SCIENCE AND TECHNOLOGY RESEARCH CENTRE
UNIVERSITAS PGRI SEMARANG - INDONESIA

ISSN: 2715-4211

Journal ASSET

Advance Sustainable Science, Engineering and Technology

Volume 2 Number 2 September 2020

URL LINK: http://journal.upgris.ac.id/index.php/asset

Advance Sustainable Science, Engineering and Technology

e-ISSN: 2715-4211

Volume 2 Number 2 September 2020

Science and Technology Research Centre, Universitas PGRI Semarang, Indonesia

Advance Sustainable Science, Engineering and Technology (ASSET) is a peer-reviewed open-access international scientific journal dedicated to the latest advancements in sciences, applied sciences and engineering, as well as relating sustainable technology. This journal aims to provide a platform for scientists and academicians all over the world to promote, share, and discuss various new issues and developments in different areas of sciences, engineering, and technology.

ASSET papers will be published twice a year by Universitas PGRI Semarang, Central Java, Indonesia.

Advance Sustainable Science, Engineering and Technology
e-ISSN: 2715-4211
Volume 2 Number 2 September 2020
Science and Technology Research Centre, Universitas PGRI Semarang, Indonesia

Editorial Preface

Advance Sustainable Science, Engineering and Technology (ASSET)
Volume 2 Number 2 September 2020

It is our great pleasure to present the Volume 2 Number 2 Advance Sustainable Science, Environmental Engineering and Technology (ASSET). This issue also commemorates one year of ASSET. We very much appreciate our associate editors and our editorial board members, with their cumulative experience, this journal brings a substantial representation of the field of science, engineering and technology. Without the service and dedication of our editorial board, ASSET would have never existed.

This issue includes seven manuscripts. Kresnasari and Darajati's article: *Feeding Habits of Marsela Fish (Parachromis managuensis) In Penjalin Reservoir Brebes, Central Java*. Their study aims to determine the eating habits of Marsela fish, the composition of the type of food and its relationship to the abundance of plankton in the waters. Dwiningsih's aticle: *Development of Single Nucleotide Polymorphism (SNP) Markers in Tropical Crops*. This review describes about how SNP can be discovered in the plant genomes and the application of SNP in plant breeding, especially in tropical crops such as rice, maize, peas, potato, tomato, cassava, taro, etc. Agung's article: *Growth Performance of Daphnia sp. Cultured in Different Concentration of Rice Washing Water*. Their study aimed to investigate the effect of the administration of rice washing water in culture medium on the growth performance of Daphnia sp. Setyawati and Herlambang's article: *Mapping Exclusive Breastfeeding Coverage and Toddler Stunting Prevalence in Indonesia Based On Web Geographic Information System*. The purpose of this research is to map the spread of stunting toddlers and exclusive breast milk coverage in Indonesia. Lunggani's article: *Characterization of Yellow Pigmented Bacteria Associated with Gracilaria sp*. The research aims to isolated bacteria from Gracilaria sp., screened their symbiont bacteria that could potentially produce pigments. Hakim's article: *The Potential of Mechanic Vibration for Generating Electric Energy*. The purpose for their study is to convert mechanical vibration into electrical Power. The final article, Nurdyansyah and Widyastuti's article: *Comparison of Antioxidant Activity of Ethanolic, Methanolic, n-Hexan, and Aqueous Extract of Parkia speciosa Peel based on Half -Maximal Inhibitory Concentration Through Free Radical Inhibition*. The objectives of their study was to determine the half maximum inhibitory concentration (IC50) from four types of Parkia speciosa peel extracts (ethanol, methanol, nhexane, and aqueous) through DPPH free radical inhibition.

We thank all of the 26 authors affiliated from University of Arkansas, USA; Universitas Sultan Ageng Tirtayasa, Indonesia; Universitas Dian Nuswantoro, Indoensia; Universitas Jenderal Soedirman, Indonesia; Universitas Diponegoro, Indonesia; and Universitas PGRI Semarang, Indonesia who have contributed to this issue.

Last but not least, I very much appreciate your support as we strive to make ASSET the most authoritative journal on the latest advancements in sciences, applied sciences and engineering, as well

as relating sustainable technology. We hope that this issue can provide valuable information for better understanding.

September 2020
Asst. Prof. Mega Novita
Asst. Prof. Rizky Muliani Dwi Ujianti

Advance Sustainable Science, Engineering and Technology
e-ISSN: 2715-4211
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Feeding Habits of Marsela Fish (*Parachromis managuensis*) In Penjalin Reservoir Brebes, Central Java

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Abstract. The presence of introduced fish populations in the Penjalin Reservoir is increasing, one of the dominant ones being caught is Marsela (*Parachromis managuensis*). It is feared that this fish population will continue to increase and will have a negative impact on other organisms, especially native fish communities. This study aims to determine the eating habits of Marsela fish, the composition of the type of food and its relationship to the abundance of plankton in the waters. This research was conducted in September-October 2018. The method used was CPUE with a sampling technique that is Simple Random Sampling. Sampling of fish in one day is done 6 times, namely at 05:00, 11:00, 17:30, 21:00, 00:00, and 03:00. Fish samples obtained are then grouped according to length and then an analysis of the contents of the stomach. The results showed that in the Penjalin Reservoir the percentage of phytoplankton presence was 96% with the highest abundance found in the Bacillariophyta group. Marsela is an omnivorous fish with popular foods are Chlorophyta and Charophyta. However, in adulthood Marsela fish also eat animals.

Keywords: Marsela Fish (*Parachromis managuensis*), Feeding Habit, Penjalin Reservoir

1. Introduction

The fish community in Penjalin Reservoir is currently dominated by predatory fish, especially by Manila Fish or Marsela Fish (*Parachromis managuensis*). In 2013, there were 6 species of fish caught in the

Penjalin Reservoir, namely Betutu Fish (*Oxyeleotris marmorata*), Nila (*Oreochromis niloticus*), Nilem (*Osteochilus vittatus*), Tawes (*Barbonymus gonionotus*), and Beunteur (*Puntius binotatus*), each of which is 45 fish, 19 fish, 1 fish, 1 fish, 5 fish, 5 fish and dominated by 129 Marsela fish (*P. managuensis*) (Hedianto *et al*., 2013). Then in 2016 in the same place found as many as 217 Marsela Fish (*P. managuensis*) (Hamiyati, 2016). This fish is the result of unintentional introductions and has high tolerance characteristics in a waters.

In general, the shape of the Marsela (*P. managuensis*) body is elongated and slender, the mouth is oblique, the back edge has a line extending down to the bottom of the anterior edge of the eye, has a reddish-red (purple-red) and black spot on the eye body and fins, there is a black line right along the body, has a green color on the back (dorsal) and yellow on the abdomen (ventral), and the lining on the edges of his eyes are red (Figure 1).

The classification of Marsella Fish (*P. managuensis*) according to Gunther (1867) in Agasen *et al,* (2006) namely:

Phylum: Chordata

Subfilum: Vertebrates

Class: Actinopterygii

Order: Perciformes

Family: Cichlidae

Genus: Parachomis (Agassiz, 1859)

Species: Parachomis managuensis (Gunther, 1867)

Figure 1. Marsela Fish (*P. managuensis*)

According to Conkel (1993), Marsela Fish (*P. managuensis*) can develop both in warm and murky waters with bottom waters in the form of mud or litter and high levels of eutrophication. If the trophic status of the waters of the Penjalin Reservoir changes to eutrophic, it is feared that the population of Marsela Fish (*P. managuensis*) will become very dominant (invasive alien species). If this happens, then ecologically, it has a negative impact on other organisms, especially native fish communities (Umar and Sulaiman, 2013). While from an economic standpoint, this incident will reduce the income of fishermen. Marsela fish are classified as economically low fish for the surrounding community, even though the abundance is high in nature.

Spreading fish or restocking is an effort to improve the ecological balance in the Penjalin Reservoir, especially from native fish species. However, it should be noted that the high number of Marsela fish (*P. managuensis*) as predators is feared that the types of fish that will be stocked actually become prey. The best first step towards restoring ecology in the Penjalin Reservoir is to control the Marsela Fish (*P. managuensis*) population. According to Hedianto *et al*., (2013), one of the causes of the low population of Tawes as native fish in the Penjalin Reservoir is the presence of high predation pressure and territorial competition by Marsela Fish (*P. managuensis*).

Food is one of the important factors in an organism in determining the extent of spread of a species and controlling the size of a population (Astuti *et al*., 2013). To control an introduced fish population some information is needed, for example the availability of natural food resources. The lack of information about Marsela Fish (*P. managuensis*), prompts the author to conduct a study of the eating habits of Marsela Fish (*P. managuensis*) in Penjalin Reservoir.

2. Methods

The method used is CPUE with the technique in sampling which is Simple Random Sampling. Sampling of fish is done in 1 day as many as 6 times namely at 05:00, 11:00, 17:30, 21:00, 00:00, and 3:00 in September - October 2018.

The fish caught were measured by total length using calipers, while the weights of fish were weighed using digital scales. In observing fish food habits is done by observing the contents of the stomach of the fish which is split and removed its contents then separated according to the type of food. Furthermore, each water sample and fish's stomach contents were identified and then the number of plankton per milliliter was calculated using the formula from Lackey Drop Microtansec Counting (APHA, 1989 in Nurruhwati *et al*., 2017), namely:

$$N = \frac{Q1}{Q2} x \frac{V1}{V2} x \frac{1}{p} x \frac{1}{w}$$

Information:

N: average number of plankers in the preparation

Q1: Extensive glass cover

Q2: wide field of view

V1: volume of water in a container

V2: volume of water under the glass cover

P: number of fields observed

W: marsela fish hull volume

Calculation of natural feed for gastric contents uses a selectivity index (E) to compare natural food between gastric contents and food in waters. This index calculation uses the method developed by Krebs (1989) in Kurnia *et al*., (2017) with the formula:

$$E = \frac{ri - pi}{ri + pi}$$

Information:

ri: the relative number of different types of organisms that are eaten

pi: the relative number of kinds of organisms in waters

The selectivity index (E) is used to compare the natural food of Marsella Fish (*P. managuensis*) in each group of lengths between the contents of the fish's hull and the feed in the waters, where:

+1: fish tend to have the habit of eating these types.

0: fish tend not to choose natural food in the environment.

-1: fish tend not to have the habit of eating these types.

3. Results and Discussion

1. Distribution of Fish Frequency Length

Marsela fish (*P. managuensis*) caught from Penjalin Reservoir totaled 47 tails. This number was captured from all observation points. Fish that have been measured length and weight are then grouped manually into three long groups. The long range of Marsella Fish (*P. managuensis*) is presented in Table 1.

Table 1. Long-Range Groups of Marsella Fish (*P. managuensis*) in The Penjalin Reservoir

Group	Length range (cm)	Amount	Kategori
1	9,7 – 11	14	Small
2	11,1 – 12,9	17	Medium
3	13 – 20	16	Big

Next Marsela Fish (*P. managuensis*) were dissected for sampling the eating habits in the digestive tract and analyzing the selectivity index. The results of the analysis were compared and explained descriptively how different each group of Marsela Fish (*P. managuensis*) lengths was.

2. Plankton Abundance in the Weirs Reservoir Waters

Natural feed in Penjalin Reservoirs is found in phytoplankton more than zooplankton and others. The presence of phytoplankton in a waters can indicate its fertility. Changes to a water quality can be viewed from the abundance of phytoplankton and can provide information about the condition of these waters (Hidayah, *et al*., 2014). The number of natural feed genera found in the waters of the Penjalin Reservoir is 32 plankton genera. The abundance of natural feed waters of the Penjalin Reservoir is presented in Figure 2

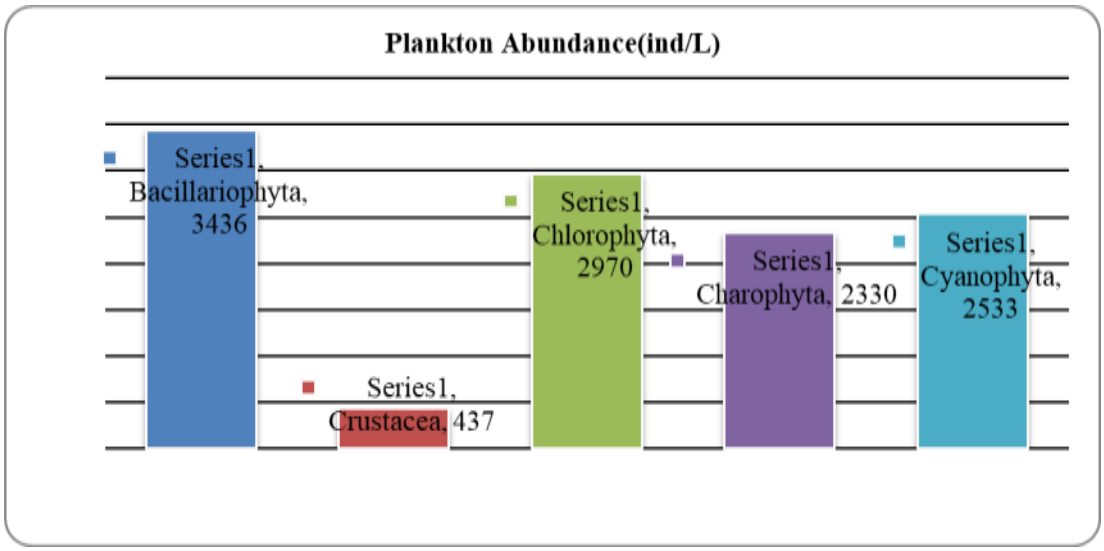

Figure 2. Planktonic Abundance (ind / L) of Natural Feed In The Penjalin Reservoir

The abundance of planktonic natural feed in the waters of the Penjalin Reservoir was 11706 ind / L, with a percentage of the presence of phytoplankton of 96%. In the same place, a 2014 study by Wijaya (2015) showed an abundance of phytoplankton ranging from 2,380-3,355 ind / L. Based on this abundance, the Penjalin Reservoir is included in the category of Mesotrophic waters which is a category with a moderate fertility rate. According to Landner (1978) in Hardiyanto *et al*., (2012), water fertility based on plankton abundance is divided into three, namely oligotrophic with plankton abundance level 0-2000 ind / L, mesotrophic with plankton abundance ranging from 2000-15000 ind / L and eutrophic with an abundance level of plankton more than 15000 ind / L. Water fertility in the reservoir is thought to originate from household waste, livestock and agricultural waste in the form of residual fertilizers that contain a lot of N and P elements. resulting in eutrophication in water (Simanjuntak, 2009). According to Suryanto (2011) excessive content of N and P elements can stimulate plankton growth quickly and abundantly, so that it can affect plankton abundance in water.

Based on the results of the study note that the highest abundance in Bacillariophyta. This is thought to be related to the temperature condition of the waters in the cold Penjalin Reservoir, so that the Bacillariophycea class is fertile. According to Hadiyanto *et al*., (2012), Bacillariophyceae is an algal group that is most easily found in various types of aquatic habitats, especially in relatively cold waters, because of this ability the Bacillariophyceae class can be used as a biological indicator of clean waters.

The second highest abundance is occupied by the Chlorophyta division. Cyanophyta's division ranks third. The number of species of Cyanophyta obtained in the Weir Reservoir is still relatively small compared to Bacillariophyceae and Chlorophyta. This indicates that the waters of the Penjalin Reservoir are still classified as good. According to Arum *et al*., (2017), if a waters are dominated by the Cyanophyta division, the waters are polluted waters.

3. Natural Feed Abundance in the Stomach of Marsela Fish

The number of plankton natural food genera found in the stomach of Marsela Fish (*P. managuensis*) is 24 genera. The diversity of genera found in the stomach of Marsela fish is presented in Table 2. Based on Table 2 it can be seen the diversity of planktonic natural food in the stomach of Marsela Fish (*P. managuensis*), age group 1 has 11 different genera which are entirely from the plant group. Age group 2 has 10 different genera, all of which are from the plant group. Whereas the age group 3 has 19 variants of genera consisting of 17 varieties of plant groups and 2 groups of animals.

Table 2. Number of Plankton And Other Natural Food Genera In The Stomach of Marsela Fish (*P. managuensis*) In Each Age Group

No	Divisi/Filum	Age Group 1	Age Group 2	Age Group 3
A	Plant group			
1	Chlorophyta	1	3	5
2	Charophyta	4	3	5
3	Bacillariophyta	3	3	4
4	Cyanophyta	3	1	3
	Jumlah	11	10	17
B	Animal Group			
1	Miozoa	0	0	1
2	Crustacea	0	0	1
	Jumlah	0	0	2
	Total	11	10	19

The abundance of natural food in the stomach of Marsela Fish (*P. managuensis*) based on Table 3, age group 1 has an abundance of 1251 ind / ml which consists entirely of plant groups. In the age group 2 has an abundance of 1281 ind / ml which consists entirely of groups of plants. Whereas the age group 3 had an abundance of 2064 ind / ml consisting of plant groups of 1948 ind / ml and animal groups 116 ind / ml.

The abundance of plankton natural food in the stomach of Marsela Fish (*P. managuensis*) based on Table 3 age group 1 is dominated by the divisions of Cyanophyta and Charophyta. In the age group 2 is dominated by the divisio Chlorophyta and Charophyta. Whereas the age group 3 is dominated by the divisions of Chlorophyta and Bacillariophyta. In the analysis of the gastric contents of the Marsela Fish (*P. managuensis*) age group 3 it was found to have eaten animal species, namely from the phyla group of miozoa and crustaceans.

Table 3. Abundance (ind / L) And Relative Abundance (%) of Natural Food In The Stomach of Marsela Fish (*P. managuensis*) In Each Age Group

No	Divisi/Filum	Age Group 1		Age Group 2		Age Group 3	
		A	RA (%)	A	RA (%)	A	RA (%)
A	Plant Goup						
1	Chlorophyta	29	2.32	466	36.38	640	31.01
2	Charophyta	465	37.17	408	31.85	435	21.08
3	Bacillariophyta	145	11.59	145	11.32	495	23.98
4	Cyanophyta	612	48.92	262	20.45	378	18.31
	Jumlah	1251	100	1281	100	1948	94.38
B	Animal Goup						
1	Miozoa	0	0	0	0	58	2.81
2	Crustacea	0	0	0	0	58	2.81
	Jumlah	0	0	0	0	116	5.62
	Total	1251	100	1281	100	2064	100

Note: A = Abundance, RA = Relative Abundance

According to Agasen *et al* (2006), states that Marsela Fish (*P. managuensis*) are known as predators that eat small fish and are very aggressive. For example, when Marsela Fish (*P. managuensis*) are introduced into Mexican waters it causes havoc among native fish populations and is considered a potential pest. However, based on data obtained in Table 3, it shows that Marsella Fish (*P. managuensis*) tend to be included in predators of phytoplankton. However, in Marsela Fish (*P. managuensis*) age group 3 there is natural food from animal species, thus Marsela Fish (*P. managuensis*) are included in the omnivorous group. The composition of fish eating changes with increasing size and age. Small fish tend to eat phytoplankton that are adjusted to the mouth opening. After growing bigger, the type of food consumed will change. According Effendie (2002), said that the larger the size of the fish, the more varied the types of food so that the area of the niche will be even greater.

4. Choice Index and Largest Portion in the Gastric of Marsela Fish (*P. managuensis*)

The selectivity index calculation (E) was analyzed by comparing the percentage plankton abundance found in the hull of Marsela Fish (*P. managuensis*) with the percentage plankton abundance in the waters of the Penjalin Reservoir. Based on the calculation of this selectivity index, it will be known that the food found in the hull of the fish is selected and favored or not. The results of the selectivity index and the percentage of planktonic natural feed in the age group 1 are presented in Table 4. Marsela Fish (*P. managuensis*) in the age group 1 made a positive or fond selection for Charophyta and Cyanophyta divisions. Marsela fish age group 1 tend to make negative or dislike selection of the divisions / phyla of Chlorophyta, Bacillariophyta, Miozoa and Crustacea.

The results of selectivity index and percentage of planktonic natural food of Marsela Fish (*P. managuensis*) age group 2 are presented in Table 5. Based on the table shows that Marsela Fish (*P. managuensis*) made a positive or fond selection of the Chlorophyta and Charophyta divisions. Marsela Fish (*P. managuensis*) in the age group 2 tend to make negative or dislike selection of the divisions of the phyla Bacillariophyta, Cyanophyta, Miozoa and Crustacea.

Table 4. Results of The Marsela Fish (*P. managuensis*) Choice Index Age Group 1

No	Types of Feed	r	Ri	P	Pi	E
A	Plant Group					
1	Chlorophyta	29	2.32	2970	25.37	-0.83
2	Charophyta	465	37.17	2330	19.9	0.3
3	Bacillariophyta	145	11.59	3436	29.35	-0.43
4	Cyanophyta	612	48.92	2533	21.64	0.39
B	Animal Group					
1	Miozoa	0	0	0	0	0
2	Crustacea	0	0	437	3.73	-1

r = number of types of feed contained in the stomach, p = number of types of feed contained in waters, ri = percent of a type of feed contained in the stomach, pi = percent of a type of feed contained in waters, E = choice index

Table 5. Results of The Marsela Fish (*P. managuensis*) Choice Index Age Group 2

No	Types of Feed	r	Ri	P	Pi	E
A	Plant Group					
1	Chlorophyta	466	36.38	2970	25.37	0.18
2	Charophyta	408	31.85	2330	19.9	0.23
3	Bacillariophyta	145	11.32	3436	29.35	-0.44

4	Cyanophyta	262	20.45	2533	21.64	-0.03
B	Animal Group					
1	Miozoa	0	0	0	0	0
2	Crustacea	0	0	437	3.73	-1

r = number of types of feed contained in the stomach, p = number of types of feed contained in waters, ri = percent of a type of feed contained in the stomach, pi = percent of a type of feed contained in waters, E = choice index

Results of the selectivity index and percentage of planktonic natural food of Marsela Fish (*P. managuensis*) age group 3 are presented in Table 6. Marsela Fish (*P. managuensis*) age group 3 makes a positive or fond selection of the Chlorophyta, Charophyta, and Miozoa divisions. Marsela Fish (*P. managuensis*) age group 3 tends to make negative or dislike selection of Bacillariophyta, Cyanophyta, and Crustacean divisions.

Based on the results of the selectivity index, Marsela Fish (*P. managuensis*) made a positive or fond selection of plankton from the Charophyta division. This is because the abundance of plankton in the waters of the Penjalin Reservoir is quite high. Although the presence of Bacillariophyta is abundant in waters, it is not certain that food is an important part of the composition of the fish diet. According to Effendie (2002), the types of food eaten by a fish species usually depend on their preference for certain types of food, the size and age of the fish, the season, and their habitat. In addition Syahputra *et al*., (2016), stated that the preference of fish in one type of food is influenced by the availability of food in the waters.

Table 6. Results of The Marsela Fish (*P. managuensis*) Choice Index Age Group 3

No	Types of Feed	r	ri	P	Pi	E
A	Plant Group					
1	Chlorophyta	640	31.01	2970	25.37	0.10
2	Charophyta	435	21.08	2330	19.9	0.03
3	Bacillariophyta	495	23.98	3436	29.35	-0.10
4	Cyanophyta	378	18.31	2533	21.64	-0.08
B	Animal Goup					
1	Miozoa	58	2.81	0	0	1
2	Crustacea	58	2.81	437	3.73	-0.14

r = number of types of feed contained in the stomach, p = number of types of feed contained in waters, ri = percent of a type of feed contained in the stomach, pi = percent of a type of feed contained in waters, E = choice index

4. CONCLUSION

Based on the results of research in Reservoir Reservoir, it can be concluded that:

1. The highest percentage of natural food found in phytoplankton is 96% with the highest abundance found in the Bacillariophyta group
2. Marsela Fish (*P. managuensis*) is an omnivorous fish with popular foods are Chlorophyta and Charophyta. However, in adulthood Marsela Fish (*P. managuensis*) also eat animals.

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Development of Single Nucleotide Polymorphism (SNP) Markers in Tropical Crops

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Abstract. Understanding genetic diversity, association studies, evolution analysis, quantitative trait loci, marker-assisted selection and genome-wide association in tropical crops are important for improving plant characteristics in order to increase food sustainability in tropical countries. Single nucleotide polymorphism (SNP) marker is becoming the most popular molecular marker for those studies. By using SNP marker, genes associated with important traits can be identified efficiently compared to the other molecular markers. This review describes about how SNP can be discovered in the plant genomes and the application of SNP in plant breeding, especially in tropical crops such as rice, maize, peas, potato, tomato, cassava, taro, etc.

Keywords: food sustainability, plant breeding, SNP marker, tropical crops

(Received 15 August 2020, Accepted 10 September 2020, Available Online by 30 September 2020)

1. Introduction

Gene position or genomic regions that regulate important traits in plants are discovered by using molecular markers. These markers are Single Nucleotide Polymorphisms (SNPs), Simple Sequence Repeats (SSRs), Amplified Fragment Length Polymorphisms (AFLPs), Restriction Fragment Length Polymorphisms (RFLPs), and Random Amplified Polymorphic DNAs (RAPDs). SNPs are widely used DNA markers to identify genomic regions for important traits that effectively to speed up the plant breeding. These SNPs characterized as the most abundant variations in the plant genome that are very useful in the high-resolution genotyping and produce the highest map resolution [1]. Additionally, using SNPs are more efficient and cost effective [2]. SNPs become the most popular DNA markers in the 21st century due to the development of genotyping by sequencing (GBS) technique [3]. Furthermore, important traits for plants including agronomic traits (grain yield, seed size, maturity day, etc.), morphological traits (plant height, seed color, root length, etc.), physiological traits (photosynthetic activity, stomatal conducting, etc.), abiotic (drought, heat, flooding, salt resistance, etc.) and biotic resistance (bacterial blight, mosaic virus, root-knot resistance).

Genomic information of plants obtained from genetic diversity, association studies, evolution analysis, quantitative trait loci (QTL), marker-assisted selection (MAS) and genome-wide association studies (GWAS) are very useful for increasing food sustainability. To achieve food sustainability in this

changing climate condition, plants with superior characteristics must be developed by using genetic information. Since 1900s, plant breeding has made a positive impact in increasing food production by developing superior cultivars [4, 5]. Furthermore, these superior cultivars have been developed through application effective molecular breeding by using MAS for desirable genes. These superior cultivars including increasing yield, adaptation in unstable environment, resistance to biotic and abiotic stresses, and quality improvement.

1.1. SNP discovery

SNP markers discovered by two methods, including SNP discovery from PCR and SNP discovery from High Throughput-Next generation sequencing (NGS) – RNA-Seq, RAD-Seq, GBS (Genome by Sequencing), WGS (Whole-Genome Sequencing), WGR (Whole-Genome Regression), etc. These approaches reduce complexity to lower the cost and simplify the discovery of SNP markers. In the past few years, NGS technologies have led to the discovery of thousands, even millions of SNPs. Moreover, there are many tools for SNP discovery such as BioEdit, DNASTAR Lasergene Genomics Suite, SAMtools, SOAPsnp, Stacks, Ddocent, Py RAD and GATK. SNPs are usually biallelic and thus easily assayed. In theory, a SNP is identified when a nucleotide from an accession read differs from the reference genome at the same nucleotide position. In the absence of a reference genome, this is achieved by comparing reads from different genotypes using *de novo* assembly strategies. Read assembly files generated by mapping programs are used to perform SNP calling. In practice, various empirical and statistical criteria are used to call SNPs, such as a minimum and maximum number of reads considering the read depth, the quality score and the consensus base ratio for examples. Moreover, SNP discovery is more robust when multiple and divergent genotypes are used simultaneously, creating the necessary basis to capture the genetic variability of a species.

Three kinds of SNP Genotyping platform: single SNP genotyping (using PCR with Taqman – Life Technologies or KASP genotyping – LGC Genomics), multiple SNP genotyping (with SNP chip – Illumina and Multiplex - Sequenom), and SNP genotyping by next generation sequencing (Genotyping by Sequencing/GBS and Restriction site associated DNA sequencing/RADSeq). In single SNP genotyping with Taqman – Life Technologies use forward primer, reverse primer and allele specific probes (VIC® labeled and 6-FAMTM labeled). Furthermore, in single SNP genotyping with Kompetitive Allele Specific PCR (KASP) genotyping – LGC Genomics are based in competitive allele-specific PCR and enable bi-allelic scoring of SNPs and insertions and deletions (Indels) at specific loci. The process in KASP genotyping is the SNP-specific KASP Assay mix and the universal KASP Master mix are added to DNA samples, a thermal cycling reaction in then performed, followed by an end-point fluorescent read. Moreover, in SNP genotyping by next generation sequencing with Genotyping by Sequencing/GBS, DNA sample info entered to webform, if the project approved, DNA samples shipped then GBS libraries made, followed by DNA sequencing data then compare with reference genome and getting data file SNPs. SNP identification in crops with GBS is more effective due to low cost and having simple methodology [6]. In SNP genotyping by next generation sequencing with Restriction site associated DNA sequencing/RADSeq can identify and score thousands of SNPs, randomly distributed across the target genome, from a group of individuals using Illumina technology (the use of restriction enzymes to cut DNA into fragments) and molecular identifiers (MID) to associate sequence reads to particular individuals. RADSeq can be used to detect restriction site presence-absence polymorphisms (by identifying a SNP that is present in one set of individuals but absent in another, indicating a variation in the restriction site).

Recent emergence of NGS technologies such as 454 Life Sciences (Roche Applied Science, Indianapolis, IN), HiSeq (Illumina, San Diego, CA), SOLiD and Ion Torrent (Life Technologies Corporation, Carlsbad, CA) has eliminated the problems associated with low throughput and high cost of SNP discovery. In addition, transcriptome resequencing using NGS technologies allows rapid and inexpensive SNP discovery within genes and avoids highly repetitive regions of a genome.

Unlike the above-mentioned techniques, recently developed genome complexity reduction technologies such as Complexity Reduction of Polymorphic Sequences (CRoPS) (Keygene N.V., Wageningen, The Netherlands) and Restriction Site Associated DNA (RAD) (Floragenics, Eugene, OR,

USA) are computationally well equipped and capable of filtering out duplicated SNPs. These systems were successfully applied to discover SNPs in crops with and without reference genome sequences. Although GBS has the potential to discover several million SNPs, one of the major drawbacks of this technique is large numbers of missing data. To solve this problem, computational biologists developed data imputation models such as BEAGLE v3.0.2 and IMPUTE v2, to bring imputed data as close as possible to the real data.

Table 1. Summary of commercially available SNP genotyping platforms [7]

Platform	Provider	Assay type	Technology	Throughput	Multiplexing
Taqman	Applied Biosystem	PCR	Taqman probe	~1536/day	~256
SNPlex	Applied Biosystem	PCR	Capillary electrophoresis	~1536/3 days	~48
BioMark HD	Fluidigm	PCR	Microfluid-based chips	~96/3 h	~96
KASPar	LGC	PCR	FRET quenching oligos	~96/day	Not vailable
Axiom Biobank	Affymetrix	Hybridization	Oligo nucleotide array	~96/5 days	~650K
Infinium II	Illumina	Hybridization	Bead array	~128/5 days	~700K
GoldenGate	Illumina	Primer extension	Bead array	~172/3 days	~1536
iPlex	Sequenome	Primer extension	Mass spectrometry	~3840/2.5 days	~40

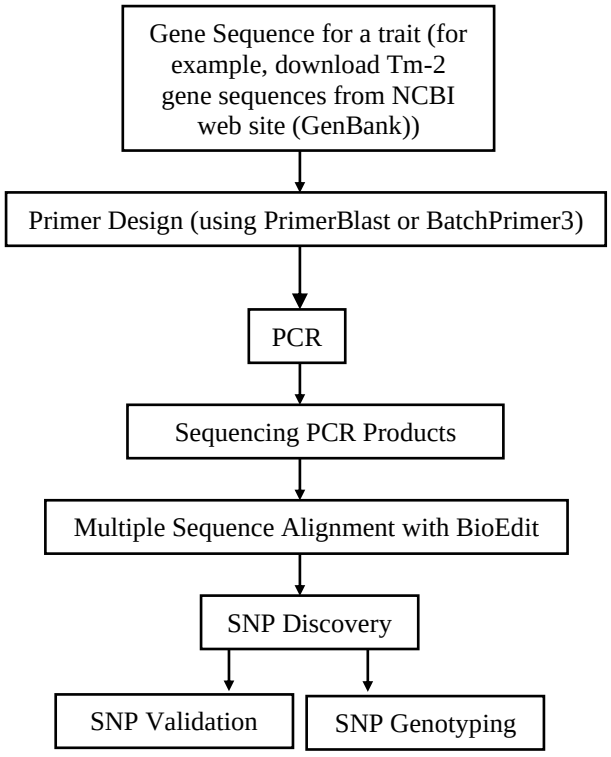

Figure 1. The procedure of SNP Discovery by PCR

Table 2. Representative GBS protocols published in peer-reviewed journals

Method	Restriction Enzyme	Insert size	Barcodes	Sequencing platform	Sequencing mode	Reference
RAD-seq (Restriction association DNA sequencing)	Sbf1 or EcoR1	Size-selection	~96	Illumina	Paired-end	[8]
MSG (Multiplex shotgun genotyping)	MseI	Size-selection	~384	Illumina	Single-end	[9]
GBS (Genotype by sequencing)	ApeKI	<350bp	~384	Illumina	Paired-end	[10]
Double-digested RAD-seq	EcoR1 and Msp1	Size-selection	~48	Illumina	Paired-end	[11]
Double-digested GBS	Pst1 and Msp1	<350bp	~384	Illumina	Paired-end	[12]
Ion Torrent GBS	Pst1 and Msp1	<350bp	~384	Ion Torrent	Paired-end	[13]
SBG (Sequence-based genotyping)	EcoR1 and Mse1 Pst1 and Mse1	Size-selection	~32	Illumina	Paired-end	[14]
REST-seq (Restriction fragment sequencing)	Taq1 and Tru1	Size-selection	~305	Ion Torrent	Paired-end	[15]
Restriction enzyme sequence comparative analysis	Mse1 or NlaIII	Size-selection	~96	Illumina	Paired-end	[16]

1.2. SNP Validation.

The discovered SNPs must be validated to identify the true SNPs and get an idea of the percentage of potentially false SNPs resulting from an SNP discovery exercise. Validation can serve as an iterative and informative process to modify and optimize the SNP filtering criteria to improve SNP calling. For example, a subset of 144 SNPs from a total of 2,113,120 SNPs were validated using the Ilumina GoldenGate assay on 160 accessions in apple.

1.3. SNP Genotyping.

SNP genotyping is the downstream application of SNP discovery to identify genetic variations. SNP applications including phylogenic analysis, marker-assisted selection, genetic mapping of quantitative trait loci (QTL), bulked segregant analysis, genome selection, and genome-wide association studies (GWAS).

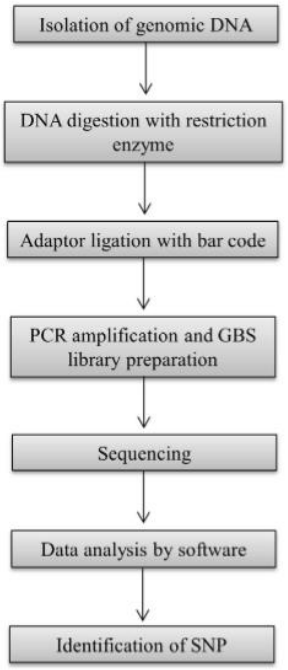

Figure 2. Overview of SNP discovery in plants through genotyping by sequencing (GBS) system [6]

In addition, analytical methods to discover novel SNPs and detect known SNPs include: capillary electrophoresis; mass spectrometry; single-strand conformation polymorphism (SSCP); single-base extension; electrochemical analysis; denaturating HPLC (High-performance liquid chromatography) and gel electrophoresis; restriction fragment length polymorphism; and hybridization analysis.

1.4. SNP discovery in tropical crops

In potato (*Solanum tuberosum*), 575,340 SNPs were identified within three cultivars: 'Atlantic', 'Premier Russet' and 'Snowden' using Illumina sequencing. DNA was extracted from 248 potato lines using the Qiagen Qiaxtractor DX system (Qiagen Inc., Valencia, CA). Samples were loaded at 50 ng/µl on an Illumina BeadXpress Analyzer (Illumina inc., San Diego, CA) and data were analyzed using the Illumina GenomeStudio software. Cluster positions for three marker classes (AA, AB, and BB) were manually determined for each marker within the Illumina GenomeStudio software. The SNPs identified in this study will enable more efficient marker-assisted breeding efforts in potato.

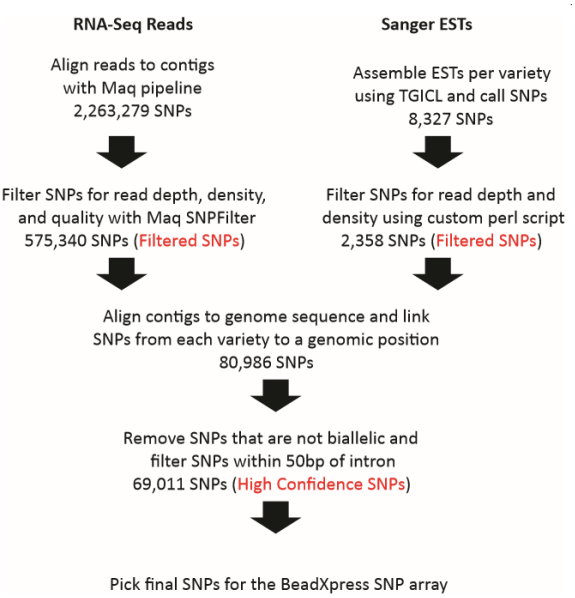

Figure 3. The workflow of SNP discovery in potato transcriptomes and design of the BeadXpress SNP array [17]

The discovery of ~10,000 SNPs and nearly 1,000 indels in eggplant (Solanum melongena L.), equivalent to a SNP frequency of 0.8 per Kb and an indel frequency of 0.07 per Kb using RAD tag sequencing and the Illumina GoldenGate assay. This is the workflow for eggplant RAD tags sequencing and gene annotation based on Barchi et al. (2011) [18] (Figure 4).

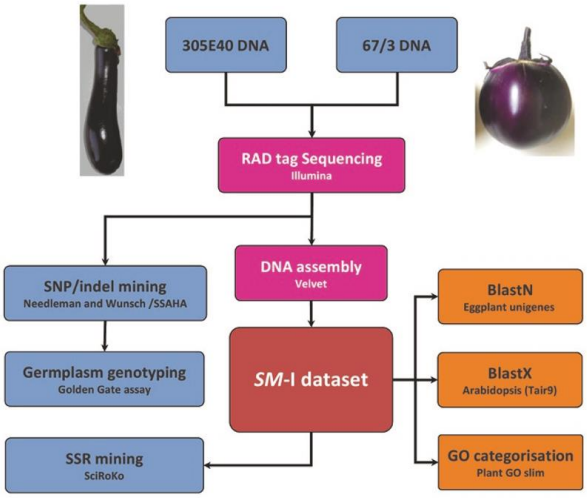

Figure 4. The workflow for eggplant RAD tags sequencing and gene annotation based on Barchi et al. (2011) [18]

A set of 100,000 to 250,000 SNPs were discovered in sorghum (Sorghum bicolor L.) by High Throughput-Next generation sequencing (NGS) – RAD-Seq [19]. In common bean (Phaseolus vulgaris L.), 827 non-genic SNPs were discovered using the GoldenGate technology of Illumina [20]. Moreover, in chickpea (Cicer arietinum L.), 1022 SNPs were identified based on Illumina GoldenGate Genotyping Technology [21]. About 533 SNPs were discovered in Camelina sativa by Illumina GoldenGate [22]. In quinoa (Chenopodium quinoa Willd.), 14,178 SNPs were identified based on KASPar genotyping chemistry and were detected using the Fluidigm dynamic array platform [23]. In rice (Oryza sativa), more than four million SNPs from around 500 rice landraces were identified by Yu et al. (2014) [24] using GBS. In Nipponbare genome 0.64 SNP was found per one kb, while in Dongjin genome contains

0.45 SNP/kb. Moreover, Huang et al. (2009) [25] detected 122,791 SNPs in indica cv."9311" and japonica cv. "Nipponbare" that was average 3.2 SNPs/kb.

1.5. SNP Identification for a trait

SNPs are involving in genetic and genome mapping, association studies, genetic diversity analysis, and tagging important genes. In figure 5 is one of the example of association SNPs with a trait (plant height).

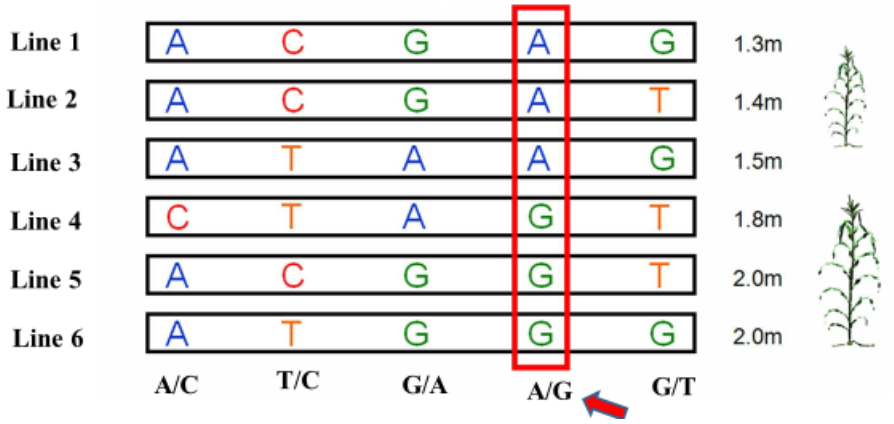

Figure 5. SNP identification for plant height

In this example, each SNP conducts a test of association with a trait (plant height). In here, SNP (A/G) associated with plant height. In this significant SNP/trait association suggests SNP has direct biological function (functional polymorphism) and SNP in Linkage Disequilibrium with functional polymorphisms. Furthermore, association studies can determine whether a genetic variant is associated with a trait (for example, disease). Based on Shi et al. (2011) [26], SNPs have been discovered and verified in tomato and successfully used in selection of resistance to viruses, bacterial speck and bacterial spot. Below is the example of four tomatoes that resistance and susceptible to root-knot nematode (figure 6). SNP (G/C) associated with root-knot nematode. Mi is root-knot nematode resistance and mi is root-knot nematode susceptible.

Motella/LA2823	Mi	AAGTAGACGAGGTTAGTAAAAT
Mogeor/LA3471	Mi	AAGTAGACGAGGTTAGTAAAAT
NY07-464	mi	AAGTAGACGACGTTAGTAAAAT
LA3130	mi	AAGTAGACGACGTTAGTAAAAT

Figure 6. SNP identification for resistance and susceptible to root-knot nematode in tomatoes [26]

1.6. Application of SNP markers in molecular breeding

SNP is becoming to be the most useful as molecular marker in genome mapping, comparative mapping, framework mapping, varietal/line identification, map-based cloning, genome selection, association studies, diversity analysis, genetic variation, population structure, evolution analysis, bulk segregant analysis, tagging genes for economically important traits, accelerated cloning of gene/QTL of interest, marker-assisted selection (MAS) and genome-wide association studies (GWAS) in crops because of their abundance and automated high throughput genotyping. MAS has many advantages than conventional phenotypic selection because MAS is simpler than phenotypic screening which can save time, resources and effort; selection can be carried out at the seedling stage; and single plants can be accurately selected. Moreover, SNP markers can be used in molecular breeding in population genetics, such as disease association and pathogen detection.

In tomato (Solanuum lycopersicum L. syn Lycopersicon esculentum Mill.) breeding, SNP markers were used in selection Tomato Mosaic Virus (ToMV) and Tomato spotted wilt virus (TSWV) resistance genes [11]. In this research, SNP markers were used in association study, to create a genetic test that will screen for a disease in which the disease-causing gene had already been identified. Then, collect

leaves samples from a group of plants affected by the disease and analyze their DNA for SNP patterns. Furthermore, compare these patterns to patterns obtained by analyzing the DNA from group of plants unaffected by the disease. This comparison can detect differences between the SNP patterns of the two groups of plants. Therefore, SNP marker has become important and useful in the selection of disease resistance genes. These markers will provide breeders with a tool in selection of Tm-2 and Tm-22 resistance genes of ToMV in tomato breeding program. SNPs can differentiate resistance and susceptible allele at Tm-2 locus. In addition, SNP markers also can differentiate Sw5-b and Sw5-a resistance genes and sw5-b susceptible gene of TSWV.

Furthermore, in molecular breeding, SNP markers also can be used in quantitative trait locus (QTL)/gene discovery. For example, in maize (Zea mays), SNP markers have facilitated the dissection of complex traits such as flowering time by using 5000 Recombinant Inbred Lines (RILs) and genotyping with 1,200 SNP markers. Next, the genetic architecture of flowering time was discovered and controlled by small additive QTL rather than a single large-effect QTL [27]. In addition, based on Poland et al. (2011) [28], by using 5000 RILs and 1.6 million SNP markers, 29 QTL were discovered and candidate genes for northern leaf blight disease were identified. Moreover, a study from Pioneer Hi-Bred International Inc. (a private breeding program) by using their proprietary SNP markers developed by them self, reported identifying a high-oil QTL (qHO6) affecting maize seed oil and oleic acid contents. This QTL encodes an acyl-CoA:diacylglycerol acyltransferase (DGAT1-2), which catalyzes the final step of oil synthesis [30]. According to Kump et al. (2011) [30], the genetic structure of northern leaf blight, southern leaf blight, and leaf architecture was studied using ~1.6 million SNPs in maize. Additionally, five SNP primers which are associated with northern leaf blight resistant gene (Ht1) were identified by Junta et al. (2020) [31]. These SNP primers are MZSNP-0055106, MZSNP-0065744, MZSNP-0070164, MZSNP0063922, and MZSNP-0073150 that located on chromosome 2.

In rice (Oryza sativa) breeding, a GWAS was performed using ~3.6 million SNPs from ~50,000 rice accessions identified genomic regions associated with 14 agronomic traits [32]. These traits including morphological characteristics (tiller number and leaf angle), yield components (grain width, grain length, grain weight and spikelet number), grain quality (gelatinization temperature and amylose content), coloration (apiculus color, pericarp color and hull color) and physiological features (heading date, drought tolerant and degree of seed shattering). Furthermore, according to McNally et al. (2009) [33], SNPs revealed the breeding history and relationships among the 20 rice varieties; some SNPs are associated with agronomic traits that used in rice improvement. These comprehensive SNP data provide a foundation for deep exploration of rice diversity and gene–trait relationships and their use for future rice improvement. Based on Ayres (2000) [34], SNP markers were used to identify Waxy gene (control amylose synthesis by coding starch synthase enzyme) and sd-1 (semi dwarfing gene). In addition, 1536 SNP markers used for identify genetic diversity on Malaysian rice varieties [35]. Meanwhile, only 932 SNPs that have high quality alleles and amplified across the rice varieties.

In wheat (Triticum aestivum), SNP markers were mapped between the known flanking markers for Fhb1 (Fusarium head blight resistance gene). These new markers would be useful for MAS and fine mapping towards cloning Fhb1 gene [36]. Moreover, SNP markers have also been used to study the evolution of genes such as WAG-2 in wheat [37]. Identification of Pin b gene (Puroindolin b) for grain hardiness [38] and Rht1 and Rht2 (semi dwarfing gene) also using SNP markers [39].

In soybean (Glycine max), SNP markers were used to increase the efficiency and cost effectiveness through MAS and enhance the resolution within the target locus. The gene Rag1 (Soybean aphid resistance gene) was mapped between two SNP markers that corresponded to a physical distance of 115kb and identified several candidate genes [40]. In another aphid resistance gene, Rag2, originally mapped to a 10cM interval, was fine mapped to a 54kb interval using SNP markers that were developed by resequencing of target intervals and sequence-tagged sites [41]. Ha et al. (2007) [42] identified SNP markers tightly linked to a QTL conferring resistance to southern root-knot nematode by developing these SNP markers from the bacterial artificial chromosome (BAC) ends and SSR-containing genomic DNA clones. According to Shi et al. (2009) [43], SNP markers were useful in identification and selection of Soybean Mosaic Virus (SMV) resistance genes. SMV is the most destructive viral disease in soybean.

Then, genetic resistance is the primary method of controlling this disease. These genes are Rsv1, Rsv3, and Rsv4. Furthermore, SNP markers were developed for those genes. There are 2 SNP markers were identified from the 3gG2 gene (the resistance allele at Rsv1 locus and the gene has been cloned and sequenced) at Rsv1 locus, 4 SNPs near Rsv1, 5 near Rsv3, and 5 near Rsv4 were validated. SNP markers also were used to identify Rhg1 and Rhg4 (Soybean Cyst Nematode resistance allele) [44].

In sugar beet (Beta vulgaris), SNP markers were developed to map QTL for Beet necrotic yellow vein virus resistance genes, Rz4 [45] and Rz5 I [46]. Additionally, in cowpea (Vigna unguiculata), a consensus genetic map was developed based on EST-derived SNPs that a very important resource for genomic and QTL mapping studies [47]. In Arabidopsis, SNP markers were utilized to clone the VTC2 gene based on the fine mapping and map based cloning approaches. Jander et al. (2002) [48] fine mapped the gene interval from ~980kb region to a 20kb interval and additional nine candidate genes were identified in that interval and subsequently the underlying mutation was discovered.

In oil palm (Elaeis guineensis), SNP markers were used for genetic diversity [49]. The genetic evaluation of oil palm germplasm collections is important to insight into the variability among populations. The information obtained is also useful for incorporating new genetic materials into current breeding programs. Furthermore, genetic diversity information among populations is important to identify selected palms with economically important traits which are being incorporated into the current breeding programs; these include high oil yield, low height increment, large kernel, long stalk, low levels of lipase, and high levels of carotene, vitamin E, iodine, and oleic acid. In this research, 219 oil palms from two natural Angolan populations are used and a total of 62 SNP markers were designed from oil palm genomic sequences and converted to cleaved amplified polymorphic sequence (CAPS). Based on cluster analysis using unweighted pair group method with arithmetic, the 219 oil palms fell into two clusters or genetic groups that do not coincide with the geographic populations. Cluster I consisted of eight palms and cluster II included 211 palms. Furthermore, molecular variance analysis revealed that only 7% of the total genetic variation was explained by the variation between populations and 93% of the total genetic variation was attributed to variation within populations. Moreover, levels of genetic variation in plants are directly associated with breeding system. This information can be applied in selecting a sampling strategy for genetic conservation. In addition, SNP markers also use for identifying fumarate hydratase 1 (FUM1) gene located on chromosome 4, which associated with nitrogen uptake in oil palm [50].

Genetic diversity study in cassava (Manihot esculenta Crantz) was done by using 5600 informative SNP markers obtained from GBS analysis [51]. The result showed three main clusters from 96 cassava genotypes, at similarity of 0.41. This genetic diversity suggests that cassava genotypes might contains alleles with high additive genetic variance that very important for the genetic improvement, conservation, and provide database for parental selection in order to develop hybrid vigor cassava. Furthermore, genetic diversity study of 70 taro (Colocasia esculenta) accessions of Hawaiian, South Pacific, Pulauan, and mainland Asian origins was done by using 2400 SNP markers [52]. The disease resistant gene in taro was revealed by using this genetic diversity study.

In pea (Pisum sativum), SNP markers were applied in fine mapping within chosen QTL confidence intervals and marker assisted breeding for important traits in pea improvement [53]. Moreover, in eggplant (Solanum melongena), the genetic diversity revealed by SNP markers [17]. In this research, 384 of the 2,201 highest quality SNPs (score > 0.6) were applied to genotype 23 eggplant germplasms that have variation in fruit shape and skin color. About 94 SNP markers in common bean (Phaseolus vulgaris L.), were used in genetic diversity. These markers were evaluated from 70 cultivated and wild accessions according to their gene pool, race and country of origin [20]. In Chickpea (Cicer arietinum), 1022 SNPs were used to make high-resolution genetic linkage map [21]. Furthermore, in Camelina sativa, 533 SNPs were applied to mapped potential candidate genes and to assess genetic variation among a collection of 175 accessions. The SNPs will provide useful tools for future crop improvement of C. sativa as an industrial oilseed [22].

SNP markers were used on GWAS and candidate gene association (CGA) studies. A GWAS approach was applied to understand the genetic architecture of complex traits, for example complex

diseases of northern and southern corn leaf blights [30]. As GWAS requires large number of molecular markers, the utility of GWAS in dissection of molecular basis of traits in polyploid crops such as wheat, and cotton has been fairly limited due to the insufficient number of polymorphic markers and the absence of reference genome.

2. Conclusion

Genomic information of plants obtained from genetic diversity, association studies, evolution analysis, quantitative trait loci (QTL), marker-assisted selection (MAS) and genome-wide association studies (GWAS) are very useful for increasing food sustainability. To achieve food sustainability in this changing climate condition, plants with superior characteristics must be developed by using genomic information. Genes associated with important traits can be identified efficiently with SNP markers. Genomic information of many tropical crops have been identified in order to improve their characteristics.

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Growth Performance of *Daphnia* sp. Cultured in Different Concentration of Rice Washing Water

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Abstract. This study aimed to investigate the effect of the administration of rice washing water in culture medium on the growth performance of *Daphnia* sp. This research using three different doses of rice washing water i.e 1 mL/L, 3mL/L, and 5 mL/L. *Daphnia* sp. was cultured with an initial density of 20 ind/L. Observed parameters include growth parameters (population density, size and specific growth rate) and water quality. This result showed that a concentration of 3 mL/L created the highest population of *Daphnia* sp. density about 620±20 ind/L, number small size (young stage) 81.2%, and the highest specific growth rate about 56.68±0.55%. The water quality content of DO, temperature and pH during this study were in the good range of *Daphnia* sp. life and reproduction. The research has a conclusion that rice washing water can be used to nutritional sources of *Daphnia* sp. In the future, it is necessary to make further observations about the reproductive performance of *Daphnia* sp, given rice washing water through clone culture.

Keywords: Live food, Population density, Rice washing water, Size of *Daphia* sp., Specific growth rate

(Received 15 August 2020, Accepted 10 September 2020, Available Online by 30 September 2020)

1. Introduction

Aquaculture is the production of aquatic organisms and plants on controlled conditions [1]. Presently, Aquaculture is one of the promising agriculture activities that growing rapidly around the

world and has the potential to improve food security [2]. The success of aquaculture production depends on the all phase in aquaculture include broodstock holding, hatchery production of fry, nursing system, and grow out to market size [3]. Especially in fish larval farming on the hatchery, the obstacle is the availability and quality of live food at the right amount and size that suitable for the mouth size of the fish larva at the time of the first feeding.

Live food is used as a carrier of nutrition for the larval stage of aquatic animals [4]. *Daphnia* sp. is one of the potential live feeds that has been cultivated to meet the nutritional needs of freshwater fish hatcheries. The advantages of using *Daphnia* sp. for live feed in hatcheries due to its low cost and nutritional content [5]. The growth and development of *Daphnia sp*. are affected by nutrition, therefore alternative nutritional source that can increase the *Daphnia* sp. population is needed to maintain the availability of *Daphnia* sp. as live feed for the fish larvae. Rice washing water is one of the potential alternative nutritional sources for *Daphnia* sp. According to Laila [6], rice washing water is containing optimal nutrition to support the growth of *Daphnia* include Vitamin B1, Vitamin B3, Vitamin B6, Fe, natrium, phospor, and kalium. This study aimed to investigate the effect of the administration of rice washing water on growth population, size, specific growth rate, and water quality of *Daphnia* sp.

2. Methods

2.1. Research on Weather Predictions

The rice washing water is obtained from the washing of rice with water at a ratio of 1:1 (w/v). After that, rice washing water were was allowed for 5 minutes [7].

2.2. Preparation of Daphnia sp.

Daphnia sp. used in this study were isolated from a fish pond in Serang Regency, Banten. The stock was cultured in an aquarium with a capacity of 25 L and feed with yeast *Saccharomyces cerevisiae* [8]. The individuals used in this study were adult stages that had a lenght of 2 mm±0.4 mm and a width of 1±0.2 mm.

2.3. Rearing of Dapnia sp.

This study was conducted at Aquaculture Laboratory, Department of Fisheries, Faculty of Agriculture, University of Sultan Ageng Tirtayasa. A total of 20 ind / L *Daphnia* sp. were reared using an aquarium with a size of 30 cm × 30 cm × 30 cm (l × w × t). Each aquarium is filled with 3 L of water an added aeration of 0.12 L/min. The study used three treatments of adding rice washing water in the culture media of *Daphnia* sp. (mL/L), i.e. 1 (treatment A), 3 (treatment B), and 5 (treatment C). Each tratment was repeated three times. *Daphnia* sp. culture is carried out for eigth days. The water (20-25%) of this culture was replaced every three days. Water quality, namely temperature, dissolved oxygen (DO), and pH, is measured every two days to maintain water quality. Temperature is measured using a thermometer (°C), DO with the use of DO meter, and pH using pH meter.

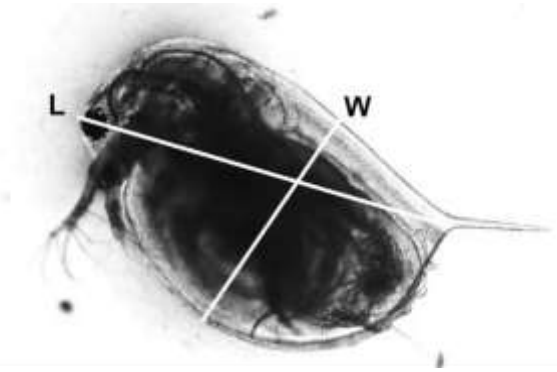

Figure 1. Character length (L) and width (W) in *Daphnia* sp.

2.4. Observation of Dapnia sp.

Observation for the abundance of *Daphnia* sp. was conducted every day to counting the population of *Daphnia* sp. The size of *Daphnia* sp. was observed on the last day of culture. Length and width measurements are performed using ImageJ software. Size is characterized into three groups namely, small (S), medium (M), and large (L), based on length and width (Figure 1). Size S has length 0.82-0.92 mm and width 0.46-0.55 mm, size M has length 0.93-2.14 mm and width 0.82-1.25 mm, and size L has length 2.15-3.19 mm and width 1.26-1.56 mm.

2.5. Data analysis

The population density and specific growth rate data were analyzed using Analysis of Variance (ANOVA) and for those with differences were subjected to Duncan Multiple Range test with a 95% confidence interval. The percentage of size *Daphnia* sp. and water quality were analyzed descriptively.

3. Results and Discussion

3.1. The population density and size of Daphnia sp.

The Effect of three different dosage addition of rice washing water on the population density of *Daphnia sp*. is presented in Figure 2.

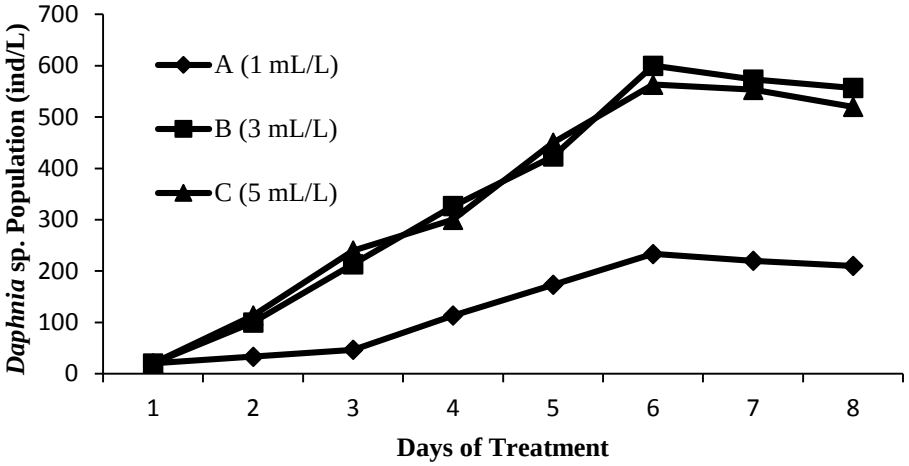

Figure 2. The Effect of three different rice washing water concentration on population density of *Daphnia sp.*

The densities of *Daphnia* sp. were cultivated on medium containing rice washing water with a concentration of 1, 3 and 5 ml/ L increased until the 6th day, then decreased on the 7th and 8th days. The highest peak density of *Daphnia* sp. given rice washing water was achieved on the 6th day. Similarly, the previous studies showed that that the peak growth density of *Daphnia* sp. occurred 6 days after the administration of tofu and bran fermentation [9]. Treatment B showed the highest density of *Daphnia* sp. which was 620±20 ind/L that was significantly different ($P<0.05$) from treatment A which was 180±50 ind/L and C was 580±15 ind/L. According to Lawrence [10], the growth and development of *Daphnia* sp. were affected by nutrient, age, temperature, and filtered particle shape. The result showed that *Daphnia* sp. can utilize nutrients from rice washing water so that it can increase the growth of the *Daphnia* sp. population.

Besides, the size of *Daphnia* sp. on the last culture day showed that all treatments produced more small size *Daphnia* sp (length 0.82-0.92 mm and width 0.46-0.55 mm). The number of small sizes or

young *Daphnia* sp. indicate that rice washing water supports reproduction of *Daphnia* sp. Treatment B produced the smallest size of 81.2% (Figure 3.). These results need to be observed further to observe the reproductive performance of *Daphnia* sp. given rice washing water through clone culture.

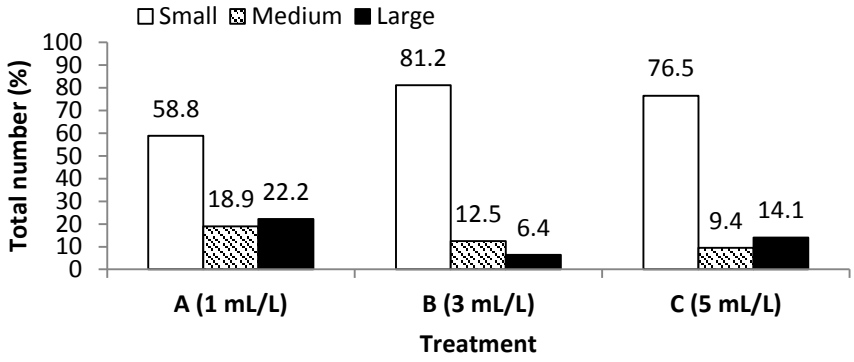

Figure 3. Percentage of the amount of *Daphnia* sp based on size on the eighth day of culture.

3.2. Specific growth rate

The Effect of the addition of three different dosages of rice washing water on specific growth rate of *Daphnia sp.* is presented in Figure 4. The result showed that the *Daphnia* sp. highest significant ($P<0.05$) survival growth rate was found in treatment B which was 56.68±0.55% followed by treatment C was 55.63±0.45%, while the lowest survival growth rate was found in treatment A was 40.67±3.74%. According to [11] *Daphnia* sp. requires adequate nutrition from food to reproduce and grow therefore resulting in a multiplied population increase that affect specific growth rate value. While in treatment A, the nutrition from rice washing water to *Daphnia* is insufficient, therefore showed a low specific growth rate.

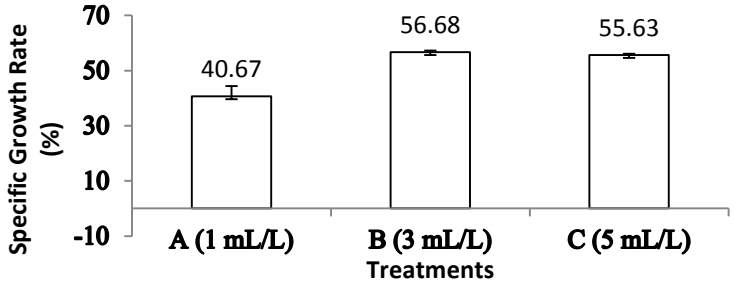

Figure 4. The Effect of three different rice washing water concentration on specific growth rate of *Daphnia* sp.

3.4. Water quality

Growth of *Daphnia* sp. influenced by water quality. In this study, water quality was maintained in the ideal range for *Daphnia* sp. The result of water quality showed in table 1.

Table 1. Water quality of rearing media with different concentration of rice washing water

Treatment	DO	Temperature	pH
A (1 mL/L)	7.45±0.08	27.57±0.07	7.58±0.04
B (3 mL/L)	7.47±0.31	27.61±0.02	7.62±0.07
C (5 mL/L)	7.6±0.00	27.63±0.06	7.61±0.11

The mean DO value in three different treatments in this study was 7.45±0.08 mg/L - 7.6±0.00 mg/L. According to [12], DO in culture media supports the respiration of *Daphnia* sp. without creating competition between *Daphnia* sp. and decomposing microbes. So, *Daphnia* sp. can utilize optimum DO for metabolism to growth and reproduction. *Daphnia* sp. can survive in DO >3 mg/L, but they grow better at a minimum of 6 mg/L.

The mean temperature value in this study was 27.57±0.07 °C - 27.63±0.06 °C. According to [13], the ideal temperature for the growth of daphnia was 25-30°C. In cladocerans, food activity depends on the temperature of the concentration of food. Therefore, better performance of reproductive and growth is expected from populations where there are nutritional conditions of abundance and appropriate temperatures [13].

The mean pH value in this study was 7.58±0.04 to 7.61±0.11. The ideal pH for the growth of *Daphnia* sp was 7-8.6 [1]. According to Darmawan [14], the pH affects the egg's life span of zooplankton microcrustaceans include Daphnia, and pH determines the toxicity of medium from ammonia. The ideal pH of *Daphnia* sp. was 7-8.6.

4. Conclusion

Rice washing water can be used as an alternative nutritional source for *Daphnia* sp. The addition of 3 mL/L rice washing water was the best concentration to the growing population, number of small size (young stage), and specific growth rate of *Daphnia* sp.

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Mapping Exclusive Breastfeeding Coverage and Toddler Stunting Prevalence in Indonesia Based on Web Geographic Information System

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Abstract. Stunting is a chronic nutritional problem in most of developing countries for quite a long time, including Indonesia. In 2018, Indonesia had 30.8% of stunting toddlers, 29.6% in 2017 and 27.54% in 2016. The trend over the last 3 years the prevalence of stunting has increased. The purpose of this research is to map the spread of stunting toddlers and exclusive breast milk coverage in Indonesia. This research is a cross sectional study using secondary data sourced from reporting compiled by the Ministry of Health of the Republic of Indonesia in 2018. WebGIS is used to compile mappings of both variables in each province through the official website of BPS. The results of this study show the information presented in WebGis seen most provinces have a prevalence of stunting in black zones (very high) and exclusive breast milk coverage in red zones (very less met than national targets).

Keywords: Stunting, Exclusive breasfeeding, Toddler, WebGIS, Indonesia

(Received 15 August 2020, Accepted 10 September 2020, Available Online by 30 September 2020)

1. Introduction

Stunting is a chronic nutritional problem faced by developing countries for quite a long time, including Indonesia. The factors that cause stunting start from the root of the problem to the direct cause. Long-term lack of food intake is the main cause of stunting. What is meant here is the low quality and quantity of feeding in infants and toddlers.[1][2]

Exclusive breast milk is the provision of nutritional intake only breast milk starting from the newborn until the age of 6 months. But for some reason, babies don't get it. They get early MP-breast milk or formula milk at that age. One of the many impacts that arises from this is toddler stunting. Long-term malnutrition, which starts as a baby, contributes greatly to a toddler's growth in the next phase. Moreover, the exclusive breastfeeding period is part of 1000 first day of life (HPK). [3][4]

In 2018, Indonesia had 30.8% of stunting toddlers, 29.6% in 2017 and 27.54% in 2016. Trend over the last 3 years the prevalence of stunting has increased.[5] Stunting becomes a strategic program to handle both sensitive and specific aspects. The results achieved from this treatment are important to report so that the public can also understand the situation of stunting in Indonesia. Reporting of stunting situations can be done using a geographic information system, as already done in the reporting of nutritional status monitoring results in Sukoharjo. The development of this system can support nutrition status monitoring activities and is expected to increase the success of nutrition improvement programs in toddlers.[6] The purpose of this research is to map the spread of stunting toddlers and exclusive breast milk coverage in Indonesia.

2. Methods

This research is a cross sectional study using secondary data sourced from reporting compiled by the Ministry of Health of the Republic of Indonesia in 2018 and published through the official website of BPS.[7] The data analyzed included the prevalence of stunting toddlers and the percentage of exclusive breastfeeding from 34 provinces in Indonesia. WebGIS is used to compile mappings of both variables in each province. The webGis drafting flow is presented in Figure 1.

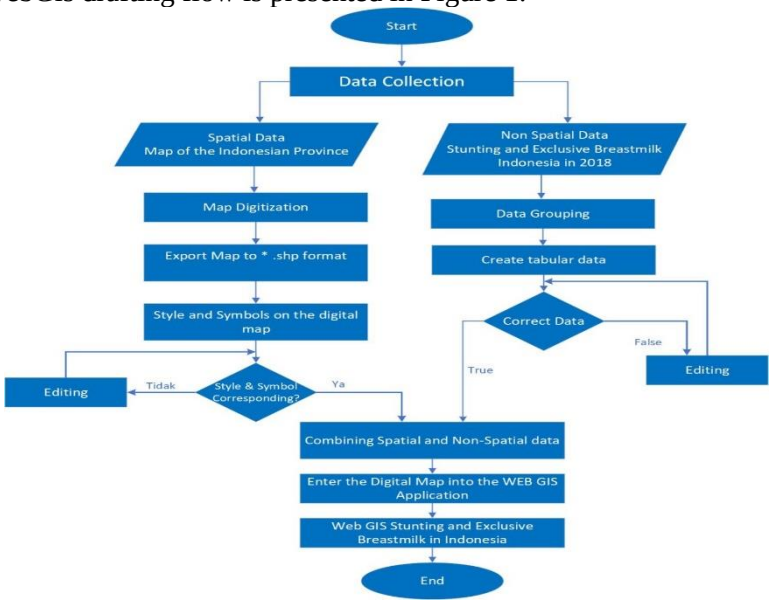

Figure 1. WebGIS Development Guide Flow Chart

Mapping results are arranged in several zones based on the prevalence and percentage of each province (Table 1) .

Table 1. Mapping zone

Zone	Stunting Prevalence	Exclusive breastmilk coverage
Green	< 10%	≥ 80%
Yellow	10 – 19,9%	60 – 80%
Red	20 – 29,9%	<60%
Black	≥ 30%	

3. Results and Discussion

Stunting toddlers were found in 34 provinces. The prevalence of stunting is categorized as a public health problem if it is "10%. [8] That is, areas with a prevalence above 10% need to make treatment efforts. So far the presentation of data is still in the form of charts or tables, whereas ad other events in the presentation of data so that the reader becomes quick to understand (figure 2).

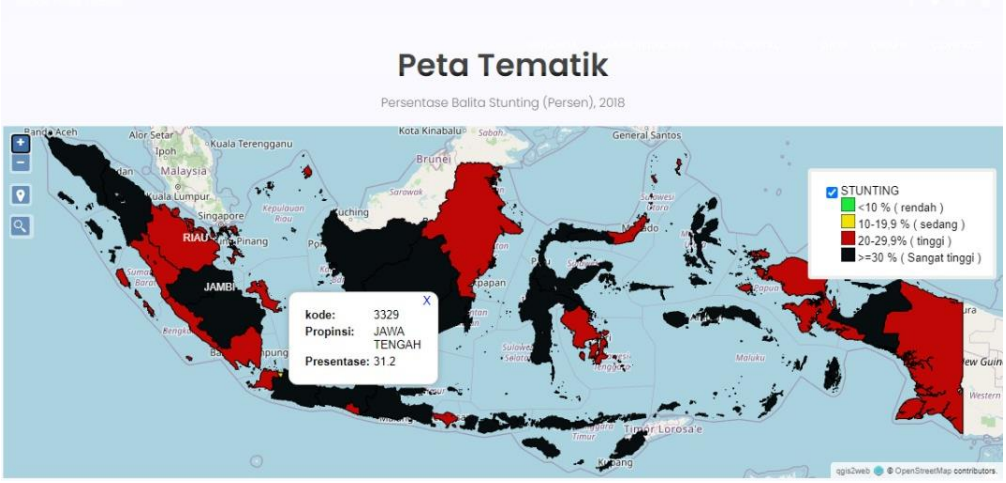

Figure 2. Mapping Toddlers Stunting in Indonesia.

Most of Indonesia is dominated by black and red zone. This means that the prevalence of stunting in Indonesia is evenly distributed. The data is clarified by table 2 as follows:

Table 2. Stunting Prevalence and Exclusive Breasfeeding Coverage di Indonesia

Province	Exclusive breastfeeding coverage	Stunting Prevalence	Province	Exclusive breastfeeding coverage	Stunting Prevalence	Province	Exclusive breastfeeding coverage	Stunting Prevalence
ACEH	33,33	37,1	JAWA TENGAH	50,56	31,2	SULAWESI UTARA	33,62	32,3
SUMATERA UTARA	25,69	32,4	DI YOGYAKARTA	55,70	32,8	SULAWESI TENGAH	41,91	35,7
SUMATERA BARAT	50,40	29,9	JAWA TIMUR	40,79	26,6	SULAWESI SELATAN	52,18	28,7
RIAU	36,29	27,4	BANTEN	36,83	21,8	SULAWESI TENGGARA	34,96	32,5
JAMBI	62,67	30,1	BALI	27,08	33,5	GORONTALO	42,19	41,6
SUMATERA SELATAN	41,56	31,7	NUSA TENGGARA BARAT	64,25	42,6	SULAWESI BARAT	61,77	34,0
BENGKULU	46,78	28,0	NUSA TENGGARA TIMUR	62,17	33,3	MALUKU	36,36	31,4
LAMPUNG	44,58	27,3	KALIMANTAN BARAT	51,37	34,0	MALUKU UTARA	64,28	27,7
KEP. BANGKA BELITUNG	34,56	23,4	KALIMANTAN TENGAH	44,11	33,1	PAPUA BARAT	35,01	33,1
KEP. RIAU	53,85	23,6	KALIMANTAN SELATAN	40,69	29,2	PAPUA	43,48	30,8
DKI JAKARTA	45,66	17,6	KALIMANTAN TIMUR	59,00	26,9			
JAWA BARAT	44,67	31,1	KALIMANTAN UTARA	46,25	25,5	INDONESIA	44,36	30,8

Mapping for exclusive breast milk coverage percentage shown in figure 3.

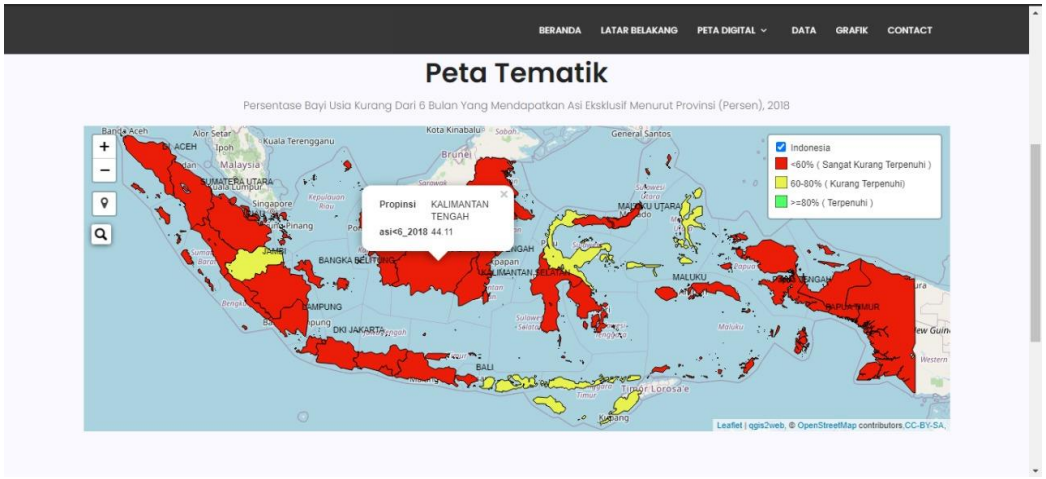

Figure 3. Mapping of Exclusive breasfeeding Percentage in Indonesia 2018.

The national target of exclusive breast milk coverage is 80%. [9] However, the data gathered from Indonesia Health Survey (Riskesdas) 2018, the coverage is below the target of all provinces in Indonesia. Figure 3 shows, there are most regions showing a coverage of <60%. This data is far from a set target.

The province with the highest exclusive breast milk coverage in North Maluku (64.28%), while for the lowest stunting prevalence in DKI Jakarta (17.6%) The province with the lowest breast milk coverage in North Sumatra (25.69%, while for the highest stunting prevalence in East Nusa Tenggara (42.6%). Exclusive breastfeeding and stunting have negative relationships, meaning that higher breastfeeding will suppress stunting in toddlers. However, when figures 2 and 3 are combined, the results are not so. Provinces with the highest exclusive breast milk coverage, the prevalence of stunting is not the lowest.

The high prevalence of stunting, prompted the government to accelerate its handling immediately. Therefore, the government established stunting intervention programs since 2018. The handling includes specific and sensitive efforts that are still ongoing gradually and targeted all provinces get it.[10]

Apart from mapping results that do not show results in line between the prevalence of stunting and exclusive breastfeeding coverage, exclusive breastfeeding is part of a specific effort. Its success involves many parties, especially those closest to the mother and baby. Breast milk is exclusively related to stunting events in toddlers. These results were shown in several studies in several regions. [3] Even in research with the uni direction hypothesis shown a positive r value, meaning that the more fulfillment of the quality and quantity of exclusive breastfeeding in toddlers, the better the nutritional status of the toddler.[11] Even in research in Mamasa shows that babies who don't get exclusive breast milk are at 61 times greater risk of stunting as toddlers. [12] However, some studies have also shown that exclusive breast milk is not related to stunting events in toddlers. [13]

4. Conclusion

The province with the highest exclusive breast milk coverage in North Maluku (64.28%), while for the lowest stunting prevalence in DKI Jakarta (17.6%) The province with the lowest breast milk coverage in North Sumatra (25.69%, while for the highest stunting prevalence in East Nusa Tenggara (42.6%). Most provinces have a prevalence of stunting in black zones (very high) and exclusive breast milk coverage in red zones (very less met than national targets).

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Characterization of Yellow Pigmented Bacteria Associated with *Gracilaria* sp.

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Abstract. Research on the kinship analysis of endophytic bacterial isolated from *Gracillaria* sp has been carried out. The presence of bacteria associated with *Gracilaria* sp. has enabled the use of these bacteria as a source of new bioactive compounds, such as biopigments. The research aims to isolated bacteria from *Gracilaria* sp., screened their symbiont bacteria that could potentially produce pigments. Sampling *Gracilaria* sp. conducted in the waters of the Island of Karimunjawa, Jepara. Furthermore, bacterial isolation was carried out, screening for pigment-producing bacteria and 16S rRNA sequence analysis. Research result showed that the symbiont bacteria isolate TK 373 produced consistent pigments after several regenerations, in several types of growth media incubated at room temperature. The results of 16S rDNA identification showed that the TK 373 isolate had the closest relationship with *Pseudoalteromonas* sp. with 98.72 % homology.

Keywords: *symbiont bacteria, pigment, Gracilaria* sp.

(Received 15 August 2020, Accepted 10 September 2020, Available Online by 30 September 2020)

1. Introduction

Indonesia gets a big advantage because it has a very large marine. These advantages include in the form of biodiversity, both in the form of community, species and genetic diversity. Indonesia's abundant natural wealth and biodiversity have prospects for this nation to develop bio-industry. One of these potential genetic resources is algae.

Various natural products from macro algae metabolites plays a very important role in the natural product discovery process [1,2,3]. Algae, has been widely used as a bioactive source metabolites such as proteins, lipids, mineral salts, polyphenols and polysaccharides which are very useful in various industries of food, pharmacy, cosmetics [4,5]. Red algae is a group of algae that has extraordinary potential, including the pigment content of red algae which has pharmaceutical, nutraceutical and cosmeceutical prospects [6, 7].

Marine microorganisms have a very large role in the life cycle at sea. One of them is as epiphytic microbes or microbes associated with marine organisms. Various scientific publications show that there are associations of microorganisms with marine organisms that also synthesize secondary metabolites such as their host organisms, including red algae symbionts [8, 9]. This is a consideration to explore

the red algae symbiont bacteria with the hope of providing a wider opportunity to get red algae bacterial associan that have biological abilities like their host.

2. Methods

2.1. Sampling site

Algae samples of *Gracilaria* sp. were collected from Karimunjawa island Indonesia. After collection, algae materials were placed onto sterile bottles containing autoclaved seawater and brought in chilled condition, to the Integrated Biotechnology Laboratory, Diponegoro University.

2.2. Isolation and purification of bacteria from algae

Fresh algae samples were washed with marine steriled water. Bacteria associated with *Gracilaria* sp. isolated according to the method of [10]. Using a sterile scalpel, seaweed tissue is cut in a size of about 0.1 cm3 and sprayed three times with sterilized sea water. These pieces are then rinsed for surface sterilization with sterile sea water. Zobell media are used to place *Gracilaria* sp tissue in an incubator at 28° C for 72 hours. Different colonies appear morphologically to be separated and purified.

2.3. Phylogenetic Analysis

Genomic DNA was extracted using the standard chelex protocol according to [11]. The 16S rRNA gene of the isolate was amplified with the bacterial universal 16S rRNA primers 27F and 1492R. The optimizations used were: 93° C for 30 seconds, 54.5° C for 30 seconds, and 72 ° C for 1.5 minutes. run 30 cycles. The presence of PCR products was confirmed by electrophoresis on 1 % agarose gels. Basic Local Alignment Search Tool (BLAST) is used to determine bacterial species that are closely related to potential bacterial isolates. Phylogenetic trees with bootstrap sampling were reconstructed by the neighbor-joining method, with 1,000 bootstrap replications was run in MEGA 7 [12, 13].

3. Results and Discussion

Efforts to obtain bacterial isolates pigmented brown algae symbionts were carried out using culture-dependent methods. This method is a physiological identification process of microbes by first isolating and purifying the bacteria from the host. Based on the results of isolation and purification, it was found that several isolates produced pigment. However, after several isolates regeneration processes were carried out, it turned out that only one isolate was able to show fertile growth and a consistent color, namely the TK 373 isolate.This isolate produced a bright yellow color when grown on Marine agar and Nutrient Agar media, incubated at room temperature (Figure 1)

The BLAST results showed that the TK 373 isolate had the highest similarity with Pseudoalteromonas with 98.72% similarity (Table 1). 97-99% similarity for 16S rRNA sequences for bacteria showed similarity at the genus level, whereas> 99% similarity in gene sequences was the criterion used to identify isolates at the species level [14]. The results of phylogenetic reconstruction of bacterial isolates based on partial gene sequences of 16S rRNA showed the closeness of TK 373 to *Pseudoalteromonas* sp. (Figure 2).

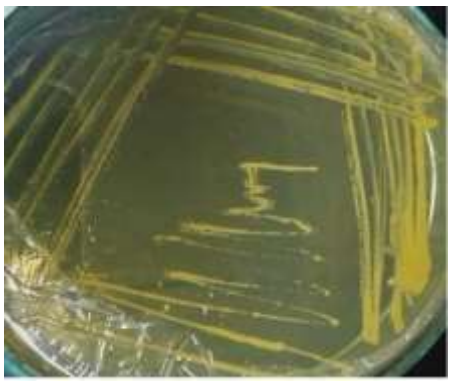

Figure 1. Colony characteristics of TK 373 isolates on ZMA media were incubated for 48 hours at room temperature

Table 1. Homology of partial gene sequences of 16S rRNA isolate TK 373 with GenBank Database

Isolate code	Homology	Query cover (%)	Score	E-value	Per Ident	Ascension number
TK 373	*Pseudoalteromonas* sp. strain S14	91%	2084	0,0	98,72 %	KX989351.1

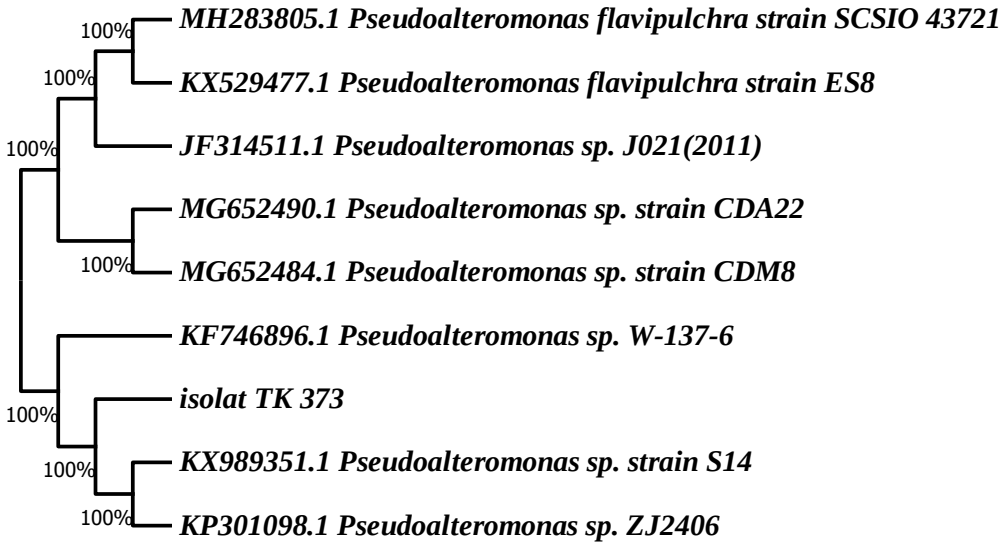

Figure 2. Reconstruction of the TK 373 phylogenetic tree with reference strains obtained from NCBI. A neighbor joining tree was calculated using partial 16S rRNA gene sequences.

Many researchers have reviewed the Pseudoalteromonas. [15] stated that pseudoalteromonas are true marine bacteria, this is because the Pseudoalteromonas require a sea water base for growth. Pseudoalteromonas are gram-negative bacteria, motile and have polar flagella, are heterotrophic, and have GC content between 38 - 50% [16]

The genus Pseudoalteromonas is an interesting topic of study. This is due to its potential and uniqueness. This potential is the productive capacity of its metabolite production, while its uniqueness is seen in terms of its association with other organisms [17]. Based on these two points of view, it can be said that their ability to associate with other organisms is the key to the abundance of potential metabolites from the Genus Pseudoalteromonas [15]. The results of this study also support this argument. Isolate TK 373 was related to Genus Pseudoalteromonas, isolated from Gracilaria sp. on sampling sites in marine waters.

The metabolite production capacity in Pseudoalteromonas is usually associated with pigmentation, for example, P. tunicata CCUG 26757 and P. rubra DSM 6842 strains produce various pigments that are involved in antibacterial and antifungal activity [18,19,20,21]

Isolate TK 373 produces a bright yellow color. This of course gives hope that these isolates are potential. Screening for bioactive potential needs to be done as part of the bioprospection of potential local isolates that can be applied in the industrial sector.

4. Conclusion

Research on molecular characterization and morphotypes of local bacterial isolates with symbionts in Gracilaria sp. has provided findings that TK 373 isolate has the closest similarity to Pseudoalteromonas, one of the potential genera where most of its members are able to produce pigments that can be explored further for its potential.

Acknowledgements

For all assistance during the research, Siu Semar Saratu Langden was greatly appreciated.

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The Potential of Mechanic Vibration for Generating Electric Energy

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Abstract. The purpose for this study is to convert mechanical vibration into electrical Power. Our target is to provide enough power for 1.2 V rechargeable battery having 160 μA. The investigation was conducted experimentally in electrical mechanical engineering laboratory. Four piezoelectric was connected to circuits in series mode called 1 module. In order to recharge the battery having voltage 1.2 volt, the measurement of the intensity of the loudspeaker was verified. According to table 1, it was found that at 2 m/s^2 of vibration is best because the value is 1.4 V nearest to 1.2 V. It was found that the mechanical vibration can be converted into electric power. It was found that to recharge 160 μ A having 1.2 V, the 20 modules are needed.

Keywords: piezoelectric, vibration, electric energy

(Received 15 August 2020, Accepted 10 September 2020, Available Online by 30 September 2020)

1. Introduction

An energy source is something around us that is capable of producing energy both small and large. Energy sources can be broadly divided into 2, namely renewable and non-renewable energy sources [1]. Energy demand in Indonesia is growing with the rapid increase in development in the fields of technology, industry and information. With the increase in energy consumption in Indonesia and the decreasing reserves of fossil energy, Indonesia is ready to develop non-renewable energy. Various new

forms of energy have developed, including wind power, steam power, biomass, and so on [2].

Today there is a shortage of energy sources and at the same time the load of modern electricity and devices based primarily and directly on electric power is increasing. This reality is a major challenge facing the government. Using multiple sources of energy instead of relying on a single source, especially if this source is a conventional source, is a necessary step. Conventional energy sources such as fuel and natural gas will end with time, so researchers are trying hard to develop and explore renewable energy sources such as the sun [3], wind [4], vibration [5], and thermal energy [6].

Most of the energy used is inaccessible energy so that accessible energy and / or other alternative energy sources are needed.

Noise is noise pollution that generally occurs in dense residential areas, industrial areas, and others. Hence, that is wasted energy. Psychological and health effects caused by the smell of deafness, feeling depressed, hypertension, and others. To prevent this, workers who are in areas with a high level of protection are required to be obliged.

Sound or oil is one of the most widely available sources of energy. Sound in fact is a mechanical wave generated from pressure oscillations through several media. Sounds that can be heard by the human sense of hearing with a frequency from about 20 Hz to 20,000 Hz are called audiosonics. In air at standard temperature and pressure, sound wavelengths range from 17 m to 17 mm.

Sound energy is the energy produced by sound vibrations as it travels through air, air, or other space. The frequency of the first sound energy source is infrasonic. Infrasonic is a very weak sound because the vibrations produced in infrasonic waves are less than 20 vibrations per second. Audiosonics is a type of sound that can be heard by humans. Ultrasonic waves are waves with sound vibrations of more than 20,000 vibrations per second [7].

Noise is noise pollution. Therefore, noise pollution needs to be minimized or used for energy sources. In this research, noise pollution is stated by a sound signal that is predicted to have the potential to be converted into energy.

2. Methods

3.1 Development Model

This research was conducted by means of experiment. This research was conducted to investigate the potential for mechanical vibrations for a source of electrical energy.

The stress of this research can be obtained from the measurement of the oscilloscope which comes from mechanical vibrations. The current is obtained by adding a load in the form of a resistor. Then the power is obtained from the multiplication of voltage and current.

3.2 Research Procedures

The research procedure in this thesis is as follows:

Piezoelectric Sensor Testing. Firstly, the sensor is installed and the vibration is set at 0.5 m/s^2 by adjusting the volume. Later on, the output (voltage) sensors 1, 2, 3 and 4 were measured. It is measured twice. After that the voltage multiplier module is installed and measured the voltage. The module consists of 4 circuits. The circuits which represent the multiplier voltage are connected to the piezoelectric sensors in series mode. The multiplier voltage is arranged from 2 Capasitors and 2 Diodes. The detail procedures are shown in Figure 1.

3.3 Population and Sample

The population in this study was the Electrical Engineering Laboratory Campus 3, Universitas PGRI Semarang. The samples in this study are several piezoelectric sensors to be used as research objects.

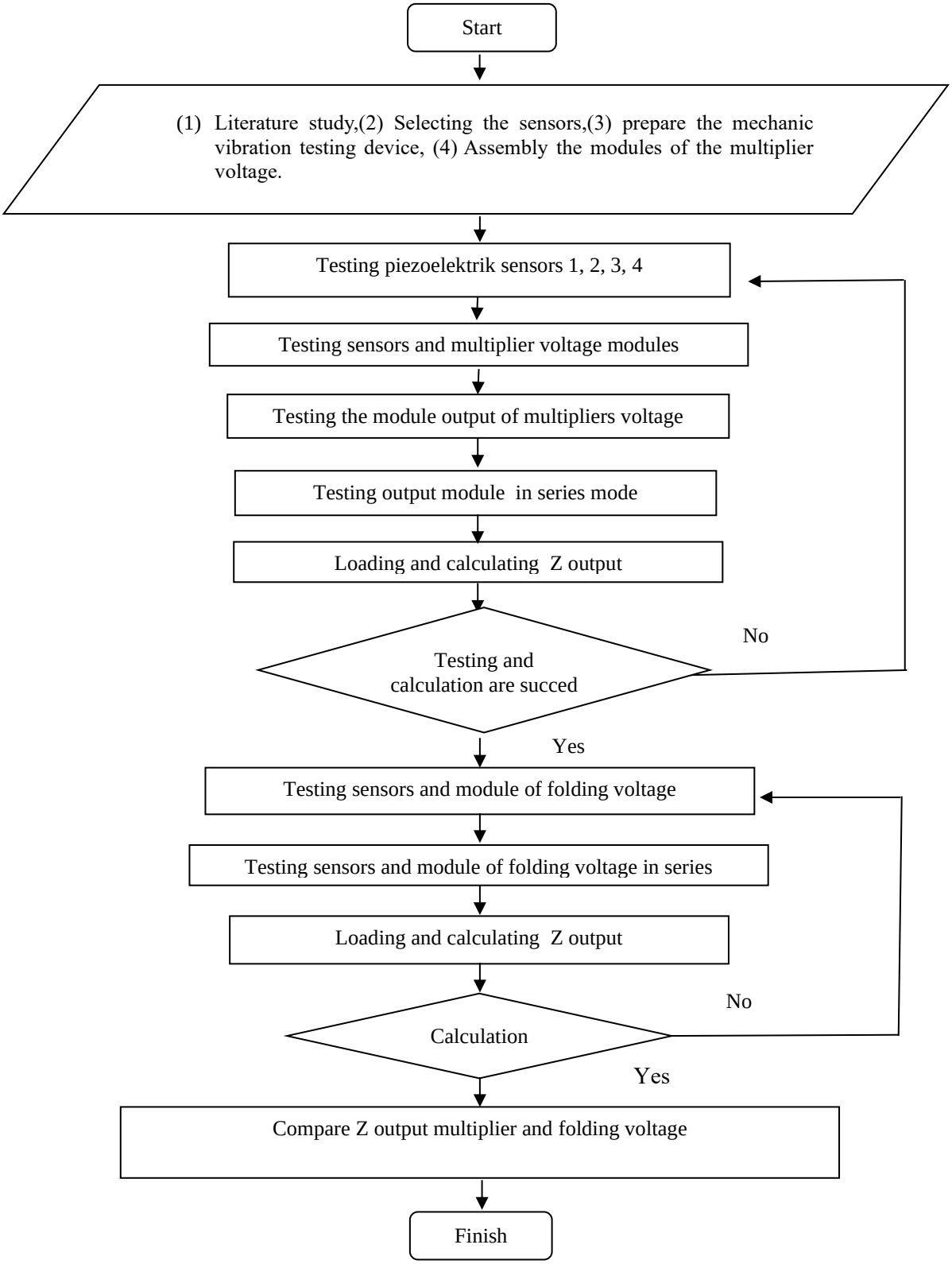

Figure 1. Flow chart of the research

3. Results and Discussion

As can be seen in Fig. 2, the experimental device is set up. It consists of loudspeaker, 4 piezoelectric sensors, voltage multiplier and loads. The mechanic vibration resulted by the electrodynamic loudspeaker converted into the electric power.

Figure 2. the experimental device

Table 1 shows the sensor testing which are arranged in 4 series circuit piezoelectric and voltage multipliers [Figure 1] for generating the electric power. These are called as a module. It is clear that the varied number of the vibration, which is the source of the energy, is five. They are 0.5 m/s^2, 1 m/s^2, 1 .5 m/s^2, 2 m/s^2 and 2.5 m/s^2. The output of the vibration resulting low voltage. To increase the voltage, a voltage multiplier is needed. It was measured and conducted three times as shown at table 1. Thus it was found the average output (output AVG) is 1.4 V for vibration 2 m/s^2.

In order to recharge the battery having voltage 1.2 volt, the measurement of the intensity of the loudspeaker was verified. According to table 1, it was found that at 2 m/s^2 of vibration is best because the value is 1.4 V nearest to 1.2 V, which is the voltage of the rechargeable battery.

Tabel 1. Testing the sensors.

Module	Vibration (m/s^2)	Output 1 DC (Volt)	Output 1 DC 2	Output DC 3	Output AVG
4 series circuit	0.5	0.24	0.24	0.24	0.24
	1	0.6	0.6	0.6	0.6
	1.5	1	1	1	1
	2	1.4	1.4	1.4	1.4
	2.5	2	2	2	2

It was found experimentally that the Output voltage V_{out} from the module shown in table 2 is as follows

Table 2. Vibration source for 2 m/s^2.

Vibration (m/s^2)	Voltage without load (V_{tb})	R (Ω)	V_{out} (V)	Z_{out} (Ω)
2	1.4	83300	0.47	164827.7
		76900	0.45	163412.5
		71400	0.43	161065.1
		66700	0.41	161056.1
		62500	0.40	158459.6

Based on table 2, we can find that the average of the output impedance Z_{out} is 161764.2 Ω using the equation below

$$Z_{out} = \left(\frac{V_{tb} - V_{out}}{V_{out}}\right) R \tag{1}$$

where Z_{out} is the output of impedance, V_{tb} is Voltage without load, V_{out} expresses voltage output and R is resistance. To recharge battery having 160 μA, the maximum current I_{max} must be found. Using equation 2, we calculated I_{max}.

$$I_{max} = V_{tb}/(Z_{out} + R_l) \tag{2}$$

Figure 3 shows the diagram module. It consists of four piezoelectric and circuits. The piezoelectric is connected to voltage multiplier called circuit. The mechanic vibration resulting voltage. However, the voltage is low. To increase it, the circuit is needed.

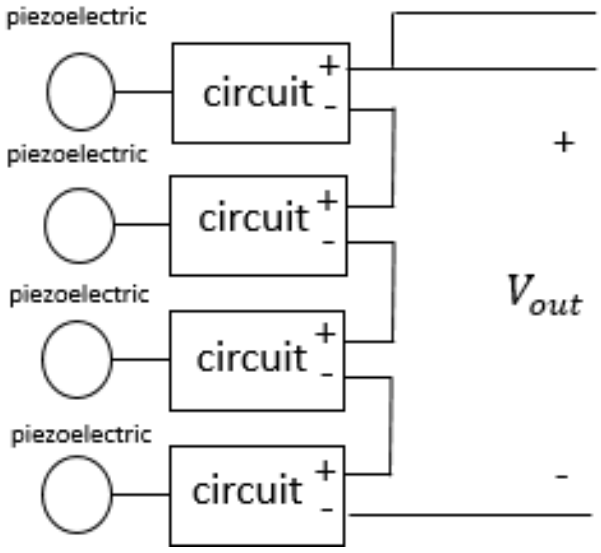

Figure 3. diagram module

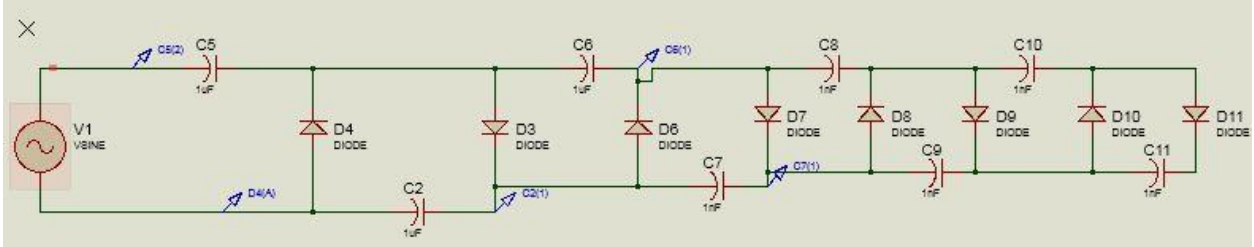

Figure 4. Schematic of Multiplier Circuit

As can be seen in Figure 4, the schematic of multiplier circuit is shown. From left side, we can see the energy source from piezoelectric having 4 series mode. It is connected to capacitor and diode. There are 8 capacitors and diodes.
Table 3 shows 1 module and 20 modules. 1 module resulting 8,65 μA. It was resulted experimentally and using equation 1 and 2. The rechargeable battery need 160 μA, so using calculation we need 20 modules.

Tabel 3. Sensor Testing

	1 Module	**20 Module**
Current	8,65 μA	160 μA

4. Conclusion

In this investigation, the mechanical vibration can be converted into electrical power. Moreover, we found that for charging 1.2 V rechargeable battery having 160 μA, we need to set up 20 modules, which have four series mode of piezoelectric and circuits.

Acknowledgements
This work was supported by LPPM Universitas PGRI Semarang.

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Comparison of Antioxidant Activity of Ethanolic, Methanolic, n-Hexan, and Aqueous Extract of Parkia speciosa Peel based on Half -Maximal Inhibitory Concentration Through Free Radical Inhibition

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Abstract. The objectives of this study was to determine the half maximum inhibitory concentration (IC_{50}) from four types of *Parkia speciosa* peel extracts (ethanol, methanol, n-hexane, and aqueous) through DPPH free radical inhibition. First Parkia's peel extract made by drying the Parkia's peel that has been sorted, then crushed and mashed with a blender. Parkia's powder then macerated for 3 replication using each type of solvent and then solvent evaporation was carried out using a rotary vacuum evaporator. The evaporated extract produced then tested for antioxidant activity using the IC_{50} method and phytochemical screening was performed to analyze the potential content of functional compounds. The results showed that all types of solvents dissolve alkaloid compounds (except water extract), flavonoids, saponins, tannins, and phenols. IC_{50} values produced from the four types of petai bark extract using methanol, ethanol, water, and n-hexane solvents sequentially were 76.92; 111; 136; and 201 ppm. Methanol extract had the lowest IC_{50} value of 76.92 ppm which resulted that the methanol extract of petai skin had a strong (active) antioxidant strength compared to others.

Keywords: Antioxidant, Exctract, Parkia's peel, IC_{50}

(Received 15 August 2020, Accepted 10 September 2020, Available Online by 30 September 2020)

1. Introduction

The use of natural materials with bioactive compounds has recently expanded. This is relating to the increase of various degenerative diseases caused by free radicals such as heart disease, arteriosclerosis, cancer, and symptoms of aging. Problems related to the function and ability of antioxidants in the body as inhibitor of cell oxidation chain due to highly reactive free radicals [1][2]. The use of traditional plants as plants that have functional values for health has been widely used and

researched in Indonesia. The content of compounds such as phenolic compounds, flavonoids, and terpenes in traditional plants functions as antioxidants, anti-inflammatory, anti-microbial and others.

Antioxidants based on the source are divided into 2 types of natural antioxidants and synthetic antioxidants [3]. Synthetic antioxidants may have negative effects if consumed for a long time. meanwhile naturalantioxidant is widely used as an ininhibitor to free radicals in the body. The limited production of endogenous antioxidants has an impact to the body to need additional exogenous antioxidants to inhibit free radicals. Therefore, exploration of natural sources of antioxidant have been continuously improved. One of the Indonesian plants source which has the potential to be explored is petai.

Petai (*Parkia speciosa*) is one of the abundant plant in Indonesia because it is easy to grow anywhere. *Parkia speciosa* peel (Parkia's peel) is a part of the petai plant which is not used and is usually thrown away as waste. Parkia's peel have potential benefit such as antioxidants, antidiabetic, and antiangiogenic. Potential benefit from Parkia'speel may become from its contain such as phenolic compounds and flavonoids in large quantities. The antioxidants contained in the extract of petai seeds and peels after being fractionated with several solvents showed that the ethyl acetate fraction of petai peel had the greatest antioxidant potential, with an IC_{50} value of 85.92 ppm. While IC_{50} from petai seeds had the greatest activity with IC_{50} value was 136.29 ppm [4].

The functional compounds found in Parkia's seed and peel extract influenced by various factors, one of them is the type of solvent used and the extraction temperature [5]. Types of solvents have different levels of polarity and solubility for certain types of compounds, while the extraction temperature determines the optimal temperature of the extraction process so that the maximum extraction is obtained. Therefore, the use of Parkia's peel as a source of natural antioxidants is very promising and it is necessary for further investigation the factors affecting antioxidant extraction process of Parkia's peels. Based on the reasons, it is necessary to carry out research on the comparative test of the antioxidant activity of the ethanol, methanol, aqueous, and n-hexane extracts of Parkias peel using the inhibitory concentration 50 (IC_{50}) test method.

2. Methods

2.1. Sample preparation

Parkia speciosa peel was separated from the seeds and then sliced to obtain uniform size. The peel then dried in a cabinet dryer at 50^{o}C, for 48 hours until completely dry. The dried peel was smoothed with a grinder and sieved with a 70 mesh sieve. The resulting powder was ready for extraction.

2.2. Extraction

The extraction process was carried out by the maceration method in an erlenmeyer and wrapped in aluminum foil. A total of 100 grams of parkiosa peel powder are macerated in Erlenmeyer for 24 hours. After 24 hours of maceration then filtered and obtained macerate, maceration is repeated up to 3 times. After the maceration process ends, the maserate or filtrate were evaporated until a concentrated sample of each type of solvent is obtained using a rotary vaccum evaporator at 40^{o}C. The concentrated sample was obtained then analyzed for phytochemical screening and testing for antioxidant DPPH.

2.3. DPPH Antioxidant Assay

Quantitative measurements of radical scavenging assay were carried out according to the method described by [5]. The quantitative measurement of radical scavenging properties was carried out in universal bottle. The reaction mixture contained 50 µl of sample at concentration ranging from 0; 20; 40; 60; 80; 100 ppm and 5 ml of a 0.04% (w/v) solution of DPPH in 80% methanol. Gallic acid was used for comparison or as a positive control. The DPPH solution in the absence of sample was used as control and the 80% methanol was used as blank. Discolourations were measured at 517 nm by using spectrophotometer (HITACHI U-1900 spectrophotometer 200V) after incubation for 30 min in the

darkroom. Measurement was performed at least in triplicate. The percentage of the DPPH free radical was calculated using the following equation:

$$\text{DPPH scavenging effect (\%)} = ((A_0 - A_1)/ A_0) \times 100$$

Where A_0 was the absorbance of the control and A_1 was the absorbance in the presence of the *Parkia speciosa* peel extract [6].

2.4. Determination of Half maximal Inhibitory concentration

The actual decrease in absorption induced by the test was compared with the positive controls. The IC_{50} (concentration providing 50% inhibition) values were calculated use the dose inhibition curve in linear range by plotting the extract concentration versus the corresponding scavenging effect.

2.5. Phytochemical Screening (Qualitative analyses)

- Alkaloid Identification
  0.5 grams of sample extract was dissolved in 5 ml hydrochloric acid, then filtered. The filtrate obtained was used as a sample solution. About 1 ml sample solution then added with 2 drops of Mayer LP reagen, a positive result showed by the formation of a yellow precipitate.
- Flavonoid Identification
  A total of 0.5 grams sample extract was added with 2 drops of NaOH solution. The formation of an intense yellow color by adding dilute acids indicates the presence of flavonoids.
- Saponin identification
  A total of 0.5 grams of sample extract was added with 2 ml of distilled water, then shaked for 10 seconds. Positive results were showed by formation of a stable foam for not less than 10 minutes
- Tanin identification
  A total of 0.5 grams of sample extract was added with 2 drops of 1 % gelatin solution in NaCl. the formation of white pellet indicates the presence of tannin
- Fenolic identification
  A total 0.5 grams of sample extract was added with 3 drops of $FeCl_3$ solution. The presence of Fenolic compound indicated by formationof greensh blue color of the sample solution.

2.6. Statistical Analysis

All the data analysis was replicated and showed as mean ± SD. Analysis of Variance were perfomed using one-way analysis of Variance (ANOVA). Significant differences between means were determined by Duncan's Test and if P value less than 0.05 were considered statistically significant. All data were analysed using SPSS 17 version programme.

3. Results and Discussion

The dried Parkiosa's peel powder was subjected to normal temperature (37^{o}C) condition of maseration process. as a result, methanolic, ethanolic, n-hexane and aqueous exctracts were obtained. The extract was analyzed for phytocemical screening, antioxidant activity based on half maximum inhibitory concentration (IC_{50}).

3.1. Yield Extract

The different types of solvents would determine the yield extract. The results of the *Parkia speciosa* peel yield extract's using various types of solvent can be seen in Table 1.

Table 1. Effect of various types of solvents on the yield percentage of the extract

Sample	powder weight (g)	final weigt ekstrak(g)	Yield (%)
Methanolic extract	20	4,56	22,80
Ethanolic extract	20	3,27	16,35

Aqueous extract	20	4,99	24,95
n-hexane extract	20	3,05	15,25

Parkia's peel dry powder was extracted by maceration for 24 hours using various types of solvents. The maserate obtained after the extraction process then concentrated with a rotary vaccum evaporator to remove the solvent, so that a concentrated extract will be obtained. In general, the extract yield ranged from 15.25 to 24.95%. The extract yield of the four types of extracts had a high enough value, in which the yield of water extract was higher than methanolic extract. This may be caused by compounds from Parkia's peel that dissolve in water more than other solvents. Methanol and ethanol are also universal solvents capable of binding or dissolving compounds derived from natural materials, both non-polar, semi-polar and polar [7].

3.2. Phytocemical screening

Phytochemical screening was carried out to determine the potential content of compounds qualitatively extracted from the material with various types of solvents, so that potential antioxidant activity can be identified. The results of phytochemical's screening are as shown in Table 2.

Tabel 2. Results of Phytochemical Screening Parkia's peel Extract using various types of solvents

Compound group	Sample extract			
	Metanolic	Ethanolic	Aqueous	n-Heksan
Alkaloid	+	+	-	+
Flavonoid	+	+	+	+
Saponin	+	+	+	+
Tanin	+	+	+	+
Fenol	+	+	+	+

The results of phytochemical screening of Parkia's peel extract with various types of solvent showed that there were compounds derived from the alkaloids (except aqueous extract), flavonoids, saponins, tannins, and phenols. Parkia's peel contains secondary metabolite compounds in the form of phenolic compounds and their derivatives that have the potential as antioxidants [8][9]. Secondary metabolic compounds that have the potential to act as antioxidants are phenolic compounds, such as phenyl propanoids, flavonoids, anthocyanins, tannins, melanins, simple monocyclic phenols, and lignins [10].

3.3. Half Maximum inhibitory concentration (IC_{50})

Quantitative antioxidant activity testing was carried out by measuring DPPH radical activity by spectrophotometry method at an absorption wavelength of 517 nm. The IC_{50} (inhibitory concentration 50) value indicates that the ability of the extract or compound to reduce or inhibit free radicals by 50%. Determination of the IC_{50} activity can be seen in Table 3.

Table 3. Determination of half maximum inhibitory concentration of each sample

Sample Concentration (ppm)	% inhibiton of methanolic extract	IC_{50}	% inhibiton of ethanolic extract	IC_{50}	% inhibiton of n-hexan extract	IC_{50}	%Inhibiton of aqueous extract	IC_{50}
0	-		-		-		-	
20	9,724		9,681		8,388		9,124	
40	20,186	76,9	17,009	111	21,063	201	16,295	136
60	33,677		25,308		35,611		21,974	
80	54,312		35,217		43,296		30,581	
100	68,325		45,748		56,002		37,129	

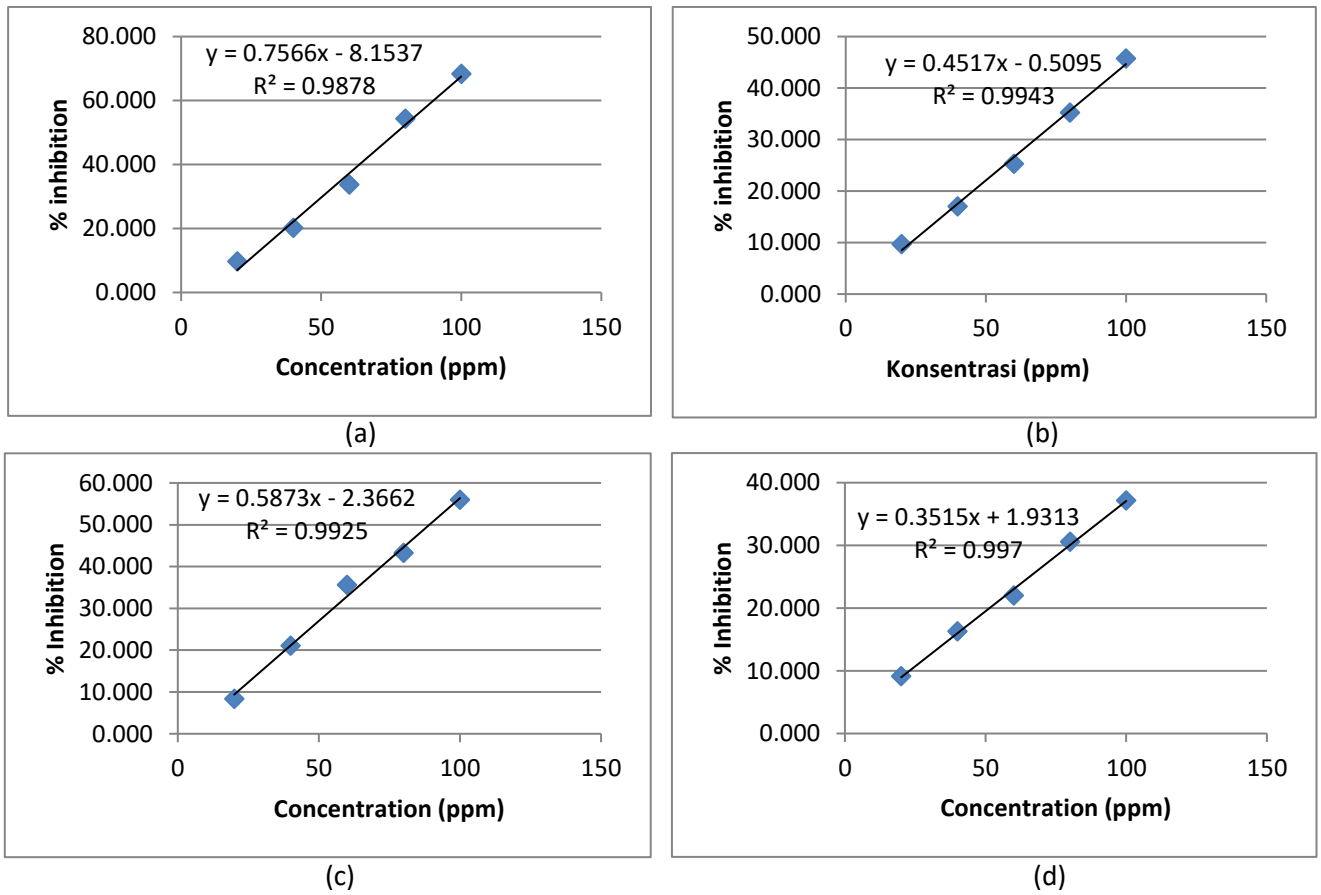

Figure 1. Regretion curve of DPPH analysis based on Typical solvent (a) methanolic extract, (b) ethanolic extract, (c) n-hexane extract, (d) aqueous extract

Antioxidant properties of Parkia's peel extracts was evaluated to find a new natural source of antioxidant. DPPH radical is a commonly used reagent for evaluation of antioxidant activity because of its stability in the radical form and simplicity of the assay [11]. The principle of this assay in the color change of DPPH solution from purple to yellow as the radical is quenched by the antioxidant [12]. The colour changes can be measured quantitatively by spectrophotometer at 517 nm.

Based on Figure 1 (a), the linear equation value for methanolic extract y = 0.756x-8.153, so the calculation of the IC_{50} value for methanoic extract obtained the following values: y = 0.756x-8.153 (for y = 50), then the x value is 76.92 ppm. The IC_{50} value of the methanol extract can be categories as active antioxidant power (50-100 ppm) [13]. Furthermore, based on the calculation of the regression curve In the ethanol extract sample (Figure 1.b), the regression equation y = 0.451x - 0.509 (assuming the value of y = 50) is obtained, the x value is 111 ppm. The IC_{50} value of the Parkia's peel ethanolic extract showed that the extract had moderate IC_{50} antioxidant activity (101-250 ppm). The value obtained is classified as having the ability to reduce moderate radicals, it happened because the possibility of the extract obtained has low purity or is in the form of crude extract.

The correlation curve between the concentration of aqueous extracts used in reducing free radical DPPH (Figure 1.d) was calculated as% inhibition against free radicals. So that the regression equation y = 0.351x + 1.931 (y value = 50) is obtained, then the x value (IC50) is 136 (ppm). The IC50 value ranging from 101-250 ppm had sufficient or moderate antioxidant reducing power [13]. While, the

calculation of IC_{50} from n-hexane extract of Parkia's peel had a value about 201 ppm. The result Showed that n-hexane extract has the highest IC_{50} value compared to the other solvents. so that in the determination of IC_{50} n-hexane extract has a low qualification of antioxidant strength (> 200 ppm).

The comparison value of IC_{50} among four typesof solvent usedin this research, the methanolic extract exhibit a significant dose dependent inhibition of DPPH activity with 50% of inhibition (IC_{50}) at concentration of 76.9 ppm (Figure 1.a). Basically, a higher DPPH radical-scavenging activity is associated with a lower IC_{50} value. There are studies have been carried out to evaluate the antioxidant activity of *Cassia* species using DPPH assay [14] and reported that, particularly *C. fistula* exhibited higher antioxidant activity compared to *C. spectabilis* [15]. Whereby the present study proof that, the *Parkia speciosa* peel extract has the potential compound(s) react as antioxidant which is suitable to develop a drugs for the prevention of human disease related to free radical mechanism.

4. Conclusion

The methanolic extract of *Parkia speciosa* peel had the highest IC_{50} activity value compared to the three types of extracts with a value of 76.92 ppm, while the ethanol extract, aqueous extract, and n-hexane extract were 111; 136; and 201 ppm, respectively. The IC_{50} value in the range between 50-100 has strong antioxidant activity, while the IC50 value in the range 101-250 includes moderate ability.

Acknowledgements

The authors are very grateful to LPPM UPGRIS for providing all facilities for this research.

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ISSN: 2715-4211