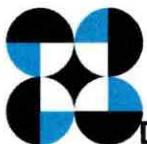


### EXECUTIVE BRIEF

Nature of Request	New for Funding																										
Project Title	<b>Transcriptome Analysis of Philippine Mangoes in Response to Anthracnose and Cecid Fly Damage</b>																										
Project Leader/ Implementing Agency	<b>Mr. John Edward L. Felipe</b> Institute of Crop Science University of the Philippines Los Baños																										
Duration Request	Two (2) Years (January 1, 2024 – December 31, 2025)																										
Funding Request	<table border="1"> <thead> <tr> <th rowspan="2">Particulars</th> <th colspan="3">Proposed Budget (PhP)</th> </tr> <tr> <th>Year 1</th> <th>Year 2</th> <th>Total</th> </tr> </thead> <tbody> <tr> <td>PS</td> <td>988,908.00</td> <td>859,308.00</td> <td>1,848,216.00</td> </tr> <tr> <td>MOOE</td> <td>2,055,237.23</td> <td>646,506.85</td> <td>2,701,744.08</td> </tr> <tr> <td>EO</td> <td>450,000.00</td> <td>-</td> <td>450,000.00</td> </tr> <tr> <td><b>TOTAL</b></td> <td><b>3,494,145.23</b></td> <td><b>1,505,814.85</b></td> <td><b>4,999,960.08</b></td> </tr> </tbody> </table>				Particulars	Proposed Budget (PhP)			Year 1	Year 2	Total	PS	988,908.00	859,308.00	1,848,216.00	MOOE	2,055,237.23	646,506.85	2,701,744.08	EO	450,000.00	-	450,000.00	<b>TOTAL</b>	<b>3,494,145.23</b>	<b>1,505,814.85</b>	<b>4,999,960.08</b>
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Counterpart Fund	PhP1,336,246.40																										
Brief Description/ Rationale	<p>Mango is an economically important fruit crop in the Philippines, with the 'Carabao' mango being the most widely cultivated and only export variety. However, the productivity, profitability, and export potential of 'Carabao' mango production in the Philippines are often hindered by low fruit quality, susceptibility to pests and diseases, such as cecid fly and anthracnose, respectively, and inconsistent yield. Developing new mango varieties with improved fruit quality and resistance to pests and diseases is a priority for the Philippine mango industry.</p> <p>To facilitate varietal improvement and increase breeding efficiency in mango, key genes and molecular mechanisms associated with the desired trait must be identified and understood. The proposed study aims to achieve this using genomics and transcriptomics tools. Specifically, the study aims to identify the underlying molecular mechanisms and genes involved in anthracnose infection and cecid fly infestation affecting postharvest fruit quality. With the recently published whole genome sequence of 'Carabao', Huani and Paho mango, utilizing RNA-seq technology for transcriptome analysis will generate a large dataset of expressed genes in selected accessions, providing a comprehensive picture of the molecular mechanisms involved in mango fruit development and ripening.</p> <p>The knowledge generated by this project will provide valuable information for molecular marker development, enabling mango breeders and researchers to develop new varieties with improved fruit quality and resistance to selected pest and diseases. Furthermore, these findings will also pave way for more advanced applications in 'Carabao' mango such as genetic engineering/gene editing, and functional and comparative genomics. The study will also contribute to a better understanding of the molecular mechanisms involved in fruit development and ripening, which is relevant not only to mango but also to other fruit crops.</p>																										

Objectives	<p><b>General:</b> To identify candidate genes involved in anthracnose and cecid fly resistance in selected Philippine mangoes using RNA-seq technology</p> <p><b>Specific:</b></p> <ol style="list-style-type: none"> <li>1. Generate a transcriptome dataset for 'Carabao' and Huani mango using RNA-seq technology;</li> <li>2. Identify differentially expressed genes associated with anthracnose disease and cecid fly resistance based on functional annotation and gene expression analysis;</li> <li>3. Validate the expression of candidate genes using quantitative qRT-PCR and evaluate their potential for use in the mango breeding program; and</li> <li>4. Develop molecular markers for the precise integration of anthracnose and/or cecid fly resistance genes in marker-assisted breeding.</li> </ol>												
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Expected Outputs	<table border="1" data-bbox="512 1006 1407 1567"> <thead> <tr> <th data-bbox="512 1006 795 1051">Outputs</th><th data-bbox="795 1006 912 1051">Year 1</th><th data-bbox="912 1006 1407 1051">Year 2</th></tr> </thead> <tbody> <tr> <td data-bbox="512 1051 795 1282">Publications</td><td data-bbox="795 1051 912 1282"></td><td data-bbox="912 1051 1407 1282"> <ul style="list-style-type: none"> <li>• At least 1 scientific article submitted in a peer reviewed journal</li> <li>• At least 1 paper presented in conference</li> </ul> </td></tr> <tr> <td data-bbox="512 1282 795 1327">People Services</td><td data-bbox="795 1282 912 1327">1 BS/MS student</td><td data-bbox="912 1282 1407 1327"></td></tr> <tr> <td data-bbox="512 1327 795 1567">Products</td><td data-bbox="795 1327 912 1567"></td><td data-bbox="912 1327 1407 1567"> <ul style="list-style-type: none"> <li>• 3 Transcriptome dataset</li> <li>• 1 RNA-seq pipeline</li> <li>• At least 1 molecular marker for MAS</li> </ul> </td></tr> </tbody> </table>	Outputs	Year 1	Year 2	Publications		<ul style="list-style-type: none"> <li>• At least 1 scientific article submitted in a peer reviewed journal</li> <li>• At least 1 paper presented in conference</li> </ul>	People Services	1 BS/MS student		Products		<ul style="list-style-type: none"> <li>• 3 Transcriptome dataset</li> <li>• 1 RNA-seq pipeline</li> <li>• At least 1 molecular marker for MAS</li> </ul>
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Potential Impacts: 2'a Social Impact  Economic Impact	<ul style="list-style-type: none"> <li>• Identified candidate genes can be used in the development of new and improved mango varieties with better fruit quality and yield, which contribute to food security and ensure a steady supply to the domestic and export markets</li> <li>• Provide training opportunities for researchers and students, which can help in building scientific capacity</li> <li>• Promote a culture of scientific inquiry and research in the Philippines</li> <li>• Identified candidate genes can be used in improving mango fruit quality which increases its export value and therefore expand its export potential; consequently this will also increase the income of mango farmers</li> </ul>												

<p><b>From Evaluating Council/ Division</b></p> <p><b>Technical Merit</b></p> <p><b>Technologies that will be generated</b></p>	<p>The proposed project is aligned with the Industry Strategic S&amp;T Program (ISP) for Mango, as a component of the DOST-PCAARRD mango breeding program, and an offshoot of the recently completed project titled, "Full Genome Sequencing of Selected Philippine Mango Species." Specifically, the proposed project will address the gap under varietal improvement wherein there is only one export variety and provide the necessary information and technology towards marker-assisted selection (MAS) for mango. The project also aims to generate molecular markers associated with anthracnose and/or cecid fly resistance.</p> <p>The knowledge generated will provide valuable information for molecular marker development, enabling mango breeders and researchers to develop new varieties with improved fruit quality and resistance to a specific pest and disease, which is a priority for the Philippine mango industry. Overall, the study will contribute to a better understanding of the molecular mechanisms involved in fruit development and ripening, which is relevant not only to mango but also to other fruit crops.</p> <p>The proposed project will identify candidate genes involved in anthracnose and/or cecid fly resistance in selected Philippine mangoes using RNA-seq technology, and generate transcriptome dataset, RNA-seq pipeline, and at least 1 molecular marker for MAS</p>
<p><b>Socio-Economic Benefit/ Environmental Impact/ Tangible Benefits</b></p>	<p>The proposed project will increase productivity and efficiency in the mango breeding program using MAS and thereby play a significant role in developing new varieties with promising traits. This will result in potential increase in income for mango farmers and other stakeholders involved in the mango industry with the use of improved varieties. Moreover, there will be reduced adverse effects on the environment due to pesticide and fungicide use when resistant varieties are developed.</p>
<p><b>Remarks/ Recommendations</b></p>	<p>The proposed GREAT re-entry project proposal is recommended for implementation and funding under DOST-PCAARRD-GIA.</p>



**DOST Form 2 (for Basic/Applied Research)**  
**DETAILED RESEARCH & DEVELOPMENT PROJECT PROPOSAL**

**(1) PROJECT PROFILE**

Program Title:

Project Title: **Transcriptome Analysis of Philippine Mangoes in Response to Anthracnose and Cecid Fly Damage**

Project Leader/Sex: **John Edward L. Felipe/Male**

Project Duration (number of months): **24 months**

Project Start Date: **January 1, 2024**

Project End Date: **December 31, 2025**

Implementing Agency (Name of University-College-Institute, Department/Organization or Company): **University of the Philippines Los Baños, College of Agriculture and Food Science, Institute of Crop Science**

Address/Telephone/Fax/Email (Barangay, Municipality, District, Province, Region): **Batong Malake, Los Baños, District 2, Laguna, Region IV-A / 09190078776 / jlfelipe@up.edu.ph**

**(2) COOPERATING AGENCY/IES** (Name/s and Address/es)

N/A

**(3) SITE(S) OF IMPLEMENTATION**

IMPLEMENTATION SITES NO.	COUNTRY	REGION	PROVINCE	DISTRICT	MUNICIPALITY	BARANGAY
1.	Philippines	IV-B	Laguna	District 2	Los Baños	Batong Malake
2.						
3.						
4.						
5.						

**(4) TYPE OF RESEARCH**

Basic  
 Applied

**(5) R&D PRIORITY AREA & PROGRAM (based on HNRDA 2017-2022)**

Agriculture, Aquatic and Natural Resources  
Commodity: Mango  
 Health  
 Priority Topic: \_\_\_\_\_  
 Industry, Energy and Emerging Technology  
Sector: \_\_\_\_\_  
 Disaster Risk Reduction and Climate Change Adaptation  
 Basic Research  
Sector: \_\_\_\_\_

**Sustainable Development Goal (SDG) Addressed**

- Zero Hunger (SDG 2)
- Good Health and Well-being (SDG 3)
- Industry, Innovation and Infrastructure (SDG 9)

**(6) EXECUTIVE SUMMARY** (not to exceed 200 words)

This proposal primarily aims to identify candidate genes associated with anthracnose and cecidfly resistance in Philippine 'Carabao' mango using RNA-seq technology, and develop molecular markers for marker-assisted breeding. The traditional breeding program for mango is slow and challenging, and molecular tools can facilitate varietal improvement and increase breeding efficiency. This project will use RNA-seq technology to identify the underlying molecular mechanisms and genes involved in disease and insect resistance traits that significantly affect the postharvest fruit quality of 'Carabao' mango. The project aims to generate a large dataset of expressed genes in selected accessions to provide a comprehensive picture of the molecular mechanisms involved in mango fruit

development and ripening. The knowledge generated will provide valuable information for molecular marker development, enabling mango breeders and researchers to develop new varieties with improved fruit quality and resistance to a specific pest and disease, which is a top priority for the Philippine mango industry. Overall, the study will contribute to a better understanding of the molecular mechanisms involved in fruit development and ripening, which is relevant not only to mango but also to other fruit crops.

## (7) INTRODUCTION

Mango (*Mangifera indica L.*) is a major fruit crop in the tropical and subtropical regions, particularly in Asia. It has been widely cultivated in India and Southeast Asia for thousands of years due to its large fruit with a soft, sweet pulp (Kuhn et al., 2017; Mukherjee & Litz, 2009). In the Philippines, mango is one of the most important fruit crops ranking third in terms of volume of production (741,694 MT) and area planted (185,900 ha) after banana and pineapple (PSA, 2021). It is also one of the leading export fruits in the country, with the 'Carabao' mango being the only variety exported (DA-BAR, 2022). This variety accounts for 80% of the country's total mango production and acclaimed as one of the best variety in the world (DA-BAR, 2022). Despite its excellent qualities, the export potential of 'Carabao' mango is not fully attained due to its short shelf life, thin peel and low fruit quality and production yield attributed to genetic factors, and susceptibility to insect pests and pathogenic diseases. Developing new mango varieties with improved fruit quality and resistance to pests and diseases is, therefore, a top priority for the Philippine mango industry.

Traditional mango breeding programs has been slow and challenging and often takes more than 20 years. This is due to a number of factors such as long juvenile stage, long generation times, high heterozygosity, low crossing rates (0.1%) from high numbers of flowers per panicle, a very high level of fruitlet drop, and only a single seed per flower resulting in a low number of fruits (Bally et al., 2009; Kuhn et al., 2017). In addition, polyembryonic cultivars such as the 'Carabao' mango produce multiple apomictic seedlings that arise from maternal nucellar tissues around the single zygotic embryo, making it difficult to differentiate from the true hybrid (Asker & Jerling, 1992) . Moreover, breeding efficiency is reduced by poor understanding of genetics of important horticultural traits, and the lack of genotypic and phenotypic diversity among the commercial mango cultivars (Kuhn et al., 2017).

Transcriptomics, the study of the entire set of RNA molecules transcribed from a genome, has emerged as a powerful tool for identifying candidate genes involved in specific biological processes, including those related to fruit quality improvement. In this proposed study, we aim to identify candidate genes associated with fruit quality traits in Philippine 'Carabao' mango using RNA -seq technology and develop molecular markers for marker-assisted breeding. The proposed study will address the gaps in knowledge regarding the underlying molecular mechanisms and genes involved in controlling fruit quality traits in 'Carabao' mango such as fruit size, peel color, texture, resistance to anthracnose and fruit fly, etc. Such knowledge will provide valuable information for mango breeders to develop new varieties with improved fruit quality and resistance to pests and diseases. We hypothesize that transcriptome analysis of 'Carabao' mango varieties will identify differentially expressed genes associated with disease and insect resistance, and other fruit quality traits. These genes can be validated using quantitative RT-PCR and used to develop molecular markers for marker-assisted breeding. Overall, this proposed research project aims to enhance our understanding of the molecular basis of fruit quality in 'Carabao' mango and contribute to the development of new mango varieties with improved traits that meet the demands of the market and ensure the sustainability of the Philippine mango industry.

### (7.1) RATIONALE/SIGNIFICANCE (not to exceed 300 words)

Mango is an economically important fruit crop in the Philippines, with the 'Carabao' mango being the most widely cultivated and only export variety. However, the productivity, profitability, and export potential of 'Carabao' mango production in the Philippines are often hindered by low fruit quality, susceptibility to pests and diseases, and inconsistent yield. Developing new mango varieties with improved fruit quality and resistance to pests and diseases is, therefore, a priority for the Philippine mango industry. To facilitate varietal improvement and increase breeding efficiency in mango, key genes and molecular mechanisms associated with the desired trait must be identified and understood. The proposed study aims to achieve this using genomics and transcriptomics tools. Specifically, the study aims to identify the underlying molecular mechanisms and genes involved in

anthracnose infection and cecid fly infestation affecting postharvest fruit quality. With the recently published whole genome sequence of 'Carabao', Huani and Paho mango, utilizing RNA-seq technology for transcriptome analysis will generate a large dataset of expressed genes in selected accessions, providing a comprehensive picture of the molecular mechanisms involved in mango fruit development and ripening. The knowledge generated by this project will provide valuable information for molecular marker development, enabling mango breeders and researchers to develop new varieties with improved fruit quality and resistance to selected pests and diseases. Furthermore, these findings will also pave way for more advanced applications in 'Carabao' mango such as genetic engineering/gene editing, and functional and comparative genomics. The study will also contribute to a better understanding of the molecular mechanisms involved in fruit development and ripening, which is relevant not only to mango but also to other fruit crops.

## (7.2) SCIENTIFIC BASIS/THEORETICAL FRAMEWORK

In recent years, transcriptome analysis has been used as a powerful tool to understand gene expression and regulatory networks in different plant varieties. Comparative transcriptome analysis can be used to identify differentially expressed genes and pathways that are involved in specific traits or processes, providing insights into the molecular basis of phenotypic variation between varieties. In mango, transcriptome analysis has been used to study the genes involved in fruit ripening (Dautt-Castro et al., 2018; Deshpande et al., 2017; Wu et al., 2014), stress and defense response (Hong et al., 2016; Luria et al., 2014), sugar metabolism (Li et al., 2020), cuticle biosynthesis (Tafolla-Arellano et al., 2017), and secondary metabolism (Bajpai et al., 2018; Khan et al., 2017; Xin et al., 2021).

However, varieties or cultivars of the same plant species often have different genetic backgrounds, which can lead to differences in gene expression and the resulting transcriptome. For example, different varieties of mango may have different levels of expression for genes involved in fruit ripening or pathogen resistance. Additionally, different varieties may respond differently to environmental stresses or stimuli, resulting in differences in gene expression and transcriptome profiles. Hence, it is important to conduct variety-specific transcriptome analysis and ideally using a suitable reference genome that can accurately represent the genetic background of the variety being studied.

With the recently sequenced full genome of the export variety 'Carabao' mango (*Mangifera indica*) and two other Philippine mango species, *M. odorata*, which shows possible resistance to fruitfly and anthracnose; and *M. altissima*, a self-pollinating species of mango, there is now a comprehensive resource for transcriptome analysis of Philippine mango varieties. The advantage of having a reference genome from the same variety or cultivar being studied in RNA-seq analysis is that it provides a high-quality reference genome that can be used as a basis for transcriptome analysis. With a reference genome, it is possible to accurately map and quantify gene expression levels, identify alternative splicing events, detect single nucleotide polymorphisms (SNPs), and perform functional annotation of the expressed genes. Additionally, a reference genome can aid in the identification of candidate genes for fruit quality improvement, such as those involved in biosynthesis and regulation of important fruit quality traits.

Using a reference genome from a different variety or cultivar may result in misalignments and bias in gene expression analysis, as genetic differences between varieties/cultivars can lead to variation in transcript sequences and expression levels. Therefore, having a reference genome from the same variety or cultivar can improve the accuracy and reliability of RNA-seq analysis results, leading to more meaningful and informative results that can aid in breeding programs for fruit quality improvement.

## (7.3) OBJECTIVES

### **General:**

To identify candidate genes involved in anthracnose and cecid fly resistance in selected Philippine mangoes using RNA-seq technology

### **Specific:**

1. Generate a transcriptome dataset for 'Carabao' and Huani mango using RNA-seq technology;

2. Identify differentially expressed genes associated with anthracnose disease and cecid fly resistance based on functional annotation and gene expression analysis;
3. Validate the expression of candidate genes using quantitative qRT-PCR and evaluate their potential for use in the mango breeding program; and
4. Develop molecular markers for the precise integration of anthracnose and cecid fly resistance genes in marker-assisted breeding.

## **(8) REVIEW OF LITERATURE**

### **Botany and Agricultural Importance of Mango**

Mango (*Mangifera indica* L.) is a perennial fruit tree and a commercially important tropical fruit crop of Asia. The mango tree is erect, with height ranging from 30 to 70 ft (10–40 m), and evergreen with symmetrical, round and broad canopy, or more upright with a relatively slender crown. Its flowers are yellowish or reddish borne in inflorescences appearing at branch terminals in dense panicles of up to 2000 glabrous or pubescent minute flowers (Mukherjee & Litz, 2009; Tharanathan et al., 2006). The minute flowers, which are only 5–10 millimeter in diameter, are monoecious, polygamous, and hermaphrodite arranged in a pseudoterminal, rigid, erect and widely branched inflorescence. Both male and perfect flowers are found in an inflorescence but the pistil aborts in male flowers. Cross-pollination is aided by flies, wild bees, wasps, moths, beetles, etc. (Lakshminarayana, 1980; Mukherjee, 1997; Singh, 1990; Tharanathan et al., 2006). The mango fruit is a fleshy, resinous drupe that varies in size, shape, color, fiber content, flavor, and taste (Mukherjee & Litz, 2009). A formation of a small conical projection developing laterally at the proximal end of the fruit, is a characteristic feature of mango known as the "beak" (Tharanathan et al., 2006). The pericarp is composed of a smooth exocarp, fleshy mesocarp, and stony endocarp. The exocarp develops into a smooth, green and waxy, leathery protective skin that changes to a pale green or yellow with red blush (depending on the cultivar) when ripe (Mukherjee & Litz, 2009; Tharanathan et al., 2006). The mesocarp is composed of the firm and fleshy edible pulp with flavors ranging from turpentine to sweet. The pulp can be fibrous or fiber free according to cultivar types. The fruit also contains chlorophyll, carotenes, anthocyanins, and xanthophylls. The chlorophyll disappears during ripening while the anthocyanins and carotenoids increase with maturity (Lakshminarayana, 1980). The endocarp develops into thick, tough, leathery, glandular covering of the ex-albuminous seed. The seed is solitary, stony, hard, large and flat, ovoid-oblong, or kidney shaped with a thin and papery seed coat. At maturity, the seed is surrounded by the fibrous endocarp. Depending on the variety, the seed may be monoembryonic or polyembryonic which can produce one or several seedlings, respectively (Mukherjee, 1997; Mukherjee & Litz, 2009; Singh, 1990; Tharanathan et al., 2006).

Mango is the fifth most cultivated fruit in the world with more than one thousand varieties grown in Asia, Central and South America, and Africa. In 2010, the global production of mango fruit reached up to over 38.67 MT fruit annually, and is concentrated mainly in Asia which accounts for 76.5% production (Mitra, 2016). Trade in mango products has also tripled from total exports valued at USD 696 million in 2005 to almost USD 2 billion in 2015 (Fernandez-Stark et al., 2017). Despite the increasing demand in developed countries, approximately 3-4% only of the global production is traded internationally and the rest is traded and consumed domestically (Mitra, 2016).

In the Philippines, mango is one of the most important fruit crops ranking third in terms of volume of production (741,694 MT) and area planted (185,900 has) after banana and pineapple (PSA, 2021). It is also one of the leading export fruits in the country, with the 'Carabao' mango being the only variety exported. In 2021, about 10.08 thousand metric tons of fresh mangoes were exported with a value of PhP 617.78 million, which corresponds to -18.3 percent decline compared to previous years (PSA, 2022).

### **Fruit Quality Traits of Mango**

Fruit quality traits are important characteristics that affect consumer acceptance, marketability, and exportability of mango fruit. The most important fruit quality traits of mango include resistance to postharvest disease and insect pests, peel color, fruit size, shape, aroma, taste, texture, and nutritional value (Islam et al., 2017; Singh and Srivastava, 2018).

Fruit color is an essential trait, and it is used as a measure of ripeness and marketability. The size and shape of the fruit determine the yield and ease of harvest. The aroma and taste of the fruit are critical for consumer acceptance, while the texture of the fruit is important for processing and storage. In addition, resistance to disease and insect pests is also an essential quality trait as mango is susceptible to various diseases and insect pests that can cause significant yield losses and affect fruit quality. Resistance to diseases and insect pests is critical for sustainable mango production (Sarwar et al., 2019). Peel thickness is another important fruit quality trait. Thick peel is desirable in mango fruit as it protects the fruit from physical damage during transportation and storage. It also helps in reducing water loss and maintains fruit freshness. However, excessive peel thickness can make it difficult to peel the fruit, reducing consumer acceptability (Islam et al., 2017).

Several factors can influence these traits, including environmental factors, cultural practices, and postharvest handling. Environmental factors, such as temperature, light, and water availability, can affect fruit size, color, and flavor (Jha et al., 2021). Cultural practices, such as pruning, fertilization, and irrigation, can affect fruit yield, size, and quality (Mitra et al., 2019). Postharvest handling practices, such as storage temperature and duration, can affect fruit shelf life, color, and texture (Siddique et al., 2020).

Fruit quality traits in mango are influenced by genetic factors as well. The inheritance of these traits is complex, with many genes and environmental factors interacting to produce the final fruit phenotype. Advances in genomics and molecular biology have allowed for the identification of candidate genes and quantitative trait loci (QTLs) associated with fruit quality traits in mango. These genes are involved in various biological processes, including sugar metabolism, pigment biosynthesis, and aroma biosynthesis (Kumar et al., 2019).

The identification of candidate genes and QTLs associated with fruit quality traits is crucial for the improvement of mango fruit quality through breeding and biotechnology. The selection and introgression of favorable alleles can improve fruit size, color, flavor, and nutritional content. Furthermore, the manipulation of gene expression using gene editing technologies such as CRISPR/Cas9 can lead to the development of new cultivars with desired fruit quality traits (Grosser et al., 2020).

### **Transcriptome Sequencing (RNA-seq)**

RNA sequencing (RNA-seq) technology is used to map and quantify transcribed RNA and has become a powerful tool for studying entire transcriptomes (Wang et al., 2009; Zhang et al., 2018). RNA-seq can identify mRNAs, non-coding RNAs, and small RNAs, as well as provide information on alternative splicing and novel isoforms of mRNA transcripts (Chen et al., 2016; Kim & Salzberg, 2011; Trapnell et al., 2009). Compared to hybridization-based methods, RNA-seq has advantages such as not requiring prior knowledge of the query genome, detecting novel transcripts, having high reproducibility, low noise level, and a broader dynamic range for quantifying gene expression levels (Hurd & Nelson, 2009; Zhao et al., 2014).

RNA sequencing has emerged as a preferred method over microarrays for gene expression profiling because it allows the entire transcriptome to be surveyed in a very quantitative and high-throughput manner (Ching et al., 2014; Wang et al., 2009). The RNA sequencing process involves purifying RNA samples, shearing them, converting them to cDNA, and sequencing them on next-generation sequencing (NGS) platforms.

One of the most widely used platforms is Illumina sequencing, which uses reversible terminators to sequence millions of short reads in parallel (Metzker, 2010). This platform has high accuracy and throughput, making it suitable for studying transcriptomes at a high resolution. Another platform is the Ion Torrent, which uses semiconductor technology to detect hydrogen ions generated during nucleotide incorporation (Rothberg et al., 2011). This platform has the advantage of fast turnaround time and low input requirements, but its accuracy decreases as the read length increases.

PacBio sequencing is another technology used for transcriptome sequencing, which utilizes single-molecule real-time sequencing to generate long reads (Eid et al., 2009). This platform has the advantage of producing full-length transcripts, enabling the identification of alternative splicing events

and isoforms. However, it has a high error rate and lower throughput compared to Illumina sequencing.

Nanopore sequencing is a newer technology that uses a nanopore-based sensor to detect changes in electrical current as individual nucleotides pass through the pore (Jain et al., 2016). This platform has the advantage of generating long reads and real-time sequencing, allowing for the detection of RNA modifications and alternative splicing events. However, it has a higher error rate and lower throughput compared to Illumina sequencing.

Despite its advantages, RNA-seq also has limitations. One major limitation is the requirement for high-quality RNA samples, as degraded RNA can lead to biases in gene expression quantification (Wang et al., 2016). Another limitation is the cost associated with sequencing and bioinformatics analysis, making it less feasible for large-scale studies (Jain & Tuteja, 2019). Additionally, RNA-seq is susceptible to sequencing biases and errors, which can impact the accuracy of quantification (Wang et al., 2016). Nevertheless, RNA-seq remains a valuable tool for exploring transcriptomes, discovering novel transcripts, and understanding alternative splicing and non-coding RNAs.

### **Analysis of Transcriptome Data**

Transcriptome analysis has become a vital tool in studying gene expression in various organisms. The analysis of transcriptome data can provide insights into the biological processes that occur within an organism, and the process can be broken down into several steps, including pre-processing of raw data, quality control of raw data, sequence alignment and assembly, functional annotation of expressed genes, and differential gene expression analysis.

Pre-processing of raw data is an essential step in transcriptome data analysis. Pre-processing involves removing low-quality reads, trimming adapter sequences, and removing contaminants. The removal of low-quality reads helps to reduce noise and improve the accuracy of downstream analysis (Wang et al., 2018). The trimming of adapter sequences helps to improve the quality of reads by removing any remaining adapter sequences that may have been present in the original reads (Andrews, 2010).

Quality control of raw data is another crucial step in transcriptome data analysis. Quality control ensures that the pre-processed data is of high quality, and any low-quality reads that were not removed during pre-processing are identified and filtered out (Jain et al., 2015). Quality control measures such as the assessment of sequencing depth, GC content, and sequence duplication levels help to ensure that the data is of high quality and suitable for downstream analysis.

Sequence alignment and assembly is the next step in transcriptome data analysis. This step involves aligning the reads to a reference genome or de novo assembly. The alignment of reads to a reference genome allows for the identification of known genes and isoforms. De novo assembly, on the other hand, is useful when no reference genome is available, and allows for the identification of novel genes and isoforms (Haas et al., 2013).

Functional annotation of expressed genes is another critical step in transcriptome data analysis. Functional annotation provides information about the biological functions and pathways associated with expressed genes. Functional annotation involves assigning Gene Ontology (GO) terms and KEGG pathways to the expressed genes (Ashburner et al., 2000; Kanehisa et al., 2008). The identification of enriched pathways and biological processes can provide insights into the biological functions of the expressed genes.

Differential gene expression analysis is the final step in transcriptome data analysis. Differential gene expression analysis involves comparing the expression levels of genes between different conditions or treatments. Differential gene expression analysis can help identify genes that are differentially expressed in response to a particular treatment or condition. The identification of differentially expressed genes can provide insights into the molecular mechanisms underlying biological processes (Trapnell et al., 2013).

## Gene Expression Analysis

Gene expression analysis is a process that involves investigating the pattern of expressed genes at the transcription level in a specific cell or under particular circumstances. To date, several molecular techniques, such as serial analysis of gene expression (SAGE), massive parallel signature sequencing (MPSS), microarray expression profiling, and RNA-seq analysis, have been used to study gene expression patterns (Frigessi et al., 2005; Jain, 2012; O'Brien et al., 2012; Wang et al., 2009). The relative abundances of RNAs transcribed from genes provide valuable information about the expression level of the corresponding genes during specific developmental stages or physiological conditions (Finotello & Di Camillo, 2015). RNA-seq combined with bioinformatics tools has become an excellent approach to identify and quantify transcript expression and their isoforms (Zhang et al., 2017).

For an RNA-seq study aimed at detecting differentially expressed genes, a well-annotated transcriptome assembly is necessary. The basic data processing pipeline includes read mapping, read counting, counts normalization, and detection of differentially expressed genes (Finotello & Di Camillo, 2015). The read mapping process involves mapping reads to the reference genome or to the transcriptome sequences reconstructed using de novo assembly strategies (Figure 1) (Lunter & Goodson, 2011). This process aims to identify the genomic location where each short read best matches the reference genome or transcript set (Langmead & Salzberg, 2012). To obtain quantitative expression data, RNA-seq reads are mapped to a reference sequence to produce SAM (Sequence Alignment/Map) files that contain information about the reference and read sequences, the mapping location of each read, and the quality of alignment (Li et al., 2009; Langmead & Salzberg, 2012). This can be performed using Bowtie, a bioinformatics tool for short read alignment that can align over 25 million reads per hour and quantify transcript abundances (Langmead et al., 2009; Li & Dewey, 2011)

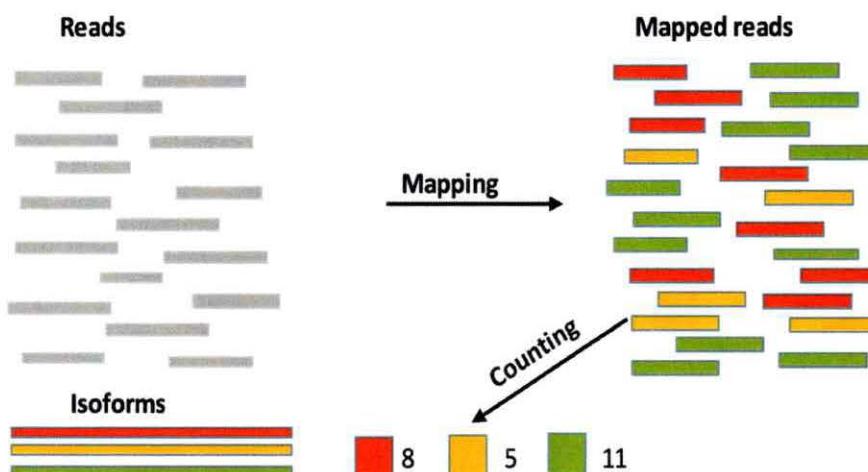


Figure 1. Read mapping and counting process. Figure taken from Krivenjeva (2018)

Once read mapping is complete, read counting is performed by counting the number of reads that have mapped to the gene and isoform levels (Finotello & Di Camillo, 2015). Since RNA-seq reads do not always map uniquely to a single gene or isoform, this process can be complicated (Li & Dewey, 2011). However, RSEM, a software tool for quantifying transcript abundances, has been developed to estimate isoform expression levels using the Expectation-Maximization algorithm (Li & Dewey, 2011; Liu, 2014). RSEM records every possible alignment and their quality score for each read, which is used to determine the count estimates.

Lastly, normalization based on transcript length and based on expression level between libraries is performed in RNA-seq data analysis. This is to remove systematic technical effects and ensure that technical bias has minimal impact on the results (Oshlack and Wakefield, 2009). This can be performed using a scaling factor called Trimmed Mean of M-values (TMM) integrated in edgeR (Empirical analysis of Differential Gene Expression in R) RNA-seq analysis package written in R language. Using appropriate normalization methods, technical bias can be minimized, ensuring the accuracy of the results.

## **Transcriptome Studies in Mango**

Transcriptome studies have been conducted to investigate the molecular mechanisms underlying various aspects of mango fruit development, ripening, and response to stresses. Azim et al. (2014) characterized the transcriptome and chloroplast genome of mango using next-generation sequencing technology and identified several genes involved in carbohydrate metabolism, lipid metabolism, and secondary metabolism pathways. Wu et al. (2014) investigated the molecular mechanisms of fruit development and ripening in mango using transcriptome and proteomic analysis and identified differentially expressed genes involved in fruit development, ripening, and senescence, as well as several proteins involved in sugar metabolism and flavor synthesis.

Luria et al. (2014) performed de novo assembly of mango fruit peel transcriptome to understand the molecular mechanisms underlying the response of mango to hot water treatment and identified several genes involved in heat shock response, pathogen defense, and oxidative stress response. Similarly, Dautt-Castro et al. (2015) analyzed the mesocarp transcriptome of mango cv. Kent to identify gene families important for ripening and identified several genes involved in ethylene biosynthesis, sugar metabolism, and cell wall modification.

Hong et al. (2016) characterized the transcriptome and expression profiles of defense genes in postharvest mango fruit against *Colletotrichum gloeosporioides* and identified several genes involved in plant-pathogen interactions and defense signaling pathways. The up-regulated genes include MYB & WRKY transcription factors, ethylene response factors (ERFs) genes, nucleotide binding site-leucine-rich repeats (NBS-LRRs) genes, nonexpressor of pathogenesis-related genes (NPRs) genes and pathogenesis-related protein (PRs) genes. Sivankalyani et al. (2016) investigated the transcriptome dynamics in mango fruit peel under chilling stress and identified several genes involved in cold acclimation, transcription factors, membrane transporters, and stress-responsive genes.

Deshpande et al. (2017) analyzed the transcriptional transitions during Alphonso mango fruit development and ripening to explain its distinct aroma and shelf life characteristics and identified several genes involved in ethylene biosynthesis and signaling, as well as genes involved in flavor synthesis. Tafolla-Arellano et al. (2017) performed transcriptome analysis of mango fruit epidermal peel to identify putative cuticle-associated genes and identified several genes involved in cuticle biosynthesis, as well as genes involved in stress response and signaling pathways.

Dautt-Castro et al. (2018) analyzed the mesocarp RNA-Seq of mango to identify the effects of quarantine postharvest treatment on gene expression and identified several genes involved in fruit development, ripening, and stress response pathways. Bajpai et al. (2018) investigated the molecular basis of anthocyanin biosynthesis in mango peel and identified several genes involved in the anthocyanin biosynthesis pathway, as well as genes involved in sugar metabolism and stress response.

Khanum et al. (2020) studied the adaptation mechanism of mango fruit to heat stress by analyzing the transcriptome and metabolic profiles and identified several genes involved in heat shock response, oxidative stress response, and secondary metabolism pathways. Li et al. (2020) generated the chromosome-scale reference genome of mango using single-molecule real-time (SMRT) sequencing technology and identified several genes involved in fruit development, ripening, and stress response pathways, as well as several genes involved in flavor synthesis.

Xin et al. (2021) performed dynamic analysis of the transcriptome and metabolic profiling of mango to understand the molecular basis of fruit ripening and softening and identified differentially expressed genes involved in ethylene biosynthesis and signaling, cell wall modification, sugar metabolism, and flavor synthesis.

## **(9) METHODOLOGY**

#### A. Sample collection, characterization, and RNA extraction

Mango fruit samples will be sourced primarily from plantation or backyard farms with an available fruit-bearing mango tree of the desired variety or species. Approximately 30 fruits with minimal to no blemishes at the hard green stage will be collected randomly from each independent tree identified, and will be transported immediately back to the laboratory for de-sapping and washing. For each accession, three biological replicates will be used in the transcriptome analysis.

For fruit quality characterization and analysis, reaction to anthracnose disease and cecid fly infestation will be evaluated. Based on the data from previous varietal characterization and evaluation study of Mango Varietal Improvement Program at UPLB IPB, Huani (*Mangifera odorata*) and one susceptible 'Carabao' (*M. indica*) strain (e.g. 'Sweet Elena') will be used for the anthracnose resistance characterization. Artificial inoculation method will be performed on fruit samples using the droplet method (Grice et al., 2022). Fruit peel tissues will be collected from 30mm around the inoculation point at regular intervals. Subsequently, total RNA will be extracted from the collected peel tissues and pooled per biological replicate for the construction of cDNA library (Figure 2).

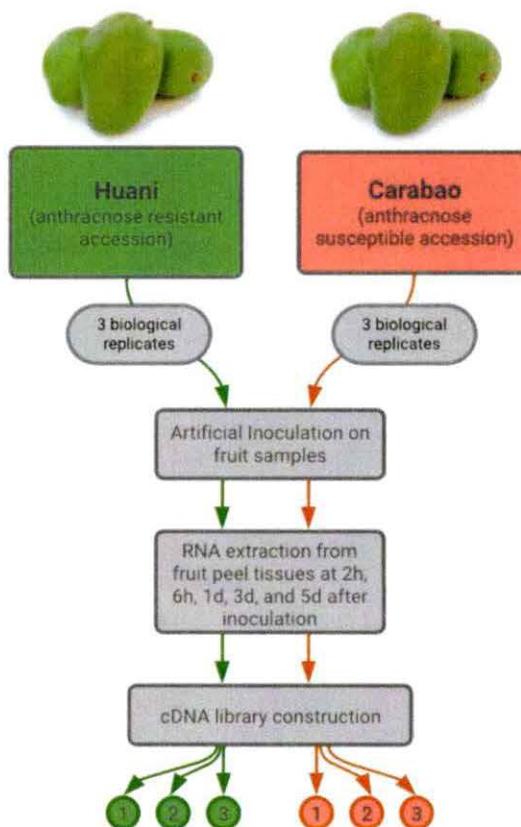


Figure 2. Schematic diagram of the experimental treatments for anthracnose resistance evaluation

For cecid fly infestation, preliminary evaluation and screening will be performed to identify two specific accessions of 'Carabao' and Huani that will be used for the comparative transcriptome analysis. The summary of the proposed experimental treatments to identify DEGs associated with cecid fly resistance is shown in Figure 3.

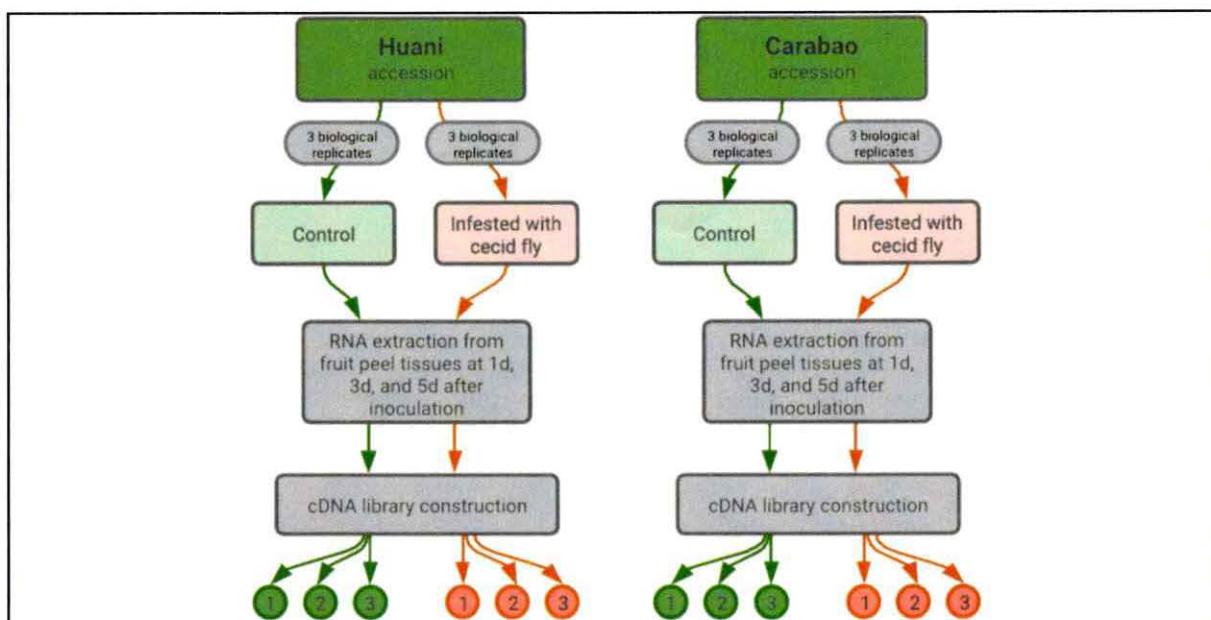


Figure 3. Schematic diagram of the experimental treatments for cecid fly resistance evaluation.

For transcriptome sequencing, high-quality total RNA will be isolated using commercially available RNA extraction kits. For gene expression analysis, total RNA may be isolated using previously described isolation protocols. RNA quality and integrity will be evaluated using agarose gel electrophoresis and spectrophotometry, and working stocks will be prepared and stored at -20°C until further use.

### B. RNA-seq library preparation and sequencing

Total RNA isolated from fruit peel samples will be used to carry out reverse transcription for synthesis of cDNA using commercially available kits. The constructed cDNA library will be sent to a selected servicing facility for sequencing on NovaSeq6000 or similar platform with at least 100 bp paired-end reads.

The raw sequencing reads will be quality checked and pre-processed using available bioinformatics tools such as FastQC and Trimmomatic to filter low quality sequences and empty reads, and to trim adapter sequences.

### C. Transcriptome assembly and functional annotation

The pre-processed sequence reads will be assembled by mapping to the recently published reference genomes of three Philippine mango species *Mangifera indica* ‘Carabao’, *Mangifera odorata* (Huani), and *Mangifera altissima* (Paho) using splice-aware aligner tools such as HISAT2, Tophat2, STAR, or Trinity software. The quality and completeness of the assembled transcripts will be checked using Qualimap or BUSCO to evaluate biases in the sequencing and/or mapping of the data. Potential candidate coding regions within transcripts will then be extracted from the assembled transcripts using available software such as Transdecoder (<https://transdecoder.github.io/>) to identify non-redundant sequences for downstream analysis. Quantification will be performed using htseq-count, featureCounts, or Quasi-mapping tools such as Salmon. Differentially expressed (DE) genes will be determined using differential expression analysis tools such as DESeq2, NOISeq, etc.

Assembled unigenes will then be searched against several protein databases, such as the Nr using the BLASTx program, the Swiss-Prot protein database (<http://www.expasy.ch/sprot>), KEGG database (Kanehisa et al., 2006) or the COG database (<http://www.ncbi.nih.gov/COG>). Functional annotation, enzyme code distribution and gene ontology (GO) mapping and InterProScan analyses

will be performed using BLAST2GO or other available bioinformatic analysis tools (e.g. EnrichR, GSEA, etc.).

#### D. Quantitative RT-PCR analysis and validation

At least 5 candidate DEGs associated with each trait of interest will be selected based on the RNA-seq data, including upregulated and down-regulated genes in various tissue or genotype source. Expression of DEGs will be validated using qRT-PCR amplification. Total RNA will be isolated and purified using available kits or according to previous studies. cDNA synthesis will be performed using commercially available kits. Gene-specific primers will be designed using Primer3 or Primer-BLAST, and primer specificity and efficiency will be validated using standard curves. RT-qPCR will be performed based on existing protocols using commercially available SYBR Green premix kits on a BioRad Real-Time PCR Detection System. Previously described endogenous genes such as *Miactin* or elongation factor 1 $\alpha$  (*EF1 $\alpha$* ) will be used as reference genes (Hong et al, 2015; Deshpande et al, 2017). All qRT-PCR reactions will be normalized using the reference gene Ct value, and the expression levels of target genes will be calculated according to the formula  $2^{-\Delta\Delta Ct}$  (Livak and Schittgen, 2001).

#### (10) TECHNOLOGY ROADMAP (if applicable) (use the attached sheet)

#### (11) EXPECTED OUTPUTS (6Ps)

##### Publications (2)

- At least 1 scientific article submitted in a peer reviewed journal
- At least 1 paper presented in conference

##### Patents/IP (N/A)

##### Products (5)

- 3 Transcriptome dataset
- 1 RNA-seq pipeline
- At least 1 molecular marker for MAS

##### People Services (1)

- Accommodate at least 1 BS/MS student

##### Places and Partnerships (N/A)

##### Policy (N/A)

#### (12) POTENTIAL OUTCOMES

- Increased productivity and efficiency in mango breeding programs using marker-assisted selection.
- Enhancement of institutional and human resources capability to sustain the crop biotechnology research and further developments.
- Provision of training opportunities for male and female researchers and students to enhance their skills and knowledge in the field of biotechnology research.
- Submission of a new proposal to study other fruit quality and production related traits of 'Carabao' mango using the same technology developed.
- Reduced adverse effects on the environment due to pesticide and fungicide use when resistant varieties are developed.
- Potential increase in income for mango farmers and other stakeholders involved in the mango industry when varieties with improved fruit quality are developed.

- Possible establishment of working partnerships between research institutions, government agencies, and private sectors involved in the mango industry.

### **(13) POTENTIAL IMPACTS (2Is)**

#### **Social Impact**

- Identified candidate genes can be used in the development of new and improved mango varieties with better fruit quality and yield, which contribute to food security and ensure a steady supply to the community
- Provide training opportunities for researchers and students, which can help in building scientific capacity
- Promote a culture of scientific inquiry and research in the Philippines

#### **Economic Impact**

- Identified candidate genes can be used in improving mango fruit quality which increases its export value and therefore expand its export potential; Consequently, this will also increase the income of mango farmers

### **(14) TARGET BENEFICIARIES**

- Researchers
- Breeders
- Mango growers and stakeholders
- Plant Scientists/Botanists
- Students

### **(15) SUSTAINABILITY PLAN (if applicable)**

Producing high-quality mangoes that meet international standards is essential for maintaining a competitive edge in global markets. This project is directed towards producing world-class Philippine mango varieties through precision breeding.

The dataset resulting from transcriptome analysis will serve as a crucial resource for identifying candidate genes and molecular markers associated with enhanced fruit quality traits in Philippine mangoes. This valuable information will not only drive advancements in the mango breeding program, but it will also lay the experimental foundation for possible gene editing applications. In the future, the project will subsequently expand its focus to encompass transcriptome analysis for other economically important traits, including pest resistance, yield, and environmental stress tolerance, to further boost the competitiveness of the mango industry and ensuring the continuity of the project.

Beyond breeding applications, transcriptomic data can also be used in developing targeted pest management strategies utilizing natural or biocontrol methods. Moreover, it can also facilitate the optimization of cultural practices based on physiological characteristics of different varieties, and the enhancement post-harvest handling techniques to extend the shelf life and elevate the export quality of mango products.

To further secure the sustainability of the project, capacity building initiatives will be undertaken to train and educate staff in advanced genomics and transcriptomics research techniques. The provision of funding for upgrading laboratory facilities and procurement of laboratory equipment will also ensure proper implementation of future research projects at the Institute of Crop Science, UPLB. The project also recognizes the importance of collaboration and diversifying of funding sources to address social and environmental challenges, and to maintain financial stability. We will proactively seek collaborations with experts and stakeholders in the academe, industry, and other government agencies or private sector entities that share our vision for advancing the Philippine mango industry.

**(16) GENDER AND DEVELOPMENT (GAD) SCORE** (refer to the attached GAD checklist)

9.34

**(17) LIMITATIONS OF THE PROJECT**

The proposed project will be limited only to the transcriptome analysis of fruit tissues and anthracnose/cedid fly resistance traits of mango. Similar studies on other fruit quality traits, yield, early maturity, and other production-related traits of 'Carabao' mango will be conducted in the future. Only 'Carabao' mango accessions available in IPB, UPLB will be used as samples in the study, but may also include samples from the recently sequenced *M. odorata* and *M. altissima*.

**(18) LIST OF RISKS AND ASSUMPTIONS RISK MANAGEMENT PLAN** (List possible risks and assumptions in attaining target outputs or objectives.)

OBJECTIVES	RISKS AND ASSUMPTIONS	ACTION PLAN
1. Generate a transcriptome dataset for 'Carabao' and Huani mango using RNA-seq technology	<ul style="list-style-type: none"> <li><b>Risk:</b> Lack of available fruit-bearing trees of specific accession during the sample collection period</li> <li><b>Risk:</b> Inability to rear cecid flies for controlled inoculation</li> <li><b>Risk:</b> Poor quality or quantity of RNA extracted from mango tissues which could lead to incomplete or unreliable transcriptome data;</li> <li><b>Risk:</b> Technical difficulties during RNA-seq library preparation</li> <li><b>Assumption:</b> The selected mango accessions represent contrasting genetic background of the 'Carabao' and Huani mango for comparative transcriptome analysis</li> </ul>	<ul style="list-style-type: none"> <li>Other available accessions from other sources will be used provided that each has contrasting genetic background or phenotypic traits (i.e. resistant and susceptible)</li> <li>Fruit samples will be collected from field inoculated trees</li> <li>Try other commercially available RNA isolation kits or optimize existing protocols to ensure high RNA quality &amp; quantity prior to sequencing;</li> <li>Include library construction in professional services to be packaged with transcriptome sequencing;</li> <li>Consult/collaborate with other researchers doing transcriptome analysis</li> </ul>
2. Identify differentially expressed genes associated with anthracnose disease and cecid fly resistance based on functional annotation and gene expression analysis	<ul style="list-style-type: none"> <li><b>Risk:</b> Failure of procurement of subscription-based bioinformatics software</li> </ul>	<ul style="list-style-type: none"> <li>Find alternative software/tools that are free and applicable to use based on previous studies</li> <li>Use complementary methods and multiple databases and software tools for annotation and analysis, compare and cross-reference results, and seek expert input or consultation</li> </ul>

3. Validate the expression of candidate genes using quantitative qRT-PCR and evaluate their potential for use in the mango breeding program	<ul style="list-style-type: none"> <li>• <b>Risk:</b> Lack of available lab materials/reagents not included in the original proposal due to unforeseen circumstances (e.g., changes in protocols for RNA isolation or gene expression analysis)</li> <li>• <b>Risk:</b> Inability to repair the existing qPCR machine</li> <li>• <b>Risk:</b> Technical difficulties or errors during RT-PCR could lead to inaccurate measurement of gene expression levels</li> <li>• <b>Assumption:</b> Variations in gene expression correspond to phenotypic variations, and DEGs are likely to be associated with target traits</li> </ul>	<ul style="list-style-type: none"> <li>• Request for re-alignment of budget to prioritize procurement of needed materials/reagents</li> <li>• Seek collaboration with other laboratories in the same institution for temporary use of equipment</li> <li>• Perform optimization of RT-qPCR protocol and replicate the gene expression analysis to ensure accurate results; Consult/collaborate with other researchers doing transcriptome analysis</li> </ul>
4. Develop molecular markers for the precise integration of anthracnose and cecid fly resistance genes in marker-assisted breeding	<ul style="list-style-type: none"> <li>• <b>Risk:</b> The identified candidate genes may not be reliable or accurate indicators of the target traits</li> <li>• Assumption: Developed molecular markers based on identified genes can distinguish desired traits/genotypes in marker-assisted selection</li> </ul>	<ul style="list-style-type: none"> <li>• Conduct supplemental association analysis using GBS data, phenotypic validation studies, or comparative genomics analysis</li> </ul>

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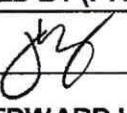
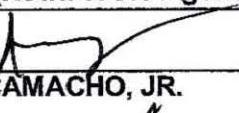
## (20) PERSONNEL REQUIREMENT

Position	Percent Time Devoted to the Project	Responsibilities
Project Leader: John Edward L. Felipe	20%	<ul style="list-style-type: none"> <li>• Lead and direct the scientific direction of the research project</li> </ul>

		<ul style="list-style-type: none"> <li>• Lead the research team to complete project outputs within the allotted timeline and budget</li> <li>• Will evaluate research results, and steer the project activities towards the achievement of project goals</li> <li>• Lead the preparation of research and administrative reports</li> <li>• Communicate research results during technical reviews and through conference presentations, scientific publications, or project reports.</li> <li>• Will prepare, together with Project Technical Assistant, the articles for publication scientific journals and presentation in scientific meetings</li> </ul>
One (1) Project Staff Level 1: Jena Joy G. Apolinario (SG-18)	20%	<ul style="list-style-type: none"> <li>• Supervise the analysis and set-up of experiments for the characterization of cecid fly resistance in mango fruit</li> <li>• Be involved in sample collections and set-up of experiments for controlled infestation</li> <li>• Provide guidance to the Project Technical Assistant on lab-based activities</li> <li>• Interpret results and provide recommendations</li> <li>• Assist in data analysis and write-up of reports and publication</li> </ul>
One (1) Project Staff Level 2: Jennifer M. Niem (SG-21)	20%	<ul style="list-style-type: none"> <li>• Supervise the analysis and set-up of experiments for the characterization of anthracnose resistance in mango fruit</li> <li>• Be involved in sample collections and set-up of experiments for controlled inoculation</li> <li>• Provide guidance to the Project Technical Assistant on lab-based activities</li> <li>• Interpret results and provide recommendations</li> <li>• Assist in data analysis and write-up of reports and publication</li> </ul>
One (1) Project Technical Assistant III	100%	<ul style="list-style-type: none"> <li>• Perform laboratory activities from collection, to extraction and preparation of RNA for sequencing</li> <li>• Assist in performing bioinformatics work, analysis of transcriptome datasets, and developing database with the project leader</li> <li>• Communicate research results through conference presentations, scientific publications, or project reports.</li> <li>• Communicate with the Project leader results of laboratory experiments as well as updates on the workflow of the project</li> </ul>

		<ul style="list-style-type: none"> <li>• Supervise and train students incorporated with the project and implement and maintain laboratory rules and regulations</li> <li>• Perform other duties that maybe assigned from time to time</li> </ul>		
One (1) Project Administrative Assistant I	100%	<ul style="list-style-type: none"> <li>• In charge of administrative matters under the guidance of the project leader</li> <li>• Oversee processing for local and international travels and trainings</li> <li>• In charge on the procurement of materials and equipment for the project</li> <li>• Assist in procurement and processing of documents</li> <li>• Prepare appointment documents of hired staff</li> <li>• Oversee project expenditures and canvassing of equipment, materials and other needs of the project</li> <li>• Render assistance in laboratory procedures as needed</li> <li>• Perform other duties that maybe assigned from time to time</li> </ul>		
<b>(21) BUDGET BY IMPLEMENTING AGENCY</b>				
IMPLEMENTING AGENCY	PS	MOOE	EO	Total
Year 1	988,908.00	2,055,237.23	450,000.00	3,494,145.23
Year 2	859,308.00	646,506.85	0	1,505,814.85
<b>TOTAL</b>	<b>1,848,216.00</b>	<b>2,701,744.08</b>	<b>450,000.00</b>	<b>4,999,960.08</b>
<b>(22) OTHER ONGOING PROJECTS BEING HANDLED BY THE PROJECT LEADER: 0</b> (number)				
Title of the Project	Funding Agency	Involvement in the Project		
NA	NA	NA		
<b>(23) OTHER SUPPORTING DOCUMENTS</b> (Please refer to page 2 for the additional necessary documents.)				

I hereby certify the truth of the foregoing and have no pending financial and/or technical obligations from the DOST and its attached Agencies. I further certify that the programs/projects being handled is within the prescribed number as stipulated in the DOST-GIA Guidelines. Any willful omission/false statement shall be a basis of disapproval and cancellation of the project.

	SUBMITTED BY (Project Leader)	ENDORSED BY (Head of the Agency)
Signature		
Printed Name	JOHN EDWARD L. FELIPE	JOSE V. CAMACHO, JR.
Designation/Title	Assistant Professor 2	Chancellor, UPLB
Date	8 November 2023	

Note: See guidelines/definitions at the back.



DOST Form 5  
A - PROJECT WORKPLAN

## (1) Program

(1) Program Title: N/A

**(2) Project Title:** Transcriptome Analysis of Philippine Mangoes in Response to Anthracnose and Cecid Fly Damage

**(3) Project Duration (number of months):**

24 months

**(4) Project Start Date:**

January 1, 2024

**(5) Project End Date:**

December 31, 2025

DOST Form 5  
B - EXPECTED OUTPUTS

## (1) Program

**Title:** N/A

**(2) Project Title:** Transcriptome Analysis of Philippine Mangoes in Response to Anthracnose and Cecid Fly Damage

**(3) Project Duration (number of months):**

24 months

**(4) Project Start Date:**

January 1, 2024

**(5) Project End Date:**

December 31, 2025

**DOST Form 5**  
**C – RISKS AND ASSUMPTIONS**

**(1) Program**

Title: N/A

**(2) Project Title:** Transcriptome Analysis of Philippine Mangoes in Response to Anthracnose and Cecid Fly Damage

**(3) Project Duration (number of months):**

24 months

**(4) Project Start Date:**

January 1, 2024

**(5) Project End Date:**

December 31, 2025

OBJECTIVES	(11) RISKS AND ASSUMPTIONS	(12) ACTION PLAN (use separate sheet if necessary)
1. Generate a transcriptome dataset for 'Carabao' and Huani mango using RNA-seq technology	<ul style="list-style-type: none"> <li><b>Risk:</b> Lack of available fruit-bearing trees of specific accession during the sample collection period</li> <li><b>Risk:</b> Inability to rear cecid flies for controlled inoculation</li> <li><b>Risk:</b> Poor quality or quantity of RNA extracted from mango tissues which could lead to incomplete or unreliable transcriptome data;</li> <li><b>Risk:</b> Technical difficulties during RNA-seq library preparation</li> <li><b>Assumption:</b> The selected mango accessions represent contrasting genetic background of the 'Carabao' and Huani mango for comparative transcriptome analysis</li> </ul>	<ul style="list-style-type: none"> <li>Other available accessions from other sources will be used provided that each has contrasting genetic background or phenotypic traits (i.e. resistant and susceptible)</li> <li>Fruit samples will be collected from field inoculated trees</li> <li>Try other commercially available RNA isolation kits or optimize existing protocols to ensure high RNA quality &amp; quantity prior to sequencing;</li> <li>Include library construction in professional services to be packaged with transcriptome sequencing; Consult/collaborate with other researchers doing transcriptome analysis</li> </ul>
2. Identify differentially expressed genes associated with anthracnose disease and cecid fly resistance based on functional annotation and gene expression analysis	<ul style="list-style-type: none"> <li><b>Risk:</b> Failure of procurement of subscription-based bioinformatics software</li> </ul>	<ul style="list-style-type: none"> <li>Find alternative software/tools that are free and applicable to use based on previous studies</li> <li>Use complementary methods and multiple databases and software tools for annotation and analysis, compare and cross-reference results, and seek expert input or consultation</li> </ul>
3. Validate the expression of candidate genes using quantitative qRT-PCR and evaluate their potential for use in the mango breeding program	<ul style="list-style-type: none"> <li><b>Risk:</b> Lack of available lab materials/reagents not included in the original proposal due to unforeseen circumstances (e.g., changes in protocols for RNA isolation or gene expression analysis)</li> <li><b>Risk:</b> Inability to repair the existing qPCR machine</li> <li><b>Risk:</b> Technical difficulties or errors during RT-PCR could lead to inaccurate measurement of gene expression levels</li> <li><b>Assumption:</b> Variations in gene expression correspond to phenotypic variations, and DEGs are likely to be associated with target traits</li> </ul>	<ul style="list-style-type: none"> <li>Request for re-alignment of budget to prioritize procurement of needed materials/reagents</li> <li>Seek collaboration with other laboratories in the same institution for temporary use of equipment</li> <li>Perform optimization of RT-qPCR protocol and replicate the gene expression analysis to ensure accurate results; Consult/collaborate with other researchers doing transcriptome analysis</li> </ul>

4. Develop molecular markers for the precise integration of anthracnose and cecid fly resistance genes in marker-assisted breeding	<ul style="list-style-type: none"><li>• <b>Risk:</b> The identified candidate genes may not be reliable or accurate indicators of the target traits</li><li>• <b>Assumption:</b> Developed molecular markers based on identified genes can distinguish desired traits/genotypes in marker-assisted selection</li></ul>	<ul style="list-style-type: none"><li>• Conduct supplemental association analysis using GBS data, phenotypic validation studies, or comparative genomics analysis</li></ul>
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**Table 1.** Breakdown of UPLB Counterpart Fund

ITEMS	Salary	% Time Allotted	Year 1 Line-Item Budget (LIB)					Year 2	Grand Total
			Q1	Q2	Q3	Q4	Y1 Total		
<b>I. Personal Services (PS)</b>									
Honoraria									
One (1) Project Leader @ P8,800/mo x 12 mos.	51,357.00	20%	30,814.20	30,814.20	30,814.20	30,814.20	123,256.80	123,256.80	246,513.60
One (1) Project Staff Level 1 @ P4,800/mo x 12 mos.	46,725.00	20%	28,035.00	28,035.00	28,035.00	28,035.00	112,140.00		112,140.00
One (1) Project Staff Level 2 @ P6,000/mo x 12 mos.	63,997.00	20%	38,398.20	38,398.20	38,398.20	38,398.20	153,592.80		153,592.80
<b>Sub-Total for PS</b>			<b>97,247.40</b>	<b>97,247.40</b>	<b>97,247.40</b>	<b>97,247.40</b>	<b>388,989.60</b>	<b>123,256.80</b>	<b>512,246.40</b>
<b>II. Maintenance and other Operating Expenses (MOOE)</b>									
Traveling Expenses									
Repairs and Maintenance of Facilities									
Repairs and Maintenance of Machinery and Equipment			25,000.00	25,000.00	25,000.00	25,000.00	100,000.00	100,000.00	200,000.00
Repairs and Maintenance of Office and Laboratory Facilities			25,000.00	25,000.00	25,000.00	25,000.00	100,000.00	100,000.00	200,000.00
Supplies and Materials Expenses									
a. Office Supplies			6,250.00	6,250.00	6,250.00	6,250.00	25,000.00	25,000.00	50,000.00
b. Laboratory Supplies			3,000.00	3,000.00	3,000.00	3,000.00	12,000.00	12,000.00	24,000.00
Utility Expenses (Water and Electricity Expenses)			43,750.00	43,750.00	43,750.00	43,750.00	175,000.00	175,000.00	350,000.00
<b>Sub-Total for MOOE</b>			<b>103,000.00</b>	<b>103,000.00</b>	<b>103,000.00</b>	<b>103,000.00</b>	<b>412,000.00</b>	<b>412,000.00</b>	<b>824,000.00</b>
<b>GRAND TOTAL</b>			<b>200,247.40</b>	<b>200,247.40</b>	<b>200,247.40</b>	<b>200,247.40</b>	<b>800,989.60</b>	<b>535,256.80</b>	<b>1,336,246.40</b>

4. Develop molecular markers for the precise integration of anthracnose and cecid fly resistance genes in marker-assisted breeding	<ul style="list-style-type: none"><li>• <b>Risk:</b> The identified candidate genes may not be reliable or accurate indicators of the target traits</li><li>• <b>Assumption:</b> Developed molecular markers based on identified genes can distinguish desired traits/genotypes in marker-assisted selection</li></ul>	<ul style="list-style-type: none"><li>• Conduct supplemental association analysis using GBS data, phenotypic validation studies, or comparative genomics analysis</li></ul>
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**Table 1.** Breakdown of Line-item Budget for the project

ITEMS	Proposed Year 1 Line-Item Budget (LIB)					Year 2	Grand Total
	Q1	Q2	Q3	Q4	Y1 Total		
<b>I. Personal Services (PS)</b>							
<i>Direct Cost</i>							
Salaries							
One (1) Project Technical Assistant III @ P34,998.00/mo x 12 mos.	104,994.00	104,994.00	104,994.00	104,994.00	419,976.00	419,976.00	839,952.00
One (1) Project Administrative Assistant I @ P27,811.00/mo x 12 mos.	83,433.00	83,433.00	83,433.00	83,433.00	333,732.00	333,732.00	667,464.00
Honoraria							
One (1) Project Leader @ P8,800/mo x 12 mos.	26,400.00	26,400.00	26,400.00	26,400.00	105,600.00	105,600.00	211,200.00
One (1) Project Staff Level 1 @ P4,800/mo x 12 mos.	14,400.00	14,400.00	14,400.00	14,400.00	57,600.00		57,600.00
One (1) Project Staff Level 2 @ P6,000/mo x 12 mos.	18,000.00	18,000.00	18,000.00	18,000.00	72,000.00		72,000.00
Sub-Total for PS	247,227.00	247,227.00	247,227.00	247,227.00	988,908.00	859,308.00	1,848,216.00
<b>II. Maintenance and other Operating Expenses (MOOE)</b>							
<i>Direct Cost</i>							
Traveling Expenses							
a. Local	60,000.00	60,000.00	15,000.00	15,000.00	150,000.00	60,000.00	210,000.00
b. International	-	-	-	-	-	-	0.00
Supplies and Materials							0.00
a. Laboratory Supplies (see attached list)	716,855.00	-	-	-	716,855.00	221,450.00	938,305.00
b. Office Supplies	20,000.00	-	-	-	20,000.00	20,000.00	40,000.00
Repairs and Maintenance of Facilities							
Repairs and Maintenance of Machinery and Equipment	200,000.00	-	-	-	200,000.00	-	200,000.00
Repairs and Maintenance of Office and Laboratory Facilities	60,000.00	-	-	-	60,000.00	-	60,000.00
Communication Expenses	15,000.00	15,000.00	15,000.00	15,000.00	60,000.00	36,000.00	96,000.00
Professional Services							
S&T Consultant for Genomics/Transcriptomics @ P6,000/mo x 12 mo	18,000.00	18,000.00	18,000.00	18,000.00	72,000.00	72,000.00	144,000.00
Transcriptome sequencing @ P27,000/sample x 18 samples	-	-	486,000.00	-	486,000.00		486,000.00
Representation Expenses	6,000.00	6,000.00	6,000.00	6,000.00	24,000.00	24,000.00	48,000.00
Subscription Expenses (Bioinformatics software)	-	-	27,000.00	27,000.00	54,000.00	108,000.00	162,000.00
Sub-Total for MOOE DC	1,095,855.00	99,000.00	567,000.00	81,000.00	1,842,855.00	541,450.00	2,384,305.00
<i>Indirect Cost</i>							
Supplies and Materials Expenses	20,146.23	5,193.41	12,213.41	4,923.41	42,476.45	21,011.37	63,487.82
Utilities	80,584.92	20,773.62	48,853.62	19,693.62	169,905.78	84,045.48	253,951.26
Sub-Total for MOOE IC	100,731.15	25,967.03	61,067.03	24,617.03	212,382.23	105,056.85	317,439.08
Sub-Total for MOOE	1,196,586.15	124,967.03	628,067.03	105,617.03	2,055,237.23	646,506.85	2,701,744.08
<b>III. Equipment Outlay (EO)</b>							
One (1) unit Laptop with at least 11-Core CPU, 14-Core GPU, 36GB Unified Memory, 1TB SSD Storage (for Bioinformatics & data analysis)	190,000.00				190,000.00		190,000.00
One (1) unit Laptop with CIS Printer (for Project management)	75,000.00				75,000.00		75,000.00
One (1) unit Camera with lens & accessories	70,000.00				70,000.00		70,000.00
One (1) unit Flake ice maker, 10 kg ice storage capacity	55,000.00				55,000.00		55,000.00
One (1) unit Liquid Nitrogen storage tank/dewar with complete accessories	60,000.00				60,000.00		60,000.00
Sub-Total for EO	450,000.00	0.00	0.00	0.00	450,000.00	0.00	450,000.00
<b>GRAND TOTAL</b>	1,893,813.15	372,194.03	875,294.03	352,844.03	3,494,145.23	1,505,814.85	4,999,960.08



## JUSTIFICATION FOR EQUIPMENT OUTLAY

Project Title: **Transcriptome Analysis of Philippine Mangoes in Response to Anthracnose and Cecid Fly Damage**  
 Total Project Duration: **2 years (24 months)**  
 Implementing Agency: **University of the Philippines Los Baños**  
 Project Leader: **John Edward L. Felipe**

Name of Equipment	No. of existing equipment	No. of units to be purchased	Price per Unit	Justification for the Purchase
Laptop (for Bioinformatics)	0	1	190,000.00	Dedicated working computer for bioinformatics and data analysis, data storage, research output preparation, training, and overall project planning and management
Laptop (for project management)	0	1	75,000.00	Dedicated working computer of the Project Technical Assistant for data collection and management, report preparation, and project activities
Camera	0	1	70,000	For documentation of experimental results and project activities
Flake ice maker	0	1	55,000	To produce readily available ice flakes to be used in RNA isolation (to maintain integrity of RNA at low temperatures)
Liquid nitrogen storage tank	0	1	60,000	For storage and use of liquid nitrogen during the RNA isolation
qPCR system	1	0		The existing machine is currently unusable but a budget for repair has been allocated in the proposed LIB.
PCR Machine/Thermal Cycler	1	0		
Gel Documentation System	2	0		
Mupid-One Horizontal Gel Electrophoresis System	2	0		
Refrigerator	3	0		
Autoclave	2	0		
Microwave Oven	1	0		
Vortex Mixer	3	0		
Micropipettors set	6	0		
Refrigerated microcentrifuge	1	0		
Analytical balance	3	0		

# John Edward L. Felipe

-  Cambria Subd., Brgy. Santo Domingo, Bay, Laguna 4033 Philippines
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## Education

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### **University of the Philippines Los Baños**

Master of Science (MS) in Molecular Biology and Biotechnology (MS MBB),  
*minor in Genetics/Plant Breeding*  
August 2017 – December 2019



### **University of the Philippines Baguio**

Bachelor of Science (BS) in Biology  
June 2010 – April 2014

## Work Experience

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### **Assistant Professor 2**

University of the Philippines Los Baños – Institute of Crop Science  
Sep 2022 – present



### **Instructor I**

Tarlac Agricultural University – College of Arts and Sciences  
Aug 2020 – July 2022 (2 yrs)

#### **Laboratory Manager**

Tissue Culture and Molecular Disease Indexing Laboratory  
Jan 2020 – July 2022 (2 yrs 7 mos)

#### **Project Staff II (Study Leader)**

NICER Project 1: Optimization of Sweetpotato Clean Planting Materials (SP-CPM) Production in Central Luzon  
Study 2: Development of polyphasic disease indexing protocol for detecting sweetpotato viruses  
Jan 2020 – Mar 2022 (2 yrs 3 mos) | Funded by DOST-GIA

#### **Project Staff**

ACEF Project: Upscaling of Clean Planting Material Propagation for Sustainable Sweet Potato Production  
Jan 2020 - Dec 2020 (1 year) | Funded by DA-ACEF



### **Graduate Research Associate (DOST-PCAARRD GREAT Program)**

University of the Philippines Los Baños – Institute of Plant Breeding  
Aug 2017 – Jul 2019 (2 yrs)



### Science Research Assistant

Tarlac Agricultural University – Rootcrops Research and Training Center

Mar 2015 - Aug 2020 (5 yrs 6 mos)

#### Project Member

Upgrading of the Tissue Culture and Molecular Disease Indexing Laboratory  
Jan – Dec 2017 (1 yr) | Funded by DOST-PCAARRD



### Laboratory Research Assistant

Tarlac Agricultural University – Rootcrops Research and Training Center

Jul 2014 - Mar 2015 (9 mos)

## Research Papers

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### 1. Refereed Journal Articles

**Felipe, J. L.**, Lachica, J. A. P., Cueva, F. M. D., Laurel, N. R., Alcasid, C. E., Sison, M. L. J., ... & Ocampo, E. T. M. (2022). Validation and molecular analysis of  $\beta$ -1, 3-GLU2 SNP marker associated with resistance to *Colletotrichum gloeosporioides* in mango (*Mangifera indica* L.). *Physiological and Molecular Plant Pathology* (118):101804, <https://doi.org/10.1016/j.pmpp.2022.101804>

Felipe, I. R. E., **Felipe, J. L.**, & Reginaldo, A. A. (2017). Ectoparasites of Commensal Rodents from Backyard Farmlands in Baguio City, Philippines. *Philippine Journal of Arts, Sciences & Technology* (2):1, Available online at: <http://pjast.tau.edu.ph/index.php/pjast/article/view/13>

### 2. Conference Papers & Presentations

#### *Oral Presentations*

**Felipe, J. L.**, Sagun, C. L., Camacho, J. V., Bajet, N. B., & Laranang, L. B. Optimization of SP-CPM Production in Central Luzon Optimization of SP-CPM Production in Central Luzon; Presented at the 33<sup>rd</sup> Annual Agency In-house Review of Completed and On-going Research and Development Projects; July 25, 2022; Tarlac Agricultural University, Camiling, Tarlac

**Felipe, J. L.**, Lachica, J. A. P., Cueva, F. M. D., Laurel, N. R., Alcasid, C. E., Sison, M. L. J., ... & Ocampo, E. T. M. Validation and molecular analysis of  $\beta$ -1,3-GLU2 SNP marker associated with resistance to anthracnose in Philippine carabao mango (*Mangifera indica* L. cv. 'Carabao'); Presented at the 2021 DOST-PCAARRD Graduate Alumni Association Inc. (DPGAA) Biennial Convention; November 3-4, 2021 via Zoom

Camacho, J. V., **Felipe, J. L.**, Bajet, N. B., & Laranang, L. B. Enhancing Sweetpotato Production through the use of Virus-free Planting Materials; Presented at the 1<sup>st</sup> International Agriculture, Biosystems and Technology Conference; July 22-23, 2021 via Zoom

Ocampo, E. T. M., **Felipe, J. L.**, Lachica, J. A. P., Laurel, N. R., Alcasid, C. E., Dela Cueva, F. M., & Valencia, L. DC. A Single Nucleotide Polymorphism (SNP) Marker Associated with Anthracnose (*Colletotrichum* sp.) Resistance in Philippine Carabao Mango (*Mangifera indica* L. cv. 'Carabao'); Presented at the 27<sup>th</sup> National Fruit Symposium; October 15-18, 2019, Bayview Park Hotel, Manila

## **Poster**

Laranang, L. B., Mariano, M. G., **Felipe, J. L.**, & Falible D. L. (2022). Upscaling of Clean Planting Material (SP-CPM) for Sustainable Sweetpotato Production; Presented at the 33<sup>rd</sup> Annual Agency In-house Review of Completed and On-going Research and Development Projects; July 25, 2022; Tarlac Agricultural University, Camiling, Tarlac

## **3. Thesis**

**Felipe, J. L.** (2020). Development of Single Nucleotide Polymorphism (SNP) Markers Associated with Anthracnose (*Colletotrichum* sp.) Resistance in Philippine Carabao Mango (*Mangifera indica* L. cv. 'Carabao'). University of the Philippines Los Baños (MS Thesis)

**Felipe, J. L.** and Estrada, I. B. (2014). Ectoparasites of Commensal Rodents from Backyard Farmlands in Baguio City, Philippines. University of the Philippines Baguio (BS Thesis)

## **Professional Trainings/ Seminars/ Workshops attended**

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Participant	<i>Training on Practical Bioinformatics Skills for Plant and Animal Whole Genome Sequence Data</i> Center for Agricultural Biotechnology (CAB), Kasetsart University, Thailand August 28 to September 1, 2023
Resource Speaker	<i>Executive Course on Agricultural Biotechnology</i> Institute of Crop Science, UPLB August 14-18, 2023
Participant	<i>Potato Seed Production Technology in Southeast Asia with Special Focus on Vietnam and Philippines</i> International Potato Center (CIP) September 28, 2021
Participant	<i>Genomics &amp; Agriculture: Tool Set of Agricultural Genomics</i> Philippine Genome Center (PGC); UP Mindanao October 1, 2021
Participant	<i>Best Practices in Writing and Publishing Your Research Paper</i> Elsevier April 23, 2021
Participant	<i>Emerging and New Plant Pathogens from the Philippines and Overview of Plant Quarantine Regulations in the Philippines</i> Plant Pathology Laboratory-Institute of Plant Breeding, UPLB April 22, 2021
Participant	<i>The Art of Article Writing, Document Sources, and Literature Mapping</i> Isabela State University April 21-22, 2021
Participant	<i>Protecting Germplasm Health from Pathogens and Pests</i> Institute of Plant Breeding, UPLB February 24, 2021
Organizer and Participant	<i>Virtual Training on Nursery Accreditation and Plant Material Certification</i> TAU-Rootcrops Center; Bureau of Plant Industry November 10, 2020

Participant	<i>S&amp;T-based Tools for Varietal Selection, Improvement, and Propagation</i> DOST-PCAARRD November 20, 2020
Participant	<i>Training-Workshop on Technical Writing for Publication in a Refereed Journal</i> DOST-PCAARRD March 9-13, 2020
Participant	<i>Training-Workshop on Research Proposal Preparation and Packaging</i> TAU, DOST-PCAARRD February 21-22, 2020
Participant	<i>Concepts in the Safety Assessment of Novel Food and Feed</i> International Life Sciences Institute (ILSI Global) e-Learning Course Completed March 31, 2019
Participant	<i>Application of Problem Formulation to Food and Feed Safety Assessments</i> International Life Sciences Institute (ILSI Global) e-Learning Course Completed April 28, 2019
Participant	<i>Confined Field Trials of Genetically Engineered Plants</i> International Life Sciences Institute (ILSI Global) e-Learning Course Completed April 29, 2019
Participant	<i>Application of Problem Formulation to the Environmental Risk Assessment of Genetically Engineered Crops</i> International Life Sciences Institute (ILSI Global) e-Learning Course Completed April 30, 2019
Participant	<i>Seminar on CRISPR-Cas9 and Genomics</i> IPB, UPLB; East-West Seed Co. January 23, 2018
Participant	<i>R Programming for Researchers: A Workshop on Data Preparation and Analysis</i> IPB, UPLB February 27, 2018
Participant	<i>Training on Banana Tissue Culture &amp; Nursery Management</i> Bioversity International, IPB-UPLB July 21-22, 2016
Participant	<i>Symposium on Predatory Journals and Conferences</i> National Academy of Science and Technology (NAST); DOST; UP Diliman March 28, 2016
Participant and Organizer	<i>Training on Sweetpotato Clean Planting Material Production and Integrated Crop Management for Aurora Province</i> Tarlac College of Agriculture October 16-17, 2014

## Awards & Fellowships

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### Graduate Research and Education Assistantship for Technology (GREAT)

#### Program Grant

DOST-PCAARRD

Aug 2017



### Best Paper Award

2021 DPGAA Biennial Convention

Nov 2021



### Publication Incentive Award

DOST-PCAARRD

March 2022

## Professional Affiliations

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### Philippine Society of Biochemistry and Molecular Biology (PSBMB)

Regular Member

Dec 2022 – Present



### National Research Council of the Philippines (NRCP)

Associate Member

Nov 2020 – Present



### Crop Science Society of the Philippines (CSSP)

Regular Member

Nov 2020 – Present



### DOST-PCAARRD Graduate Alumni Association Inc. (DPGAA)

Regular Member

Nov 2021 – Present

#### comosus L.) Fruit

Secondary Level	<b>CANOSSA COLLEGE</b> San Pablo City, Laguna Philippines 1990-1994 Second Honors
Primary Level	<b>Sto. Tomas North Central School</b> Sto. Tomas, Batangas Philippines 1984-1990 With Honors

## **WORK EXPERIENCE**

May 2023-present	Assistant Professor 5 Institute of Weed Science, Entomology, and Plant Pathology College of Agriculture and Food Science UPLB, College, Laguna
March 2021-present	<b>Curator</b> Mycology Section UPLB Museum of Natural History UPLB, College, Laguna
Feb. 2014 – April 2023	<b>University Researcher</b> Mycology Section UPLB Museum of Natural History UPLB, College, Laguna
July 2016 – March 2021	<b>PhD Research Scholar</b> School of Agriculture and Wine Sciences Charles Sturt University New South Wales, Australia
Feb. 2012 – June 2013	<b>Mycotoxin Specialist (Professional Service)</b> Postharvest Unit, CESD International Rice Research Institute, Los Banos, Laguna
Jan. 2009 – Dec. 2011	<b>Graduate Research Assistant</b> Vegetable Plant Pathology Laboratory WSU-Northwest Research and Extension Center Mount Vernon, WA USA
Jan. 2006 – Dec. 2008	<b>University Research Associate II</b> CPC Cluster (Plant Pathology) UPLB, College, Laguna Research: UPLB-DOST-PCARRD (S & T Anchor Program for

## JENNIFER MILLERA NIEM

290 San Juan, Sto. Tomas City, Batangas 4234  
Mobile no. + 63 920 695 5915  
Email Address: jmniem@up.edu.ph



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### PERSONAL INFORMATION

Nickname : Jenn  
Age : 46  
Date of Birth : February 10, 1977  
Place of Birth : San Juan, Sto. Tomas City, Batangas Philippines  
Religion : Roman Catholic  
Civil Status : Single  
Nationality : Filipino  
Home Address : 290 San Juan, Sto. Tomas City Batangas 4234 Philippines

### EDUCATIONAL BACKGROUND

Post graduate level	Doctor of Philosophy in Plant Pathology <b>Charles Sturt University</b> Wagga Wagga, New South Wales, Australia 2016-2021 <b>Research Study: Biological control of grapevine trunk diseases using bacterial endophytes from grapevines</b>
Post graduate level	Master of Science in Plant Pathology <b>Washington State University</b> Pullman, Washington, USA 2009-2011 <b>Research Study: Effects of Flooding on the Survival of Sclerotinia and Verticillium in Potato Field Soils of Western Washington</b>
Tertiary level	Bachelor of Science in Agriculture Major: Plant Pathology <b>University of the Philippines at Los Baños</b> College, Laguna, Philippines 1994-1999 <b>Research Study: Etiology and Control of Yeasty Fermentation in Fresh Cut Pineapple (<u>Ananas</u>)</b>

Batangas State University, Batangas, Philippines, April 14-15, 2016.

5. International Kasetsart University Science and Technology Annual Research Symposium. Bangkok, Thailand, March 27-28, 2014.
6. 2012 IRRI Young Scientists Conference. International Rice Research Institute, Los Baños, Laguna, November 8-9, 2012.
7. 2009 American Phytopathological Society Annual Scientific Conference. Portland, OR, USA, August 1-5, 2009.

#### **MEMBERSHIP IN SCIENTIFIC AND OTHER PROFESSIONAL ORGANIZATIONS**

UP Phytopathological Society (undergraduate organization of Plant Pathology majors: 1996-1999, member; 2006 to 2008, junior adviser)

UPCA Alumni Association (member)

Philippine Association for the Advancement of Science (Lifetime Member)

Mycological Society of the Philippines (Lifetime Member)

#### **PAPER/POSTER PRESENTED IN SCIENTIFIC CONFERENCE**

1. **J. Niem, R.** Billones-Baaijens, B. Stodart and S. Savocchia. Exploring the interactions between bacterial endophytes and trunk disease pathogens of grapevine. (oral presentation). 50<sup>th</sup> Biennial Australasian Plant Pathology Society (APPS) Conference. Melbourne, Victoria, November 25-28, 2019.
2. **J. Niem, R.** Billones-Baaijens, B. Stodart and S. Savocchia. Bacterial antagonists to control trunk diseases. (oral presentation, received the "Innovation and Science" award. 2018 CRUSH Grape and Wine Science Symposium. Waite Campus, University of Adelaide, Adelaide, South Australia, September 25-26, 2018.
3. **J. Niem, R.** Billones-Baaijens, B. Stodart and S. Savocchia. Biological control of grapevine trunk diseases using endophytic bacteria. (poster presentation). Science Protecting Plant Health Conference 2017. Australasian Plant Pathology Society (APPS). Brisbane Convention and Exhibition Centre, Brisbane, Queensland, September 26-28, 2017.
4. **Jennifer Niem**, Regina Billones-Baaijens, Benjamin Stodart and Sandra Savocchia. Exploring endophytic bacteria as potential biocontrol agents against grapevine trunk disease pathogens. (poster presentation). 10<sup>th</sup> International Workshop on Grapevine Trunk Diseases. Reims, France, July 4-7, 2017.
5. **Niem, J.** Baldovino, M, Pampolina, N., Balatibat, J., Tinio, C., Malabriga, P. Jr., Aguilon, D., Dela Cruz, N., Vibar, P., Bawingan, P., Rodruiguez, R. S., Magdaong, J., Labatos, B. V. and Flores, J. Diversity of macrofungi in Mount Mamparang, Didipio, Nueva Vizcaya (poster presentation). 18<sup>th</sup> Annual Scientific Meeting of the Mycological Society of the Philippines.  
Batangas State University, Batangas, Philippines, April 14-15, 2016.

Banana)  
**Survey, Diagnosis, and Management of Bacterial Wilt/Moko  
and Bugtok Diseases of Banana**

April 2001 – July 2004	<b>University Research Associate II</b> Dept. of Plant Pathology UPLB, College, Laguna Research: CDR-USAID funded project (Collaborative project between the Dept. of Plant Pathology, UPLB and Volcani Center, Israel <b><i>Induced Resistance in Subtropical Fruits for the Prevention of Decay Initiated from Quiescent Infecting Pathogens</i></b>
March 1999- March 2001	<b>University Research Associate I</b> Dept. of Plant Pathology UPLB, College, Laguna Research: CDR-USAID funded project <b><i>Induced Resistance in Subtropical Fruits for the Prevention of Decay Initiated from Quiescent Infecting Pathogens</i></b>

## TRAINING

Division of Food Technology  
IGV Institute for Cereal Processing  
Nuthetal, Germany  
July 16-31, 2012  
**Research: Extraction and Purification of Aflatoxin in Cereal Crops**

Dept. of Postharvest Science of Fresh Produce  
Institute for Technology and Storage of Agricultural Products  
ARO, Volcani Center, Israel  
Aug.15, 2004-Dec. 21, 2005  
**Research:** CDR-USAID funded project (Collaborative project between the Dept. of Plant  
Pathology, UPLB and Volcani Center, Israel  
***Differential susceptibility of apple cultivars to Alternaria alternata causing core rot  
disease in apple fruit***

## SEMINARS/CONFERENCE ATTENDED

1. 50<sup>th</sup> Annual Australasian Plant Pathology Society (APPS) Conference. Melbourne,  
Victoria. Nov. 25-28, 2019.
2. 2018 CRUSH Grape and Wine Science Symposium. Waite Campus, University of  
Adelaide, Adelaide, South Australia. September 25-26, 2018.
3. Science Protecting Plant Health Conference 2017. Australasian Plant Pathology  
Society (APPS). Brisbane Convention and Exhibition Centre, Brisbane, Queensland,  
September 26-28, 2017.
4. 18<sup>th</sup> Annual Scientific Meeting of the Mycological Society of the Philippines.

6. **J. Niem.** Environmental Pollution and Restoration: Role of fungi in bioremediation (oral presentation). EcoArt and Science Exhibit and Seminar: Linking Science and Art. Forest Biological Sciences, College of Forestry and Natural Resources. UPLB, College, Laguna, Philippines, December 3, 2015.
7. **J. Niem**, B. Gundersen, and DA Inglis. Field flooding for controlling soilborne potato pathogens in western Washington (oral presentation). APS /Pacific Division Annual Meeting. Embassy Suites Hotel, Sacramento, CA, June 27-29, 2012.
8. **JM Niem**, B Gundersen, and DA Inglis. Effects of flooding on soilborne potato pathogens in the Skagit Valley of Washington (oral presentation). WSU-Mount Vernon NWREC Symposium on Agriculture and Northwest Ecosystem. Mount Vernon, WA, USA, November 10, 2009.
9. **JM Niem**, EY Ardales, and MP Natural. Survey of moko and bugtok diseases of banana in Luzon. Poster presenter. Pest Management Council of the Philippines Annual Scientific Conference. Puerto Princesa City, Palawan, May 6-9, 2008.

## SCIENTIFIC PUBLICATION

1. **Niem, J.**, Billones-Baaijens, R., Stodart, B.J., Reveglia, P., Savocchia, S. 2023. Biocontrol potential of an endophytic *Pseudomonas poae* strain against the grapevine trunk disease pathogen *Neofusicoccum luteum* and its mechanism of action. *Plants*. 12: 2132. <https://doi.org/10.3390/plants12112132>.
2. Pampolina, N. M., Tadiosa, E. R., Ata, J. P., Soriano, K. J. R., Parlucha, J. A., **Niem, J. M.** 2022. Species and functional diversity of forest fungi for conservation and sustainable landscape in the Philippines. In J. J. G. Guerro, T. U. Dalisay, M. P. de Leon, M. A. O. Balendres, K. I. R. Notarte, & T. E. E. dela Cruz (Eds). *Mycology in the Tropics, Updates on Philippine Fungi*. pp. 89-135. United Kingdom: Elsevier Inc. (Academic Press).
3. Reveglia, P.; Billones-Baaijens, R.; **Niem, J.M.**; Masi, M.; Cimmino, A.; Evidente, A.; Savocchia, S. 2021. Production of phytotoxic metabolites by Botryosphaeriaceae in naturally infected and artificially inoculated grapevines. *Plants*. 10:802. <https://doi.org/10.3390/plants10040802>.
4. **Niem, J.**, Billones-Baaijens, R., Savocchia, S., Stodart, B. (2020). Diversity profiling of grapevine microbial endosphere and antagonistic potential of endophytic *Pseudomonas* against grapevine trunk diseases. *Frontiers in Microbiology*. 11:477. doi: 10.3389/fmicb.2020.00477.
5. **Niem, J.**, Billones-Baaijens, R., Savocchia, S., Stodart, B. (2020). Endophytic bacteria from grapevines: a potential biocontrol of trunk diseases. *Wine & Viticulture Journal*. 35 (4): 52-58.
6. **Niem, J.**, Billones-Baaijens, R., Savocchia, S., Stodart, B. (2019). Draft genome sequences of endophytic *Pseudomonas* spp. isolated from grapevine tissue and antagonistic to grapevine trunk disease pathogens. *Microbiology Resource Announcements*. 8: e00345–19.

7. **Niem, J.M.** and Baldovino, M.M. 2015. Initial checklist of macrofungi in the karst area of Cavinti, Laguna. *UPLB Museum Publications in Natural History*. Vol. 4: 55-61.
8. Inglis, D., Gundersen, B., **Niem, J.**, and Morse, J. 2013. Field flooding for controlling soilborne potato pathogens in Western Washington. *Washington State University Extension*. EM056E. Published online on Oct 2013 at  
<http://cru.cahe.wsu.edu/CEPublications/EM056E/EM056E.pdf>
9. **Niem, J.**, Gundersen, B., and Inglis, D. A. 2013. Effects of soil flooding on the survival of two potato pathogens, *Sclerotinia sclerotiorum* and *Verticillium dahliae*. *American Journal of Potato Research*. 90 (6): 578-590.
10. **Niem, J.**, I. Miyara, Y. Ettedgui, M. Reuveni, M. Flaishman, and D. Prusky. 2007. Core rot development in Red Delicious apples Is affected by susceptibility of the seed locule to *Alternaria alternata* colonization. *Phytopathology*. 97 (11): 1415-1421.

I hereby declare that the details and information given above are complete and true to the best of my knowledge.



JENNIFER M. NIEM

## CURRICULUM VITAE

**NAME:** JENA JOY G. APOLINARIO  
**POSITION:** Assistant Professor 1  
**OFFICE ADDRESS:** Institute of Weed Science, Entomology and Plant Pathology  
College of Agriculture and Food Sciences  
University of the Philippines Los Baños  
College, Laguna 4031 Philippines  
Tel Fax (63-49) 536-1315  
Mobile +63 995 964 7495  
Email jgapolinario1@up.edu.ph

**FIELD OF SPECIALIZATION:** Insect Ecology, Pest Management

### EDUCATIONAL BACKGROUND:

Doctor of Philosophy in Entomology *candidate*, UP Los Baños  
MS in Entomology, UP Los Baños, 2015  
BS Agriculture, Major in Entomology, UP Los Baños, 2007

### PUBLICATIONS:

ISI-Journals:3  
Number Papers Presented in International and Local Scientific Conferences: 20  
Extension materials developed: 3

### RESEARCH PROJECTS:

#### *On-going*

- Building Rural Community Capacity Towards Resiliency of Mango and Coconut Livelihoods in Luzon (PCAARRD funded, Study Leader)
- Development of area-wide management approaches for fruit flies in mango for Indonesia, Philippines, Australia and the Asia Pacific Region (ACIAR funded, Study Leader)
- Field Evaluation of Jade 200SC against major insect pests of mango (Project leader)

#### Completed Projects:

As Project Leader – 8

As Study Leader - 9

### TECHNOLOGIES DEVELOPED AND COPYRIGHTED:

Mango IRM web application (with Dr. Celia dR. Medina and Dr. Luis Rey I. Velasco)

### TECHNICAL ACCREDITATIONS

Fertilizer and Pesticide Authority

Accredited Researcher – Entomology

Accredited Researcher – Supervised Pesticide Residue Trial

Bureau of Agriculture and Fisheries Standards

Certified Researcher for Organic Biocontrol Agents

### SCHOLARSHIP AND AWARDS:

- DOST- Accelerated Science and Technology Human Resource Development Program scholarship (2011 & 2019)
- 2<sup>nd</sup> Best Poster Award. International Symposium on Insects. Kuala Lumpur, Malaysia. (2012)
- Pest Management Council of the Philippines (PMCP)-Bayer Cropscience Best Undergraduate Thesis in Entomology (2008)

## GAD Checklists 2: For the Project Identification and Design Stages

**Note: Put 'X' mark on appropriate box**

Element and items/question (col.1)	Done? (col.2)			Score for an item/ element (col.3)	Comments/ gender issues identified (col.4)
	No (2a)	Partly (2b)	Yes (2c)		
1.0 Involvement of women and men (max score: 2; for each item, 1)				<b>2</b>	
1.1 Participation of women and men in beneficiary groups in the identification of the problem (possible scores: 0, 0.5, 1.0)			x	1	
1.2 Participation of women and men in beneficiary groups in project design (possible scores: 0, 0.5, 1.0)			x	1	
2.0 Collection of sex-disaggregated data and gender-related information (possible scores: 0, 1.0, 2.0)	x			<b>0</b>	
3.0 Conduct of gender analysis and identification of gender issues (max score: 2; for each item, 1)				1	
3.1 Analysis of gender gaps and inequalities related to gender roles, perspectives and needs, or access to and control of resources (possible scores: 0, 0.5, 1.0)	x			0	
3.2 Analysis of constraints and opportunities related to women's and men's participation in the project (possible scores: 0, 0.5, 1.0)			x	1	
4.0 Gender equality goals, outcomes, and outputs (possible scores: 0, 1.0, 2.0) Does the project have clearly stated gender equality goals, objectives, outcomes or outputs?		x		1	
5.0 Matching of strategies with gender issues (possible scores: 0, 1.0, 2.0) Do the strategies and activities match the gender issues and gender equality goals identified?	x			<b>0</b>	
6.0 Gender analysis of the likely impacts of the project (max score: 2, for each item, 0.67)				<b>1.67</b>	
6.1 Are women and girl children among the direct or indirect beneficiaries? (possible scores: 0, 0.33, 0.67)			x	0.67	
6.2 Has the project considered its long-term impact on women's socioeconomic status and Empowerment? (possible scores: 0, 0.33, 0.67)			x	0.67	
6.3 Has the project included strategies for avoiding or minimizing negative impacts on women's status and welfare? (possible scores: 0, 0.33, 0.66)		x		0.33	
7.0 Monitoring targets and indicators (possible scores: 0, 1.0, 2.0) Does the project include gender equality targets and indicators to measure gender equality outputs and outcomes?	x			<b>0</b>	
8.0 Sex-disaggregated database requirements (possible scores: 0, 1.0, 2.0) Does the project M&E system require the collection of sex-disaggregated data?	x			<b>0</b>	
9.0 Resources (max score: 2; for each item, 1)				<b>2</b>	
9.1 Is the budget allotted by the project sufficient for gender equality promotion or integration? OR, will the project tap counterpart funds from LGUs/ partners for its GAD efforts? (possible scores: 0, 0.5, 1.0)			x	1	
9.2 Does the project have the expertise to promote gender equality and women's empowerment? OR, is the project committed to investing project			v	1	

staff time in building capacities within the project to integrate GAD or promote gender equality? (possible scores: 0, 0.5, 1.0)			^		
10.0 Relationship with the agency's GAD efforts (max score: 2; for each item, 0.67)				1.67	
10.1 Will the project build on or strengthen the agency/ PCW/ government's commitment to the empowerment of women? (possible scores: 0, 0.33, 0.67) IF THE AGENCY HAS NO GAD PLAN: Will the project help in formulating the implementing agency's GAD plan?			x	0.67	
10.2 Will the project build on the initiatives or actions of other organization in the area? (possible scores: 0, 0.33, 0.67)			x	0.67	
10.3 Does the project have an exit plan that will ensure the sustainability of GAD efforts and benefits? (possible scores: 0, 0.33, 0.67)		x		0.33	
TOTAL GAD SCORE FOR THE PROJECT IDENTIFICATION AND DESIGN STAGES				9.34	

#### Interpretation of the GAD score

- 0 - 3.9 GAD is invisible in the project (proposal is returned).
- 4.0 - 7.9 Proposed project has promising GAD prospects (proposal earns a "conditional pass," pending identification of gender issues and strategies and activities to address these and inclusion of the collection of sex-disaggregated data in the monitoring and evaluation plan).
- 8.0 - 14.9 Proposed project is gender-sensitive (proposal passes the GAD test) ✓
- 15.0 - 20.0 Proposed project is gender-responsive (proponent is commended).



## UNIVERSITY OF THE PHILIPPINES LOS BAÑOS

*Office of the Chancellor*

*[Signature]*  
06-16-2023  
THE CHANCELLOR

### INDORSEMENT

29 March 2023

Respectfully forwarded to Dr. Reynaldo V. Ebora, Executive Director, Philippine Council for Agriculture, Aquatic and Natural Resources Research and Development (PCAARRD), Department of Science and Technology, Los Baños, Laguna, the attached project proposal of Asst. Prof. John Edward L. Felipe, Assistant Professor 2, Institute of Crop Science, College of Agriculture and Food Science, University of the Philippines Los Baños, entitled "**Transcriptome Sequencing and Analysis of Philippine 'Carabao' Mango Varieties: Identification of Candidate Genes for Fruit Quality Improvement,**" for possible funding.

The general objective of the research project is to identify candidate genes involved in improving fruit quality in Philippine 'Carabao' mango using RNA-seq technology and develop molecular markers for mango varietal improvement program. Specifically, it aims to accomplish the following:

1. Generate a transcriptome dataset for selected Philippine 'Carabao' mango varieties using RNA-seq;
2. Identify differentially expressed genes associated with disease and insect resistance, and other fruit quality traits based on functional annotation and gene ontology analysis;
3. Validate the expression of candidate genes using quantitative RT-PCR and evaluate their potential for use in the mango breeding program;
4. Develop molecular markers linked to the identified candidate genes for marker-assisted breeding;
5. Create a public library for the annotated transcriptome of 'Carabao' mango; and
6. Prepare, and finalize project reports, and articles for publication in scientific

The proposed project will run for 2 years with a budgetary requirement of Php 4,999,960.95. Correspondingly, the implementing agency commits as its counterpart funds the amount of Php 1,153,462.40 in the form of personal services and maintenance and other operating expenses. In support of the University's strategic plan to increase the scientific productivity of UPLB, the project is expected to generate at least 1 journal article (Web of Science). Furthermore, this project is classified under UPLB AGORA Focus Area: Food Security and Sovereignty and Sustainable Development Goals: Zero Hunger, Good Health and Well-being, and Industry, Innovation and Infrastructure. This project is classified under AGORA's Focus Area: One Health and aligned to address the Sustainable Development Goal: Good Health and Well-being.

This proposal is being endorsed subject to UPLB legal office requirements herein attached.

*[Signature]*  
**FERNANDO O. PARAS, JR.**  
Vice Chancellor for Planning and Development  
and Officer-in-Charge

Attachment: a/s  
CJCJ-I-23-056