability of the system. It obtained a composite mean of 4.08.

The IT Experts evaluated the reliability of the system based on the system availability with a 4.50 mean with a descriptive rating of Excellent and system stability with a 3.67 mean with a descriptive rating of Very Good.

4.2 Assessment of Users

Table 2. Assessment of the System by Users

Assessment Criteria	Mean	Descriptive Rating
Accessibility (Composite Mean: 4.69		
Ease of Use	4.67	Excellent
Flexebility of the System	4.72	Excellent

Table 2 shows the results of users' assessment using a homogeneous parallel cluster. The users of the system were the students, IT faculty, and employees of Tarlac Agricultural University. To obtain the reliability of the evaluation, there were sixty (60) users who evaluated the system.

They evaluated the system accessibility based on ease of use with 4.67 as excellent and flexibility of the system with 4.72 as excellent. The system accessibility obtained a composite mean of 4.69 with a descriptive rating of excellent. The result indicate the uncomplicatedness of the system's operation.

5 Conclusion

The study found that the development and assessment result of the homogeneous parallel process clustering as alternatives is significant. Over the course of the review and testing, the performance of the machine was not damaged. Hence, the achievement of serviceable machines with low development costs has been established and guaranteed. Moreover, based on expert opinion and review, the use of a homogeneous parallel clustering method is strongly appropriate. The functionality of the framework was based on the efficiency of parallel clustering, and it also notes that operating is the master process and the nodes. Finally, because of the ease of service, the system's assessment is strongly appropriate to consumers in terms of usability.

In addition, the study findings have been established and could be introduced to other universities and schools in the area which will be used as an alternative computer to run application in today's technology demands. This will assist faculty, teachers and staff in researching other technical development and device efficiency.

Nevertheless, with the use of clustering strategies and encouraging e-waste management, universities and schools to alternatively develop outdated computers.
A future collection of machines with changed architectures will be selected for future work to enhance the analysis, in order to observe the effect of heterogeneity on the efficiency and growth of the clustering technique.

Lastly, to increase device reliability, an implementation can require additional measures, configurations, and performance review. Additional testing methods are also recommended to determine the efficiency of the device being built.

References

1. D. Sinha-Khetriwal, The management of electronic waste: a comparative study on India and Switzerland, M.S.
2. M Sang M.Leea & SilvanaTrimi (2016). Innovation for creating a smart future, Journal of Innovation & Knowledge, ISSN: 2444-569X, Vol: 3, Issue: 1, Page: 1-8ay
3. D.D. Williams, C.R. Graham, in International Encyclopedia of Education (Third Edition), 2010
4. Yeo C.S., Buyya R., Pourreza H., Eskicioglu R., Graham P., Sommers F. (2006) Cluster Computing: High-Performance, High-Availability, and High-Throughput Processing on a Network of Computers. In: Zomaya A.Y. (eds) Handbook of Nature-Inspired and Innovative Computing. Springer, Boston, MA. 2006.
5. Mugunthan, S. R. "Novel Cluster Rotating and Routing Strategy for software defined Wireless Sensor Networks." Journal of ISMAC 2, no. 02 (2020): 140-146.

6. Thomas Sterling, in Encyclopedia of Physical Science and Technology (Third Edition), 2003
7. Raj, Jennifer S. "Machine Learning Based Resourceful Clustering With Load Optimization for Wireless Sensor Networks." Journal of Ubiquitous Computing and Communication Technologies (UCCT) 2, no. 01 (2020): 29-38.
8. Thomas Sterling, ... Maciej Brodowicz, in High Performance Computing, 2018
9. Casey, J. (2015). Computer Hardware: Hardware Components and Internal PC Connections. Guide for undergraduate students. Technological University Dublin
10. Sachidananda Kangovi, in Peering Carrier Ethernet Networks, 2017
11. Naomi J. Alpern, Robert J. Shimonski, in Eleventh Hour Network+, 2010
12. Castaos, I & Garrido, Izaskun & Garrido, Aitor & Sevillano, M. (2009). Design and implementation of an easy-to-use automated system to build Beowulf parallel computing clusters. 1 - 6. 10.1109/ICAT.2009.5348420.
13. Van Steen, M., & Tanenbaum, A. S. (2016). A brief introduction to distributed systems. Computing, 98(10), 967–1009. doi:10.1007/s00607-016-0508-7
14. Matthew L. Massie, Brent N. Chun, and David E. Culler. 2004. The ganglia distributed monitoring system: design, implementation, and experience. Parallel Comput. 30, 7 (2004), 817--840.
15. O'Connor, D.T., and A. Kleyner. 2012. ''Practical Reliability Engineering'', 5th Edition. Chichester, UK: J. Wiley & Sons, Ltd.
16. Petrie, Helen & Bevan, Nigel. (2009). The Evaluation of Accessibility, Usability, and User Experience. C Stepanidis. 10.1201/9781420064995-c20.
17. ISO/IEC 9126, Software Engineering - Product quality, Parts 1-4, 1999-2004.

Validation and molecular analysis of *β-1,3-GLU2 SNP marker* associated with resistance to *anthracnose* in Philippine carabao mango (*Mangifera indica* L. cv. 'Carabao')

John Edward L. Felipe[1,3]*, John Albert P. Lachica[1], Fe M. Dela Cueva[1], Niño R. Laurel[1], Carolyn E. Alcasid[1], Maria Luz J. Sison[1], Lolita DC. Valencia[1], and Eureka Teresa M. Ocampo[2]

[1] *Institute of Plant Breeding and* [2] *Institute of Crop Science, College of Agriculture and Food Science, University of the Philippines Los Baños, College, Los Baños, Laguna 4031*

[3] *Department of Natural Sciences, College of Arts & Sciences, Tarlac Agricultural University, Camiling, Tarlac 2306*

* Corresponding author: jlfelipe@tau.edu.ph

ABSTRACT

In the Philippines, mango anchors a million-dollar industry which is largely dependent on the export of a sole variety, the 'Carabao' mango. Varietal improvement of the 'Carabao' mango involves the introgression of anthracnose resistance to improve its yield and export quality. Molecular genomic tools such as single nucleotide polymorphism (SNP) markers provide a platform to accelerate breeding of resistant varieties through marker-assisted selection (MAS). Here, we developed and analyzed the molecular basis of an SNP marker within the pathogenesis-related β-1,3-glucanase 2 (β-1,3-GLU2) gene putatively linked with resistance to anthracnose. This SNP is an A/G transition causing a missense mutation (I196V) in glycosyl hydrolase family 17 (GH17), a highly conserved domain involved in physiologically important processes in plants, notably in response to biotic and abiotic stresses. Structural analysis suggested that the I196V mutation resulted in conformational changes in the enzyme's (β/α)8 TIM-barrel motif and catalytic groove by causing a steric clash with V261 residue, thereby possibly affecting the overall protein stability or catalytic activity and subsequently inhibit fungal defense response mechanisms. Our findings also demonstrated the association of this SNP marker with anthracnose resistance wherein mango accessions with the mutant allele 'G' showed significantly higher disease severity post-inoculation while those with wildtype allele 'A' showed phenotypic resistance against anthracnose. The positive correlation between the type of SNP allele present and reaction of mango to *C. gloeosporioides sensu lato* coupled with the ability of the marker to discriminate SNP alleles using a simple and cost-effective allele-specific PCR assay make it suitable for MAS. The results of this study support the utilization of the developed β-1,3-GLU2 SNP marker for routine screening of anthracnose resistant phenotypes as early as the seedling stage. This will help improve mango breeding efficiency and significantly reduce the expenses in field inputs, maintenance, and evaluation of material over years.

Keywords: AS-PCR, Carabao mango, *Colletotrichum gloeosporioides sensu lato*, Genotype-by-sequencing, Glucan endo-1,3-beta-glucosidase, Single nucleotide polymorphism

1. INTRODUCTION

Mango (*Mangifera indica* L.) is a commercially important fruit crop in the tropical and subtropical regions, particularly in Asia. It anchors a million-dollar industry ranking sixth in terms global fruit production after bananas, watermelons, apples, oranges, and grapes (FAO, 2019a). In 2018, the global production of mango fruit reached up to over 52 million metric tons, with more than one thousand varieties grown in Asia, Central and South America, and Africa (FAO, 2019b). Although there is increasing demand in developed countries, only 3-4% of the global production is traded internationally and the rest is traded and consumed domestically (Mitra, 2016). Despite its excellent qualities, the export potential of mangoes are not fully attained due to its short shelf-life, thin peel and low quality and production yield attributed to susceptibility to insect pests and pathogenic diseases. The high average temperature and relative humidity in tropical regions favor the rapid development of diseases at both the pre- and postharvest stages, which directly affect fruit quality and yield (Dodd et al., 1991). One of the most serious and destructive diseases of mango is anthracnose, which is primarily caused by the fungus *Colletotrichum gloeosporioides* (Dodd et al., 1991). This fungus causes leaf blight, blossom blight, mummified fruits, and dieback by infecting leaves, flowers and juvenile fruits (Arauz, 2000). During postharvest, germination of dormant fungal spores is induced by ripening and disease development becomes apparent when black, sunken, rapidly proliferating lesions develop on marketable fruits rendering them worthless and non-marketable (Akem, 2006).

Different disease management strategies have been developed to control the disease but these often involve excessive use of fungicides, which are expensive and damaging to the environment (Dodd et al., 1991). Another management method, the hot water treatment (HWT), needs to be revalidated for its fungistatic rather than fungicidal ability (Alvindia & Acda, 2015). In the absence of cheap, safe and efficient protection measures, breeding for resistance is valuable in solving the growing problem in the mango industry. However, traditional mango breeding programs are slow and challenging due to several

factors such as long juvenile stage, long generation time, high heterozygosity, and low crossing rates (0.1%). The detection of the disease poses additional problems in traditional mango breeding programs as infected fruits show no signs of the disease until the onset of ripening.

Advancements in molecular biology provide genomic tools such as DNA markers which can aid in the selection of target traits and to accelerate the breeding process of new varieties of mango. DNA markers are sequences with a known location in the genome and they can assist in breeding selection when found associated with a desired trait. Among the DNA markers, single nucleotide polymorphisms (SNPs) are advantageous in marker-assisted selection (MAS) due to its high density and abundance across the genome (Syvänen, 2005). SNPs are single base changes in the DNA, which allow a higher probability of finding an SNP-based marker within the gene of interest. SNP marker detection can be automated, enabling high-throughput analysis appropriate for breeding programs involving large populations (J. Kumar et al., 2011; Syvänen, 2005; Y. Xu & Crouch, 2008). Although several studies have already used SNPs in linkage mapping and estimation of genetic diversity in mango (Iquebal et al., 2017; D.N. Kuhn et al., 2016; David N. Kuhn et al., 2019; Sherman et al., 2015; Singh et al., 2016; Warschefsky & von Wettberg, 2019), SNP markers associated with important horticultural traits, including disease resistance, are yet to be developed and used for marker-assisted selection in mango breeding.

In the present study, a previously identified SNP marker located within a putative defense-related gene associated with resistance against *Colletotricum gloeosporioides* infection was developed and validated for use in marker assisted selection (MAS) of anthracnose resistant mango phenotypes. We hypothesize that this SNP plays a role in *C. gloeosporioides* pathogenicity on mango fruit that may confer resistance to anthracnose disease.

2. MATERIALS AND METHODS

2.1 Plant materials

A total of 143 mango varieties and strains from commercial growing areas and research institutions in the Philippines were used in the present study. Genetic resources in this collection include 130 'Carabao' mango strains, 9 commercial varieties, and 4 unknown cultivars. Scions of mango collections showing promising traits were collected and asexually propagated by cleft-grafting and maintained at the fruit crops nursery of the Institute of Plant Breeding, University of the Philippines Los Baños (UPLB), Laguna, Philippines.

2.2 Phenotypic screening for anthracnose resistance

Preliminary screening of mango accessions for anthracnose resistance was performed using the pathogen *Colletotrichum gloeosporioides sensu lato* isolated from mango samples collected from various provinces in the Philippines through tissue planting technique. The most aggressive isolate (Mg12) was used in the study (Figure). Molecular identity of this fungal isolate was established through PCR assay using Col1/Col2 (Martinez-Culebras et al., 2003) and CgInt/ITS4 (Mills et al., 1992) primer pairs. Fungal spores were harvested by adding 5 mL sterile distilled water on 7-day old culture and scraped using flamed L-spreader. Spore suspension was filtered on a four-layered sterile gauze cloth to filter only the fungal spores. Spore count was adjusted to 10^6 spores/mL using hemacytometer. Fruits were artificially evaluated *in vitro* via spore suspension droplet inoculation technique. Samples were disinfected using 20% sodium hypochlorite for 10 minutes, rinsed with sterile distilled water twice and blot dried using sterile tissue paper. Disinfected fruits were placed in a moisture chamber with moistened sterile cotton to maintain humid condition (Alcasid et al., 2018; Torres-Calzada et al., 2013). Each fruit was pricked with sterile 1 mL syringe to create wound and inoculated with 20 µL of spore suspension (10^6 spores/mL). Reaction to mango anthracnose was observed after 12 days of incubation following the disease rating scale (Table 1. Disease rating scale used for the phenotypic screening in this study.) and disease severity was calculated using the formula previously described by Paull (2002).

Table 1. Disease rating scale used for the phenotypic screening in this study.

Rating Scale	Description	Reaction
0	No infection	Immune
1	1-5% of the total fruit surface infected	Highly Resistant
3	6-15% of the total fruit surface infected	Resistant
5	16-30% of the total fruit surface infected	Intermediate
7	31-50% of the total fruit surface infected	Susceptible
9	More than 50% infection	Highly Susceptible

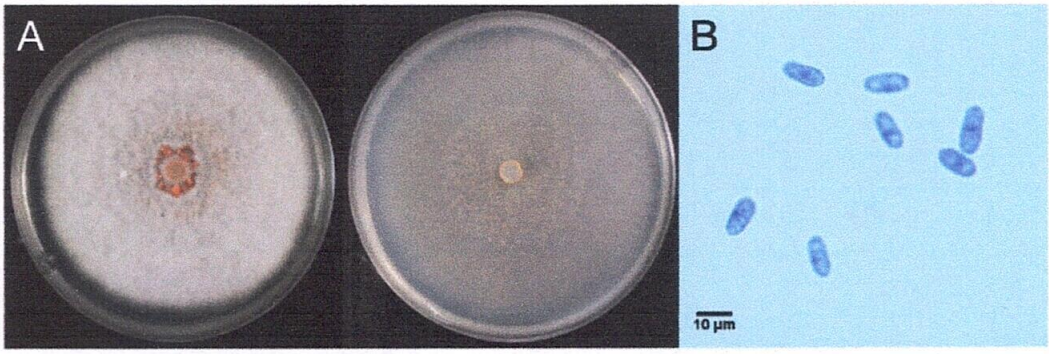


Figure 1. (A) Colony morphology of *C. gloeosporioides sensu lato* isolate MG12 and its (B) spores under 40X magnification.

Confirmatory evaluation of resistance was conducted for accessions that initially showed resistant and intermediate reactions in the preliminary trials. Approximately 120-day old mango fruits were disinfected and reactions were evaluated following the methods described above.

2.3 Genomic DNA isolation

Genomic DNA was isolated from the youngest, fully mature and green leaves following the modified CTAB protocol of Lachica et al. (2019). DNA stock solutions were quantified using Epoch Microplate Spectrophotometer (BioTek® Instruments, Inc., USA) and diluted to 50 ng/µL with sterile nanopure water. All extracted DNA and working stocks were stored at -20°C until use.

2.4 *β-1,3-GLU2* marker design and validation

A significant mango SNP (AlleleID 21881933) was recently identified by the Institute of Plant Breeding–Physiology Laboratory of UPLB (unpublished data) using Genotyping-by-Sequencing (GBS) generated by DArTseq™ platform (Diversity Arrays Technology Pty Ltd., Canberra, Australia). Gene annotation reveals that SNP 21881933 (A>G) is within a putative Glucan endo-1,3-beta-glucosidase 2 or *β-1,3-glucanase 2* (*β-1,3-GLU2*) gene. This SNP was developed into an allele-specific PCR (AS-PCR) marker for experimental validation. Gene-specific primers were designed based on the *Citrus sinensis* glucan endo-1,3-beta-glucosidase 2 (top hit) conserved regions flanking the SNP site using NCBI Primer-BLAST (Altschul et al., 1990). The gene specific forward primer (Glu2-F) was 5'—ACTGCAGCTAATTGGG—3' while the reverse primer (Glu2-R) was 5'—GGTTTGTAGTATCATTTGCT—3'. In addition, an allele-specific forward primer (AS-F), 5'—GTTCGTATCTCATGCTCAAAG—3', with a 3'-terminal nucleotide corresponding to the target mutant SNP allele "G" was designed using the Web-based Allele Specific Primer (WASP) tool (Wangkumhang et al., 2007). All primers were synthesized by Integrated DNA Technologies (IDT), Singapore.

PCR reactions were performed in a final mix of 10 µL using the iNtRON PCR Kit (iNtRON Biotechnology, Inc., South Korea) containing 1X PCR Buffer A, 0.2 mM dNTPs, 0.5 U i-Taq™ DNA Polymerase, 0.2 mM of each primer, 10 ng of template DNA, and sterile nanopure water. The following optimized thermal cycling conditions were performed on T100 Thermal Cyclers (BioRad® Laboratories, USA): 2 min at 94 °C, followed by 30 cycles of 20 s at 94 °C, 10 s at 50 °C (annealing), 30 s at 72 °C, and 1 cycle of 5 min at 72 °C. PCR products were visualized in 2% agarose gel electrophoresis using the GelDoc™ XR+ Gel Documentation System (BioRad® Laboratories, USA). The presence of the SNP allele 'G' was based on the detection of a 507-bp fragment in the AS-PCR assay. Amplifications that did not contain this fragment indicates the presence of the SNP allele 'A'. An 805-bp fragment, serving as internal control, indicates the successful amplification of the *β-1,3-GLU2* gene fragment. Analysis of variance was performed for the available phenotypic data, and the association between the SNP alleles and reaction to anthracnose (% disease severity) was analyzed by performing a Pearson correlation and linear regression analysis using IBM SPSS Statistics v23.0 software (SPSS, Chicago, USA).

2.5 Sequence analysis and molecular modelling of mango *β-1,3-GLU2 and SNP 21881933*

Amplified *β-1,3-GLU2* gene fragments from identified resistant and susceptible accessions were sequenced using Sanger technology (1st BASE, Selangor, Malaysia) to further validate the presence of the SNP and the identity of the target gene. Multiple sequence alignment (MSA) and identification of open reading frames (ORF) were performed using MEGA7 (S. Kumar et al., 2016) and UniPro UGENE v33.0 (Okonechnikov et al., 2012), respectively. Protein sequence analysis utilized the standard protein BLAST (BLASTp) tool and the Conserved Domain Database (CDD) of NCBI. Analysis and identification of conserved and functional residues were performed using ConSurf (Glaser et al., 2003; Landau et al., 2005). ORFs were translated using ExPasy (Gasteiger et al., 2003) and protein structure modeling was performed using Phyre2

(Protein Homology/analogY Recognition Engine version 2.0) (Kelley et al., 2015). The coordinates corresponding to the crystal structure of the glycoallergen endo-beta-1,3-glucanase (Hev b 2) from *Hevea brasiliensis* (top hit) was used to generate the model of *β-1,3-GLU2* in this study. UCSF Chimera software version 1.15 (http://www.cgl.ucsf.edu/chimera) was utilized for 3D model visualization, and analysis of molecular structures and mutation analysis, using default settings and criteria.

3. RESULTS

3.1 Phenotypic evaluation of anthracnose resistance

Isolate Mg12 used in this study amplified a single band product using the Col1/Col2 primer pair establishing that the isolate belongs to genus *Colletotrichum*. Using CgInt/ITS4 primer pair, a 450 bp product was amplified corresponding to the expected amplicon size for *Colletotrichum gloeosporioides sensu lato*. No amplification was observed in the control group.

Of the total 143 mango accessions evaluated, thirty (30) initially exhibited intermediate resistance (IR) and resistant (R) reactions, while the rest showed susceptible (S) and highly susceptible (HS) reactions in the preliminary screenings. Confirmatory evaluations showed that nine (9) accessions exhibited potential resistance to mango anthracnose after three trials. None of the 30 accessions exhibited immune reaction or 0% infection. The remaining 21 accessions showed intermediate and susceptible reactions to mango anthracnose (Table 2).

Table 2. Confirmatory evaluation of 30 mango accessions which initially showed intermediate resistance (IR) and resistant (R) reactions to *Colletotrichum gloeosporioides sensu lato*.

Sample	Accession	Variety	Source	Mean Disease Severity (%)
1	ADMINX	Unknown	Laguna	14
2	12-182	Haden	Laguna	11
3	12-070	Unknown	Laguna	14.2
4	16-016	Haden	Guimaras	11
5	Haden Hawaii	Haden	Laguna	11
6	Tommy Atkins	Tommy Atkins	Laguna	12
7	12-265	Haden	Davao	11
8	Gouveia	Gouveia	Guimaras	15
9	12-112	Carabao	La Union	12.5
10	12-052	Carabao	Laguna	36.67
11	12-080	Carabao	Quezon	30
12	12-088	Carabao	Zambales	29
13	12-171	Carabao	Laguna	25
14	GES 73	GES 73	Guimaras	25
15	12-209	Carabao	Quezon	25.33
16	12-013	Carabao	Laguna	44.67
17	12-014	Carabao	Laguna	43
18	12-027	Carabao	Laguna	31
19	12-075	Carabao	Quezon	36
20	12-081	Carabao	Quezon	33.67
21	12-090	Carabao	Zambales	37
22	12-104	Carabao	La Union	36.67
23	12-106	Carabao	La Union	33
24	12-111	Carabao	La Union	36
25	12-166	Carabao	Laguna	47
26	12-170	Carabao	Laguna	37
27	12-179	Carabao	Laguna	42
28	12-092	Carabao	Zambales	56
29	12-094	Carabao	Zambales	56
30	12-178	Carabao	Laguna	58

3.2 Experimental validation by AS-PCR

The AS-PCR assay optimized in this study yields a polymorphic banding pattern that can be used to differentiate genotypes between mango accessions. Results show that a 507-bp allele G-specific PCR product was amplified in 22 of the 30 accessions tested using the allele-specific primer pair (AS-F and Glu2-R). The remaining 8 accessions, namely 'ADMINX', '12-182', '12-070', '16-016', 'Haden Hawaii', 'Tommy Atkins', '12-265', and 'Gouveia', did not amplify this PCR product (Figure). The specific nucleotide at the 3' end of the AS-F primer will only allow amplification in the presence of the allele G and consequently prevents the amplification of a mismatched allele A. This means that the 22 accessions that amplified the 507-bp PCR product contain the G allele, while the 8 other accessions contain the A allele. To further ensure the accuracy of the AS-PCR, gene-specific primers Glu2-F and Glu2-R were introduced in the reaction to amplify an 805-bp *β-1,3-GLU2* gene fragment. This PCR product was successfully amplified in all tested accessions indicating a successful PCR reaction and confirming the presence of the *β-1,3-GLU2* gene in all samples.

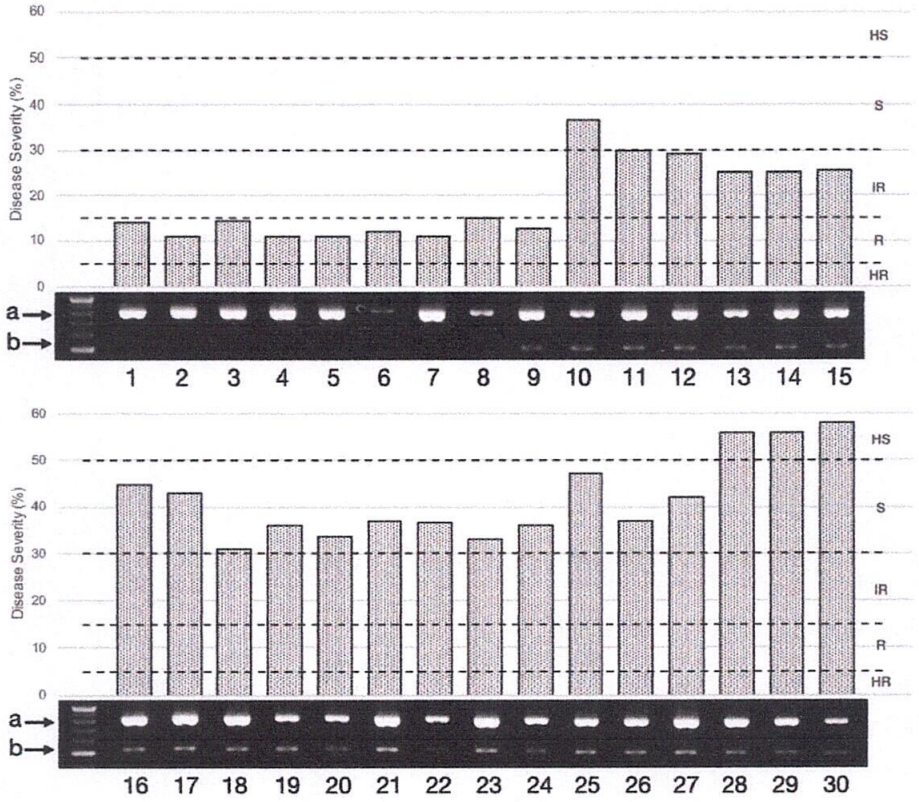


Figure 3. Association of anthracnose resistance phenotypes (% disease severity) with the banding patterns from the SNP 21881933 derived AS-PCR assay of different mango accessions. The upper band (a) is the amplification of the *β-1,3-GLU2* fragment and serves as a positive control. The lower band (b) is an allele-specific amplification. Lanes 1-9 are identified resistant accessions while lanes 10-30 are identified susceptible to intermediately resistant accessions based on Table 2. (HS) highly susceptible; (S) susceptible; (IR) intermediate; (R) resistant; (HR) highly resistant

3.3 Association of SNP 21881933 alleles with phenotypic anthracnose resistance

All but one of the 9 resistant accessions tested showed a consistent genotype–phenotype relationship wherein resistance to anthracnose was associated with the *β-1,3-GLU2* allele A. On the other hand, intermediate resistance and susceptibility to anthracnose were associated with the *β-1,3-GLU2* allele G, as shown by its presence in all accessions with higher % disease severity (>30%). Only the resistant accession '12-112' yielded an inconsistent association between genotype and phenotype, which may be due to a different mode of resistance. Moreover, analysis of phenotype variance (one-way ANOVA) showed that the level of resistance is significantly different between the alleles A and G genotypes ($P < 0.01$). In terms of genotype-phenotype association analysis, Pearson correlation and regression analysis showed that SNP 21881933 alleles is significantly correlated to anthracnose resistance phenotypes ($r = 0.74$; $P < 0.01$).

3.4 Molecular analysis of SNP 21881933 and mango *β-1,3-GLU2* gene

PCR products amplified by the gene-specific primers in this study were sequenced to confirm its identity and the presence of SNP 21881933 in mango accessions. All amplicon sequences significantly matched to a *β-1,3-glucanase 2* (*β-1,3-GLU2*) protein in the NCBI RefSeq database. Sequence comparisons with *β-1,3-GLU2* of closely related fruit tree crops confirms

an A > G nucleotide transition at the SNP 21881933 site (Figure 1-A). This leads to a missense mutation in the translated *β-1,3-GLU2* protein with an isoleucine to valine substitution within the conserved Glycosyl hydrolase family 17 (GH17) domain (Figure 1-B). ConSurf analysis predicts that this specific isoleucine residue (I196) is a structural residue that is highly conserved among closely related fruit tree crops (Figure 1-C, D).

The predicted protein structure of mango *β-1,3-GLU2*, which was modelled based on the crystal structure of *H. brasiliensis* endo-beta-1,3-glucanase (Hev b 2), exhibits the canonical $(\beta/\alpha)_8$ TIM-barrel motif and the catalytic groove across the protein surface found in other glucan endohydrolases (Receveur-Bréchot et al., 2006) (Figure 1-E). Superimposition of the structure models with A and G alleles also revealed changes in the predicted folding pattern of residues 82-90 (α-helix), 119-127 (extended loop), and 293-298 (extended loop) of the mutant *β-1,3-GLU2* (Figure 1-F). Based on the predicted model, the I196V mutation is situated near the catalytic groove where the binding site of substrates containing β-1,3-linked glucose residues is situated (Figure 1-G). Since I196 is predicted to be a structural residue, replacement with a valine residue at this position might result to structural changes in the catalytic groove that may affect the substrate binding specificity of the protein. To investigate the possible effects of this mutation, I196V was introduced using UCSF Chimera v.1.15 and predicted a significant clash with V261 residue having an overlap of 0.780 Å and distance of 2.980 Å (Figure 1-I).

4. DISCUSSION

Fruit tree breeding methods conventionally rely on phenotypic evaluations of parental lines to be used for varietal improvement. Such selection practices are subjective and are highly variable depending on the environment of the plant. Marker-assisted selection (MAS) provides a more rapid, accurate and discriminative way in identifying individuals as parentals for breeding and development of plant varieties with desired agronomic characteristics. In this study, an SNP marker within the β-1,3-glucanase 2 (*β-1,3-GLU2*) gene in mango was found to be associated with resistance to anthracnose and developed for MAS using a simple AS-PCR assay.

β-1,3-glucanases (E.C. 3.2.1.39) are enzymes widely found in bacteria, fungi, viruses, and plants that catalyze the hydrolysis of 1,3-β-D-glucosidic linkages between β-1,3-glucans – a major component of fungal and plant cell walls (Torres et al., 2015; X. Xu et al., 2016). In plants, β-1,3-Glucanase genes (GLUs) forms complex and diverse gene families playing important roles in physiological and developmental processes, including pathogen defense mechanisms (Doxey et al., 2007). GLUs and other pathogenesis-related proteins like chitinase and phenylalanine ammonia-lyase (PAL) are considered key enzymes in the control of plant disease in resistant systems (Zeng et al., 2006). Specifically, plant GLUs defend against pathogen infections by hydrolyzing β-1,3-glucans in fungal cell walls or by promoting the release of cell wall-associated immune elicitors that further stimulate defense reactions (X. Xu et al., 2016).

Previous studies have shown evidences of β-1,3-glucanase's role in *Colletotrichum* infection. In maize, nine GLUs were significantly upregulated in leaves during *Colletotricum graminicola* infection as a result of pathogen-associated molecular patterns (PAMP)-triggered defense response (Oliveira-Garcia & Deising, 2013). Similarly, strawberry plants infected with *C. fragariae* or *C. acutatum* induced the expression of two GLUs to over a thousand fold (Shi et al., 2006). In mango, Zhang et al. (2013) previously reported that β-aminobutyric acid (BABA) treatment in mango fruits significantly enhanced the activities of β-1,3-glucanases and effectively suppressed anthracnose caused by *C. gloeosporioides* during storage at 25°C. Increased β-1,3-glucanase activities was also attributed to reduction of postharvest anthracnose and enhancement of disease resistance in mango fruit after exogenous nitric oxide (NO) treatment (Hu et al., 2014). These previous findings indicate that β-1,3-glucanases significantly respond to *Colletotrichum* infection and possibly elicit defense response mechanisms involving the enhancement of defense-related enzyme activities to confer resistance to the pathogen.

Our results describe a possible mechanism of resistance against *C. gloeosporioides* in mango involving β-1,3-glucanases. A positive correlation between the presence of SNP 21881933 mutation and increased susceptibility to the disease implies that this specific mutation might have significantly reduced the activity of β-1,3-glucanase 2 enzymes, which have a direct role in defense response. As a PR protein, β-1,3-glucanases have been shown to directly defend against fungi infection by hydrolyzing fungal cell walls leading to fungal cell lysis (de la Cruz et al., 1995; Sandhu et al., 2017). While the mutant allele G found in susceptible accessions could have deleterious effect on the enzyme function rendering it vulnerable against infection and disease development, the β-1,3-GLU2 allele A, on the other hand, could be inferred as a biologically-active protein retaining its hydrolyzing function. This conclusion is supported by the fact that there is a significantly higher disease severity in all accessions with the G allele post-inoculation, while accessions with A allele had significantly reduced infection.

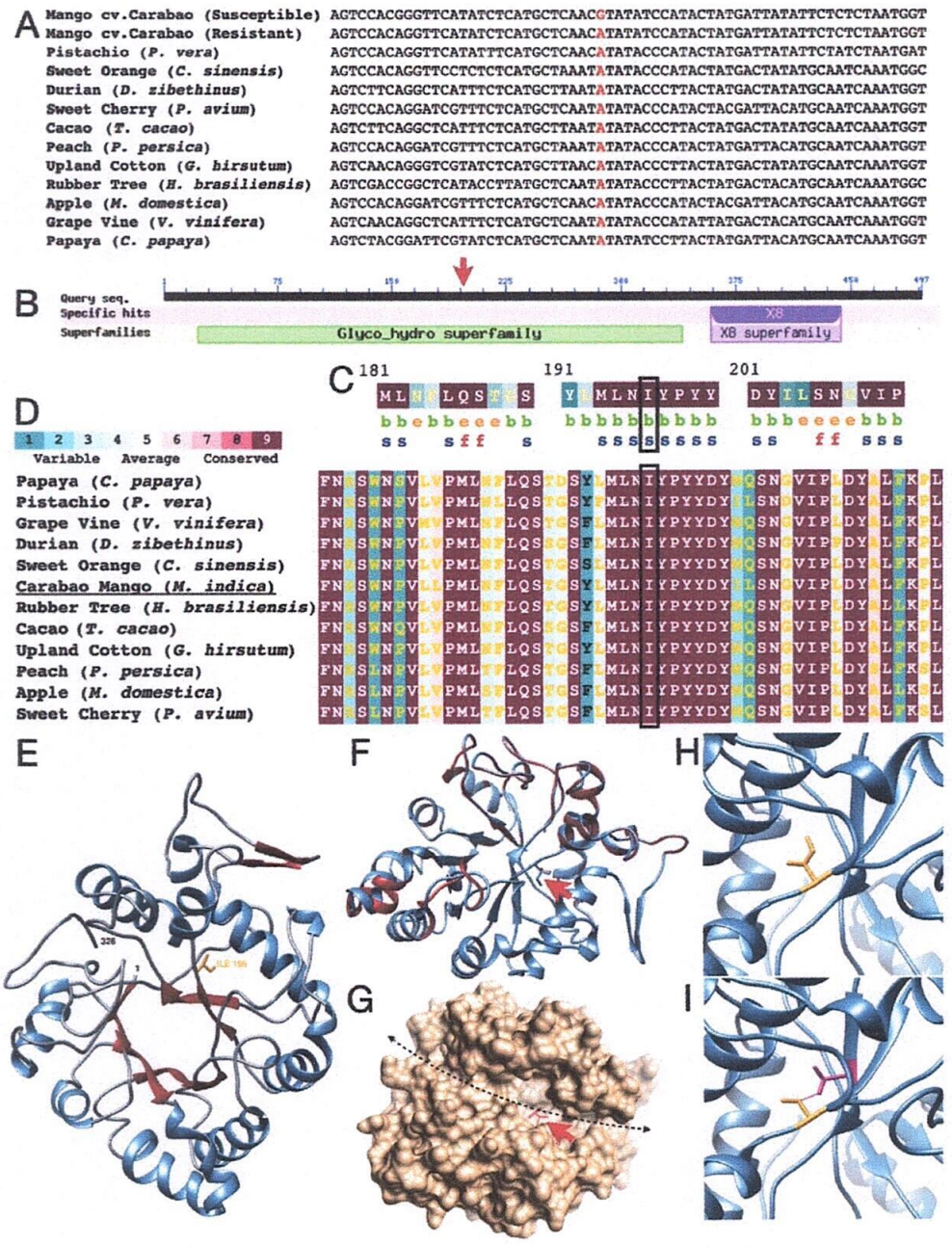


Figure 1. SNP 21881933 is a A > G transition in mango *β-1,3-GLU2* leading to an Isoleucine to Valine substitution. (A) MSA showing the SNP 21881933 site at position 6:17813013 (indicated in red) in mango *β-1,3-GLU2* and its homologs. The adenine [A] base is conserved between mango, apple, cacao and other fruit crops. (B) The isoleucine to valine substitution (I196V) caused by SNP 21881933 is predicted to be within the conserved protein domain family Glycosyl hydrolases family 17 (GH17; pfam00332) through the NCBI conserved domain search service (CD-search). A red arrow indicates site of I196V mutation relative to the GH17 domain. (C) ConSurf analysis of the mango *β-1,3-GLU2* protein sequence shows that 196I is highly conserved and predicted to be a buried residue (b), and thus likely to be a structural residue (s). (D) 196I in mango is highly conserved in β-1,3-glucanases of other fruit tree crops based on ConSurf Color-Coded MSA. Yellow letters in (B,C) indicates insufficient data where calculation of conservation was performed on less than 10% of the sequences. (E) Complete 3D structural model of the wildtype mango *β-1,3-GLU2* protein based on Phyre2 modelling from the *H. brasiliensis* β-1,3-glucanase crystal structure. The β-sheet strands (red) surrounded by the α-helices (blue) form the (β/α)8 TIM-barrel motif referred to in the text. Numbers 1 and 326 indicate the N- and C-terminal ends of the polypeptide chain, respectively. 'ILE 196' indicates the position of the isoleucin residue at the mutation site. (F) Superimposition of the structural models from mango *β-1,3-GLU2* with allele A (blue) and allele G (red) reveals some structural differences, with 97.52% identity. A red arrow points to the I196V substitution caused by SNP 21881933. (G) Molecular surface model of the mango WT *β-1,3-GLU2* protein showing the proximity of 196I residue (red arrow) to the catalytic groove indicated by the curved dashed line. (H,I) The effect of the mutation on the *β-1,3-GLU2* atomic interactions (clashes/contacts) is shown in H and I. (H) In the wildtype A allele, Ile196 residue (yellow) does not interact with any other residues within 5.0 Å. (I) In the SNP 21881933 mutation, Val196 (yellow) forms a steric clash (pink line) with Val261 (magenta) indicating a direct unfavorable interaction.

Missense mutations caused by SNPs occurring at gene coding regions generally affect protein stability, protein-protein interactions, and critical components of biological reaction (Zhe Zhang et al., 2012). SNP 21881933 described in this study has been found to be located within the highly conserved Glycosyl hydrolases family 17 (GH17) domain. This domain family is involved in physiologically important processes in plants, such as response to biotic and abiotic stresses, defense against herbivores, activation of phytohormones, lignification, and cell wall remodeling (Opassiri et al., 2006). Interestingly, another conserved domain called C-terminal X8 family carbohydrate-binding domain is also present in the expressed *β-1,3-GLU2* gene close to the SNP site. This domain is primarily involved in carbohydrate binding and cleaving (1,3)-beta-D-glucosidic linkages along with GH17 family (Marchler-Bauer et al., 2014). These suggest that the SNP is situated near or within the active site region of the enzyme and mutations within this domain could affect any of these functions. Any conformational change altering the active sites of proteins, as well as mutations quite close to it, can affect biochemical reactions because catalytic reactions are very sensitive to the precise geometry of these active sites for both of the products and reactants (Zhe Zhang et al., 2012).

To analyze the possible structural effects of the SNP mutation, we generated the predicted protein structure of the isolated mango *β-1,3-GLU2* and introduced the mutation in silico. Based on the three-dimensional structure model, the resulting I196V mutation is situated within the typical eightfold β/α TIM-barrel motif and the catalytic groove across the protein surface strictly conserved in other glucan endohydrolases of the GH17 family. This motif consists of an internal crown of eight β-strands connected to an outer crown of eight α-helices. According to Receveur-Bréchot et al. (2006), the active site of these enzymes consists of two glutamate residues in strands *β4 and β7* acting as proton donor nucleophile residue, respectively. These correspond to residues E50 and E240 in mango *β-1,3-GLU2*. Moreover, the three-dimensional molecular surface model showed that the I196V substitution constitute a part of the catalytic groove where β-1,3 linked glucan trisaccharide substrates are accommodated (Receveur-Bréchot et al., 2006). These observations suggest that I196, being a conserved structural residue, is necessary in maintaining the precise conformation of the enzyme's active site.

We hypothesized that the replacement of I196 mango *β-1,3-GLU2* with a valine residue caused contacts/clashes with the surrounding residues. Based on mutation analysis, a steric clash between V196 and V261 side chains was predicted with an overlap of less than 0.8 Å. This indicates a direct unfavorable interaction wherein atoms are too close together allowing van der Waals forces between the two residues. Such molecular interactions could result to structural perturbations. Since the I196V mutation is proximal to the active site and catalytic groove of mango *β-1,3-GLU2*, a clash between V196 and V261 could affect conformational stability and topology of the region possibly inhibiting the interaction of the substrate with the active site.

Together, these results suggest that the mutation caused by SNP 21881933 and its predicted structural changes most likely affected the catalytic activity of the wildtype *β-1,3-GLU2* in defense response against *C. gloeosporioides* infection. Although our findings provided an insight in the structural impact of the SNP mutation, functional studies are needed in order to confirm and elucidate further these mechanisms of resistance. Moreover, despite a significant positive correlation between *β-1,3-GLU2* alleles and degree of *C. gloeosporioides* infection, the biological function of β-1,3-glucanases alone cannot be conclusively associated to anthracnose resistance. Plant defense response against fungi is complex and requires involvement of regulatory factors that determines the susceptibility or resistance of plant to a particular pathogen.

The developed *β-1,3-GLU2* SNP marker reported in this study will enable marker-assisted selection of anthracnose-resistant mango as early as the seedling stage. We recommend this functional marker for routine genotyping of parental and hybrid mango to facilitate and improve the efficiency of mango breeding programs.

5. CONCLUSION

In crop production where specific quality traits dictate either significant economic losses or gains, more efficient breeding strategies for food crops are needed. Advancements in molecular biology provide genomic tools such as molecular markers which can aid in the selection of target traits to shorten selection times and to accelerate the breeding process of new varieties of mango. As demonstrated by this study, SNP markers can be used to identify mango with phenotypic anthracnose resistance as early as the seedling stage to significantly reduce the expenses in field inputs, maintenance, and evaluation of material over years. AS-PCR is a simple and effective method to employ such markers, but these can be also used in high-throughput genotyping technologies for large-scale screening and analysis. Our results show that *β-1,3-GLU2* is a reliable marker for screening and developing anthracnose resistant breeding lines that can complement the conventional mango breeding approach. However, due to the limited sample size and phenotypic data available in this study, it is recommended

to further employ the developed marker in a segregating population with a large sample size to strengthen the genotype-phenotype association analysis.

6. FUNDING

This work was supported by the Philippine Department of Science and Technology – Philippine Council for Agriculture, Aquatic and Natural Resources Research and Development (DOST-PCAARRD) under the project "Identification of Molecular Markers Associated with Red Blush, Thick Peel, and Resistance to Anthracnose and Fruitfly in Mango"; and through the Graduate Research and Education Assistantship for Technology (GREAT) Program.

7. REFERENCES

Akem, C. N. (2006). Mango Anthracnose Disease: Presen Status and Future Research Priorities. *Plant Pathology Journal*, *5*((3)), 266–273. https://doi.org/10.3923/ppj.2006.266.273

Alcasid, C., Dela Cueva, F., & Velncia, L. (2018). Intra-varietal response of Philippines 'Carabao' mango (Mangifera indica L.) against stem-end rot caused by Lasiodiplodia theobromae (Pat.) Griff. & Maubi. *Journal of Tropical Plant Pathology*, *54*, 45–53.

Altschul, S. F., Gish, W., Miller, W., Myers, E. W., & Lipman, D. J. (1990). Basic local alignment search tool. *Journal of Molecular Biology*, *215*(3), 403–410.

Alvindia, D. G., & Acda, M. A. (2015). Revisiting the efficacy of hot water treatment in managing anthracnose and stem-end rot diseases of mango cv. "Carabao." *Crop Protection*, *67*, 96e101-101. https://doi.org/10.1016/j.cropro.2014.09.016

Arauz, L. F. (2000). Mango Anthracnose: Economic Impact and Current Options for Integrated Management. *Plant Disease*, *84*(6), 600–611.

de la Cruz, J., Pintor-Toro, J. A., Benítez, T., Llobell, A., & Romero, L. C. (1995). A novel endo-beta-1,3-glucanase, BGN13.1, involved in the mycoparasitism of Trichoderma harzianum. *Journal of Bacteriology*, *177*(23), 6937–6945. https://doi.org/10.1128/jb.177.23.6937-6945.1995

Dodd, J. C., Estrada, A. B., Matcham, J., Jeffries, P., & Jeger, M. J. (1991). The effect of climatic factors on Colletotrichum gloeosporioides, causal agent of mango anthracnose, in the Philippines. *Plant Pathology*, *40*(4), 568–575.

Doxey, A. C., Yaish, M. W. F., Moffatt, B. A., Griffith, M., & McConkey, B. J. (2007). Functional divergence in the Arabidopsis β-1,3-glucanase gene family inferred by phylogenetic reconstruction of expression states. *Molecular Biology and Evolution*, *24*(4), 1045–1055. https://doi.org/10.1093/molbev/msm024

FAO. (2019a). *Global Fruit Production in 2019, by Selected Variety (in Million Metric Tons)*. Statistica. https://www.statista.com/statistics/264001/worldwide-production-of-fruit-by-variety/%0A

FAO. (2019b). *Major tropical fruits - Statistical compendium 2018*.

Gasteiger, E., Gattiker, A., Hoogland, C., Ivanyi, I., Appel, R. D., & Bairoch, A. (2003). ExPASy: the proteomics server for in-depth protein knowledge and analysis. *Nucleic Acids Research*, *31*(13), 3784–3788.

Glaser, F., Pupko, T., Paz, I., Bell, R. E., Bechor-Shental, D., Martz, E., & Ben-Tal, N. (2003). ConSurf: Identification of Functional Regions in Proteins by Surface-Mapping of Phylogenetic Information. *Bioinformatics*, *19*(1), 163–164. https://doi.org/10.1093/bioinformatics/19.1.163

Hu, M., Yang, D., Huber, D. J., Jiang, Y., Li, M., Gao, Z., & Zhang, Z. (2014). Reduction of postharvest anthracnose and enhancement of disease resistance in ripening mango fruit by nitric oxide treatment. *Postharvest Biology and Technology*, *97*, 115–122. https://doi.org/10.1016/j.postharvbio.2014.06.013

Iquebal, M. A., Jaiswal, S., Mahato, A. K., Jayaswal, P. K., Angadi, U. B., Kumar, N., Sharma, N., Singh, A. K., Srivastav, M., Prakash, J., Singh, S. K., Khan, K., Mishra, R. K., Rajan, S., Bajpai, A., Sandhya, B. S., Nischita, P., Ravishankar, K. V., Dinesh, M. R., … Singh, N. K. (2017). MiSNPDb: A web-based genomic resources of tropical ecology fruit mango (Mangifera indica L.) for phylogeography and varietal differentiation. *Scientific Reports*, *7*(1), 1–9. https://doi.org/10.1038/s41598-017-14998-2

Kelley, L. A., Mezulis, S., Yates, C. M., Wass, M. N., & Sternberg, M. J. E. (2015). The Phyre2 web portal for protein modeling, prediction and analysis. *Nature Protocols*, *10*(6), 845.

Kuhn, D.N., Dillon, N. L., Innes, D. J., Wu, L.-S., & Mockaitis, K. (2016). Development of single nucleotide polymorphism (SNP) markers from the mango (Mangifera indica) transcriptome for mapping and estimation of genetic diversity. *Acta Horticulturae*, *1111*, 315–322. https://doi.org/10.17660/ActaHortic.2016.1111.45

Kuhn, David N., Dillon, N., Bally, I., Groh, A., Rahaman, J., Warschefsky, E., Freeman, B., Innes, D., & Chambers, A. H. (2019). Estimation of genetic diversity and relatedness in a mango germplasm collection using SNP markers and a simplified visual analysis method. *Scientia Horticulturae*, *252*(March), 156–168. https://doi.org/10.1016/j.scienta.2019.03.037

Kumar, J., Choudhary, A. K., Solanki, R. K., & Pratap, A. (2011). Towards marker-assisted selection in pulses: A review. *Plant Breeding*, *130*(3), 297–313. https://doi.org/10.1111/j.1439-0523.2011.01851.x

Kumar, S., Stecher, G., & Tamura, K. (2016). MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. *Molecular Biology and Evolution*, *33*(7), 1870–1874.

Lachica, J. A. P., Vilela, J. A., Santos, M. M. L., & M, E. T. (2019). *SNP Discovery and Genetic Clustering of Philippine ' Carabao ' Mango (Mangifera indica L. cv. 'Carabao') using Genotype-By-Sequencing (DArTseq). 44*(April), 10–17.

Landau, M., Mayrose, I., Rosenberg, Y., Glaser, F., Martz, E., Pupko, T., & Ben-Tal, N. (2005). ConSurf 2005: the projection of evolutionary conservation scores of residues on protein structures. *Nucleic Acids Research*, *33*(Web Server), W299–W302. https://doi.org/10.1093/nar/gki370

Marchler-Bauer, A., Derbyshire, M. K., Gonzales, N. R., Lu, S., Chitsaz, F., Geer, L. Y., Geer, R. C., He, J., Gwadz, M., & Hurwitz, D. I. (2014). CDD: NCBI's conserved domain database. *Nucleic Acids Research*, *43*(D1), D222–D226.

Martinez-Culebras, P. V, Querol Querol, A. A., M B Suarez-Fernandez Suarez-Fernandez, M. B., M D Garcia-Lopez Garcia-Lopez, M. D., E Barrio Barrio, and E., & Addresses, A. (2003). Phylogenetic Relationships Among Colletotrichum Pathogens of Strawberry and Design of PCR Primers for their Identification. *J. Phytopathology*, *151*, 153–143. www.blackwell.de/synergy

Mills, P. R., Sreenivasaprasad, S., & Brown, A. E. (1992). Detection and differentiation of Colletotrichum gloeosporioides isolates using PCR. *FEMS Microbiology Letters*, *98*(1–3), 137–143. https://doi.org/10.1111/j.1574-6968.1992.tb05503.x

Mitra, S. K. (2016). Mango production in the world - Present situation and future prospect. *Acta Horticulturae*, *1111*, 287–296. https://doi.org/10.17660/ActaHortic.2016.1111.41

Okonechnikov, K., Golosova, O., & Fursov, M. (2012). UGENE team. 2012. *Unipro UGENE: A Unified Bioinformatics Toolkit. Bioinformatics*, *28*(8), 1166–1167.

Oliveira-Garcia, E., & Deising, H. B. (2013). Infection Structure-Specific Expression of -1,3-Glucan Synthase Is Essential for Pathogenicity of Colletotrichum graminicola and Evasion of -Glucan-Triggered Immunity in Maize. *The Plant Cell*, *25*(6), 2356–2378. https://doi.org/10.1105/tpc.112.103499

Opassiri, R., Pomthong, B., Onkoksoong, T., Akiyama, T., Esen, A., & Cairns, J. R. K. (2006). Analysis of rice glycosyl hydrolase family 1 and expression of Os4bglu12 β-glucosidase. *BMC Plant Biology*, *6*(1), 33.

Paull, R. (2002). Advances in postharvest technology for tropical and subtropical fruits. *Proceedings of International Technical and Trade Seminar on Tropical and Subtropical Fruits*, 157–167.

Receveur-Bréchot, V., Czjzek, M., Barre, A., Roussel, A., Peumans, W. J., Van Damme, E. J. M., & Rougé, P. (2006). Crystal structure at 1.45-Å resolution of the major allergen endo-β-1,3-glucanase of banana as a molecular basis for the latex-fruit syndrome. *Proteins: Structure, Function and Genetics*, *63*(1), 235–242. https://doi.org/10.1002/prot.20876

Sandhu, J. S., Sidhu, M. K., & Yadav, I. S. (2017). *Control of Fungal Diseases in Agricultural Crops by Chitinase and Glucanase Transgenes* (pp. 163–212). https://doi.org/10.1007/978-3-319-48006-0_6

Sherman, A., Rubinstein, M., Eshed, R., Benita, M., Ish-Shalom, M., Sharabi-Schwager, M., Rozen, A., Saada, D., Cohen, Y., & Ophir, R. (2015). Mango (Mangifera indica L.) germplasm diversity based on single nucleotide polymorphisms derived from the transcriptome. *BMC Plant Biology*, *15*(1), 1–11. https://doi.org/10.1186/s12870-015-0663-6

Shi, Y., Zhang, Y., & Shih, D. S. (2006). Cloning and expression analysis of two β-1,3-glucanase genes from Strawberry. *Journal of Plant Physiology*, *163*(9), 956–967. https://doi.org/10.1016/j.jplph.2005.09.007

Singh, N. K., Jayaswal, P. K., Mahato, A. K., Singh, A., Singh, S., Singh, N., Rai, V., Amitha, M. S., Gaikwad, K., Sharma, N., Lal, S., Srivastava, M., Prakash, J., Kalidindi, U., Singh, S. K., Singh, A. K., Khan, K., Mishra, R. K., Rajan, S., … Sharma, T. R. (2016). Origin, Diversity and Genome Sequence of Mango (Mangifera indica L.). *Indian Journal of History of Science*, *51*(2.2), 355–368. https://doi.org/10.16943/ijhs/2016/v51i2.2/48449

Syvänen, A. C. (2005). Toward genome-wide SNP genotyping. *Nature Genetics*, *37*, S5–S10.

Torres-Calzada, C., Tapia-Tussell, R., Higuera-Ciapara, I., & Perez-Brito, D. (2013). Morphological, pathological and genetic diversity of Colletotrichum species responsible for anthracnose in papaya (Carica papaya L). *European Journal of Plant Pathology*, *135*(1), 67–79. https://doi.org/10.1007/s10658-012-0065-7

Torres, M., Palomares, O., Quiralte, J., Pauli, G., Rodríguez, R., & Villalba, M. (2015). An enzymatically active β-1,3-glucanase from ash pollen with allergenic properties: A particular member in the oleaceae family. *PLoS ONE*, *10*(7), 1–16. https://doi.org/10.1371/journal.pone.0133066

Wangkumhang, P., Chaichoompu, K., Ngamphiw, C., Ruangrit, U., Chanprasert, J., Assawamakin, A., & Tongsima, S. (2007). *WASP : a Web-based Allele-Specific PCR assay designing tool for detecting SNPs and mutations*. *9*, 1–9. https://doi.org/10.1186/1471-2164-8-275

Warschefsky, E. J., & von Wettberg, E. J. B. (2019). Population genomic analysis of mango (*Mangifera indica*) suggests a complex history of domestication. *New Phytologist*, *222*(4), 2023–2037. https://doi.org/10.1111/nph.15731

Xu, X., Feng, Y., Fang, S., Xu, J., Wang, X., & Guo, W. (2016). Genome-wide characterization of the β-1,3-glucanase gene family in Gossypium by comparative analysis. *Scientific Reports*, *6*(June), 1–15. https://doi.org/10.1038/srep29044

Xu, Y., & Crouch, J. H. (2008). Marker-assisted selection in plant breeding: From publications to practice. *Crop Science*, *48*(2), 391–407. https://doi.org/10.2135/cropsci2007.04.0191

Zeng, K., Cao, J., & Jiang, W. (2006). Enhancing disease resistance in harvested mango (Mangifera indica L. cv. 'Matisu') fruit by salicylic acid. *Journal of the Science of Food and Agriculture*, *86*(5), 694–698. https://doi.org/10.1002/jsfa.2397

Zhang, Zhe, Miteva, M. A., Wang, L., & Alexov, E. (2012). Analyzing effects of naturally occurring missense mutations. *Computational and Mathematical Methods in Medicine*, *2012*. https://doi.org/10.1155/2012/805827

Zhang, Zhengke, Yang, D., Yang, B., Gao, Z., Li, M., Jiang, Y., & Hu, M. (2013). β-Aminobutyric acid induces resistance of mango fruit to postharvest anthracnose caused by Colletotrichum gloeosporioides and enhances activity of fruit defense mechanisms. *Scientia Horticulturae*, *160*, 78–84. https://doi.org/10.1016/j.scienta.2013.05.023

Climate-Resilient agri-fisheries (CRA) assessment, targeting & prioritization for the adaptation and mitigation initiative for tarlac province

Cite as: AIP Conference Proceedings **2472**, 040012 (2022); https://doi.org/10.1063/5.0094762
Published Online: 19 August 2022

Lilibeth B. Laranang, Ermalyn D. Galo and Orlando H. Locading

Climate-Resilient Agri-fisheries (CRA) Assessment, Targeting & Prioritization for the Adaptation and Mitigation Initiative for Tarlac Province

Lilibeth B. Laranang[1,a)], Ermalyn D. Galo[2,b)], and Orlando H. Locading, Jr.[3,c)]

[1] *Rootcrops Research and Training Center, Tarlac Agricultural University, Philippines*
[2] *College of Engineering and Technology, Tarlac Agricultural University, Philippines*
[3]*College of Business and Management, Tarlac Agricultural University, Philippines*

[a)] lilibeth717@gmail.com
[b)] Corresponding author: ermalyngalo@gmail.com
[c)] ohlocading@tau.edu.ph

Abstract. This study is part of the Adaptation and Mitigation Initiative in Agri-fisheries (AMIA) Project of the Department of Agriculture (DA) and International Center for Tropical Agriculture (CIAT) to operationalize the goal of making agriculture and its stakeholders adapt and mitigate the effects of climate change. The outputs of this study were vital in the implementation of the next phase of AMIA: climate-risk vulnerability (CRVA) map and investment brief. The CRVA map was developed to identify the vulnerable areas in Tarlac. The CRVA map considered three factors: climate-risks sensitivity, exposure, and adaptive capacity given a 15-15-70 weighted percentages, respectively. The municipalities of Ramos, La Paz, Bamban and Victoria were identified as highly vulnerable to the effects of climate change. Three investment briefs were prepared for policy makers for possible funding and implementation. They were developed by identifying climate-resilient agricultural practices in the province which are the use of climate smart varieties, crop rotation-minimum tillage combination, and alternate wetting and drying method. These practices were evaluated using the CBA Tool and were found to be sustainable. Social net present value was also determined taking into account the externalities, which was given a value through interview of experts.

INTRODUCTION

The Philippines is highly vulnerable to the adverse impacts of climate change as the country's backbone is agriculture [1]. The effect of intense and longer droughts, increase in temperature, erratic rainfall distribution and natural hazards that affects earth's ecosystem as well as health, livelihood, social systems and economy. Agriculture is one of the most vulnerable sectors on the impact of climate change and farmers who are directly dependent on their lands for survival are the most affected. Climate change mitigation and adaptation to climate change is crucial particularly in agriculture sector, thus, different adaptive strategies are being done to cope up with its impacts. One of the activities in the strategic actions of food security for 2011-2028 is to enhance site-specific knowledge on the vulnerability of agriculture and fisheries [2].

The Adaptation and Mitigation Initiative in Agriculture (AMIA) seeks to enable the Department of Agriculture (DA) to plan and implement strategies to support local communities in managing climate risks – from extreme weather events to long-term climatic shifts. Spearheaded by the DA System-wide Climate Change Office (DA SWCCO), AMIA Phase 1 in 2015-16 to implement activities to strengthen DA's capacity to mainstream climate change adaptation and mitigation strategies locally and in national scale. With AMIA Phase 2, making climate-resilient agri-fisheries (CRA) an operational approach through field-level action that directly involves, and influences on the livelihoods of farming communities is one of the next big task for the program. AMIA2 aims to invest in the launching of CRA communities in Tarlac province as the initial target site for action learning, supported by an integrated package of climate services within a broader food value chain setting. The program is launching an integrated and multi-stakeholder effort to operationalize CRA at the community level in 10 target regions. Successful implementation of AMIA2 at the regional level requires the strong collaboration and support of key research and development institutions within the region. This proposed project enables AMIA2 to establish and mobilize regional teams, each led by a local State University/College (SUC), and in partnership with the corresponding Department of Agriculture - Regional Field Office (DA-RFOs).

Climate-resilient agriculture/agri-fisheries (CRA) aims to achieve food security and broader development goals under a changing climate and increasing food demand [3]. With the three pillars: productivity, adaptation, and mitigation, CRA initiates sustainably increase productivity, enhance resilience, and require planning to address tradeoffs and synergies [4]. To enable to assess, plan and pilot climate-risk prone agri-fisheries communities in pursuing sustainable livelihoods while effectively managing the impacts of climate variability. Thus, one of the Region to initiates the CRA strategic framework was Region 3 or Central Luzon specifically in

The 5th Innovation and Analytics Conference & Exhibition (IACE 2021)
AIP Conf. Proc. 2472, 040012-1–040012-7; https://doi.org/10.1063/5.0094762
Published by AIP Publishing. 978-0-7354-4387-7/$30.00

Tarlac province to identify key climate risk and vulnerable areas and to assess the current status of CRA as well as the cost and benefits of these practices and technologies. Therefore, the study aims to establish and mobilized team in Region III for AMIA 2 in order to operationalized AMIA strategies in managing climate risk in Tarlac province. Specifically is to enhanced capacities of AMIA partner organizations in the Region, developed geospatially referenced data on climate-risks in Tarlac, generate profile on community's CRA strategies, and perform costs-benefits & trade-offs for these CRA practices. The result of the study will serve as guide in piloting community action research in establishing community-level research and development interventions.

.

MATERIALS AND METHODS

Study Area

The study was established in the province of Tarlac. The study covered an area of 273,660 hectare and is located between latitude 15º 10'15" N to 15º 52'52" N, longitude 120º 8'4" E to 120º 46'27" E. The study area has flat to undulating topography, with the eastern part of the province being plain and the western part to be hilly to mountainous. Tarlac has two distinct seasons, the wet and the dry seasons. It has unimodal rainfall pattern, having high monsoon peaks in the wet season (WS) and negligible rainfall in the dry season (DS). Recorded annual rainfall varies from 2,030 mm to 4,060 mm in the northwestern portion [5].

Tarlac is basically an agriculture-based economy, located in the heart of Central Luzon with a total land area of 305,345 ha, constitutes 16.75% of the regional land area and 1.0 % of the total national land area with 112,997.57 hectares concentrated on agricultural production. Rice and corn are the top 2 commodities planted in the province planted in 2 to 3 cropping a year. There are 102,178.06 ha planted to rice, which are irrigated, rainfed and in upland areas. On the other hand, there are 16,458.98 ha planted to corn. Of these, only a small portion, are planted with white corn while the rest are planted with the yellow corn. With this vast track of land concentrated in agriculture, Tarlac likewise grows lowland vegetables and root crops. Of the lowland vegetables grown in the region, tomato occupies the largest area with 215.81 has while sweet potato is the largely grown rootcrops with a production area of 3,641.58 ha. Both crops are grown after rice usually during the onset of the dry season when rice has been harvested. Orchard occupies 10,498.65 hectares planted with our local fruit trees. The most common is mango that is planted in an area totaling to 25,660.03 hectares [6].

Framework of AMIA 2 Project

The project seeks to contribute to the overall AMIA2 program framework, by contributing specific outputs to targeted national-level research projects. It has four key components: (1) Capacity strengthening for CRA research & development, (2) Geospatial assessment of climate risks, (3) Stakeholders' participation in climate adaptation planning, and (4) Documenting & analyzing CRA practices. These project components were designed to be directly aligned with the research agenda of three AMIA2 projects: 1) climate-risk vulnerability assessment (CRVA), 2) decision-support platform for CRA, and 3) institutional and policy innovations. Figure 1 shows the framework for this study.

Component 1 - Capacity strengthening for CRA research & development

The regional project team participated in a series of trainings, workshops and learning events organized by AMIA2 projects. These were focused on three key methodologies: 1) CRVA, 2) CRA prioritization, and 3) CRA M&E. The project provided training support to key research and development stakeholders in the region, by organizing an intra-regional training that covers key learning contents from the national-level trainings. The CRA monitoring and evaluation was later included in the phase two of the AMIA2 which is currently handled by the Department of Agriculture Regional Field Office 3 in Victoria, Tarlac. The town was one of the identified vulnerable areas in the province.

Component 2 - Geospatial assessment of climate risks

The project team collected and organized geo-referenced data on vulnerability to climate risks of the region's agri-fisheries sector. These datasets, from both primary and secondary sources, were used on the methodological guidelines provided by the AMIA2 CRVA project – covering climate-risk exposure, sensitivity and adaptive capacity. Preliminary analysis – using GIS software and climate modelling tools – was undertaken at the regional level. The project team also participated in a national-team level joint analysis of cross-regional data.

Component 3 - Stakeholders' participation in climate adaptation planning

The regional project team organized a series of stakeholders' meetings and focus group discussions to collect supplementary data and validate preliminary results of CRVA, as well as in identifying CRA prioritization and planning. These activities were guided by process facilitation using the MaxEnt and CBA Tool developed by the AMIA2 projects on CRVA and CRA decision-support platform.

Component 4 - Documenting & analyzing CRA practices

A semi-structured survey with local stakeholders was conducted to identify and document CRA practices, as well as collect existing CRA-relevant statistical and other secondary data. Focus group discussion with farmers, farmer-leaders, technician, municipal and provincial agriculturists, and representative of provincial government of Tarlac were done on four separate occasions to gather data, validate and present the results, and revalidation of results. These data was systematized and analysed using cost-benefit and trade-off analyses tools as input to AMIA2 CRA prioritization and investment planning. A CBA Tool was made available online by the CIAT to facilitate the computation of cost, benefits, and others. These contributed to developing knowledge products, such as searchable online portal, under the AMIA2 project on CRVA decision-support platform.

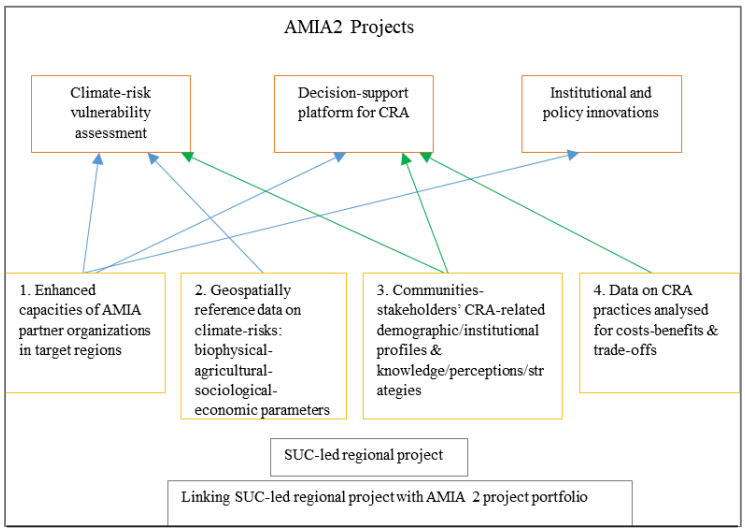


FIGURE 1. Framework of the Study

Data Acquisition

Collection of secondary data for exposure-sensitivity and secondary-primary data for adaptive capacity was done from different agencies such as the Provincial Agriculture Office, Municipal Agriculture Offices, and data from the AMIA2 outputs. Data collection started with gathering of primary and secondary data, that includes those from focus group discussion (FGD), key informant interviews (KII) and municipality surveys.

CRVA Framework

The vulnerability mapping was done which follows the Climate-Risk Vulnerability Assessment (CRVA) framework. It starts with the identification of the vulnerability determinants (hazard, sensitivity, and adaptive capacity) and their respective indicators. This framework was the standardized procedure followed by different SUCs involved in the project. Data collection started in gathering the primary and secondary data, that includes those from focus group discussion (FGD), key informant interviews (KII) and municipality surveys. The identified priority crops of the province are rice, corn, tomato, and mango. Sweet potato was an additional crop prioritized by the team because it is one of the crops planted on the second cropping season after rice and corn.

CRA Practices

Key informant survey on climate-resilient agri-fisheries (CRA) practices in the province of Tarlac. These were gathered from a series of Focus Group Discussions with municipal agriculturists, representatives of the Office of the Provincial Agriculturists and Local Farmer Technicians. From the list of CRA practices, key

informants identified three priority practices. The interview guide provided by CIAT was modified to fit the scope of the study before it was given to the group of key informants. Data gathered were used in the evaluation of CRA practices using the CBA Tool. CRA practices with the incorporation of externalities was given priority in the study.

RESULTS AND DISCUSSIONS

Enhanced Capacities of AMIA Partner Organizations in the Region

Series of trainings, workshops and learning events were organized for AMIA2 project teams in an intra-regional training that covers key learning contents from the national-level trainings as shown in Table 1.

Climate Risk Vulnerability Assessment (CRVA)

The vulnerability mapping was based on the Climate-Risk Vulnerability Assessment (CRVA) framework. It started with the identification of the vulnerability determinants (sensitivity, hazards, and adaptive capacity) and their respective indicators.

Sensitivity Analysis

In developing the impact of climate change to crop suitability, a crop distribution model was used and the factors associated are the 20 bioclimatic variables and the existing crop location. Sensitivity index was used in the sensitivity analysis to determine the sensitivity of crops to changes in temperature and precipitation.

The aggregated sensitivity of crops for rice, maize/corn, tomato, mango, and sweetpotato are given equal weights of 20% as shown in Figure 2 (a). It appears that the sensitivity index of the province generally ranges from -5% to -50% which means that it is sensitive to highly sensitive as influenced by the bioclimatic variables. On the other hand, improved varieties of crops, water conservation and soil conservation technologies that are being practiced by farmers mitigate the effect on crop yield.

Hazards Vulnerability

The natural hazards that were added to come up with the hazard vulnerability index are tropical cyclone/typhoon, flood, drought and erosion which are given the corresponding weights of 35%, 35%, 27% and 3%, respectively. The areas of San Manuel, Anao, Moncada, and La Paz are highly vulnerable to hazards as shown in Figure 2 (b). These areas experiences flooding on the onset of monsoon rain especially during typhoon because of geographically low areas of the province and become the catchment basin of the nearby provinces.

Adaptive Capacity Analysis

The adaptive capacity indicators considered in the assessment of the province's readiness to withstand the effects of climate change. These seven capitals are economic, natural, human, physical, anticipatory, social and institutional. Figure 2 (c) on the adaptive capacity illustrates the economic, natural, human, physical, anticipatory, social and institutional capital of every municipality. These seven (7) capitals of the adaptive capacity were given the same weight to come up with the aggregated adaptive capacity map.

The adaptive capacity of the province shows the readiness to adapt to climate risk. Concepcion and Gerona were found to have very high adaptive capacity while the municipalities of Ramos, San Jose, La Paz, and Bamban have very low adaptive capacity.

Climate-Risk Vulnerability

The climate-risk vulnerability map was developed by adding the sensitivity index, hazard index and the adaptive capacity index with their corresponding weights. A national experts' meeting composed of agriculturists, policy makers, and scientist, agreed the 15-15-70 percentage of weight for the sensitivity, hazard and adaptive capacity, respectively. The formula used in the development of climate-risk vulnerability map is stated in Equation 1:

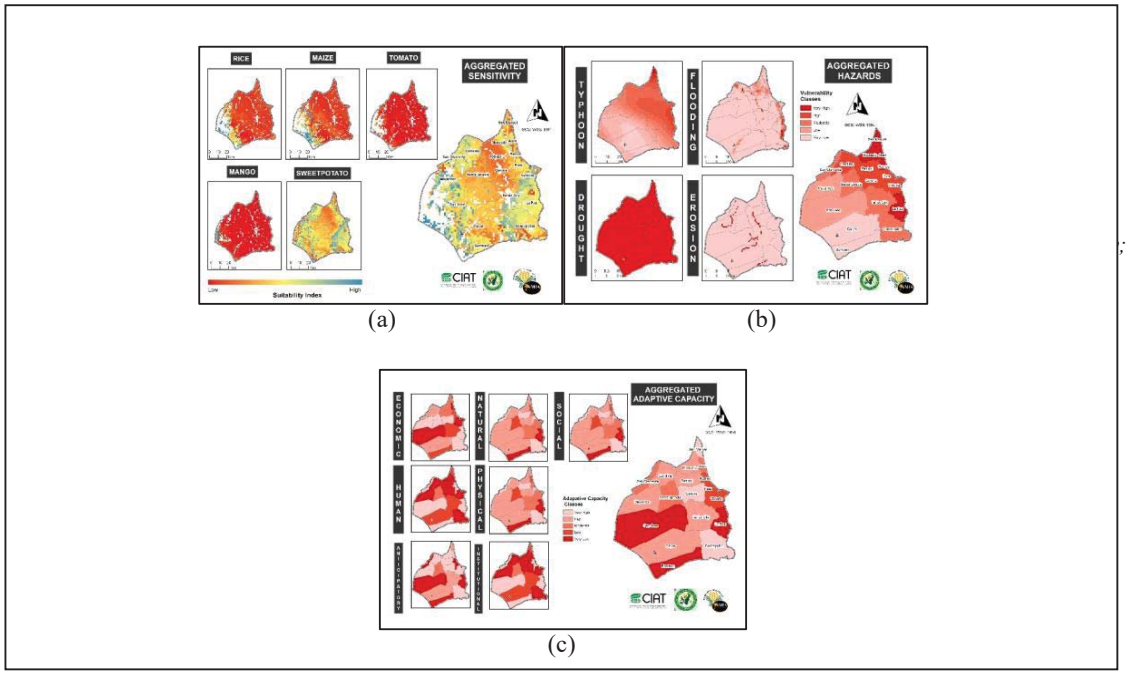
$$\begin{aligned}\text{Climate - risk vulnerability} = {} & \text{Sensitivity index} * 0.15 + \\ & \text{Hazard index} * 0.15 + \\ & \text{Adaptive capacity index} * 0.70\end{aligned} \quad (1)$$

Figure 3 show the vulnerability to climate-risk is very high in the municipalities of Ramos, Bamban and La Paz; high in San Jose, Victoria and Pura; lowest in Tarlac City. The factor that has the major contribution in the vulnerability assessment is the adaptive capacity given a weight of 70% compared to sensitivity and hazards with 15% weights each. The perspective of giving a high percentage to the adaptive capacity is the thought of the ability of every municipality being able to cope with extreme events like temperature, rainfall, typhoon, flood, drought, erosion and other natural hazards because these municipalities are equipped with facilities and structures, and services for the adaptation.

The maps developed in the CRVA assessment was presented to a focus group discussion (FGD) with the stakeholders from the Provincial Agriculture Office (PAO), Municipal Agriculture Office (MAO), Farmers and other agencies. From the FGD and field visit conducted, the participants agreed that the maps developed are similar to the actual situation in their municipality. Vulnerable areas due to risks of climate was mentioned in the study of Dikitanan, et al. (2017).

TABLE 1. Capability Building Training, Seminars and Workshop Attended by the Regional Team

Title	Date	Venue
Training on Climate Risk Vulnerability Assessment	June 6-8, 2016	Torre Venezia Hotel, Quezon City
Cost- Benefit Analysis (CBA) on Climate Resilient Agriculture Practices	August 6-8, 2016	Torre Venezia Hotel, Quezon City
Methodology for Evaluating Social and Environmental Benefits, in Agricultural Systems	December 2, 2017	Tarlac Agricultural University, Camiling, Tarlac
Climate Risk Vulnerability Assessment (CRVA) Mapping & and Adaptive Capacity Mapping	January 10-12, 2017	SEARCA, UPLB, Los Banos, Laguna
AMIA2-CIAT Project: Results Sharing and Validation Workshop on CRVA & CRA Decision Support	February 6-7,2017	Parklane International Hotel, Cebu City
Workshop on Finalizing Results on CRA and Prioritization and Extended CBA	March 1-3, 2017	B Hotel, Quezon City
Completion Review of BAR Funded Climate Change Projects	May31-June 2, 2017	Partido State University, Goa, Camarines Sur
AMIA2-CIAT Project: Workshop on Outcome Monitoring and Evaluation of Community-Based Action Research	June 21-22, 2017	Sequoia Hotel, Quezon City



(a) (b)

(c)

FIGURE 2. Maps on sensitivity, hazards and adaptive capacity to produce CRVA map: (a) Aggregated Sensitivity; (b) Aggregated Hazards; and (c) Aggregated Adaptive Capacity

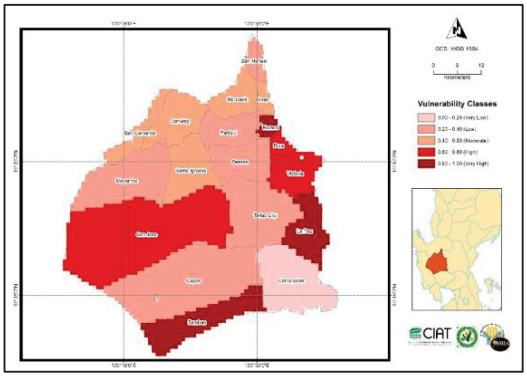


FIGURE 3. Climate-Risk Vulnerability Map of Tarlac

Cost-Benefit Analysis (CBA) of CRA Practices

The identified climate-resilient practices in the province of Tarlac which are the following: Organic agriculture, climate smart varieties/lines, water conservation technology, adaptive crop calendar/crop switching, soil conservation technology, intercropping/crop rotation, community-based management, crop insurance/weather adverse condition insurance, and improved housing for animals. The identification of CRA practices were gathered through interview. The list was trimmed and validated through a series of FGDs with municipal agriculturists, representatives of the Office of the Provincial Agriculturists, local farmer technicians and farmers. CRA practices have also been priorities in the study of Dikitanan, et al. (2017).

From the list of CRA practices, key informants identified three priority practices: climate smart varieties, crop rotation, and water conservation technology particularly the alternate wet and dry method (AWD). The interview guide provided by CIAT was modified to fit the scope of the study before it was given to the group of key informants. Data gathered were used in the evaluation of CRA practices using the CBA Tool. Validation of data was done by seventy one (71) municipal agriculturists, farmer leaders and farmer technicians participated in the Focus Group Discussions. The cost and benefits derived from the CRA practices were confirmed. These data were used to analyse the profitability and sustainability of the CRA practice. The three prioritized practices are the climate smart varieties, crop rotation and water conservation technology because of the immediate effects and perceived potential benefits to the farmers, food security and, mitigation and adaptation to climate change. Investment prioritization brief were also prepared for policy makers to support the climate-resilient agriculture practices in the provinces as shown in Figure 4. Researchers and extensions' personnel must use the investment brief for dissemination and reference for further validation or research in a specific area.

Climate Smart Varieties

Yield of crops is affected by extreme changes in climatic conditions such as flooding, and drought and attack of insect pests and diseases. Farmers and agricultural technologists favor climate smart varieties because of its high yield and its capacity to withstand varied climatic conditions. The Green Super Rice lines (GSR 8, 15, 21, and 22) earned special mention among farmers who have experienced growing it due to its resiliency especially during typhoons and floods, drought, and attack of insect pests and diseases. Farmers who planted GSR lines attested the resilience of these lines to typhoon and submergence. Yield was not likewise affected.

Crop Rotation

Crop rotation was chosen mainly because farmers could switch from rice to other crops (corn, sweet potato, etc.) depending on availability of water, soil conditions, and other climactic factors. Crop rotation also means reduction in pest occurrence due to non-availability of the host.

Water Conservation Technology

To address problems on scarcity of water supply or limited access to water sources in the upland, rainfed, and other areas, water conservation technologies are resorted to. The Alternate Wet and Dry (AWD) Method was specially mentioned because it utilizes materials readily available and can be constructed easily by the farmer. Some parts of the province depend on shallow tube wells and Tarlac Ground (TG) water as their main

source of irrigation. In these areas, one of the main expenses incurred by farmers is gasoline that is used to run pumps. This practice was chosen because it allows farmers to irrigate at the proper time. The intermittent drying of fields enables the farmers to save on time and money.

FIGURE 4. Investment Brief for Climate Smart Agriculture Practices

CONCLUSION

The two major output of this study are: climate-risks vulnerability map and investments briefs. The map was used to identify pilot area of climate smart village. The Department of Agriculture Regional Field Office 3 chose the municipality of Victoria as the pilot climate-smart village in the province of Tarlac. DA RFO3 based the selection on the result of the climate-risk vulnerability mapping and willingness of the local government unit to support the project. Victoria is considered as one of the high vulnerable municipalities that is sensitive to changes in climate, vulnerable to hazards and has less adaptive capacity to cope up with the phenomenon. Victoria is a 2nd class municipality of the province of Tarlac with 26 barangays and is located at the eastern part of the province. Majority of the total land area is devoted to agricultural activities and livestock production.

The climate resilient agriculture practices identified in the prioritization is being adopted in areas in the province for further testing and evaluation in the suitability of a CRA practices in an area. The investment briefs were presented to Department of Agriculture and Office of the President as a support for decision- and policy-making.

ACKNOWLEDGEMENT

We would like to give thanks to Department of Agriculture (DA) System-wide Climate Change Office (SWCCO) and International Center for Tropical Agriculture (CIAT) for the AMIA2 project.

REFERENCES

1. L.E. Ngilangil, S.O. Olivar and M.L.A. Ballesil. E-International Scientific Research Journal **5**(3), 74(2013).
2. Environmental Management Bureau-Climate Change Division. *National Climate Change Action Plan 2011-2028*, http://climate.emb.gov.ph.
3. R. Dikitanan, G. Grosjean, A. Nowak, and J. Leyte. *Climate-Resilient Agriculture in Philippines. CSA Country Profiles for ASIA Series,* http://cgspace.cgiar.org
4. Food and Agriculture Organization of the United Nations (FAO). *Climate-Smart Agriculture. Policies, Practices and Financing for Food Security, Adaptation and Mitigation*, http://fao.org.
5. E.M. De Guzman, A.N. Espino, Jr., M.E. Agulto and V. Malamug, *A GIS-Aided Decision Support System for Small Farm Reservoirs*, MS thesis. (Central Luzon State University, 2013).
6. Provincial Agricultural Office. "Tarlac Agricultural Profile". Tarlac Province (2016).

Civil Engineering and Architecture 10(4): 1526-1537, 2022
DOI: 10.13189/cea.2022.100422 http://www.hrpub.org

Small Farm Reservoir Suitability Analysis in Tarlac Province, Philippines

Ermalyn DG. Galo

College of Engineering and Technology, Tarlac Agricultural University, Philippines

Received October 19, 2021; Revised December 17, 2021; Accepted January 21, 2022

Cite This Paper in the following Citation Styles

(a): *[1] Ermalyn DG. Galo , "Small Farm Reservoir Suitability Analysis in Tarlac Province, Philippines," Civil Engineering and Architecture, Vol. 10, No. 4, pp. 1526-1537, 2022. DOI: 10.13189/cea.2022.100422.*

(b): *Ermalyn DG. Galo (2022). Small Farm Reservoir Suitability Analysis in Tarlac Province, Philippines. Civil Engineering and Architecture, 10(4), 1526-1537. DOI: 10.13189/cea.2022.100422.*

Abstract Small farm reservoir (SFR) suitability analysis is useful in water resources management and development assistance of government and non-government agencies for farmers and farmer-groups. The researcher utilizes the geographic information system to analyze the suitable areas for the construction and management of small water impounding to store and conserve rainwater in rainfed areas. The factors on rainfall, soil texture, slope, land use, irrigation status, groundwater availability and distance from river were considered for the suitability mapping of SFRs. The following factors have their corresponding weights which are derived from using the analytical hierarchy process (AHP) procedure. The testing of the model was done by determining the suitability value (S) of each sample SFR. The research findings showed the areas in the province potentially suitable for SFRs of the total land area of Tarlac: 47% are not suitable, 25% are marginally suitable, 13% are moderately suitable and 15% are highly suitable.

Keywords Suitability, Small Farm Reservoir, Geographic Information System, Rainfed Areas, AHP

1. Introduction

The Philippines has 41% total rainfed cropped area that mostly relies on rainfall; however its availability is lesser in dry season (Moya *et al.,* 1994). In addition, development of facilities for conventional irrigation is unlikely because of undulating topography, surface drainage and monetary constraints. Rainfed farmers suffer frequently from drought because of the inadequate water together with poor management practices of irrigation water. To mitigate the effect of drought in these areas, farmers with small farms are collecting rainfall and runoff and storing rainwater in small farm reservoir to be used for the wet and dry season crops (Guerra *et al*., 1994). Small farm reservoir (SFR) is an earth dam structure used to harvest and store rainfall and runoff for irrigation. It is the smallest version of small water impounding project with an embankment height of less than 4 meter (Ines *et al*, 2018). Studies showed that small farm reservoirs (SFRs) serve as an economically viable means for storing and conserving rainwater to lessen the effect of drought and cropping intensification in rainfed drought-prone areas. However, information about this technology is very limited making a hindrance to researchers, technical implementers and government agencies in utilizing its maximum potential in rainfed areas. Generating information system about SFRs with the aid of geographic information system (GIS) technology can be used as a basis for areas suited for SFRs as an effective water management scheme for individual farmer and farmer groups to improve crop production. Furthermore in the water resources development planning strategies of the government for the national, regional and local levels, as GIS has often used for the geographic concerns on agriculture. Thus, the objective of the study was to generate suitability maps for SFR construction in the province of Tarlac.

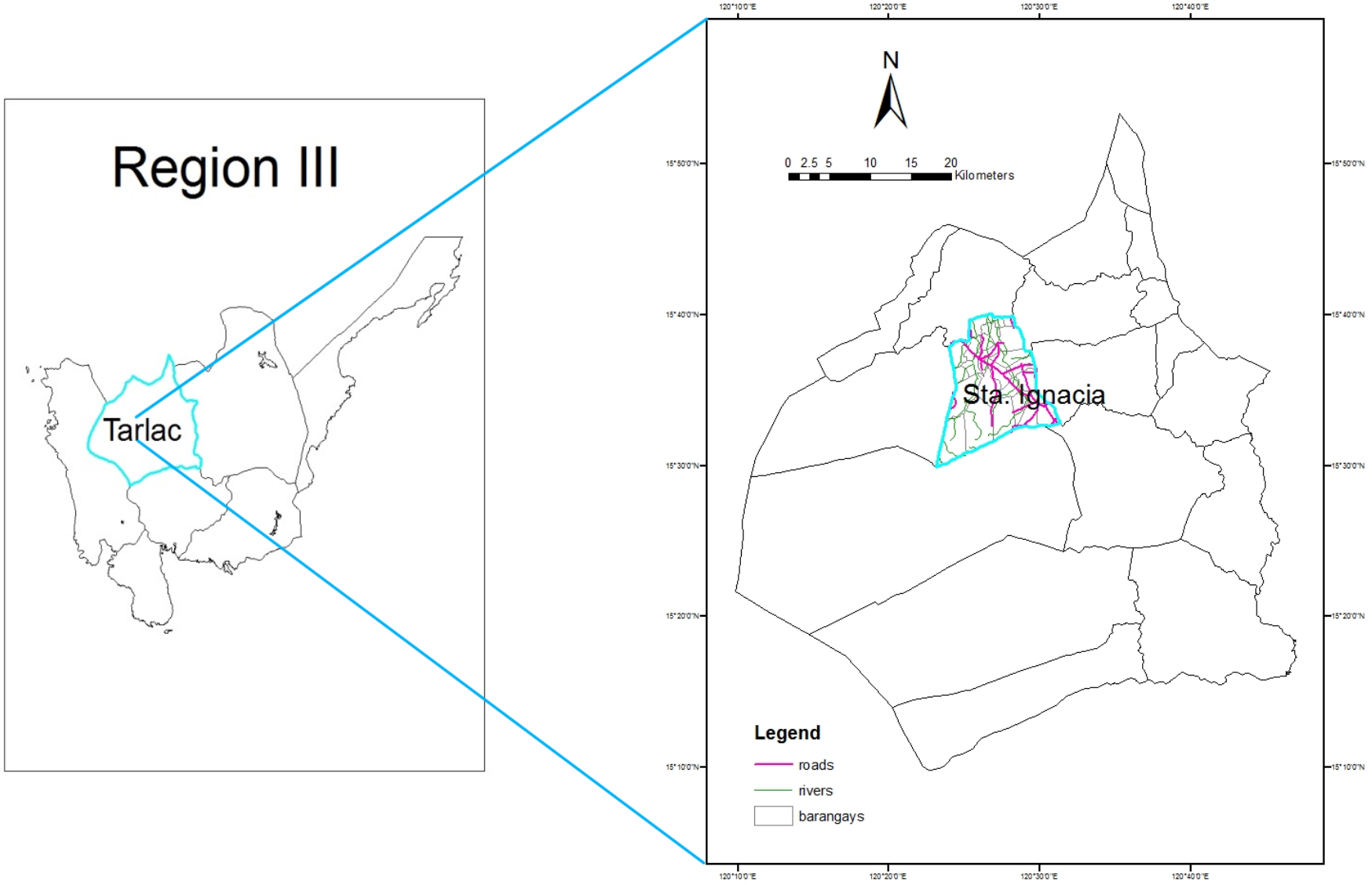


Figure 1. Map of Tarlac Province, Philippines

2. Materials and Methods

Study Area

The study was established in the province of Tarlac (Figure 1). The study covers an area of 273,660 hectares and is located between latitude 15º 10'15" N to 15º 52'52" N, longitude 120º 8'4" E to 120º 46'27" E. The study area has flat to undulating topography, with the eastern part of the province being plain and the western part to be hilly to mountainous. Tarlac has two distinct seasons, the wet and the dry seasons. It has unimodal rainfall pattern, having high monsoon rains in wet season (WS) and lesser amount of rainfall in dry season (DS). Recorded annual rainfall varies from 2,030 mm to 4,060 mm in the northwestern portion.

Data Acquisition

Rainfall map, soil texture map, slope map, land use map, irrigation status map, groundwater availability map and distance from river map were acquired from corresponding agencies namely in local agrometeorological station, Mines and Geosciences Bureau (MGB), National Mapping and Resource Information Authority (NAMRIA), National Irrigation Administration (NIA), National Water Resources Board (NWRB) and Department of Agriculture – Bureau of Agricultural Research (DA-BAR), respectively.

Suitability Factors

The data on rainfall, soil texture, slope, land use, irrigation status, groundwater availability and distance from river were used as the factors in the suitability mapping of SFRs. In the study of Cacayan *et.al* (2019), the factors considered are average annual rainfall, soil texture, slope and irrigation status while the past study of Galang *et.al* (1994), the criteria used at macrolevel are land use, slope, road network, municipal boundaries; however rainfall and soil type are excluded in the study. On the study of De Guzman (2013), the factors on rainfall, soil texture, slope, land use, irrigation status, groundwater availability and distance from river were used. The corresponding weights of these factors and its suitability ratings were determined. The factor maps derived from the seven thematic maps were integrated to the GIS (ArcGIS) software to develop a final suitability map to show the potential sites for SFRs construction. The methodology for identifying potential sites for SFRs is summarized in Figure 2.

Suitability Model for Evaluation of the Potential SFR Sites

Identification of the potential areas involves finding the areas that will satisfy a chosen set of criteria for establishment of SFR. Testing considers the impact of the system adoption.

The small farm reservoir suitability model (S) (equation 1) was derived from combining the factors with their corresponding weights for determining the potential areas for SFR in the final suitability map. Every location in the map had a suitability value. The formula below was used in calculating the suitability value of each grid cells:

S = [(Rainfall x rf) + (Soil texture x stf) + (Groundwater availability x gf) + (Slope x sf) + (Land use x lf) + (Irrigation status x if) (Distance x af)] (1)

where:

S = Suitability value for small farm reservoir
Rainfall = Rainfall factor map
Soil texture = Soil texture factor map
Slope = Slope factor map
Land use = Land use factor map
Irrigation status = Irrigation status factor map
Groundwater availability = Groundwater availability factor map
Distance = distance from river factor map
rf = rainfall weight
stf = soil texture weight
sf = slope weight
lf = land use weight
if = irrigation status weight
gf = groundwater availability weight
af = distance from river weight

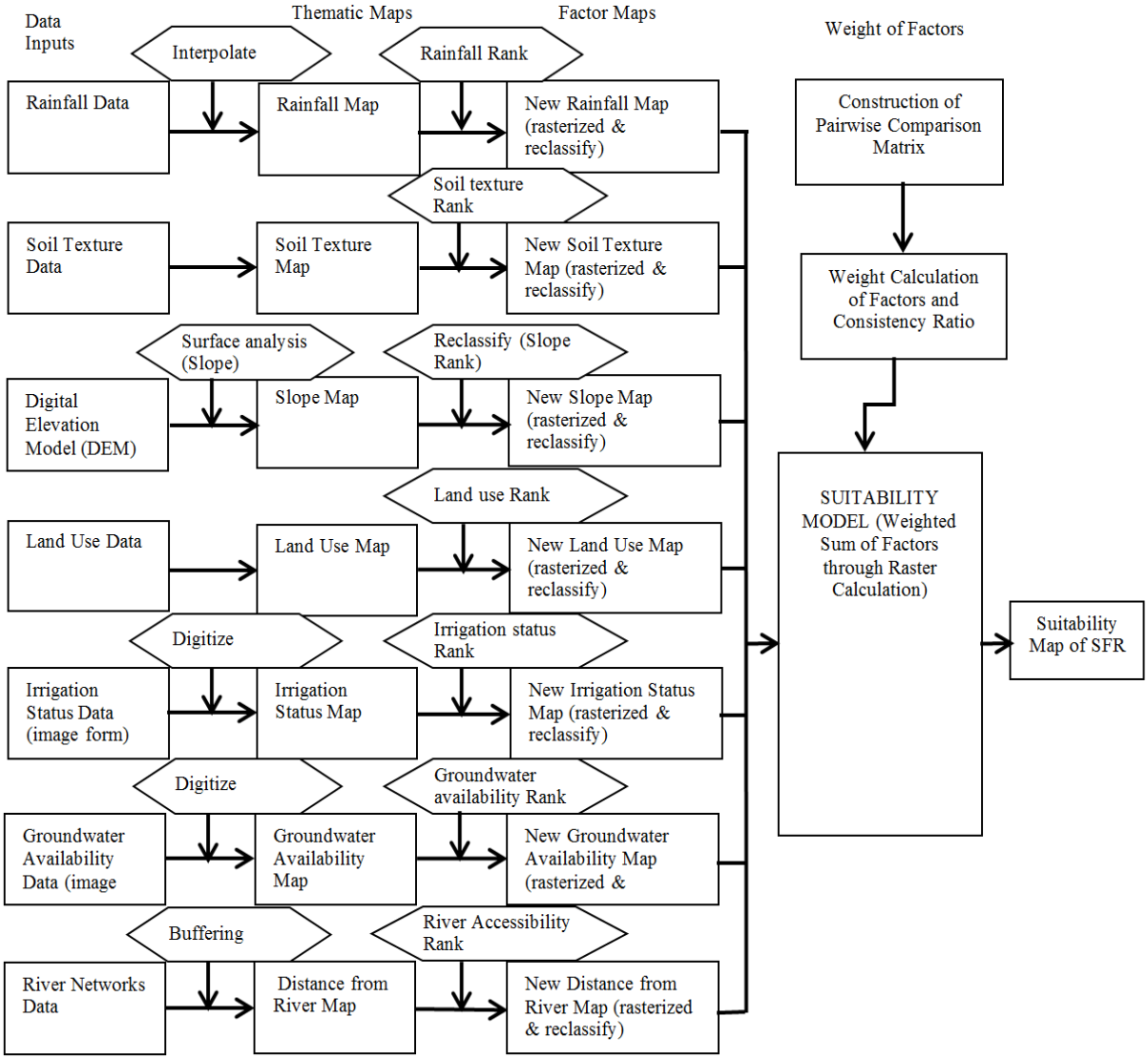


Figure 2. Methodology for identifying potential sites for SFRs

Table 1. Ranking of factors for SFRs

Factors	Description	Suitability Scale
Rainfall [a]	<1000 mm	1
	1000 - 1200 mm	2
	>1200 mm	3
Soil texture [b]	other class {sand, silt loam, silt, clay, Mountain soil (undifferentiated), Angeles soil (undifferentiated), Tarlac soils}	0
	sandy loam	1
	sandy clay loam	2
	clayloam & silty clayloam	3
Slope [c]	3- 8% (gently sloping to undulating)	3
	0 - 3% (level to nearly level)	2
	8 - 18% (undulating to rolling)	2
	18 - 30% (rolling to moderately steep)	1
	>30% (steep to very steep)	0
Land use	other land uses {built-up, closed forest, forest plantation, inland water, open forest, wooded lands}	0
	barren land	1
	Grassland	1
	cultivated land	3
Irrigation status	non-irrigated area	3
	irrigated area [d]	0
Groundwater availability [e]	deep well areas	3
	shallow well areas	0
	difficult areas [f]	0
Distance from river	> 200 m	3
	100 - 200 m	2
	50 -100 m	1
	0 -50 m	0

Note: [a] Rainfall description based from the category used by Galang et al. (1994)

[b] Soil texture description based from different soil types used by BSWM (1997) wherein only soil types under loamy soils is considered

[c] Slope class used by Galang et al. (1994) based from the slope category of DA-BAR

[d] Irrigated area of BBMP acquired from NIA-Tarlac

[e] Groundwater availability map acquired from NWRB

[f] Forested area with deep well areas that unsuitable for groundwater extraction

Ranking of Factor

Each map layer has individual values in each class. To be able to perform arithmetic operation, values must be assigned from a numeric evaluation scale referred to as suitability scale or preference from best to worst. Each factor was ranked by how suitable it is and is done through the process of reclassifying.

Table 1 shows the ranking of the factors for potential areas of SFRs wherein suitability scale of 0-3 was used, 3 being the highest value. Ranking of factors was based from the following four suitability ratings: not suitable (0), marginally suitable (1), moderately suitable (2) and highly suitable (3).

Weighting of Factor

Some factors are more important than the others in the suitability model. Therefore, percent influence or weight is assigned to each factor based from its importance. Calculating the weight of each factor was done using analytical hierarchy process (AHP). From the AHP procedures of Coyle (1989), the three steps used are as follows: (1) construction of a single pair-wise comparison matrix; (2) calculating the list of relative weights, importance, or value of the factors; and, (3) calculating and checking of the Consistency Ratio (CR).

The study of Al-Ruzouq *et al.* (2019) used the AHP in determining the importance of parameters such as precipitation, drainage stream density, geomorphology,

geology, curve number, total dissolve solids, elevation, slope and major fracture Euclidean distance for dam site suitability mapping and analysis.

Suitability Rating

Table 2 shows the suitability rating having its corresponding ranges of each class. The interpretation of suitability classes for each factor was classified on a scale from 0 to 3 as follows: not suitable, marginally suitable, moderately suitable, and highly suitable.

Table 2. Suitability rating

Suitability Levels	Range
Not suitable	0.0000 - 2.0000
Marginally Suitable	2.0001 - 2.5000
Moderately suitable	2.5001 - 2.7500
Highly suitable	2.7501 - 3.0000

Testing of the Suitability Model

The selected SFRs locations in the study area were overlayed in the final suitability map for SFRs and the testing of the model (equation 1) was done by determining the suitability value (S) of each sample SFR. There are one hundred fifty (150) SFR samples that are randomly selected on the municipality of Sta. Ignacia, Tarlac. Locations of SFRs are done using global positioning system (GPS).

3. Results and Discussion

Finding the best location for SFRs was accomplished in the suitability mapping of SFR sites. Each factor map used was reclassified according to its suitability and these maps were combined with their corresponding percent influence to produce the final suitability map of SFRs. Suitability value of every location in the map was obtained after the creation of the final suitability map for SFRs.

Thematic Maps

The thematic maps (rainfall, soil texture, slope, land use, irrigation status, groundwater availability and distance from river) used as the factors of the study that are essential for identifying the potential sites for SFR are presented in Figure 4. These are the preliminary maps used with their corresponding attributes.

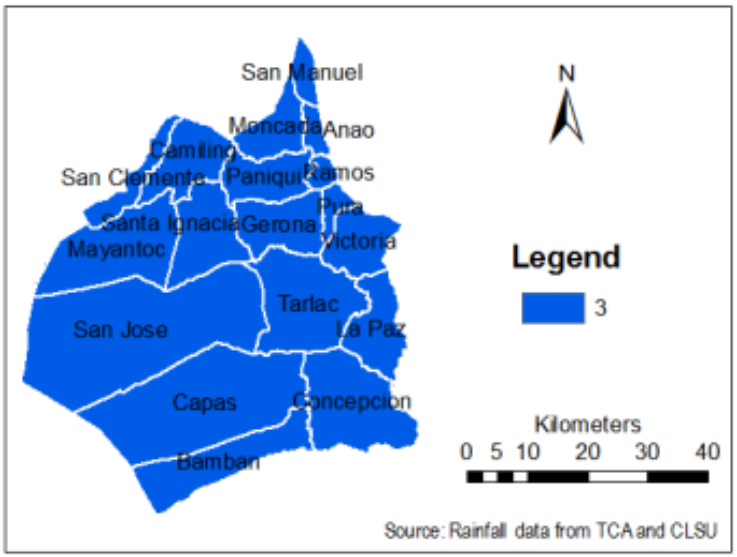
Factor Maps

Each thematic map having individual values in each class was assigned a value from the numeric evaluation scale known as suitability scale or preferences, from best to worst, to be able to perform arithmetic operation in the suitability analysis. These thematic maps are ranked according to suitability through reclassification. Ranking of the factors was done by assigning a scale of 0 to 3, 3 being the highest value. The factor maps were the resulting maps after reclassification of the thematic maps shown in Figure 5.

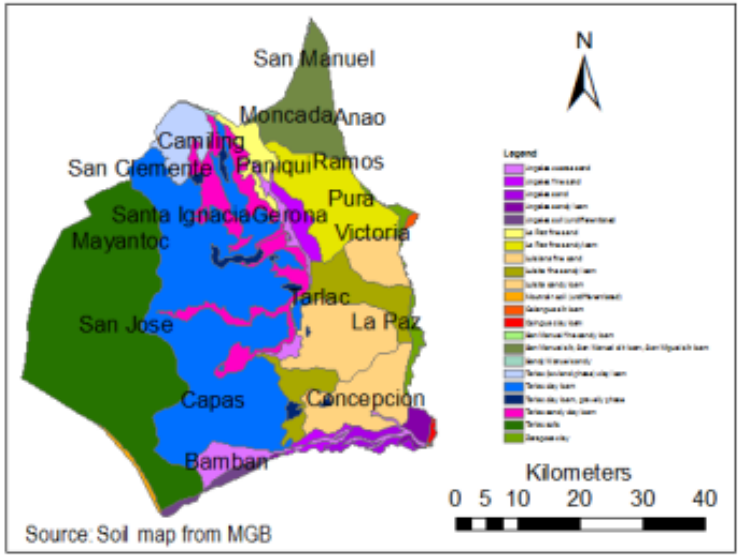
Determination of the Relative Important Weights of Factor

Assigning weights or percent influence to each factor was needed because of the fact that some factors are more important in the suitability model than others. This was done through analytical hierarchy process (AHP). If the factors are of equal importance then assign the same weight to each one.

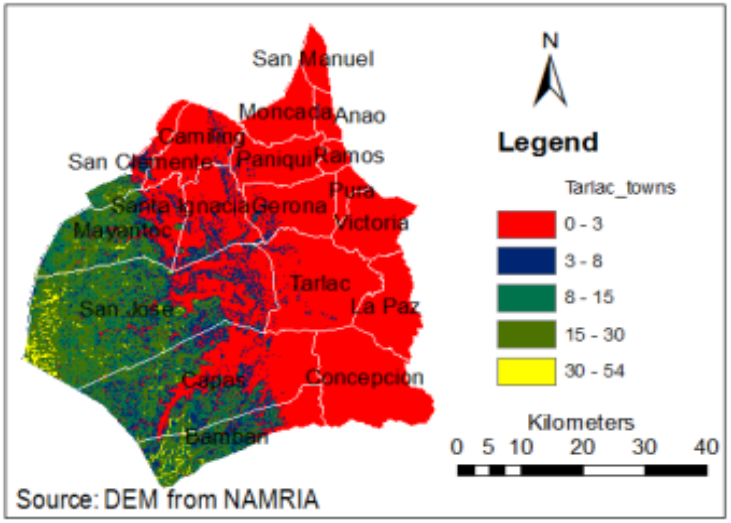
In SFR adoption, the factor considered as most preferable is land use, and the next are rainfall, irrigation status and distance from river followed by soil texture and lastly, slope. Table 3 shows the pair-wise comparison matrix for assessing the relative important weights of each factor in creating the suitable areas for SFRs.


San Manuel
Moncada
Anao
Camiling
San Clemente
Paniqui
Ramos
Pura
Santa Ignacia
Gerona
Victoria
Mayantoc
Tarlac
La Paz
San Jose
Capas
Concepcion
Bamban
N
Legend
3
Kilometers
0 5 10 20 30 40
Source: Rainfall data from TCA and CLSU

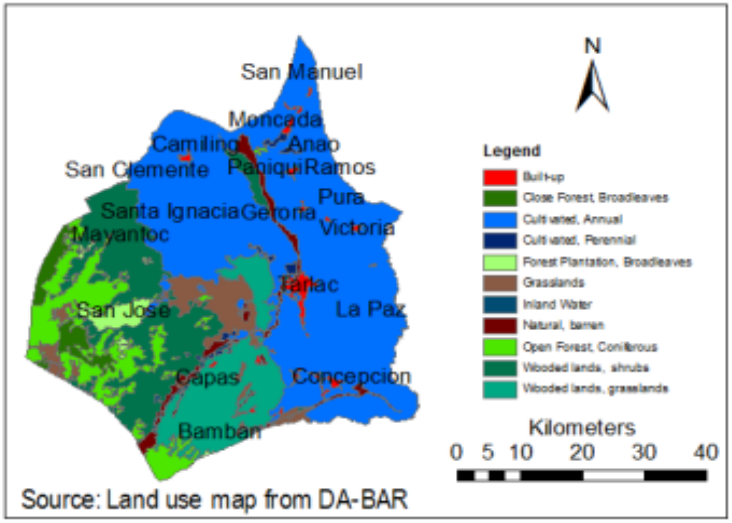
(a)


San Manuel
Moncada
Anao
Camiling
San Clemente
Paniqui
Ramos
Pura
Santa Ignacia
Gerona
Victoria
Mayantoc
Tarlac
La Paz
San Jose
Capas
Concepcion
Bamban
N
Legend
Kilometers
0 5 10 20 30 40
Source: Soil map from MGB

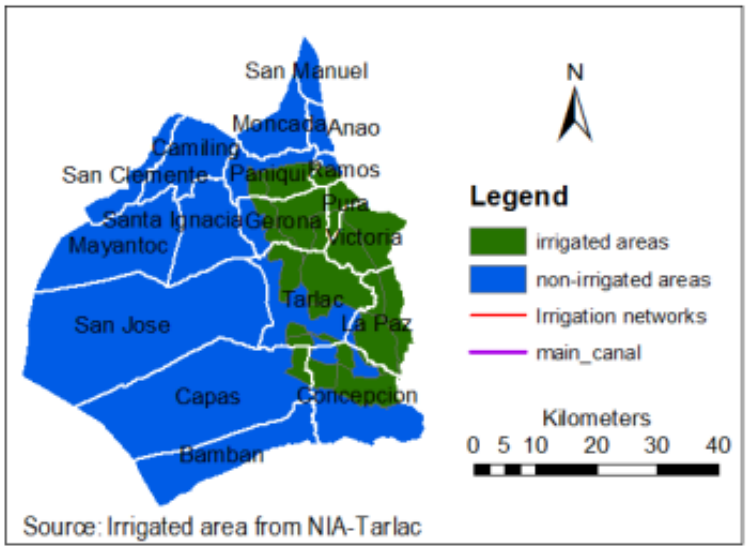
(b)


San Manuel
Moncada
Anao
Camiling
San Clemente
Paniqui
Ramos
Pura
Santa Ignacia
Gerona
Victoria
Mayantoc
Tarlac
La Paz
San Jose
Capas
Concepcion
Bamban
N
Legend
Tarlac_towns
0 - 3
3 - 8
8 - 15
15 - 30
30 - 54
Kilometers
0 5 10 20 30 40
Source: DEM from NAMRIA

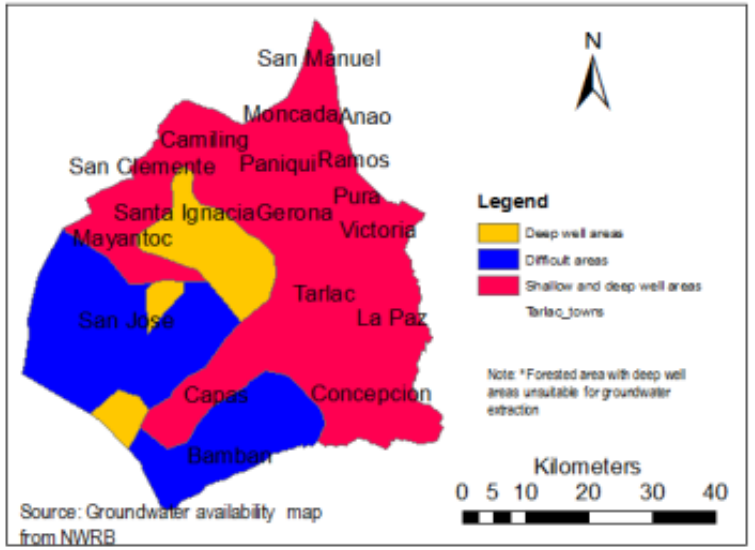
(c)


San Manuel
Moncada
Anao
Camiling
San Clemente
Paniqui
Ramos
Pura
Santa Ignacia
Gerona
Victoria
Mayantoc
Tarlac
La Paz
San Jose
Capas
Concepcion
Bamban
N
Legend
Built-up
Close Forest, Broadleaves
Cultivated, Annual
Cultivated, Perennial
Forest Plantation, Broadleaves
Grasslands
Inland Water
Natural, barren
Open Forest, Coniferous
Wooded lands, shrubs
Wooded lands, grasslands
Kilometers
0 5 10 20 30 40
Source: Land use map from DA-BAR

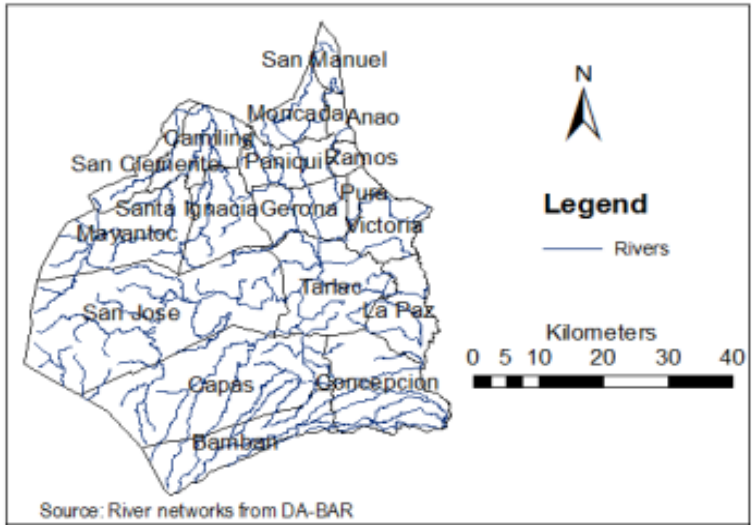
(d)



(e)

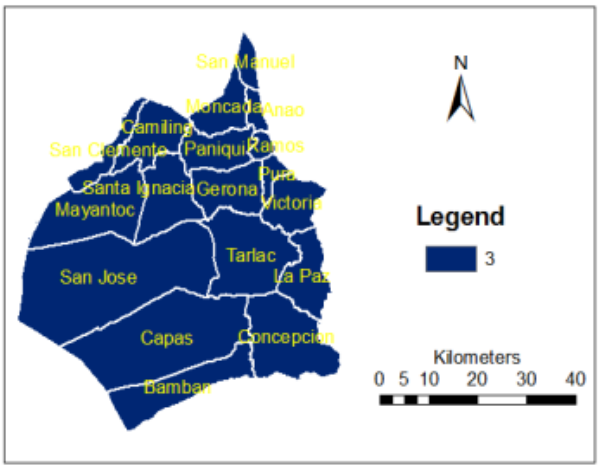


(f)

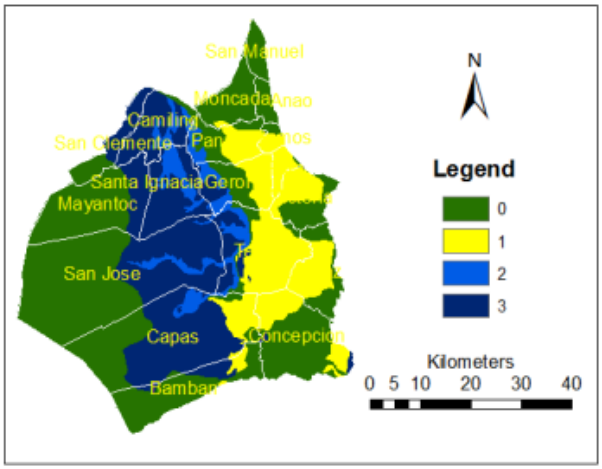


(g)

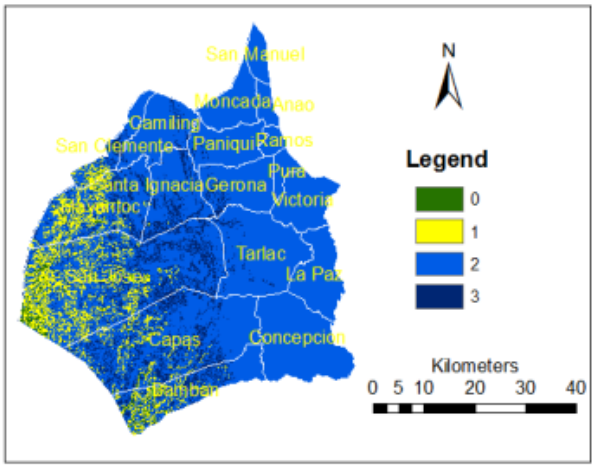
Figure 4. The thematic maps used in suitability mapping (a) Rainfall map (b) Soil texture map (c) Slope map (d) Land use map (e) Irrigation status map (f) Groundwater availability map and (g) River network


San Manuel
Moncada
Anao
Camiling
San Clemente
Paniqui
Ramos
Pura
Santa Ignacia
Gerona
Victoria
Mayantoc
Tarlac
La Paz
San Jose
Capas
Concepcion
Bamban
N
Legend
3
Kilometers
0 5 10 20 30 40

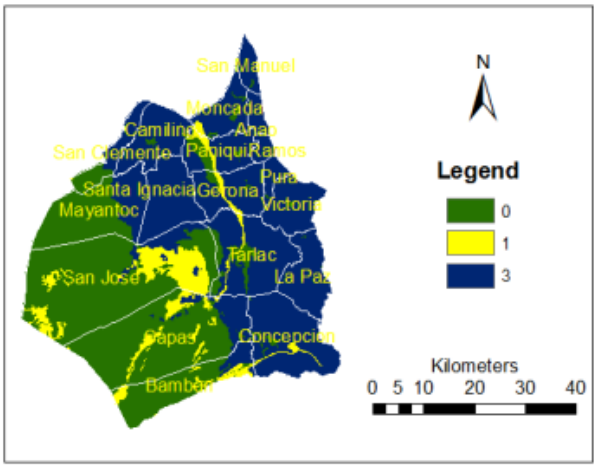
(a)


San Manuel
Moncada
Anao
Camiling
Mayantoc
San Jose
Capas
Concepcion
Bamban
N
Legend
0
1
2
3
Kilometers
0 5 10 20 30 40

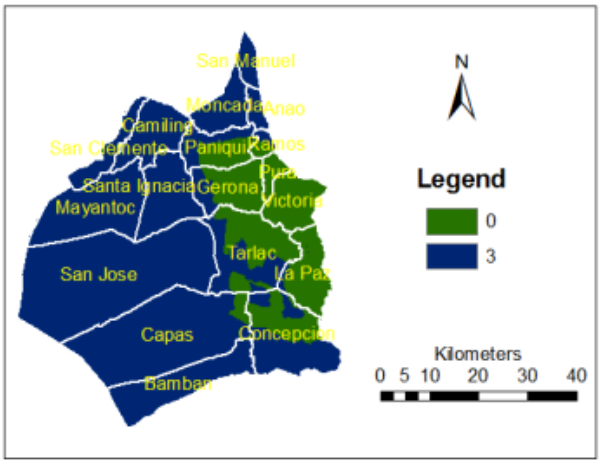
(b)


San Manuel
Moncada
Anao
Camiling
San Clemente
Paniqui
Ramos
Pura
Santa Ignacia
Gerona
Victoria
Mayantoc
Tarlac
La Paz
San Jose
Capas
Concepcion
Bamban
N
Legend
0
1
2
3
Kilometers
0 5 10 20 30 40

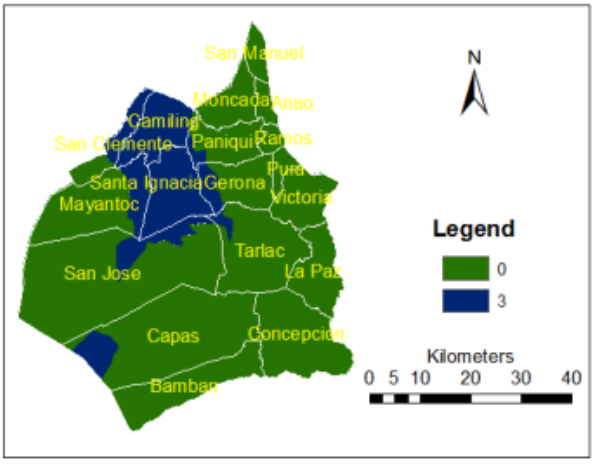
(c)


San Manuel
Moncada
Anao
Camiling
San Clemente
Paniqui
Ramos
Pura
Santa Ignacia
Gerona
Victoria
Mayantoc
Tarlac
La Paz
San Jose
Capas
Concepcion
Bamban
N
Legend
0
1
3
Kilometers
0 5 10 20 30 40

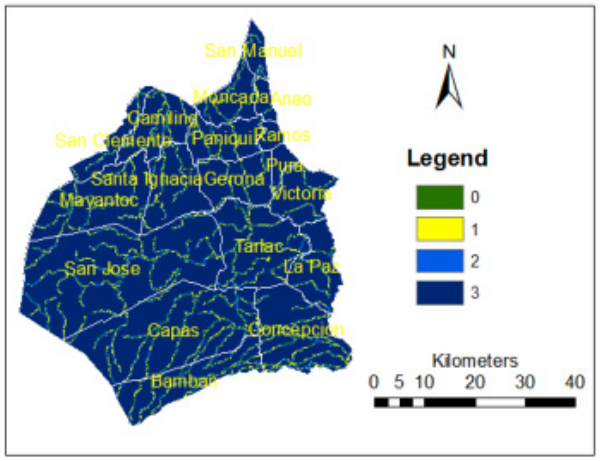
(d)


San Manuel
Moncada
Anao
Camiling
San Clemente
Paniqui
Ramos
Pura
Santa Ignacia
Gerona
Victoria
Mayantoc
Tarlac
La Paz
San Jose
Capas
Concepcion
Bamban
N
Legend
0
3
Kilometers
0 5 10 20 30 40

(e)


San Manuel
Moncada
Anao
Camiling
San Clemente
Paniqui
Ramos
Pura
Santa Ignacia
Gerona
Victoria
Mayantoc
Tarlac
La Paz
San Jose
Capas
Concepcion
Bamban
N
Legend
0
3
Kilometers
0 5 10 20 30 40

(f)



(g)

Figure 5. The different factor maps produced from the thematic maps (a) Rainfall factor map (b) Soil texture factor map (c) Slope factor map (d) Land use factor map (e) Irrigation status factor map (f) Groundwater availability factor map and (g) Distance from river factor map

Table 3. Pair-wise comparison matrix for assessing the relative weight of factors

	Rainfall	Soil texture	Slope	Land use	Irrigation status	Groundwater availability	Distance from river	RIW
Rainfall	1	5	7	1/3	1	3	1	0.167
Soil texture	1/5	1	3	1/7	1/5	1/3	1/5	0.038
Slope	1/7	1/3	1	1/9	1/7	1/5	1/7	0.022
Land use	3	7	9	1	3	5	3	0.366
Irrigation status	1	5	7	1/3	1	3	1	0.167
Groundwater availability	1/3	3	5	1/5	1/3	1	1/3	0.073
Distance from river	1	5	7	1/3	1	3	1	0.167

Consistency ratio (CR): 0.03

RIW = Relative Important Weight

Final Suitability for SFRs

Overlaying of the different factor maps produced the suitable sites for SFRs. Combining these factor maps was done by the use of raster calculator from spatial analyst tool after reclassifying each map. Each factor maps was multiplied with its corresponding weights and added together to produce the final suitability map.

The formula in equation 2 used in the suitability model was substituted by the computed values of weights or percent influence to each factor as shown below:

S = [(rainfall factor x 0.167) + (soil texture x 0.038) + (slope x 0.022) + (land use x 0.366) + (irrigation status x 0.167) + (groundwater availability 0.073) + (distance x 0.167)]

Figure 6 shows the suitability map for SFR sites in Tarlac classified into four suitability classes; 0-not suitable, 1-marginally suitable, 2-moderately suitable and 3-highly suitable. Not suitable areas have the highest value of 142,353 hectare or 47% of the total land area of the province. Marginally suitable areas were 73,839 hectare or 25% of the total area of the province. Moderately suitable areas had the smallest area of 40,129 hectare or 13% of the total land area of the province. Highly suitable areas for SFRs got 44,813 hectare or 15% of the total land area of the province.

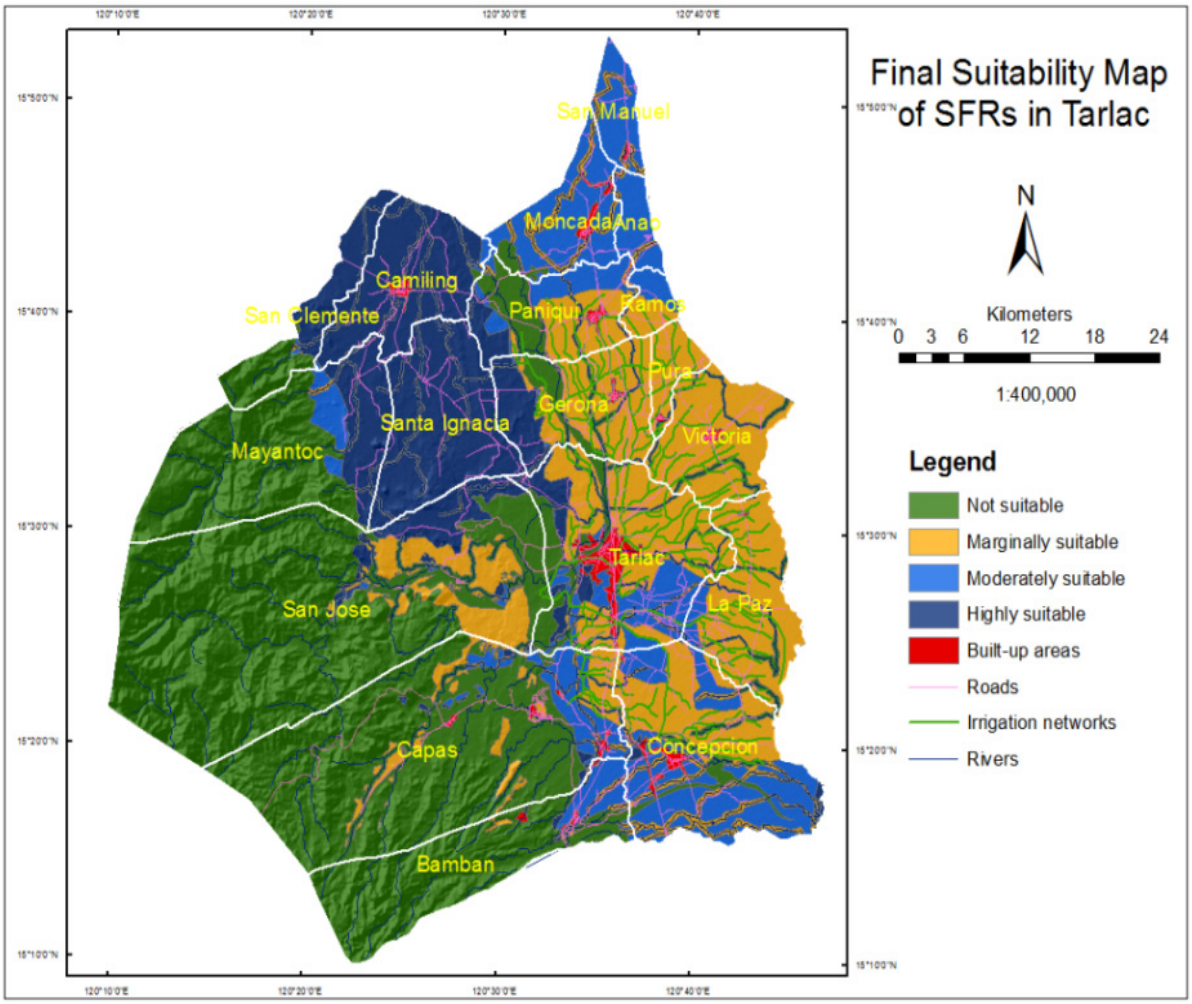


Figure 6. Final Suitability Map of SFRs in Tarlac

Testing of the Suitability Model

Testing of the suitability model was done by determining the suitability (S) value of each SFR overlay in the final suitability map. The summary of the suitability value of each SFR is shown in Table 4.

Table 4. Summary of the suitability (S) value of selected SFRs

Suitability Class	Range of Suitability Value (S)	Frequency (n=150)	Percentage (%)
Not suitable	0.0000 -2.0000	11	8
Marginally suitable	2.0001 -2.5000	0	0
Moderately suitable	2.5001 -2.7500	2	1
Highly suitable	2.7501 -3.0000	137	91

4. Conclusions

Based on the result, the usage of SFRs as a source of water for irrigation in wet and dry season intensifies cropping in drought-prone rainfed areas. The availability of information system on SFRs can be used by authorities or sectors responsible for water resources management and development.

GIS-aided decision support system for SFRs can be a viable means in determining the areas suited for SFR construction as well as the location of existing SFRs to maximize their full utilization.

REFERENCES

[1] Al-Ruzouq, R. Shanableh A., Yilmaz A. G, Idris A., Mukherjee S., Khalil M. A, and Gibril MB. A. (2019).https://www.researchgate.net/publication/335727798_Dam_Site_Suitability_Mapping_and_Analysis_Using_an_Integrated_GIS_and_Machine_Learning_Approach/link/5d7803724585151ee4adee1d/download.

[2] Cacayan Jr., A. O. Apdohan, A. G., Bacobo, A. E., and Ruta, J. L. (2019). Identifying suitable areas for small farm reservoir in Agusan Del Norte using geographic information system. Available at: https://www.researchgate.net/publication/338134754_IDENTIFYING_SUITABLE_AREAS_FO

R_SMALL_FARM_RESERVOIR_IN_AGUSAN_DEL_NORTE_USING_GEOGRAPHIC_INFORMATION_SYSTEM.

[3] Coyle, R. (1989). Practical strategy. Open access material. AHP.

[4] De Guzman, E. M. (2013). A GIS-aided decision support system for small farm reservoir. Unpublished Masteral Thesis in Agricultural Engineering, Institute of Graduate Studies, Central Luzon State University, Nueva Ecija, Philippines.

[5] Galang, A., Bhuiyan, S. and Hunt, E. (1994). Identification of potential areas for use of the on-farm reservoir system for drought alleviation. In: Bhuiyan, S.I. (Ed.), On-farm Reservoir Systems for Rainfed Ricelands. International Rice Research Institute. Manila, Philippines. pp. 25–38. Available at: http://books.irri.org/9712200663_content.pdf.

[6] Guerra, L., Watson, P, and Bhuiyan, S. (1994). Hydrological characteristics of on-farm reservoirs (OFRs) in rainfed rice growing areas. Modified version of the paper originally published in Agricultural Water Management. In: Bhuiyan, S.I. (Ed.), On-farm Reservoir Systems for Rainfed Ricelands. International Rice Research Institute. Manila, Philippines, pp.12–24. Available at: http://books.irri.org/9712200663_content.pdf.

[7] Ines, R. L., Tuazon, J. B., Daag, M. N. (2018). Utilization of Small Farm Reservoir (SFR) for Upland Agriculture of Bataan, Philippines, (pp 1-6) International Journal of Applied Agricultural Sciences. Available at: https://www.semanticscholar.org/paper/Utilization-of-Small-Farm-Reservoir-(SFR)-for-of-Ines-Tuazon/aaec07e42d7a6a5ac729cb17e083f8b75c669827.

[8] Moya, T., Dela Vina, W. and Bhuiyan, S. (1994). Potential of on-farm reservoir use for increasing productivity of rainfed rice areas: the Philippine case. Modified version of the paper originally published in Agricultural Water Management. In: Bhuiyan, S.I. (Ed.), On-farm Reservoir Systems for Rainfed Ricelands. International Rice Research Institute. Manila, Philippines, pp.61–71. Available at: http://books.irri.org/9712200663_content.pdf.

Survey of Physical, Chemical and Microbial Water Quality of Irrigation Sources in Tarlac, Philippines

Edmar N. Franquera, Cielito A. Beltran, Ma. Asuncion G. Beltran and Ruth Thesa B. Franquera

Abstract The main sources of irrigation water for irrigating crops comes from major rivers. Usually these water sources which can be used for irrigating various crops could be very vulnerable to contamination. The aim of the study was to determine the physical, chemical and microbial water quality of the different irrigation sources in Tarlac and to compare it with the existing water quality guidelines stipulated in the DENR AO 08 Series of 2016. The water samples collected from the surface water of different rivers were subjected to laboratory analysis. Higher TSS was found to be during wet season as compared during the dry season. Higher COD was found both in dry and wet seasons in Benig river. All of the major rivers have a less than 0.05 mg/l lead and 0.0002 mg/l mercury based from the result of the laboratory analysis. The highest dissolved oxygen was found to be within the Tarlac River both during the dry and wet season. Comparing with the National standards from the DENR the major rivers of Tarlac surpasses the minimum standards of classification of water bodies with dissolved oxygen ranging from 2 to 6 mg/l. The lowest dissolved oxygen was found in Concepcion River during the dry season (5.0 mg/l) and in Rio Chico River (4.8 mg/l) during the wet season. Higher total dissolved solids were observed in the different rivers during the dry season which ranges from 300 to 560 mg/l as compared during the wet season which ranges from 169 to 540 mg/l respectively. The nitrate concentrations of the different rivers in Tarlac shows to be within the range of the National Standards of the DENR. Higher concentrations of *E. coli* and fecal coliform count were also noted within the different rivers of Tarlac.

Keywords Water quality · River · Irrigation · Tarlac

E. N. Franquera (✉) · C. A. Beltran (✉) · Ma. A. G. Beltran (✉) · R. T. B. Franquera (✉)
Tarlac Agricultural University, Malacampa, Camiling, Tarlac, Philippines
e-mail: edmarfranquera123@gmail.com

C. A. Beltran
e-mail: tolitsbeltran@yahoo.com

Ma. A. G. Beltran
e-mail: marizonbeltran@yahoo.com

R. T. B. Franquera
e-mail: edmarfranquera@yahoo.com

R. Sun and L. Fei (eds.), *Sustainable Development of Water and Environment*, Environmental Science and Engineering,
https://doi.org/10.1007/978-3-030-16729-5_2

1 Introduction

Water is life. All living organisms on earth need fresh water. The major user of freshwater in most countries is agriculture. The largest single user of freshwater in the world today which consumes an average of 70% globally is accounted in agriculture.[1] However, the availability of freshwater is already decreasing due to water pollution. Agriculture is considered to be a casualty of water pollution but it also causes and contributes to water pollution due to excess nutrients by too much application of fertilizers, excessive use of pesticides and other pollutants. Globally, agriculture is also considered to be the major cause of degradation of surface including groundwater resources as a result of erosion, excessive farming contaminating freshwater like wastewater coming from large poultries and piggeries, chemical run off and other indiscriminate human activities and improper agricultural management practices. Waste coming from swine is significant source of fecal pollution leading to water pollution by contaminating of ground and surface water from lagoon overflow and the use of lagoon surface water for irrigation. Thus, it is important to test a system or test a technology such as potential aquatic plants to decontaminate the wastewaters so that this will resolve the problem.

In the Philippines, agriculture wastewater is one of the major sources of water pollution which accounted 37%.[2] In addition, only 10% of wastewater is treated while 58% of groundwater is contaminated. Regions which had unsatisfactory ratings for their water quality criteria include National Capital Region (NCR), Southern Tagalog Region, Central Luzon (Region 3) and Central Visayas. Hence, there is a need to address the global implications of water quality and there is a need for wastewater treatments. In central Luzon, the agricultural land area is 653,607 km^2 and 9.1% contributed to the agricultural BOD generation, 9.0% industrial BOD generation and 9.9% domestic BOD generation leading to water quality degradation and contamination.[3]

Generally, the availability of clean freshwater is becoming a primary limitation to human activities expansion and also the scope or capacity of our agricultural lands to feed the tremendous population growth not only in the Philippines but globally. There are an estimated 2.2 million metric tons of organic water pollution that occur in the Philippines each year and the annual economic losses caused by water pollution are estimated at Php67 Billion which is equivalent to more or less US$1.3 billion.[4] Hence, this study aims to quantify the physical, chemical and microbiological water qualities of the different river waters in Tarlac, Philippines.

[1] www.fao.org. Last accessed 30 Nov 2017.

[2] www.greenpeace.org. Last accessed 30 Nov 2017.

[3] www.wipo.int/wipo_ip_mnl_15_t4. Last accessed 27 Nov 2017.

[4] www.wepa-db.net.philippines.overview. Last accessed 30 Nov 2017.

2 Methodology

2.1 *Gathering/Collection of Data of Existing Irrigation Water Sources in Tarlac*

The existing data on the type of irrigation systems and the irrigation sources were gathered. This was done in collaboration with National Irrigation Administration (NIA). The water qualities that were gathered were compared to the existing standards of the Department of Environment and Natural Resources (DENR).

2.2 *Water Sample Collection*

Representative water samples were collected in seven major rivers of Tarlac based from the data of the National Irrigation Administration (NIA) and the Department of Environment and Natural Resources and the collection was done from 9:00 AM in the morning until 4:00 PM in the afternoon. A total of six liters of water samples were collected in each sampling sites based from the recommendation of the Department of Science and Technology. The water sampling collection was done on the onset of 2018 dry and wet season productions of rice.

2.3 *Water Quality Analysis*

Collected water samples were analyzed for its physical, chemical and microbiological qualities (Total suspended solids, chemical oxygen demand, total coliform bacteria, *E. coli*, lead and mercury content). These parameters were analyzed using the standard methods in analysis of water samples. Portable instruments were used for the analysis of the following parameters such as dissolved oxygen (portable oxygen meter), pH (HM pH-200) total dissolved solids and electrical conductivity (HM COM-100). For the nitrate quantification a Horiba portable nitrate meter was used.

2.4 *Analysis of Data*

Laboratory results from the collected water samples were analyzed and compared with the Water Quality Guidelines and General Effluent Standards of 2016 based on the Department of Environment and Natural resources (DENR) Administrative Order No. 08 Series of 2016.

3 Results and Discussions

See Table 1.

3.1 *Total Soluble Solids and Chemical Oxygen Demand*

Table 2 presents the data of the different major rivers of Tarlac in terms of the total soluble solids and chemical oxygen demand. Results showed that the different river water has a varied total suspended solids and chemical oxygen demand. Higher TSS was found to be during wet season as compared during the dry season. This was also evident in terms of the chemical oxygen demand except for the two rivers, the Rio Chico and the Camiling river which exhibited a lower COD during the wet season with less than. For the TSS, based from the standard water qualifications, Tarlac and Concepcion rivers exceeded the numerical value which a body of water could be classified ranging only from 25 to 110 but for the two rivers it has both 169 mg/l total suspended solids during the wet season. Higher COD was found both in dry and wet seasons in Benig river with 27 and 22 mg/l respectively. Result of the COD laboratory test from the Benig river was also in consonance with the result of research conducted by Fernandez and David (2008) [5] which also shows high COD in Benig River. This implies that the higher COD in the sampling area, the higher level of water pollution. The wastewater discharge coming from the different industries within the area such as the presence of piggery farms could contribute to the higher COD of the water samples which maybe contributed to the deterioration of water quality within the sampling area (Al-Badaii et al. 2013).

3.2 *Heavy Metals (Lead and Mercury)*

The heavy metal concentrations (lead and mercury) in the different major rivers of Tarlac are presented in Table 3. All of the major rivers have a less than 0.05 mg/l lead and 0.0002 mg/l mercury based from the result of the laboratory analysis. Compared to the standards for the water quality the result both of the lead and mercury content of all the major rivers showed lesser than that of the standards. This implies that the rivers were not contaminated with heavy metals. This could be due to the non-presence of mining sites within the areas where the different rivers were located. Heavy metals were considered to be toxic and dangerous. The presence of higher concentrations of heavy metals in rivers as source of irrigation for the crops could lead also to the decline in production and these heavy metals could bio accumulate affecting also the humans whom will consume the crops irrigated with higher concentrations of heavy

[5] www.bgr.bund.de.Veranstaltungen. Last accessed 15 Dec 2017.

Table 1 Water quality guidelines (DENR AO 08 Series 2016)

Parameter	Water body qualifications								
	AA	A	B	C	D	SA	SB	SC	SD
Dissolved oxygen (mg/l)	5	5	5	5	2	6	6	5	2
Fecal coliform (MPN/100 ml)	<1.1	<1.1	100	200	400	<1.1	100	200	400
Nitrate (mg/l)	7	7	7	7	15	10	10	10	15
pH	6.5–8.5	6.5–8.5	6.5–8.5	6.5–9.0	6.5–9.0	7.0–8.5	7.0–8.5	6.5–8.5	6.5–9.0
TSS	25	50	65	80	110	25	50	80	110
Lead (mg/l)	0.01	0.01	0.01	0.05	0.1	0.01	0.01	0.05	0.01
Mercury (mg/l)	0.001	0.001	0.001	0.002	0.004	0.001	0.001	0.002	0.004

Table 2 Total soluble solids and chemical oxygen demand data of different major rivers of Tarlac province, Philippines during wet and dry season of 2018

River	Total suspended solids (mg/l)		Chemical oxygen demand (mg/l)	
	Dry season	Wet season	Dry season	Wet season
Benig	32	40	27	22
Tarlac	40	169	10	14
Bamban	58	32	11	15
Concepcion	52	169	21	19
Lapaz	223	91	11	28
Rio Chico	103	66	10	<10
Camiling	17	45	6.9	<10

Table 3 Heavy metals concentration of different major rivers of Tarlac province, Philippines during wet and dry season of 2018

River	Lead (mg/l)		Mercury (mg/l)	
	Dry season	Wet season	Dry season	Wet season
Benig	<0.05	<0.05	<0.0002	<0.0002
Tarlac	<0.05	<0.05	<0.0002	<0.0002
Bamban	<0.05	<0.05	<0.0002	<0.0002
Concepcion	<0.05	<0.05	<0.0002	<0.0002
Lapaz	<0.05	<0.05	<0.0002	<0.0002
Rio Chico	<0.05	<0.05	<0.0002	<0.0002
Camiling	<0.05	<0.05	<0.0002	<0.0002

metals. When crops were irrigated with water contaminated with heavy metals, the soils will also be polluted (Verma and Dwivedi 2013).

3.3 Dissolved Oxygen and pH

Table 4 presents the data on the dissolved oxygen and pH of the different major rivers of Tarlac province Philippines. Based from the result the highest dissolved oxygen was found to be within the Tarlac River both during the dry and wet season with 16.0 and 14.8 mg/l respectively.

The lowest dissolved oxygen was found in Concepcion River during the dry season (5.0 mg/l) and in Rio Chico River (4.8 mg/l) during the wet season. Comparing with the National standards from the DENR the major rivers of Tarlac surpasses the minimum standards of classification of water bodies with dissolved oxygen ranging from 2 to 6 mg/l. Low DO is also caused by fertilizer and manure runoff from streets, lawns and farms. The growth of too much algae which could be due to the overuse of fertilizers and the presence of fecal matters causes the speeding up of using the

Table 4 Dissolve oxygen and pH of different major rivers of Tarlac province, Philippines during wet and dry season of 2018

River	Dissolved oxygen (mg/l)		pH	
	Dry season	Wet season	Dry season	Wet season
Benig	5.3	5.4	8.0	8.26
Tarlac	16.0	14.8	8.1	8.29
Bamban	9.2	6.0	8.0	7.96
Concepcion	5.0	5.0	7.0	6.78
Lapaz	8.0	5.0	7.2	7.98
Rio Chico	7.9	4.8	7.3	7.96
Camiling	15.0	14.0	8.0	8.26

oxygen quickly resulting to a lower DO.[6] The dissolved oxygen which drops below 5.0 mg/l causes stress to many aquatic lives. However based from the results, all of the rivers surpass or equal to 5.0 mg/l except for the Rio Chico River during the wet season with 4.8 mg/l.[7] In terms of pH, the major rivers of Tarlac are within the minimum and maximum standard of pH range within the DENR standards. The pH ranges from 6.78 to 8.29 during the wet season and 7.0–8.1 during the dry season.

3.4 Total Dissolved Solids and Electrical Conductivity

Higher total dissolved solids were observed in the different rivers during the dry season which ranges from 300 to 560 mg/l as compared during the wet season which ranges from 169 to 540 mg/l respectively. Too high or too low concentrations of TDS may limit the growth and may lead to the death of many aquatic organisms.[8] The reduction of water clarity, which contributes to a decrease in photosynthesis and lead to an increase in water temperature, could be due to the high concentrations of TDS. The EC during the dry season ranges from 389 to 423 while during the wet season it ranges from 280 to 420 respectively (Table 5).

3.5 Nitrate

The nitrate concentrations of the different rivers in Tarlac shows to be within the range indicated in Table 1. During the dry season, the nitrate concentrations from

[6] http://www.ririvers.org/wsp/CLASS_3/DissolvedOxygen.htm. Last accessed 30 Nov 2017.

[7] http://www.mymobilebay.com/stationdata/whatisDO.htm. Last accessed 30 Nov 2017.

[8] http://www.ei.lehigh.edu/envirosci/watershed/wq/wqbackground/tdsbg.html. Last accessed 15 Dec 2017.

Table 5 Total dissolved solids and electrical conductivity of different major rivers of Tarlac province, Philippines during wet and dry season of 2018

River	Total dissolved solids (mg/l)		Electrical conductivity (μS)	
	Dry season	Wet season	Dry season	Wet season
Benig	323	218	400	323
Tarlac	308	169	420	416
Bamban	300	254	418	375
Concepcion	560	540	423	420
Lapaz	300	220	400	291
Rio Chico	305	250	412	281
Camiling	320	200	389	280

Table 6 Nitrate content of different major rivers of Tarlac province, Philippines during wet and dry season of 2018

River	Nitrate (mg/l)	
	Dry season	Wet season
Benig	14	59
Tarlac	10	48
Bamban	10	17
Concepcion	10	48
Lapaz	14	38
Rio Chico	10	45
Camiling	10	38

the different major rivers had a range of 10–14 mg/l. While during the dry season, it ranges from 17 to 59 mg/l with Benig River as the highest. The higher nutrient concentrations within the area could be due to the wastewater from the swine farm lagoons which may be discharged from the nearby farms within the area. Less than 5 mg/l N has little effect, even on nitrogen sensitive crops, but may stimulate nuisance growth of algae and aquatic plants in streams, lakes, canals and drainage ditches (Table 6).[9]

3.6 *Fecal Coliform and* E. coli

In terms of the microbiological parameters such as fecal coliforms and *E. coli*, the different river waters of Tarlac was higher than the standards particularly in Benig River with 11,000 MPN/100 ml and within the Concepcion river which exceeds the National standards for safe water with fecal coliform count of 140,000. Higher concentrations of *E. coli* were also noted in Benig and Concepcion River both with

[9]http://www.fao.org/docrep/003/T0234E/T0234E06.htm. Last accessed 15 Dec 2017.

Table 7 Fecal coliform and *E. coli* concentration of different major rivers of Tarlac province, Philippines during wet and dry season of 2018

River	Fecal coliform (MPN/100 ml)	*E. coli* (MPN/100 ml)
	Wet season	Wet season
Benig	11,000	1700
Tarlac	390	21
Bamban	270	17
Concepcion	140,000	1700
Lapaz	2600	170
Rio Chico	2800	330
Camiling	330	<1.8

1700 MPN/100 ml. The high concentrations within the said rivers could be due to the wastewater discharged from the nearby areas contributing to the higher Fecal coliform and *E. coli* in the said areas of concern. The higher concentrations as observed in the two rivers could have a potential to reduce the water quality thus reducing also the recreational value (Table 7).[10]

4 Conclusions

The water samples collected from major rivers of Tarlac revealed that there were variations in the results in terms of the different parameters used to quantify the concentrations of the physical, chemical and microbiological quality of the river waters for irrigation purposes. Based from the result, the different river waters were also in accordance with the National Standards set by the Department of Environment and Natural Resources (DENR).

Acknowledgements The authors would like to acknowledge the Department of Agriculture Regional Field Office (DA-RFO 3) for funding this research and sincere acknowledgement was also to the management of the Tarlac Agricultural University.

References

Al-Badaii F, Shuhaimi-Othman M, Gasim MB (2013) Water quality assessment of the Semenyih River, Selangor, Malaysia. J Chem Article ID 871056, 10 p. https://doi.org/10.1155/2013/871056

Fernandez XD, David ME (2008) Water quality assessment of the benig river: implication to environmental management accessed through https://www.bgr.bund.de/EN/Themen/Wasser/Veranstaltungen/symp_sanitat-gwprotect/poster_fernandez_pdf.pdf?__blob=publicationFile&v=2 December 2017

[10]https://pubs.usgs.gov/wri/wri004139/pdf/wrir00-4139.pdf. Last accessed 15 Dec 2017.

Verma R, Dwivedi P (2013) Heavy metal water pollution—a case study. Recent Res Sci Technol 5(5):98–99. ISSN: 2076-5061. Available Online http://recent-science.com/

Open Access **Baghdad Science Journal** **P-ISSN: 2078-8665**
2021, Vol. 18 No.1 (Suppl. March) **E-ISSN: 2411-7986**

DOI: http://dx.doi.org/10.21123/bsj.2021.18.1(Suppl.).0722

Determining the Quality and Quantity of Bioethanol Production using Golden Shower (*Cassica fistula*) Fruit

Amy Lizbeth J. Rico

College of Engineering and Technology, Tarlac Agricultural University, Philippines
E-mail: amylizbethjrico@gmail.com
ORCID ID: https://orcid.org/0000-0001-8206-2827

Received 10/12/2020, Accepted 11/3/2021, Published 30/3/2021

Abstract:

Depletion of fossil fuel is one of the main reasons why the bioethanol has become popular. It is a renewable energy source. In order to meet the great demand of bioethanol, it is best that the bioethanol production is from cheap raw materials. Since the golden shower fruit is not being utilized and is considered as waste material, hence, this study was conducted to make use of the large volume of the residue as feedstock to test its potential for bioethanol extraction.The main goal of this study is to obtain the most volume of bioethanol from the golden shower fruit liquid residue by the factors, days of fermentation (3, 5, and 7 days) and sugar concentration (15, 20 and 25 brix) of the liquid residue. Also, part of the study is to compute the cost of production in extracting bioethanol from the golden shower fruit. Each treatment was replicated three (3) times. The Two-Factorial Analysis of Variance (ANOVA) of the Complete Randomized Design (CRD) was used to analyze the treatments. Treatments means were compared using the Duncan's Multiple Range Test (DMRT).

Keywords: Bioethanol, Extraction, Fermentation, Renewable Energy, Waste.

Introduction:

In recent years, one of the serious problems that our country as well as the world has been facing is the energy crisis. Furthermore, the demand for fossil fuels has increased over the past few decades.

Bioethanol, which is considered one of the renewable energy sources, production became popular because of the rapid decrease of the fossil fuels. Because of this, exhaust gases of bio ethanol are much cleaner combustion that fossil fuels. Bioethanol production can lead to healthy environment because of the lower emission of air pollutants as well as the carbon neutral. Its production may also lead to free sulfur and aromatic. Sugar feedstock from starch is the main ingredient in the production of bioethanol globally. She also stated that out of the total production, 61% is accounted to sugar crops while the remaining 39% is derived from starch as feedstock. In the Philippines, sugarcane is the main component for the production of ethanol while in the United States; corn is considered the main component in their bioethanol production. Nevertheless, considering the increasing demand for human food, using this feedstock for bioethanol production may be a competitor for human and livestock consumption.

In order to meet the great demand of bioethanol, it is best that the bioethanol production is from cheap raw materials. In this case, production of bioethanol should be derived from cheap raw materials such as agricultural wastes, fruit wastes, vegetable wastes, municipal and industrial wastes.

Materials that contain sugar such molasses, sugarcane (cane juice or cane syrup), cereal crops, sugar beet and sweet sorghum and other materials that contain sugar are fermented to produce bioethanol. Increasing focus on using the lignocellulosic biomass became the result of the development in the biotechnology (1). In these studies, the lignocellulosic biomass is used in the production of liquid fuels and other chemical that is used in bioethanol production. Many biomass substrates have high content of cellulose and hemicellulose and have been enumerated to be of great potential for bioethanol production. The pretreatment step of the raw materials is the main challenge in the conversion of ethanol from biomass. In the pretreatment step, the structure of

the ligocellulosic complex needs to be degraded (2). The steps that need to be done in the pretreatment are the removal of lignin, the partial or total hydrolysis of the hemicellulose, the decrease in the fraction of crystalline cellulose, and subsequently, the hydrolysis step. In the hydrolysis step, in order to obtain glucose that is converted into ethanol by microorganisms, the cellulose undergoes enzymatic hydrolysis (3). Eventually, ethanol is the result of the conversion of the sugars that is released during the hydrolysis of hemicellulose (4). Industrially, two processes can be used in the hydrolysis and fermentation of the pretreated materials; the separate hydrolysis and fermentation (SHF). Hydrolysis or fermentation can also be done in one single step as identified to be simultaneous saccharification and fermentation (SSF) (5). There are species that have been reported that has the ability to directly ferment cellulose into ethanol. These species are *Neurospora, Monilia, Paecilomyces and Fusarium sp* (6).

A flowering plant in the family Fabaceae is the golden shower or the Cassia fistula. The golden shower tree is a medium-sized tree which grows to 10-20 meters tall and is fast growing tree. Since the fruit of golden shower is considered as waste material, hence, this study was conducted to make use of the large volume of the residue as feedstock to test its potential for bioethanol extraction.

The objective of the study is to obtain bioethanol from golden shower fruit residues. Specifically, it aims to determine the volume of ethanol produced based on the duration of fermentation and sugar content of the feedstock, and to determine the cost of bioethanol production from golden shower fruit.

Materials and Methods:

The flow diagram in the production of bioethanol from golden shower fruit is shown in Fig. 1. The following is the process used in the production of bioethanol from golden shower fruit.

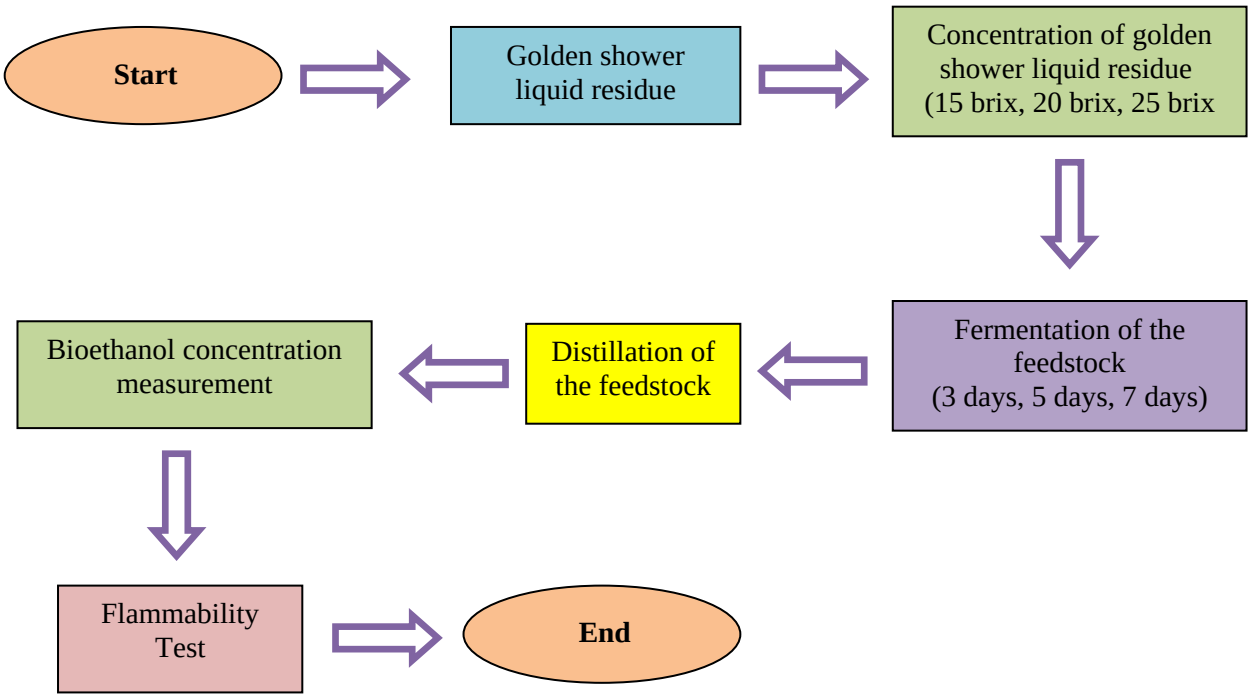


Figure 1. Flow diagram of the production of bioethanol from golden shower fruit.

Collection of Raw Materials

The golden shower fruit was collected from a farm in Camiling, Tarlac. Only the matured fruits are collected. The collected golden shower fruit were chopped using a multi-crop chopper available in the University. Twenty – five kilograms (25 kg) of chopped golden shower fruit with enough amount of water were boiled in a container (Fig. 2) for 6 hours. The boiled golden shower fruit was placed in a screen and poured with water to remove the gelatinous texture from the impurities. The poured water was collected and served as the liquid residue for the study.

Figure 2. Golden shower fruit was boiled with enough amount of water.

Concentration Process

The initial sugar content of the golden shower fruit residue was measured using the refractometer. The refractometer was calibrated first by putting few drops of distilled water on the prism. The screw calibration was turned to adjust it to zero reading.

After calibrating the refractometer, a few drops of a sample from the liquid residue was placed on the prism and the sugar content was obtained and recorded. If the sugar content is below the desired sugar content, the golden shower fruit residue was heated until the desired sugar content of the feedstock was obtained.

The golden shower fruit liquid residue was divided into three parts. Each part was allowed to boil. The first batch of golden shower fruit liquid residue was boiled for 1.5 hours (1 hour and 30 minutes) to obtain the 15 brix. Wood and biomass stove was used as the source of heat. The golden shower fruit liquid residue was stirred continuously and the concentration of sugar was checked every 30 minutes using the refractometer.

The second part underwent through the same process, boiled and stirred continuously, until 20 brix was obtained. Also, sugar content was monitored at 30 minutes interval. After 2.5 hours, sugar content of 20 brix was obtained.

The third part was also boiled and stirred continuously until the sugar content reached 25 brix. Also, the sugar content was obtained every 30 minutes while boiling. After 3 hours, sugar content of 25 brix reading was obtained from the refractometer.

Fermentation

After attaining the desired sugar content in each part, the feedstock was set aside to cool down. For each sugar content level, nine liters of feedstock were obtained. One liter of the feedstock was measured (using the graduated cylinder) was placed in container.

Meanwhile, the optimum weight of yeast per liter of feedstock is 1.5 g/L. Yeast was weighed using the electronic scale. The yeast weighing 1.5 grams was dissolved in warm water and mixed continuously until bubbles appeared. The bubbles in the mixture indicate that the yeast is already active (7). In every container, a mixture of 1.5 grams of yeast was transferred into the liter of feedstock. An airlock using the hose and water seal (Fig. 3) were connected to the container to avoid the entry of air in the feedstock during the fermentation process. Absence of air should be done in the fermentation process to be able to produce bioethanol, otherwise, ethanoic acid will be produced (8).

Figure 3. Airlock of the feedstock during the fermentation process.

The samples were placed in a shaded area. For each sugar content level, three liters were subjected 3 days of fermentation, other 3 liters to 5 days of fermentation, the remaining 3 liters were fermented for 7 days. The fermentation performance was monitored by weighing the fermentation bottles every 6 hours of fermentation. The weight loss is due to the product of fermentation, ethanol and carbon dioxide (9).

Distillation

In the extraction of bioethanol, distillation of the fermented feedstock took place. A reflux distiller was used to distill the feedstock. The sugar concentration of the fermented broth was checked in the refractometer before fermentation. In the fermentation process, the sugar is broken down with the aid of the yeast enzyme zymase. The gas that bubbles into the air is the carbon dioxide while the alcohol in the mixture with the water is the ethanol.

On the third day of fermentation, the feed stocks were placed in a reflux distiller and heated using the electric stove. The hose for the condenser of the distiller was connected to a tap water with a flow rate of 600 ml/min. The water flow rate was measured by filling a container with a tap water

within a minute. The amount of water was measured using a graduated cylinder. Too much flow of water in the condenser made it hard to reach a higher temperature to produce bioethanol. The desired temperature range for bioethanol production is 78 ºC to 98 ºC. A thermostat is connected to the distiller to determine the temperature. After 20 minutes of heating, 78 ºC was obtained and first drop of distillate was obtained. The distillation process was stopped when no distillate was collected. The collected distillates were measured using the graduated cylinder. The obtained volume was recorded. The same procedure was done for the 5 days and 7 days fermentation.

Concentration of Ethanol

The concentration of ethanol of the distillates was measured using the hydrometer. The collected distillates for each treatment were placed in a graduated cylinder. The hydrometer was allowed to float on the distillate as shown in Fig. 4. The concentration of the bioethanol was read and then recorded.

Figure 4. Concentration of ethanol determination using the hydrometer.

Flammability Test

To test the flammability of the obtained distillates, few drops of distillates from each sample were placed on the floor and lit using a lighter.

Discussion:

After the collection of the golden shower liquid residue, the sugar content was determined. The sugar content of the samples will determine the ability of the samples to be converted to bioethanol. The obtained initial sugar content of the liquid residue was 3 brix. Fig. 5 shows the initial brix reading.

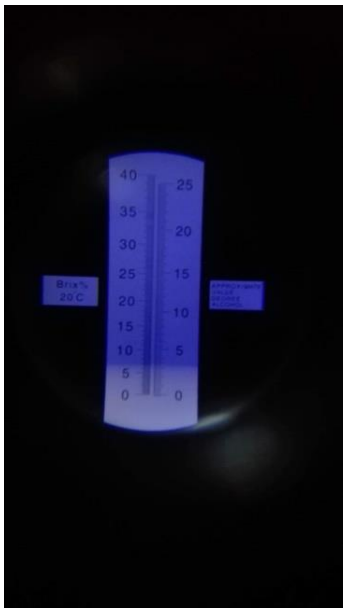


Figure 5. Initial brix reading using the refractometer.

The first batch of liquid residue was concentrated for 1.5 hours. After 60 minutes of boiling and continuous stirring, the sugar concentration of the liquid residue became 10 brix. The golden shower fruit liquid residue became 15 brix after another 90 minutes of continuous stirring. The second batch of liquid residue was concentrated for 2.5 hours. After 1 hour of the same procedure, the sugar content reading became 14 brix. After 2 hours and 30 minutes passed, the concentration of the sugar became 20 brix. The last batch of liquid residue was concentrated for 3 hours with the same procedure. After 150 minutes, the concentration of sugar in the broth became 18 brix. The sugar concentration became 25 brix after 180 minutes.

During fermentation, the weight of the samples was decreasing. The decrease in weight of the samples indicated active fermentation of the yeast. The sugar concentration of the feed stocks was read using the refractometer. The difference of the sugar content reading before and after fermentation shows the alcohol converted during the fermentation process.

Based from the results, the average extracted bioethanol from the samples is 77.96 mL. Treatment T1A3 (25 brix x 7 days) gave the highest extracted bioethanol from the fermented feedstock with an amount of 139.33 mL while treatment T3A1 (15 brix x 3 days) gave the least extracted bioethanol from the fermented feedstock with an amount of 41.33 mL. The results obtained are similar to the study conducted stating that the

ethanol content increased with increasing fermentation time in both 27 ºC and 32 ºC, though, the increase was higher at 32 ºC. The highest ethanol content was obtained on day 5 in temperature at 32 ºC (10).

Table 1 shows the obtained bioethanol using the reflux distiller using the combination of different days of fermentation and different sugar concentration. Analysis of Variance revealed significant differences on the extracted amount of bioethanol from different treatments. T1A3 and T3A1 were significantly different to all treatments. All other treatments were not significantly different from each other.

Table 1. Extracted bioethanol (mL) from the feedstock.

Treatment	R1	R2	R3	Total	Mean	
T1A1 (25 brix, 3 days)	70.0	65.0	72.0	207.0	69.00	d
T1A2 (25 brix, 5 days)	92.0	87.0	85.0	264.0	88.00	bc
T1A3 (25 brix, 7 days)	123	143	152.0	418.0	139.33	a
T2A1 (20 brix, 3 days)	65.0	60.0	69.0	194.0	64.67	ef
T2A2 (20 brix, 5 days)	75.0	72.0	80.0	227.0	75.67	cd
T2A3 (20 brix, 7days)	90.0	110	97.0	297.0	99.00	b
T3A1 (15 brix, 3 days)	40.0	45.0	39.0	124.0	41.33	h
T3A2 (15 brix, 5 days)	50.0	62.0	55.0	167.0	55.67	fg
T3A3 (15 brix, 7 days)	65.0	72.0	70.0	207.0	69.00	de
Grand total				**2105.0**		

Note: Means with the same letter are not significantly different at 1% level by DMRT.

Table 2 shows that the different level of sugar content has significant effect on the bioethanol produced. The treatment using 25 brix has the largest amount of bioethanol produced followed by 20 brix and 15 brix has the least amount of bioethanol produced. Also, the days of fermentation affect significantly on the amount of bioethanol extracted. Treatments under 7 days fermentation produced the largest amount of bioethanol. It is followed by 5 days fermentation and 3 days fermentation.

Table 2. Mean amount of bioethanol produced in mL.

Treatment	A1	A2	A3	Total	Mean	
T1	207.0	264.0	418.0	889.0	296.3	a
T2	194.0	227.0	297.0	718.0	239.3	b
T3	124.0	167.0	207.0	498.0	166.0	c
Total	525.0	658.0	922.0			

Note: Means with the same letter are not significantly different at 1% level by DMRT.

The concentration of the extracted ethanol was measured using a hydrometer. The results of concentration ranges from 95% - 97% due to the efficiency of the reflux distiller used in the study.

The bioethanol produced was lit using the lighter. The flame produced by the samples was blue which indicates complete combustion process. Figure 6 shows the flame produced from the extracted bioethanol.

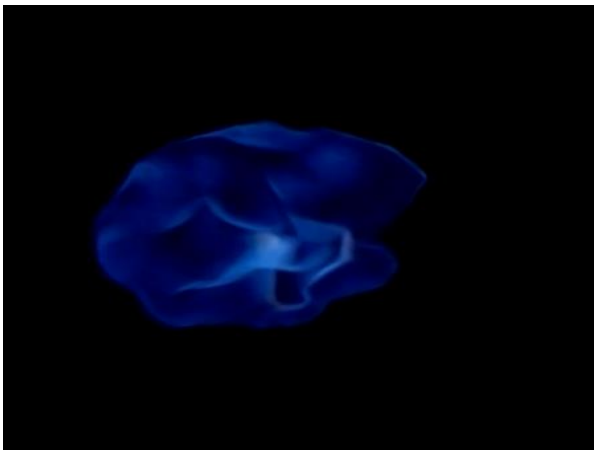


Figure 6. The flame produced by the extracted bioethanol.

The materials used in the extraction of bioethanol from golden shower fruit and the cost of production of bioethanol from golden shower fruit entailed an amount of Php50.89 in every liter of bioethanol obtained.

Acknowledgment:

The cooperation of the staff of the TAU SMART Agriculture Center is appreciated.

Author's declaration:

- Conflicts of Interest: None.
- I hereby confirm that all the Figures and Tables in the manuscript are mine. Besides, the Figures and images, which are not mine, have been given the permission for re-publication attached with the manuscript.

- Ethical Clearance: The project was approved by the local ethical committee in Tarlac Agricultural University.

References:

1. Heux S, Sablayrolles JM, Cachon R, Dequin S. Engineering a Saccharomyces cerevisiae wine yeast that exhibits reduced ethanol production during fermentation under controlled microoxygenation conditions. Appl Environ Microbiol. 2006. Sep;72(9):5822-8
2. Cardona CA, Sanchez OJ. Fuel Ethanol Production: Process Design Trends and Integration Opportunities. Bioresour Technol. 2007 Sep;98(12):2415-57. doi: 10.1016/j.biortech.2007.01.002. Epub 2007 Mar 1. PMID: 17336061.
3. Miyashita M, Akamatsu M, Sakai K, Sakai H. Improving foam stability of ethanol/water mixture with anionic surfactant and long-chain alcohol. Chemistry Letters. 2020 May;49(5):453-456. https://doi.org/10.1246/cl.200058
4. Li H, Wu M, Xu L, Hou J, Guo T, Bao X, et al. Evaluation of industrial Saccharomyces cerevisiae strains as the chassis cell for second-generation bioethanol production. Microb Biotechnol. 2020. Mar;8(2):266-74. doi: 10.1111/1751-7915.12245.
5. Peter NM, Scheffran J, Widholm J. Designing Plants to Meet the Feedstock Needs. Plant Biotechnology for Sustainable Production of Energy and Co-products. Springer Berlin Heidelberg; 2010. p. 57-84. ISBN 978-3-642-13440-1.
6. Goettemoeller J, Adrian G. Sustainable Ethanol: Biofuels, Biorefineries, Cellulosic Biomass, Flex-Fuel Vehicles, and Sustainable Farming for Energy Independence. 2017. p. 42. ISBN 978-0-9786293-0-4.
7. Gavin T, Sinnott R K. Chemical Engineering Design: Principles, Practice and Economics of Plant and Process Design. Butterworth-Heinemann. 2007. ISBN 0-7506-8423-2.
8. Amarasekara AS, Wiredu B. Sulfonic Acid Group Functionalized Ionic Liquid Catalyzed Hydrolysis of Cellulose in Water: Structure Activity Relationships. Sustainable Energy. 2014; 2(3):102-107. doi: 10.12691/rse-2-3-4..
9. Tamunaidu P, Matsui N, Okimori Y, Saka S. Nipa (Nypa fruticans) sap as a potential feedstock for ethanol production. Biomass & Bioenergy, 52, 96-102. 2013
10. Fahrizal F, Abubakar Y, Muzaifa M. The Effects of Temperature and Length of Fermentation on Bioethanol Production from Arenga Plant (Arenga pinnata MERR). 2016. Int J Adv Sci Eng Inf Technol 3(3):244.

تحديد نوعية وكمية انتاج الايثانول الحيوي باستخدام فاكهة الدش الذهبي (ناسور كاسيا)

إيمي ليزبيث ج.ريكو

كلية الهندسة والتكنولوجيا، تارلاك، الجامعة الزراعية، الفلبين

الخلاصة:

يعد استنفاد الوقود الأحفوري أحد الأسباب الرئيسة وراء انتشار الإيثانول الحيوي حيث إنه مصدر للطاقة المتجددة. ومن أجل تلبية الطلب الكبير على الإيثانول الحيوي ، فمن الأفضل أن يكون إنتاجه من مواد خام رخيصة. لكن بالنظر لعدم استخدام فاكهة الدش الذهبية بل اعتبارها مادة نفايات ، فقد أجريت هذه الدراسة للاستفادة من الحجم الكبير من المخلفات كمادة وسيطة لاختبار امكانيتها لاستخراج الإيثانول الحيوي.الهدف الرئيسي من هذه الدراسة هو الحصول على أكبر حجم من الإيثانول الحيوي من بقايا سائلة فاكهة الدش الذهبي حسب العوامل وأيام التخمير (3 و 5 و 7 أيام) وتركيز السكر (15 و 20 و 25 بريكس). ان جزء من الدراسة كذلك هو حساب تكلفة الإنتاج في استخراج الإيثانول الحيوي من هذه الفاكهة.تم تكرار كل علاج ثلاث (3) مرات عن طريق استخدام التحليل ثنائي العوامل للتباين للتصميم العشوائي الكامل لتحليل المعالجات. وقورنت العلاجات باستخدام اختبار دنكان متعدد النطاق.

الكلمات المفتاحية: الايثانول الحيوي، الاستخراج، التخمير، الطاقة المتجددة، النفايات.