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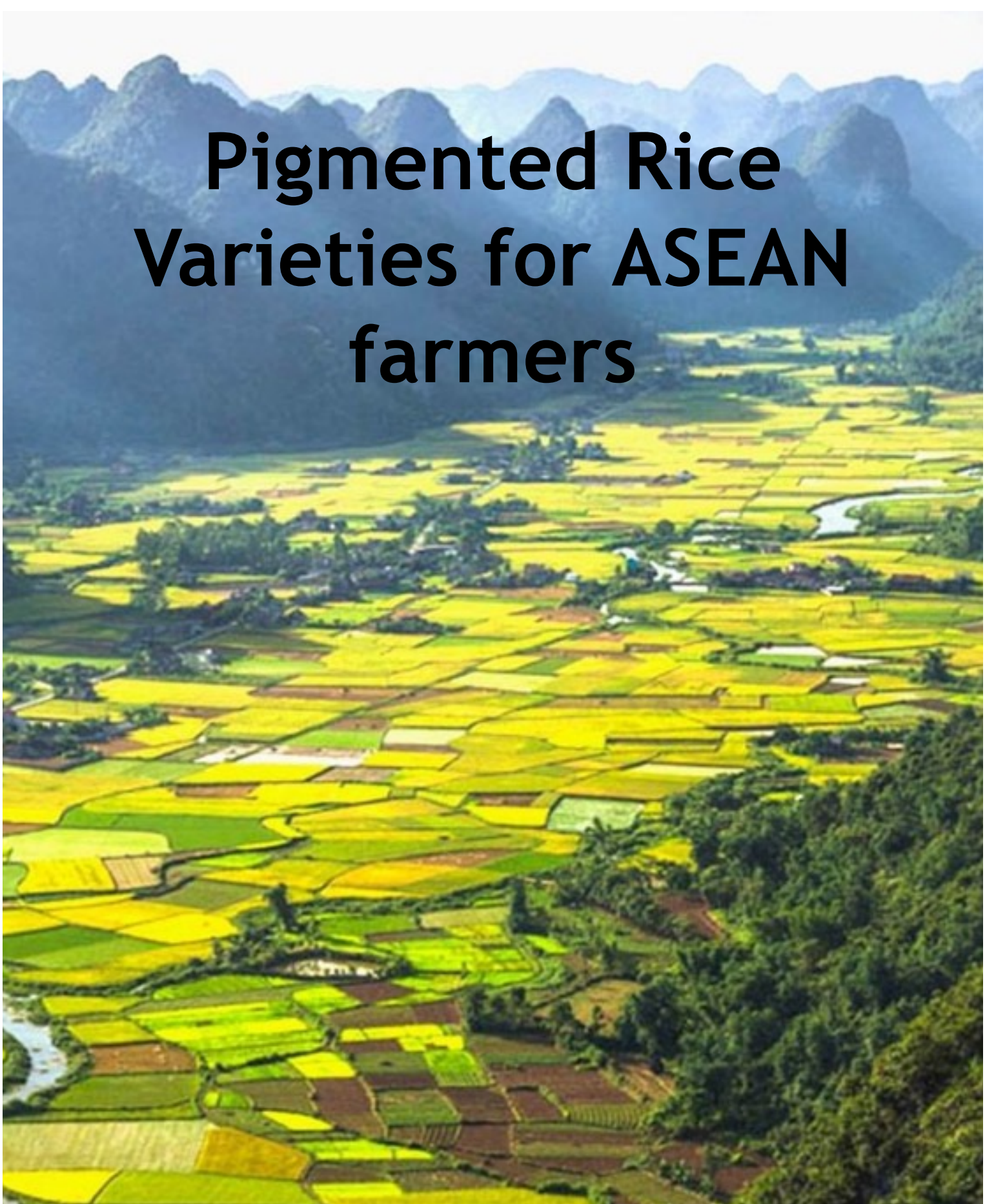
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Pigmented Rice Varieties for ASEAN farmers





Research and Innovation for Development in ASEAN (RIDA)

Final report

Project title: Pigmented Rice Varieties for ASEAN farmers.

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1. Executive summary.

Pigmented rice landraces have been cultivated for centuries in Southeast Asia, valued for their unique nutraceutical properties. Despite their benefits, these traditional varieties face significant challenges such as productivity fluctuations and limited market access. This project leverages genomic-assisted breeding to enhance the agronomic performance and nutritional attributes of pigmented rice, aiming to improve the livelihoods of rural communities in the ASEAN region. Traditional pigmented rice varieties, while nutritionally beneficial, suffer from inconsistent yields and are vulnerable to environmental stressors. This unpredictability affects the economic stability of rural farmers who depend on rice cultivation for their livelihoods. Enhancing the resilience and productivity of these rice varieties is crucial for the socio-economic development of these regions.

The research objectives of this project are centred on advancing the understanding and improvement of pigmented rice landraces. The first aim is to assemble complete genomes of ten high-performing pigmented rice varieties by employing advanced long-read sequencing technologies. Building on this genetic foundation, the next goal is to develop molecular assays that facilitate genome-assisted selection, enabling the enhancement of both agronomic traits and nutritional quality in these rice varieties. To complement the genomic studies, the project seeks to conduct a thorough metabolic characterisation alongside an agronomic evaluation of the selected rice varieties to better understand their performance and potential. Finally, the findings from this research will be shared broadly, coupled with targeted training programs designed for local farmers, breeders, and researchers to ensure practical application and widespread benefit. The methodology for this project involves a multifaceted approach to achieve its objectives. For genome assembly, Oxford Nanopore Technologies will be utilised to generate gapless assemblies of the selected pigmented rice varieties, providing a comprehensive genetic blueprint. Following this, Diversity Arrays Technology (DART) assays will be developed to identify genetic markers linked to desirable traits, enabling targeted improvement efforts. To explore the biochemical profiles of these rice varieties, advanced metabolic characterisation will be conducted using state-of-the-art GC/LC-MS techniques at Warwick Scientific Services, focusing on secondary

metabolites. Complementing the genetic and metabolic studies, agronomic evaluation will be carried out through field trials in Thailand and Indonesia, where the improved rice varieties will be assessed for their performance under diverse environmental conditions. This integrated approach ensures both scientific depth and practical applicability. The anticipated outcomes of this project are both scientific and practical. High-quality genome assemblies of pigmented rice landraces will be achieved, laying a robust foundation for genetic analysis and improvement. Additionally, molecular markers for genome-assisted selection will be developed and validated, providing tools for targeted breeding efforts. A comprehensive metabolic profiling of rice seeds will offer insights into their biochemical composition, while a carefully designed breeding strategy will aim to enhance yield and resilience in the selected varieties.

The broader implications of this research extend beyond the immediate scientific advancements. By improving the productivity and nutritional quality of pigmented rice, the project addresses key challenges related to food security, economic stability, and public health, particularly in rural communities across Southeast Asia. Furthermore, the scalability of these genomic-assisted approaches opens avenues for regional and global applications, potentially transforming agricultural practices and benefiting farming communities worldwide.

The project is supported by a multidisciplinary team and a network of collaborators, bringing together leading experts and state-of-the-art facilities from across the globe. The Principal Investigator, Prof. Jose Gutierrez-Marcos (University of Warwick, UK), spearheads the project, working alongside Co-Investigators Dr. Yekti Asih Purwestri (Universitas Gadjah Mada, Indonesia) and Dr. Chakkree Lekklar (Rajanagarindra University, Thailand). Additional expertise is provided by project collaborators, including Prof. Alisdair Fernie (Max-Planck-Institute of Molecular Plant Physiology, Germany), Dr. Andrzej Kilian (Diversity Arrays Technology, Australia), and Dr. Daniel Tunner (Oxford Nanopore Technologies, UK). The project also involves Dr. Sasongko Sasongko, Director of Jogja Benih, and Dr. Kristantini Kristantini from the Indonesia Assessment Institute of Agriculture Technology, both contributing to technology development and local implementation. The team leverages cutting-edge expertise and facilities from partner institutions. The University of Warwick offers state-of-the-art resources for genomics, proteomics, and computational research. Universitas Gadjah Mada provides advanced laboratories for molecular and biochemical studies, while Rajanagarindra University supports the project with greenhouses and field stations for crop research. The Max-Planck-Institute of Molecular Plant Physiology specializes in molecular and functional analysis of plant metabolism. Diversity Arrays Technology brings its expertise in molecular marker development and genotyping,

complemented by Oxford Nanopore Technologies' proficiency in nanopore sequencing. Local dissemination and innovation are supported by Jogja Benih, collaborating with the Indonesian Ministry of Agriculture, and the Indonesia Assessment Institute of Agriculture Technology, which focuses on the development and assessment of agricultural innovations. Together, this diverse team and infrastructure ensure the project's success from research to real-world application.

2. Introduction.

Rice has been a staple food in tropical and subtropical East Asia for nearly 14,000 years. Despite significant advances in rice breeding, local farmers in Southeast Asian countries continue to cultivate indigenous pigmented rice landraces. These varieties, while beneficial for their nutraceutical properties and local adaptation, face unpredictable productivity, posing a significant challenge to the socio-economic development and well-being of rural areas in the ASEAN region. This analysis explores the importance of enhancing the agronomical and nutritional characteristics of pigmented rice varieties through genomic-assisted breeding programs, aiming to provide a comprehensive understanding of its impact on agricultural productivity and rural socio-economic development.

The domestication and cultivation of rice in Southeast Asia have a rich history that spans almost 14 millennia. Within this period, three main varietal groups have been predominantly cultivated: japonica, indica, and javanica. Traditional farming practices in rural areas have played a crucial role in preserving a vast portion of rice genetic diversity across these groups. This genetic diversity includes over two hundred pigmented rice varieties, with approximately half being cultivated by small-hold farmers in the ASEAN region (Harwood, 2019). These pigmented rice varieties have adapted to various geographical distributions, spanning both lowlands and highlands, leading to unique environmental adaptations that result in a wide range of cultivated landraces (Fig. 1). One of the most significant advantages of pigmented rice varieties is their rich nutraceutical properties. These varieties are high in natural flavonoids, particularly phenolics and anthocyanins, which are potent antioxidants (Chen et al., 2021). Scientific studies have demonstrated that these compounds can reduce hyperglycemia, hyperlipidemia, and tumorigenesis (Chaudhary et al., 2019). Moreover, pigmented rice grains have a glycemic index lower than that of white rice, enhancing their appeal as a "functional food" (Jiamyangyuen & Srisuwan, 2017). Despite these health benefits, the productivity of current pigmented rice landraces remains unpredictable, which

substantially impacts the economic stability and development of rural farming communities in Southeast Asia.

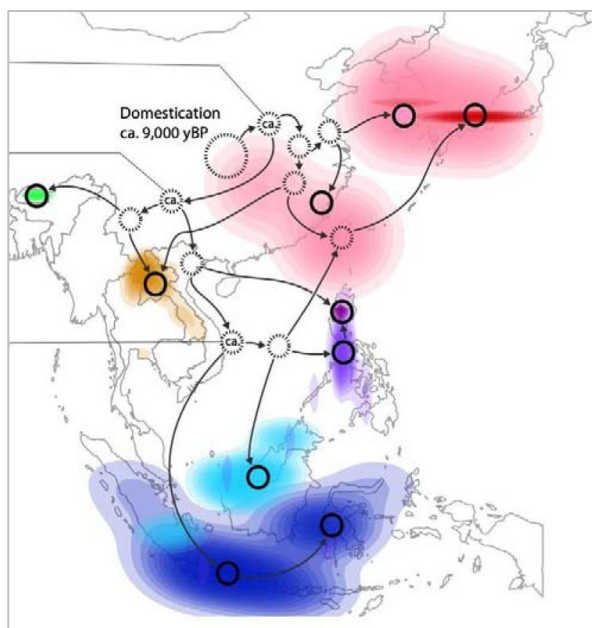


Figure 1. Proposed dispersal map of japonica white and pigmented rice landraces (Gutaker et al., 2020).

The unpredictable productivity of pigmented rice landraces presents a significant challenge for farmers in the region. This stochastic productivity affects the economic development of rural areas where stable agricultural yields are essential for economic stability and growth (Yoshida et al., 2019). Improving the consistency and reliability of these rice varieties' productivity is crucial for the socio-economic development of ASEAN rural communities, ensuring that local farmers can depend on their crops for both subsistence and income. To address the challenges faced by farmers cultivating pigmented rice varieties, genomic-assisted breeding programs offer a promising solution. These programs leverage advanced technologies to enhance both the agronomical and nutritional characteristics of rice varieties, thereby improving their productivity and health benefits. The first objective of these programs is to assemble the genomes of ten high-performing pigmented rice landraces. By utilizing long-read sequencing technologies, such as those developed by Oxford Nanopore Technologies (ONT), researchers aim to generate gapless genome assemblies, also known as telomere-to-telomere assemblies, for these landraces (Zhang et al., 2021). These comprehensive genome assemblies will provide a detailed genetic blueprint, enabling the identification of key traits that contribute to robust agronomic performance.

The second objective is to develop molecular assays that can inform and guide breeding programs. These assays will help identify specific genetic markers associated with desirable

traits, such as high nutritional and antioxidant content, and improved seed yield (Huang et al., 2018). By using these molecular tools, breeders can select and propagate rice varieties that exhibit these beneficial traits, thereby enhancing the overall quality and productivity of pigmented rice. This genomic-assisted approach not only addresses the current challenges of stochastic productivity but also ensures that the improved rice varieties retain their valuable nutraceutical properties. The implementation of genomic-assisted breeding programs has the potential to bring numerous benefits to the agricultural landscape of Southeast Asia. Enhancing the agronomical traits of pigmented rice varieties will lead to more reliable and consistent yields, providing farmers with a stable source of income and contributing to the overall economic development of rural areas (Sweeney & McCouch, 2007). Additionally, improving the nutritional qualities of these rice varieties will offer significant health benefits to consumers, addressing issues related to malnutrition and diet-related diseases. The combination of improved productivity and enhanced health benefits positions pigmented rice as a valuable crop for both farmers and consumers in the ASEAN region.



Figure 2. *Pigmented rice varieties commercially available in Thailand (Napasintuwong, O. 2020).*

The importance of improving the agronomical and nutritional characteristics of pigmented rice varieties extends beyond immediate agricultural and economic benefits. It is predicted that the cost of staple foods will increase significantly over the next five decades, leading to potential rises in malnutrition, diseases, and political instability (Godfray et al., 2010). In this context, the development of high-performing pigmented rice varieties is not only an agricultural imperative but also a critical strategy for ensuring food security and socio-economic stability in Southeast Asia. Over the past century, plant breeding has been fundamental in driving agricultural revolutions. However, to address the challenges of the 21st century, it is essential to dramatically increase food production, particularly in densely populated regions like Southeast Asia. Farmers in ASEAN countries have played a crucial

role in the domestication and cultivation of wild rice varieties, preserving a rich genetic diversity that holds immense potential for future breeding programs (Fuller et al., 2010). By tapping into this genetic and metabolic variation, researchers and breeders can develop pigmented rice varieties that offer both high productivity and significant health benefits.

Future work in this area should focus on expanding the genomic research to include a wider range of pigmented rice varieties, ensuring that the genetic diversity within these landraces is fully explored and utilized (Xia et al., 2018). Additionally, collaborations with local farmers and agricultural institutions in ASEAN countries will be vital for the successful implementation of breeding programs. By integrating traditional knowledge with advanced genomic technologies, it is possible to develop rice varieties that are not only high-performing but also culturally and environmentally appropriate for the regions where they are cultivated. Furthermore, ongoing research should investigate the environmental adaptations of pigmented rice varieties, identifying traits that contribute to their resilience in different ecological niches. Understanding these adaptations will enable breeders to develop rice varieties that are better suited to the changing climatic conditions predicted for the region (Bhatia et al., 2020). This holistic approach to breeding, which considers both genetic and environmental factors, will be essential for creating sustainable agricultural systems that can withstand future challenges.

Improving the agronomical and nutritional characteristics of pigmented rice varieties grown by farmers in Southeast Asia is of paramount importance for the region's agricultural productivity and socio-economic development. By leveraging advanced genomic technologies and implementing genomic-assisted breeding programs, it is possible to enhance the productivity and health benefits of these rice varieties, providing significant advantages to both farmers and consumers. The successful development of high-performing pigmented rice varieties will contribute to food security, economic stability, and improved health outcomes in the ASEAN region, ensuring a sustainable and prosperous future for its rural communities.

3. Background of the Research.

The domestication and cultivation of rice in Southeast Asia have a rich history that spans almost 14 millennia. Within this period, three main varietal groups have been predominantly cultivated: japonica, indica, and javanica. Traditional farming practices in rural areas have played a crucial role in preserving a vast portion of rice genetic diversity across these groups.

This genetic diversity includes over two hundred pigmented rice varieties, with approximately half being cultivated by small-hold farmers in the ASEAN region (Harwood, 2019). These pigmented rice varieties have adapted to various geographical distributions, spanning both lowlands and highlands, leading to unique environmental adaptations that result in a wide range of cultivated landraces. One of the most significant advantages of pigmented rice varieties is their rich nutraceutical properties. These varieties are high in natural flavonoids, particularly phenolics and anthocyanins, which are potent antioxidants (Chen et al., 2021). Scientific studies have demonstrated that these compounds can reduce hyperglycemia, hyperlipidemia, and tumorigenesis (Chaudhary et al., 2019). Moreover, pigmented rice grains have a glycemic index lower than that of white rice, enhancing their appeal as a "functional food" (Jiamyangyuen & Srisuwan, 2017). Despite these health benefits, the productivity of current pigmented rice landraces remains unpredictable, which substantially impacts the economic stability and development of rural farming communities in Southeast Asia.

The unpredictable productivity of pigmented rice landraces presents a significant challenge for farmers in the region. This stochastic productivity affects the economic development of rural areas where stable agricultural yields are essential for economic stability and growth (Yoshida et al., 2019). Improving the consistency and reliability of these rice varieties' productivity is crucial for the socio-economic development of ASEAN rural communities, ensuring that local farmers can depend on their crops for both subsistence and income. To address the challenges faced by farmers cultivating pigmented rice varieties, genomic-assisted breeding programs offer a promising solution. These programs leverage advanced technologies to enhance both the agronomical and nutritional characteristics of rice varieties, thereby improving their productivity and health benefits.

4. Advanced tools to decipher complex genomic information.

The ability to sequence DNA and RNA with high accuracy and resolution has revolutionized the field of genomics, facilitating numerous advances in biological research and medical diagnostics. While short-read sequencing technologies have been predominant in the past, their limitations in dealing with repetitive and complex genomic regions have spurred the development of long-read sequencing technologies. Oxford Nanopore Technologies (ONT) has emerged as a leader in this space, providing innovative tools that enable the sequencing of long DNA and RNA molecules, thus offering a more comprehensive view of the genome.

The history of sequencing technologies can be traced back to the developed of the chain-termination method. This method was the cornerstone of the Human Genome Project and enabled the first complete sequencing of the human genome. However, this sequencing technology is limited by short read lengths and high costs, making it unsuitable for high-throughput applications (Sanger et al., 1977; Mardis, 2013).

The advent of next-generation sequencing (NGS) technologies in the mid-2000s addressed some of these limitations by enabling massively parallel sequencing, thereby increasing throughput and reducing costs. However, NGS technologies, which rely on short-read sequences, struggle with repetitive and complex genomic regions, making it difficult to assemble complete genomes and identify structural variations (Metzker, 2010; Mardis, 2008). Long-read sequencing technologies, developed more recently, overcome these limitations by producing read lengths that can span kilobases to megabases of DNA, allowing for more accurate assembly of genomes and detection of structural variants. Among these technologies, those developed by Oxford Nanopore Technologies have gained significant attention due to their unique approach and capabilities (Jain et al., 2016; Rhoads & Au, 2015).

Oxford Nanopore Technologies (ONT) was developed with the aim of developing portable, scalable, and affordable sequencing technologies. ONT's core technology is based on nanopore sequencing, which involves passing single-stranded DNA or RNA molecules through a nanopore and measuring the changes in ionic current as different nucleotides pass through the pore. These changes are then used to determine the sequence of the nucleotides (Clarke et al., 2009; Deamer et al., 2016). One of the key innovations of ONT is the use of biological nanopores, such as those derived from alpha-hemolysin or *Mycobacterium smegmatis* porin A (MspA), which are inserted into a synthetic membrane. The passage of nucleotides through the nanopore disrupts the ionic current in a characteristic manner, allowing for real-time sequencing of long DNA or RNA molecules. ONT's devices, including the portable MinION, the high-throughput PromethION, and the compact Flongle, cater to a wide range of sequencing needs and applications (Jain et al., 2016; Mikheyev & Tin, 2014).



Figure 3. Long-read sequencing Oxford Nanopore platform used for deciphering the genome of black rice landraces.

The core technology of ONT is its use of nanopores for sequencing, which offers several advantages over traditional sequencing methods. These nanopores can read long DNA or RNA molecules without the need for fragmentation, preserving the native structure of the molecules. This capability is particularly useful for sequencing repetitive regions and complex genomic rearrangements, which are challenging for short-read technologies (Kasianowicz et al., 1996; Branton et al., 2008). One of the most significant technical innovations of ONT is the real-time nature of its sequencing technology. Unlike traditional sequencing methods, which require extensive preprocessing and batch processing of samples, ONT's nanopore sequencing provides immediate data output as the DNA or RNA molecules pass through the nanopore. This real-time capability is crucial for applications requiring rapid results, such as pathogen detection, outbreak surveillance, and clinical diagnostics (Deamer et al., 2016; Rang et al., 2018). ONT's devices are also designed to be highly portable and scalable. The MinION, for example, is a portable sequencer that can be connected to a laptop via USB and used in field settings, making it ideal for applications such as environmental monitoring and infectious disease outbreak response. The PromethION, on the other hand, is a high-throughput sequencer designed for large-scale genomic projects, capable of generating terabases of data in a single run. The Flongle, a smaller and more affordable device, provides an entry point for users with lower throughput needs (Jain et al., 2016; Laver et al., 2015). The ability to generate long reads has significantly impacted various areas of genomic research. One of the primary advantages of long-read sequencing is its ability to accurately

assemble genomes, particularly those with high levels of complexity or repetitive regions. This capability has been instrumental in the assembly of complete genomes for a wide range of organisms, including previously uncharacterized microorganisms, plants, and complex eukaryotic genomes (Michael et al., 2018; Koren et al., 2013). In the field of genomics, long-read sequencing offers a more detailed view of the genetic alterations that drive the development of multicellular organisms. Traditional short-read sequencing methods often miss structural variations and complex rearrangements that can be critical for understanding complex biology. Long-read sequencing enables the detection of these variations with higher accuracy, providing insights that can inform treatment strategies and improve research outcomes (Magi et al., 2018; De Coster et al., 2019). Additionally, long-read sequencing has proven valuable in the study of epigenetics, particularly in understanding DNA methylation patterns. ONT's technology allows for the direct detection of DNA modifications, providing a more comprehensive view of the epigenetic landscape and its role in gene regulation and disease (Simpson et al., 2017; Rand et al., 2017). In agricultural settings, the high accuracy and comprehensive nature of the data obtained from long-read sequencing technologies are paving the way for advancements in genetic improvement. One of the most significant applications is in the detection of genetic mutations, including those in repetitive or GC-rich regions that are challenging for short-read technologies. This capability enhances accuracy and supports the development of targeted genetic selection (Wang et al., 2015; Mantere et al., 2019). ONT's long-read sequencing technologies are also being used for plant and animal pathogen detection and surveillance. The portability and rapid turnaround time of devices like the MinION make them suitable for point-of-care testing and field-based applications. During the pathogen outbreaks, for example, the MinION was used to rapidly sequence viral genomes, providing critical information for response and treatment efforts (Quick et al., 2016; Faria et al., 2016). Moreover, long-read sequencing has potential applications in the understanding of chromosomal rearrangements. Many of these variations are caused by structural variations or complex rearrangements that are difficult to detect with short-read sequencing. Long-read sequencing provides a more accurate and comprehensive view of the genome, facilitating the identification of the genetic basis of these structural differences and enabling more effective genomic interpretation (Cretu Stancu et al., 2017; Huddleston et al., 2017).

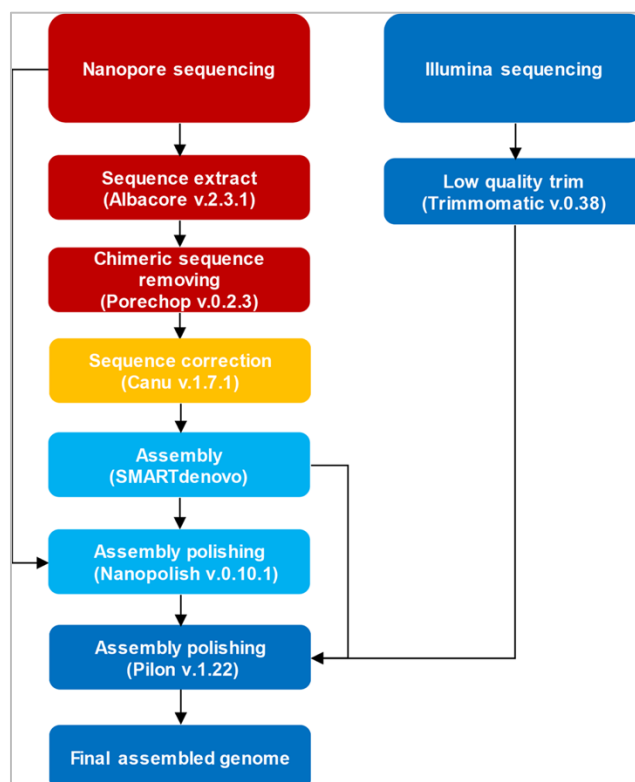


Figure 4. Nanopore data analysis overview used to assemble and polish the genome of black rice landraces.

Despite the numerous advantages of long-read sequencing technologies, several challenges remain. One of the primary challenges is the accuracy of nanopore sequencing. While the accuracy of ONT's base-calling algorithms has improved significantly over the years, it still lags behind that of some short-read platforms. Efforts are ongoing to enhance base-calling algorithms and develop robust error correction methods to address this issue (Loman et al., 2015; Goodwin et al., 2016). The relatively high cost of long-read sequencing, particularly for large-scale projects, is another barrier. While the cost per base has decreased over time, it remains higher than that of short-read sequencing. Reducing costs and increasing throughput will be critical for making long-read sequencing more accessible to a broader range of researchers and applications (Wenger et al., 2019; Payne et al., 2019). Furthermore, the large volumes of data generated by long-read sequencing present challenges for data storage, processing, and analysis. Developing efficient algorithms and computational tools to handle and interpret these data is essential for maximizing the potential of long-read sequencing technologies (Amarasinghe et al., 2020; Li et al., 2018). Several areas hold promise for the future development of long-read sequencing technologies. One area of focus is the integration of long-read sequencing with other technologies, such as CRISPR-based methods, to enable targeted sequencing of specific genomic regions. This approach could enhance the resolution and accuracy of genomic

analyses, particularly for complex and repetitive regions (Gilpatrick et al., 2020; Shendure et al., 2017). Another promising area is the development of novel nanopore chemistries and modifications to improve sequencing accuracy and throughput. Advances in nanopore design and engineering could further enhance the performance of ONT's technologies, making them even more powerful tools for genomic research (Eisenstein, 2020; Rang et al., 2018).

5. Aim of the project.

The project aims to assemble the genomes of high-performing pigmented rice landraces utilizing long-read sequencing technologies, such as those developed by Oxford Nanopore Technologies (ONT). Using this knowledge, we will determine the levels of genetic variation and link this information to the composition of secondary metabolites present in seeds. This information will allow the design of prediction models for genomic selection, to improve both agronomic and metabolic traits.

6. Relevance of Advanced Genomic Approaches.

Genomic-assisted approaches not only address the current challenges of stochastic productivity but also ensure that the improved varieties retain their valuable agronomic characteristics. Enhancing the agronomical traits of varieties that are not genetically selected will lead to more reliable and consistent yields, providing growers with a stable source of income and contributing to the overall economic development of rural areas (Sweeney & McCouch, 2007; Wang et al., 2018; Tester & Langridge, 2010). Additionally, improving the nutritional qualities of cultivated landraces will offer significant health benefits to consumers, addressing issues related to malnutrition and diet-related diseases (Chen et al., 2021; Saltzman et al., 2013; Peng et al., 2019). These approaches hold profound significance for the targeted beneficiaries, primarily rural communities reliant on agricultural production. Through the utilisation of cutting-edge long-read sequencing technologies, it is now possible to design selection breeding programs to enhance crop productivity, empowering producers with resilient and high-yielding varieties tailored to local growth conditions (Jain et al., 2016; Zhang et al., 2021). This strategy not only improves income stability but also preserves indigenous agricultural practices and biodiversity (Harwood, 2019; Esquinas-Alcázar, 2005). The enhanced nutritional value of new varieties contributes to the overall well-being of rural communities, addressing issues of food security and public health (Chaudhary et al., 2019; Jarma et al., 2021).

Genome-assisted improvement programs have wider implications for sector-relevant innovation and development in Southeast Asia. Traditional landraces, which are cultivated across Thailand and Indonesia, each facing similar challenges related to agricultural productivity and sustainability, will serve as a model for innovation in the agricultural sector across the region, with potential scalability of the innovation showcased (Fuller et al., 2010; Xia et al., 2018). Collaborative efforts among ASEAN member states will facilitate the dissemination of successful breeding strategies and technological advancements, promoting a unified approach to agricultural development (Kumar et al., 2020; Tripathi et al., 2019).

While the immediate focus of this project is on Thailand and Indonesia, its implications extend to global food security efforts. The successful implementation of genomic-assisted breeding programs can serve as a blueprint for similar initiatives worldwide, addressing challenges related to climate change, population growth, and food scarcity (Zhang et al., 2021; Peng et al., 2019). By emphasizing the preservation of genetic diversity and integrating genomic data with traditional breeding practices, this project offers a model that can be adapted to various crops and regions (Wang et al., 2018; Louwaars et al., 2009). However, several challenges must be addressed to ensure the successful implementation and scalability of genomic-assisted breeding programs. These include the need for robust infrastructure, technical expertise, and fair management of data and intellectual property rights (Louwaars et al., 2009; Fan et al., 2020). Future research should focus on understanding the long-term impacts of genomic-assisted breeding on agricultural ecosystems and food systems (Tester & Langridge, 2010; Esquinas-Alcázar, 2005).

7. Recent advances in the metabolic profile of rice seeds.

Recent advances in Mass spectrometry (MS) have enabled the comprehensive analysis of small molecules in biological systems, with particular focus on the development of high-throughput analytical technologies. These advancements have significantly enhanced our ability to identify and quantify bioactive compounds in plants, with a growing emphasis on beneficial metabolites found in seeds (Fig. 5). Seeds are rich reservoirs of metabolites, both primary and secondary, which play essential roles in plant growth, defence, and adaptation. Moreover, these compounds often provide significant health benefits when consumed by humans, highlighting the importance of advanced analytical methods for their characterisation (Butelli et al., 2008). Mass spectrometry has become a cornerstone in metabolomics due to its exceptional sensitivity, specificity, and versatility in analysing complex biological samples. The basic principle of MS involves ionising molecules,

separating them based on their mass-to-charge ratio, and detecting them with high precision. Advances in MS instrumentation, such as time-of-flight (TOF), Orbitrap analysers, and hybrid systems like quadrupole-orbitrap, have further improved the resolution and accuracy of metabolite detection (Fig 5A-B). Coupling MS with separation techniques such as gas chromatography (GC) or liquid chromatography (LC) enhances the ability to resolve complex mixtures (Fig 5C), which is critical when analysing the diverse chemical composition of plant metabolites (Chen et al., 2019).

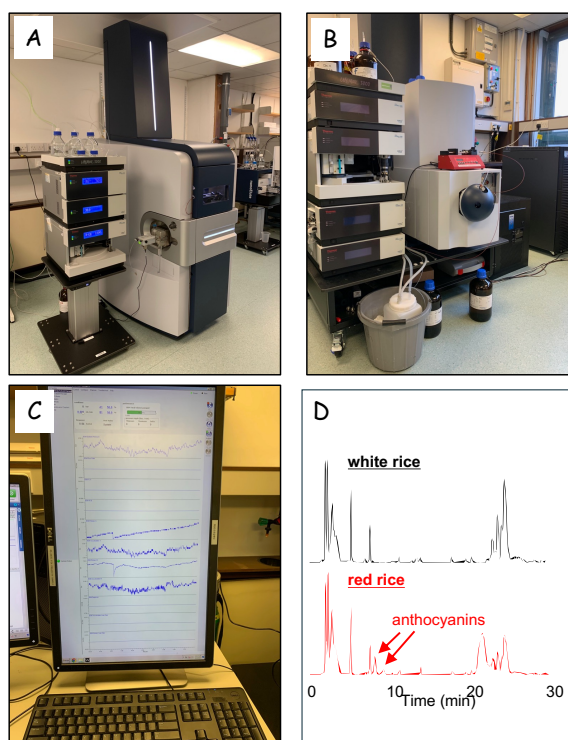


Figure 5. High-throughput metabolite analysis using chromatography coupled to mass spectrometry. (A) timsTOFF Impact II Mass Spectrometer. (B) MS profile of targeted metabolites. (C) Quantification of metabolites from white and red rice seeds. (D) Chromatogram showing anthocyanins in red rice seeds.

Recent developments have focused on ultra-high-performance liquid chromatography (UHPLC) and tandem mass spectrometry (MS/MS), which allow comprehensive metabolomic profiling with increased sensitivity and throughput (Fig. 5B). These techniques are complemented by data-independent acquisition methods that enable unbiased detection of a wide range of metabolites in a single experiment. Such capabilities are particularly valuable in untargeted metabolomics, where the goal is to obtain a global snapshot of the metabolome to discover new or underexplored compounds. In contrast, targeted metabolomics focuses on the precise quantification of specific metabolites, often guided by the use of internal standards and well-optimized protocols (Xu et al., 2019).

Plant metabolomics has increasingly targeted seeds due to their role as a concentrated source of bioactive compounds. These include primary metabolites such as carbohydrates,

amino acids, and lipids, which are essential for energy storage and germination, as well as secondary metabolites like phenolics, flavonoids, terpenoids, and alkaloids, which contribute to plant defence mechanisms and provide health-promoting properties. Among plant seeds, rice (*Oryza sativa*) has been a focal point for metabolomic studies due to its global significance as a staple food crop and its genetic and chemical diversity, particularly among pigmented rice varieties such as black, red, and purple rice (Fang et al., 2020).

Research into the metabolomic profiles of rice seeds has revealed the richness of their phytochemical composition. Pigmented rice varieties are particularly notable for their elevated levels of anthocyanins and proanthocyanidins, secondary metabolites that are responsible for their distinctive colouration and are associated with numerous health benefits (Fig. 5D). Anthocyanins, such as cyanidin-3-glucoside and peonidin derivatives, have been extensively studied for their antioxidant, anti-inflammatory, and anti-carcinogenic properties. Proanthocyanidins, another class of polyphenols, contribute to cardiovascular health through their ability to scavenge free radicals and modulate oxidative stress (Liu et al., 2010).

The use of mass spectrometry, often in combination with LC or GC, has proven indispensable in characterizing these metabolites. High-resolution MS systems have been employed to identify specific anthocyanin derivatives in pigmented rice, elucidating their molecular structures through MS/MS fragmentation patterns. These studies have not only confirmed the health-promoting properties of these compounds but have also linked them to the rice ability to withstand environmental stresses such as drought and high salinity. Untargeted metabolomic approaches have further expanded the understanding of the metabolite landscape in rice, unveiling novel bioactive compounds and highlighting the metabolic differences between pigmented and non-pigmented varieties. Multivariate statistical analyses, applied to MS data, have allowed researchers to correlate specific metabolomic signatures with phenotypic traits, including stress tolerance and nutritional value (Zhao et al., 2020).

Pigmented rice seeds have also been investigated using spatial metabolomics techniques, such as matrix-assisted laser desorption ionisation (MALDI) imaging mass spectrometry. This method provides detailed spatial distributions of metabolites within the seed, revealing that bioactive phenolic compounds are often localized in the aleurone layer and pericarp, regions that are particularly enriched in pigmented varieties. Such insights are valuable not only for understanding the functional roles of these metabolites but also for optimizing processing techniques to retain their health benefits in rice-based products (Xu et al., 2019). Advances in metabolomics have broad applications beyond basic research. The identification and characterisation of beneficial metabolites in rice seeds support the development of functional foods that are enriched with bioactive compounds, thereby

addressing consumer demand for health-promoting dietary options. Furthermore, metabolomic insights into the biochemical pathways underlying stress resistance can guide the breeding of rice varieties that are more resilient to environmental challenges, enhancing agricultural sustainability. Personalized nutrition, another emerging field, stands to benefit from metabolomic profiling by enabling the tailoring of dietary recommendations based on individual metabolic responses to specific rice varieties.

Despite these advances, challenges remain in the field of plant metabolomics. The complexity and dynamic nature of metabolomes requires sophisticated analytical tools and robust bioinformatics platforms for data processing, annotation, and interpretation. Comprehensive databases play a crucial role in metabolite identification, but gaps in coverage for plant-specific metabolites highlight the need for continued development. Furthermore, the reproducibility of metabolomic studies is often hampered by variability in sample preparation, extraction protocols, and instrumental conditions, necessitating standardized methodologies (Chen et al., 2019).

Recent advances in the application of mass spectrometry in plant metabolic determination have provided unparalleled insights into the diverse and beneficial metabolites present in seeds, with rice serving as a prominent model. Research on pigmented rice varieties has highlighted their potential as sources of health-promoting anthocyanins and proanthocyanidins, which contribute to their antioxidant and stress-resistant properties. Advances in analytical techniques have enabled the precise characterisation of these compounds and their spatial distribution within seeds. These findings pave the way for the development of functional foods, stress-resilient crops, and personalised dietary strategies. Continued innovation in instrumentation and bioinformatics will be essential to overcome existing challenges and unlock the full potential of metabolomic analysis in plant science.

8. Results.

Objective 1: Molecular Characterisation of Pigmented Rice Landraces

Task 1.1: Genome Assembly of Pigmented Rice Genomes

To achieve a comprehensive molecular characterisation of pigmented rice landraces, we began with the extraction of high molecular weight DNA exceeding 100 kb in length from ten distinct pigmented rice varieties known for their high anthocyanin content and resistance to drought and high salinity. The extracted DNA was subjected to a stringent size selection process to ensure that only the longest possible DNA molecules were loaded onto the PromethION nanopore sequencer.

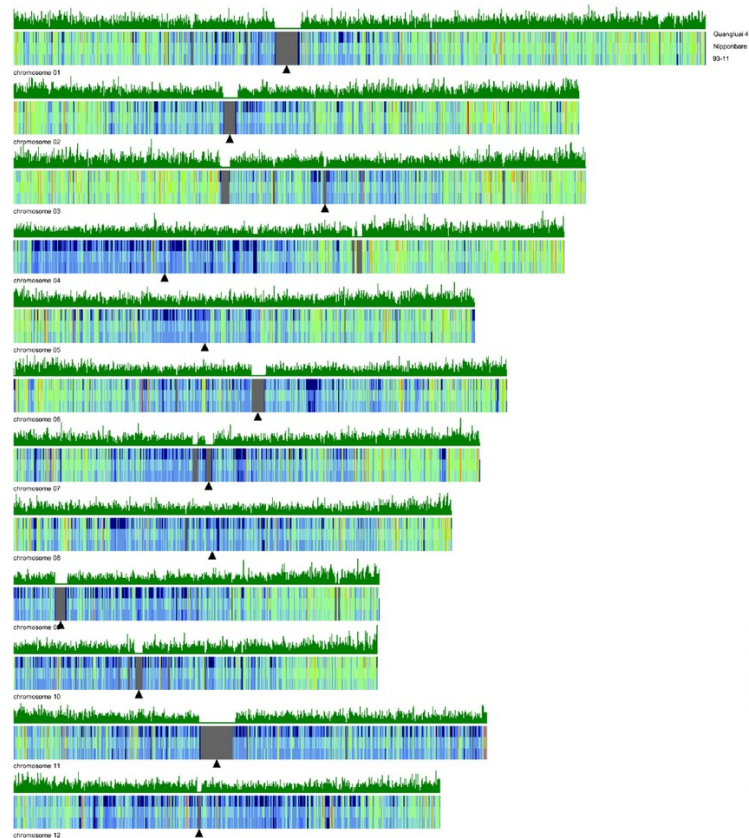


Figure. 5: Genome assembly of pigmented rice landraces. Schematic representation of gene density (green) and repetitive sequences (blue) in the different chromosomes.

Our target was to achieve a sequencing depth of over 100x, with read lengths of at least 15 kb (Fig. 5). The sequencing process generated raw FAST5 data files, which we used for base calling and genome assembly employing a range of methodologies, including Canu, Flye, and Falcon. The initial genome assemblies were further scaffolded using Ragoo and evaluated for contiguity against established benchmarking standards. We then iteratively polished these assemblies using Racon, incorporating sequences with lower read lengths but higher accuracy. This meticulous process aimed to produce assemblies with an accuracy exceeding 99.9%. This entire pipeline was repeated for each of the pigmented rice landraces, resulting in a diverse panel of high-quality genome assemblies. Using this information we have estimated the density of sequence polymorphisms (Fig. 6), which constituted the platform to identify landrace-specific molecular markers. We have developed for this project specific computational pipelines to accelerate this task, and we are now in a good position to train other researchers from collaborating laboratories.

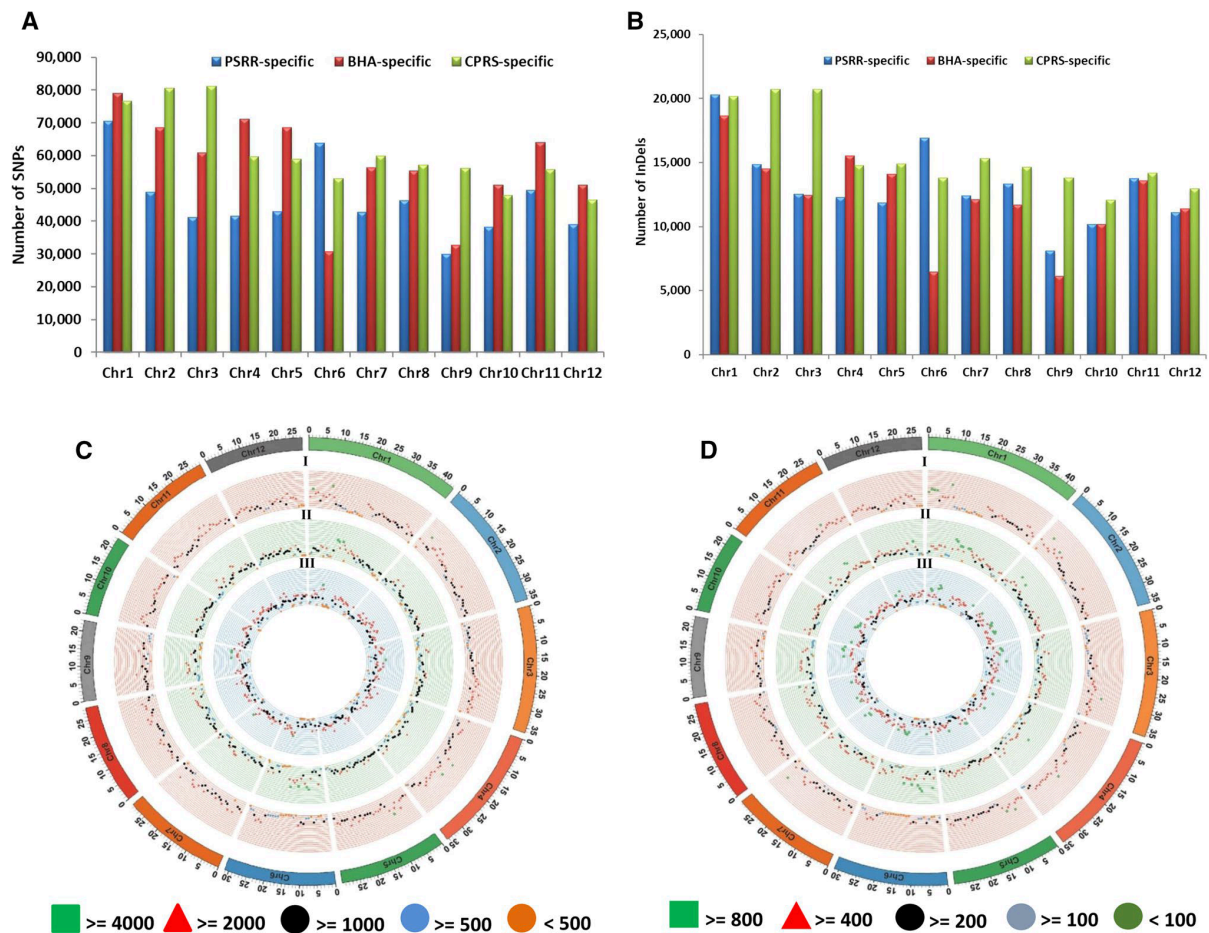


Figure 6: Genome assembly of two pigmented rice varieties allows the mapping of genotype-specific SNPs (a) and InDels (b). Genotype-specific SNPs and InDels are indicated. Circos plots showing the distribution of SNPs (c) and InDels (d) identified on each chromosome (1 Mb window size). The three inner circles represent the distribution of polymorphisms and are shown by different colours.

This part of the research has yielded significant findings that provide deep insights into the genetics and evolution of pigmented rice landraces. Through cutting-edge genomic techniques, we have successfully achieved gapless genome assemblies for ten diverse pigmented rice landraces. These high-resolution assemblies represent a significant advancement, offering an unparalleled view of the genetic structure of these rice varieties. In analysing these genomes, we identified over 2 million sequence variants across all genotypes within the study group and constructed a visualisation platform (genome browser) that allow users to visualise and extract sequence variant information (Fig. 7). These genetic differences are crucial for understanding the diversity among the selected landraces, highlighting unique traits that may contribute to their agronomic and nutritional qualities. To expand the context, these findings were further compared against 66 publicly available genome assemblies. This comparative analysis uncovered an even broader genetic

landscape, identifying more than 25 million sequence variants in total. Such extensive data provide a rich resource for exploring the genetic underpinnings of rice diversity. Importantly, this wealth of genomic information has enabled the team to trace the domestication trajectory of pigmented rice landraces.

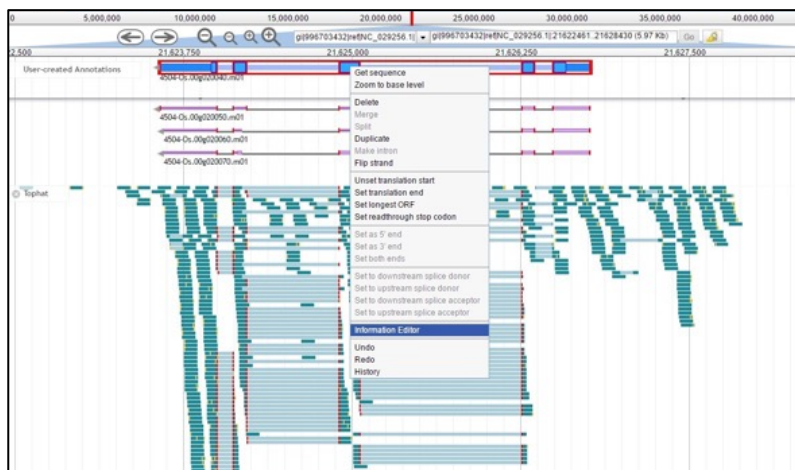


Figure. 7: Genome browser designed to visualise the sequence polymorphisms identified in the genome of pigmented rice landraces.

By examining the genetic relationships and lineage histories, the research has shed light on how these varieties have evolved under human cultivation. This work not only clarifies the domestication pathways of pigmented rice but also uncovers their genetic connections to widely cultivated rice varieties, revealing how traditional landraces contribute to modern rice biodiversity.

Task 1.2: Secondary Metabolite Composition of Seeds and Agronomic Performance of Pigmented Rice Landraces

Following the genome assembly, we proceeded with the extraction and analysis of secondary metabolites from lyophilised seeds of the selected pigmented rice landraces (Fig. 8, top panel). Metabolites were extracted with the addition of a standard before derivatisation and analysed using gas and liquid chromatography-time of flight-mass spectrometry (GC/LC-MS) at Warwick Scientific Services (Fig. 8, bottom panel).

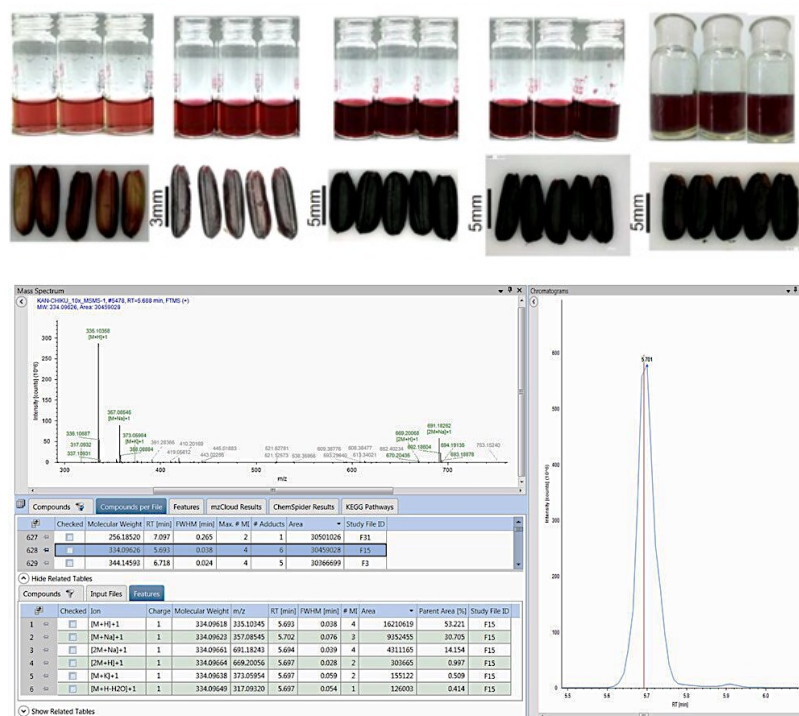


Figure. 8: Isolation and quantification of soluble metabolites from seeds of different pigmented rice landraces. Top panel, isolation of metabolites from lyophilised dry seed samples; Bottom panel, chromatogram showing the detection and quantification of different metabolites.

The spectral data obtained were cross-referenced by our project partners using an in-house metabolome database, facilitating the identification and quantification of primary and secondary metabolites (Fig.9). The results were expressed relative to the internal standard and per dry seed weight. The existing equipment and computational pipelines in the PI's laboratory allowed for an efficient and accurate analysis of the metabolites. This comprehensive metabolite profiling revealed unique seed metabolites specific to the selected ASEAN pigmented rice landraces, which are now being integrated into our genomic-selection breeding program as part of Objective 2. This detailed metabolomic analysis provides a foundational understanding of the metabolic composition of these rice varieties, paving the way for further genomic selection and breeding efforts.

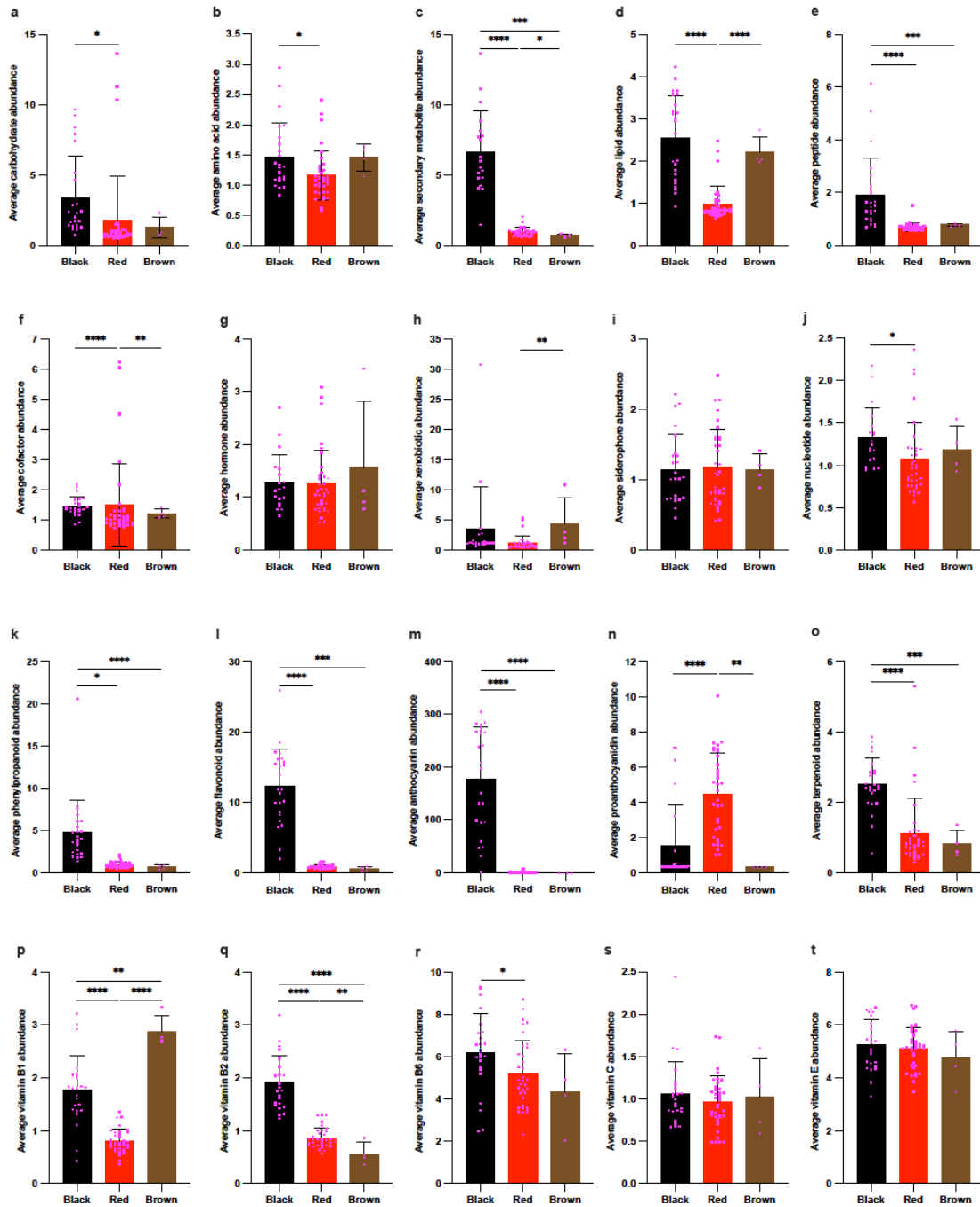


Figure 9: Metabolite abundance among pigmented rice varieties analysed. Unpaired *t*-test comparing black and red, black and brown, and red and brown rice. a) Carbohydrates. b) Amino acids. c) Secondary metabolites. d) Lipids. e) Peptides. f) Cofactors. g) Hormones. h) Xenobiotics. i) Siderophores. j) Nucleotides. k) Phenylpropanoids. l) Flavonoids. m) Anthocyanins. n) Proanthocyanidins. o) Terpenoids. p) Vitamin B1, q) Vitamin B2, r) Vitamin B6, s) Vitamin C, and t) Vitamin E. Asterisks represent significant differences: *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$; ****, $p < 0.0001$.

Objective 2: Development of Markers for Genome-Assisted Selection of Pigmented Rice Varieties with High Antioxidant Content and Improved Seed Yield

Task 2.1: Molecular Marker Development for Genome-Assisted Selection

Utilizing the high-quality genome assemblies generated in Objective 1, we have identified the molecular variations present in our collection of pigmented rice landraces. This identification process enabled the engineering of Diversity Arrays Technology (DArT) assays, which are crucial for calculating genomic estimated breeding values (GEBVs). These GEBVs, in turn, inform prediction models for genomic selection, integrating both agronomic and metabolic data to identify rice varieties with desirable traits such as high antioxidant content and improved seed yield. The development of these molecular markers involved rigorous validation processes to ensure their efficacy in predicting the desired traits. By leveraging the detailed genomic information from the assembled genomes, we created a robust framework for genomic-assisted selection that can be applied to our breeding programs.

The project outputs present transformative advancements in the field of genomic research and application. The comprehensive genome data generated enabled us the precise identification of sequence variants, providing a critical foundation for understanding genetic diversity and guiding targeted breeding efforts. A key technological breakthrough is the development of microfluidic methods for capturing sequence variants for sequencing (Fig. 10). This innovative approach allows for the efficient quantification of genetic variants, significantly enhancing the accuracy and depth of genetic analysis.

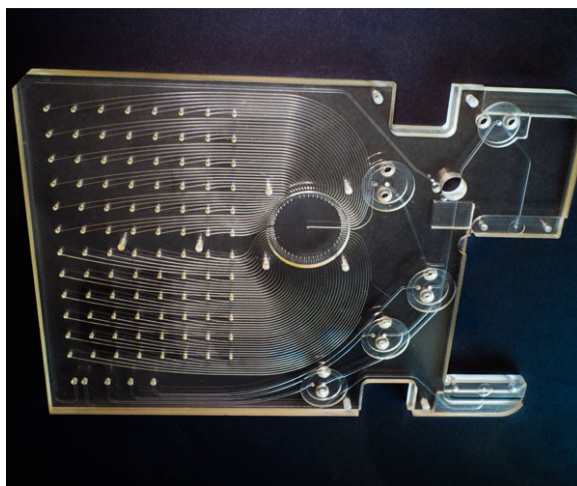


Figure 10: Microfluidic platforms can be utilised for the separation and capture of target sequences reducing the costs of sequence variant validation and quantification by next-generation sequencing.

Moreover, the methodology boasts reduced costs and high-throughput capabilities, making it an economically viable solution for large-scale genomic studies. Another advantage of this approach is its accessibility; the system can be implemented with limited equipment, making

it suitable for laboratories in resource-constrained settings. This democratizes access to advanced genomic tools, empowering researchers and breeders across diverse regions. To complement these technological advancements, robust computational tools have been established for data analysis. These tools streamline the interpretation of complex genomic datasets, ensuring that the wealth of information can be efficiently translated into actionable insights for improving crop varieties and addressing agricultural challenges. Together, these outputs highlight a significant leap forward in making genomic technologies more accessible, scalable, and impactful.

Task 2.2: Validation of Molecular Markers for Genomic-Assisted Selection

To validate the developed molecular markers, our collaborating laboratories conducted extensive field tests to measure the agronomic performance of the selected landraces under various conditions. These field tests utilized GEBVs to guide the selection of the best breeding strategies. The DArT analytical pipeline, developed by our project partner, provided precise genotype information and facilitated the construction of accurate genomic selection models. Our partner in Indonesia amplified seeds at their field station and evaluated their yield performance. The promising results from these evaluations indicated a significant improvement in both yield and antioxidant content. Based on these findings, we initiated the process of registering these improved varieties with the Indonesian Ministry of Agriculture and the Thailand Departments of Agriculture. By the end of 2024, we anticipate that elite pigmented rice lines will be selected. We aim for seeds to be registered, and distributed to small breeding companies across all ASEAN countries. These efforts are expected to enhance the agricultural productivity and socio-economic development of rural farming communities in the region.

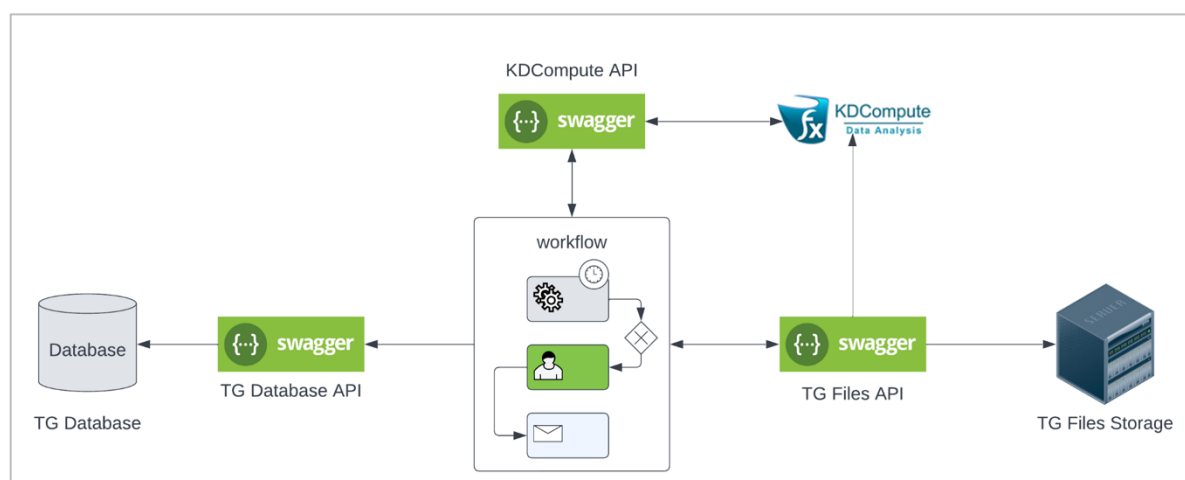


Figure 11. Data analysis workflow for DArT targeted genotyping platform (Diversity Arrays Technology).

Deliverables: The molecular characterisation and secondary metabolite analysis provided a detailed genomic and metabolic composition of the selected pigmented rice landraces. This comprehensive data set forms the basis for our genomic-assisted breeding program, aimed at developing rice varieties with enhanced agronomical and nutritional traits. Through the development and validation of molecular markers, we have established a solid framework for genome-assisted selection. The selection and registration of elite pigmented rice lines are underway, with an expected release of improved seeds to small breeders and farmers by the end of 2024. This initiative holds the promise of significantly advancing rice cultivation in Southeast Asia, offering both economic and health benefits to the local farming communities.

Task 2.3: Development of a Genomic-Assisted Selection program for elite pigmented rice varieties.

The development of a genomic-assisted selection program for elite pigmented rice varieties will represent a transformative approach to rice breeding, leveraging advanced genomic and metabolomic tools to enhance agronomic traits, nutritional quality, and stress resilience. Pigmented rice varieties, such as black, red, and purple rice, have been recognised as highly valued for their rich bioactive compounds. These varieties also contain higher levels of essential micronutrients such as iron, zinc, and selenium, making them nutritionally superior to non-pigmented varieties. Integrating genomic technologies into breeding programs provides a unique opportunity to improve these traits while addressing challenges related to yield and adaptability.

The application of genomic selection in pigmented rice has been limited by the lack of molecular markers associated with desirable traits, such as grain metabolite content and stress tolerance. Our identification of over 2M molecular markers of which over 0.3M have been experimentally validated, thus enabling genome-wide association studies (GWAS) and quantitative trait loci (QTL) mapping, which are instrumental in linking phenotypic traits with their underlying genetic determinants. Recent studies have revealed significant loci governing anthocyanin production and other phenylpropanoid pathway metabolites in pigmented rice, highlighting candidate genes for targeted improvement (Mahfouz et al., 2023). Our genome sequencing resources also facilitate the assembly of comprehensive genomic datasets, enabling precise editing of key genes. We have initiated genome editing experiments to modify genes regulating flowering time and metabolite composition in black rice varieties, to reduce their maturation period and enhance their agronomic suitability.

Our metabolomic profiling complements genomic approaches by providing insights into the biochemical pathways that produce health-promoting metabolites. Advanced mass

spectrometry techniques have enabled the quantification of significant differences in flavonoid and phenolic profiles among pigmented varieties, with black rice exhibiting the highest levels of anthocyanins and antioxidant activity. Integrating this metabolomic data into genomic selection pipelines allows breeders to prioritise varieties with optimal metabolic profiles alongside agronomic traits (Sedeek et al., 2023).

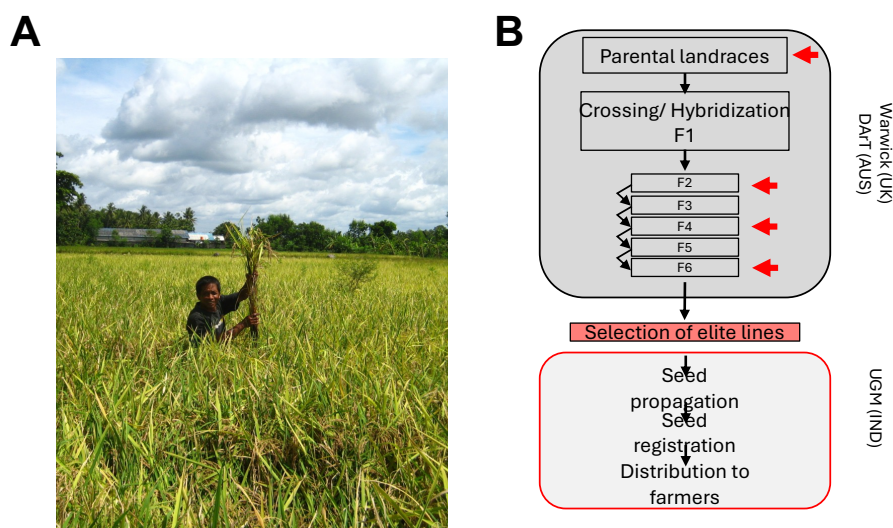


Figure 12. Genomic-assisted breeding of pigmented rice varieties. (A) Experimental determination of yield factors in different pigmented rice landraces. (B) Breeding plan for the genetic improvement of selected pigmented rice landraces using molecular (metabolic and genomic) data. Red box, action plan for the registration and propagation of elite varieties.

The opportunities presented by genomic-assisted breeding in pigmented rice are substantial. Nutritional improvements, such as biofortification with iron and zinc, directly address global malnutrition challenges, particularly in regions where rice is a dietary staple. Enhanced stress tolerance traits, including resistance to drought and salinity, increase crop resilience in the face of climate change. Additionally, consumer interest in functional foods positions pigmented rice varieties as a premium product in the global market, offering economic incentives for farmers. However, these advancements are not without limitations. The genetic complexity of traits such as grain metabolites and bioactive compound biosynthesis requires extensive multi-omics integration, which is resource-intensive and demands significant expertise. Furthermore, there is a risk of losing valuable traditional traits when prioritising specific genomic modifications. The long development cycles of some pigmented rice varieties, such as those with extended maturation periods, pose additional challenges in aligning genetic improvements with practical agricultural timelines. Moreover, the acceptance of genetically edited organisms is a potential barrier in some regions, potentially limiting the adoption of edited varieties despite their advantages (Mbanjo et al., 2020).

In summary, our designed genomic-assisted selection programs offer a robust framework for advancing elite pigmented rice varieties by combining genetic, metabolomic, and agronomic data to achieve sustainable improvements. The integration of cutting-edge technologies has already demonstrated success in enhancing nutritional profiles and stress tolerance while addressing yield constraints. Despite challenges, continued innovation and collaboration among researchers, farmers, and policymakers are essential to fully realize the potential of these programs in meeting the dual goals of food security and improved public health.

9. Discussion.

The results of this project hold profound significance for the targeted beneficiaries in the study sites, primarily rural communities in Southeast Asia reliant on rice cultivation for sustenance and livelihoods. Despite the longstanding tradition of cultivating traditional pigmented rice landraces in the region, these communities have grappled with challenges such as productivity fluctuations and limited access to markets due to the inherent vulnerabilities of these landraces (Harwood, 2019). However, through the utilisation of cutting-edge long-read sequencing technologies, the project aims to address these challenges by enhancing the agronomic performance and nutritional attributes of these landraces (Jain et al., 2016). By assembling complete genomes of selected pigmented rice landraces and developing molecular assays for breeding programs, the project seeks to empower farmers with resilient and high-yielding varieties tailored to local conditions (Zhang et al., 2021). This not only improves agricultural productivity and income stability but also preserves indigenous agricultural practices and biodiversity (Esquinas-Alcázar, 2005). The enhanced nutritional value of these varieties contributes to the overall well-being of rural communities, addressing issues of food security and public health (Chaudhary et al., 2019; Jarma et al., 2021). Research has shown that biofortified crops can significantly impact nutritional outcomes in developing regions, and this project aligns with those findings by potentially providing nutrient-rich rice varieties (Saltzman et al., 2013). The increased productivity and stability of improved pigmented rice varieties can reduce the economic vulnerability of farmers. According to Peng et al. (2019), enhancing crop resilience through genomic interventions is crucial for sustaining agricultural outputs under changing climatic conditions. This project's approach of leveraging genomic data to breed superior rice varieties represents a forward-thinking strategy that addresses both current and future challenges faced by Southeast Asian farmers. By equipping farmers with more reliable and

nutritious crop options, the project supports the broader goals of rural development and poverty alleviation (Fan et al., 2020).

Implications for Sector-Relevant Innovation and Development in Southeast Asia

The reflections from this project extend beyond the immediate study sites to wider implications for sector-relevant innovation and development in Southeast Asia. Traditional pigmented rice landraces are cultivated across various Southeast Asian countries, each facing similar challenges related to agricultural productivity and sustainability (Fuller et al., 2010). The success of this project in enhancing the resilience and nutritional value of these landraces serves as a model for innovation in the agricultural sector across the region. Countries like Vietnam and the Philippines, which have significant rice-growing regions, can benefit from adopting similar genomic-assisted breeding initiatives to improve the adaptability and productivity of their rice varieties. The integration of advanced genomic techniques in agriculture has shown promising results in various contexts. For instance, Wang et al. (2018) highlighted the potential of genomic selection in improving crop yields and stress resistance, suggesting a valuable pathway for similar efforts in Southeast Asia. Moreover, initiatives to develop molecular markers for genome-assisted selection can be scaled up and replicated in other crops, contributing to broader agricultural innovation and food security efforts in Southeast Asia. This aligns with the findings of Kumar et al. (2020), who emphasized the importance of genomic tools in enhancing crop breeding programs. By applying these technologies across different crops, Southeast Asian countries can achieve more resilient agricultural systems capable of withstanding environmental stresses and market fluctuations. The project also underscores the critical role of regional cooperation and knowledge sharing. Collaborative efforts among ASEAN member states can facilitate the dissemination of successful breeding strategies and technological advancements, promoting a unified approach to agricultural development (Tripathi et al., 2019). The ASEAN Economic Community and the ASEAN Socio-Cultural Community provide existing frameworks that can support these collaborative endeavors, ensuring that innovations are effectively integrated into regional agricultural practices.

Potential Scalability of Innovation at ASEAN Level

The potential scalability of the innovation showcased in the project presents promising opportunities at the ASEAN level. By leveraging existing regional frameworks and partnerships, such as the ASEAN Economic Community and the ASEAN Socio-Cultural Community, the project can catalyze collaborative efforts to mainstream genomic-assisted breeding and sustainable agriculture practices across ASEAN member states. The

importance of regional cooperation in agricultural innovation is well-documented, with studies like that of Tripathi et al. (2019) highlighting the benefits of coordinated efforts in enhancing food security and agricultural resilience. Engagement with ASEAN stakeholders is integral to fostering regional cooperation and knowledge exchange. Collaborative workshops and forums can facilitate the dissemination of best practices and lessons learned from the project while also fostering cross-country learning and collaboration. The development of molecular markers and breeding programs can be integrated into regional agricultural policies and initiatives, further promoting innovation and resilience in the agricultural sector at the ASEAN level (Fan et al., 2020).

A coordinated approach at the ASEAN level can also enhance resource allocation and research funding for agricultural innovation. According to Fan et al. (2020), regional alliances can play a significant role in pooling resources and expertise, thereby accelerating the development and implementation of advanced agricultural technologies. This project exemplifies how genomic-assisted breeding can serve as a cornerstone for broader agricultural strategies aimed at achieving food security and economic development in Southeast Asia. Furthermore, the project's emphasis on sustainable agriculture aligns with global trends towards environmentally friendly farming practices. By promoting the cultivation of resilient and high-yielding rice varieties, the project supports the goals of sustainable development and climate change mitigation (Peng et al., 2019). The integration of genomic technologies in agriculture not only enhances crop productivity but also contributes to the sustainability of agricultural ecosystems, as noted by Jarma et al. (2021).

Broader Implications for Global Food Security

While the immediate focus of this project is on Southeast Asia, its implications extend to global food security efforts. The successful implementation of genomic-assisted breeding programs can serve as a blueprint for similar initiatives worldwide. As global populations continue to rise and climate change impacts agricultural productivity, the need for innovative solutions in crop breeding becomes increasingly urgent (Zhang et al., 2021). Projects like this highlight the potential of cutting-edge technologies to address these challenges and ensure a stable food supply for future generations. The project's approach of integrating genomic data with traditional breeding practices offers a model that can be adapted to various crops and regions. This adaptability is crucial for addressing the diverse agricultural challenges faced by different countries. According to Zhang et al. (2021), the flexibility of genomic tools makes them suitable for a wide range of applications, from enhancing staple crops to developing specialty crops with unique nutritional profiles. Additionally, the project emphasizes the importance of preserving genetic diversity in crop breeding. The use of

pigmented rice landraces, which possess unique genetic traits, underscores the value of maintaining diverse crop gene pools. This diversity is essential for developing crops that can withstand environmental stresses and adapt to changing conditions. The conservation of genetic resources is a key component of sustainable agriculture (Esquinas-Alcázar, 2005).

10. Challenges and Future Directions.

Despite the promising outcomes of this project, several challenges must be addressed to ensure the successful implementation and scalability of genomic-assisted breeding programs. One major challenge is the need for robust infrastructure and technical expertise in genomic technologies. Developing countries may face limitations in terms of laboratory facilities, skilled personnel, and funding for advanced research (Louwaars et al., 2009). Addressing these challenges requires concerted efforts from both national governments and international organisations to build capacity and provide the necessary resources for genomic research. Furthermore, the integration of genomic data into breeding programs necessitates careful management of data and intellectual property rights. Ensuring that farmers and local communities benefit equitably from the innovations developed through genomic research is crucial. This involves creating fair and transparent policies for data sharing and access to improved crop varieties. According to Louwaars et al. (2009), equitable benefit-sharing mechanisms are essential for the sustainability of agricultural innovations and the protection of farmers' rights. Future research should also focus on understanding the long-term impacts of genomic-assisted breeding on agricultural ecosystems and food systems. While immediate benefits such as increased yields and enhanced nutritional value are evident, it is important to monitor the broader ecological and socio-economic effects of these innovations. Studies on the sustainability and resilience of genetically improved crops can provide valuable insights for refining breeding strategies and ensuring their long-term success (Tester & Langridge, 2010).

11. Barriers and limitations to the adoption of pigmented rice varieties.

The adoption of pigmented rice varieties, such as black, red, and purple rice, in Southeast Asia presents a complex scenario influenced by various genetic, economic, socio-cultural, and environmental factors. The scientific benefits of pigmented rice, including its superior nutritional content (rich in antioxidants, vitamins, and minerals), the potential for improving food security, and its resistance to certain biotic and abiotic stresses, need to be considered against practical challenges faced by both farmers and consumers. Several potential barriers could hinder the large-scale adoption of these rice varieties, focusing on the interplay between scientific research, socio-economic factors, and the broader commercial landscape.

- **Scientific and Genetic Considerations:** Yield and productivity are the major barriers to the wide adoption of pigmented rice varieties is their productivity compared to conventional white rice varieties. Most pigmented rice varieties are traditional landraces, which often have lower yields than high-yielding modern cultivars. Farmers are naturally inclined to choose varieties that maximize output per hectare, particularly in regions where rice farming is a major livelihood. The genetic research into improving the yield potential of pigmented rice varieties is ongoing, but without parity in productivity, these varieties remain less attractive to farmers. Recent studies have focused on the genetic traits that could enhance the yield of pigmented rice varieties, recognising that breeding programs have been slow to incorporate these traits into commercially viable cultivars (Xie, Y., et al., 2019). Resilience to pests and environmental stress is another potential limitation to the adoption of new varieties. Although some pigmented rice varieties possess natural resistance to pests and environmental stresses like drought or salinity, there is a significant variability in their performance under different growing conditions (Li, J., et al., 2020). This inconsistency makes them a less reliable choice for farmers operating in uncertain climates. Breeding programs aiming to enhance the resilience of pigmented rice varieties to biotic and abiotic stressors are crucial for their broader adoption.

- **Economic Barriers:** The significant costs of shifting from conventional to pigmented rice varieties require an investment in new seeds, farming practices, and sometimes infrastructure, such as drying and processing facilities. For smallholder farmers, who form the majority in Southeast Asia, the financial risk of adopting new rice varieties may be too high. This is exacerbated by the fact that pigmented rice varieties typically fetch a premium price only in niche markets, meaning the market size and profit margins are not guaranteed.

The market for pigmented rice remains small and fragmented, often limited to local sectors. International demand, while growing, is still dwarfed by the global appetite for white and jasmine rice, which dominate exports from Southeast Asian countries like Thailand and Vietnam.

The value chain for pigmented rice is not as well developed, meaning farmers face barriers in terms of getting their produce to market (Guan, Y., et al., 2021). There are often fewer intermediaries and less infrastructure in place to support the storage, transportation, and sale of pigmented rice compared to conventional varieties. Pigmented rice varieties are seen as speciality products, and their prices can be highly volatile, depending on factors like local demand, the success of annual harvests, and global trends in health-conscious eating. Farmers are likely to be concerned about this price instability, preferring the relative certainty of conventional white rice markets.

There are limited financial products tailored to support the cultivation of alternative rice varieties. Most agricultural loans and subsidies in Southeast Asia are geared towards staple white rice varieties, making it difficult for farmers to secure the necessary funding to make the switch (Ahuja, V., et al., 2020). Developing credit systems that specifically target the cultivation and marketing of pigmented rice would be one step towards overcoming financial barriers.

- Cultural and Socio-Economic Considerations: In Southeast Asia, rice consumption is deeply rooted in cultural traditions. White rice has long been the staple food, and consumer preferences for it are strong, especially among older generations. Pigmented rice, with its distinctive colour, taste, and texture, can be perceived as too unconventional, making it difficult to promote to the mass market (Pham, N., et al., 2019). Marketing efforts to highlight the health benefits of pigmented rice varieties have been somewhat successful in urban areas and among health-conscious consumers, but they have not yet penetrated rural or traditional consumer bases.

The average age of rice farmers in Southeast Asia is rising, and older farmers tend to be more resistant to adopting new agricultural practices (Huynh, Q., et al., 2021). The risk-averse nature of these farmers means they are less likely to invest in pigmented rice varieties, particularly when the market is uncertain, and productivity benefits are not immediately clear. Younger farmers may be more open to experimentation, but they often lack the resources or decision-making power within family farms to make the necessary changes (Huynh, Q., et al., 2021). In many Southeast Asian societies, rice is more than just a food crop; it is a symbol of cultural identity and social cohesion (Wang, J., et al., 2020). Traditional ceremonies, family meals, and religious rituals often centre around white rice,

making it difficult to introduce alternatives without facing cultural resistance. This is particularly true in rural areas where agricultural practices are closely tied to cultural heritage.

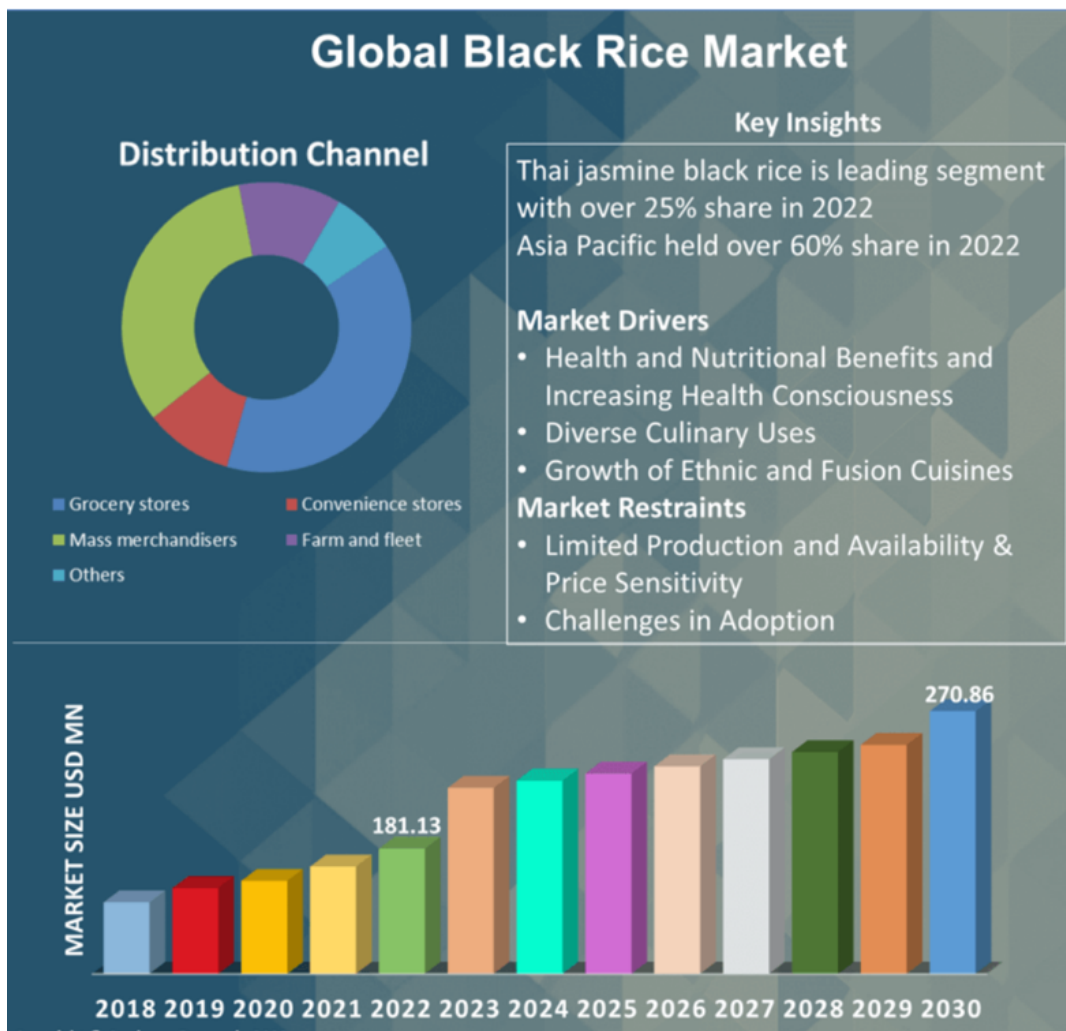


Figure 13. Significant expansion of the global black rice market is largely influenced by the steady increase in population growth (Credence Research, 2023).

- Policy and Institutional Barriers: Governments in Southeast Asia have historically focused on the production of high-yielding white rice varieties to ensure food security. This has led to policies and subsidies that favour the cultivation of white rice over pigmented varieties (Thongrak, S., et al., 2018). Without policy shifts that recognize the potential of pigmented rice to contribute to both food security and income diversification, adoption will likely remain low. While genetic research into pigmented rice varieties has made progress, there is still a gap in terms of developing varieties that are both high-yielding and nutritionally superior. National agricultural research institutes need more funding and support to focus on this area, as current efforts are primarily concentrated on conventional varieties. Additionally, more

research will be needed on the specific economic and environmental conditions in which pigmented rice varieties can thrive (Gomez, E., et al., 2021).

- **Sustainability and Environmental Factors:** Pigmented rice varieties often have specific environmental requirements that are not fully compatible with the broader rice-growing regions of Southeast Asia. Some varieties of black rice are suited to upland conditions, whereas most rice in Southeast Asia is grown in lowland, irrigated systems (Singh, R., et al., 2020). This mismatch between the preferred growing conditions of pigmented rice varieties and the available agricultural landscapes further limits their adoption (Singh, R., et al., 2020). As climate change continues to affect agricultural production, there may be increasing opportunities to promote pigmented rice varieties that are more resilient to extreme weather patterns, such as drought or salinity. However, the development of climate-resilient pigmented rice varieties is still in its early stages, and without accelerated research efforts, these opportunities may remain untapped (Nelson, G., et al., 2019).

In summary, the barriers to the adoption of pigmented rice varieties in Southeast Asia are multifaceted, involving a complex interplay of genetic, economic, socio-cultural, and policy-related factors. While pigmented rice varieties hold significant potential for improving nutrition and providing economic opportunities, particularly in niche markets, the challenges related to productivity, market development, consumer preferences, and financial support need to be addressed. Further research and targeted policy interventions are crucial if these varieties are to make a meaningful impact on food security and farmer livelihoods in the region.

12. Sustainability of the project.

The sustainability of this is crucial for its long-term success, particularly in ensuring that the project's outcomes can contribute to food security, economic stability, and improved agricultural resilience in Southeast Asia. We have developed a sustainability strategy that focuses on leveraging preliminary data, developing a clear two-year plan for future growth, and identifying potential sources of funding, particularly in collaboration with partners in Indonesia and Thailand.

The preliminary data generated from the assembly of complete genomes and metabolic characterisation of pigmented rice varieties will serve as the foundation for a sustainable breeding program. By providing a detailed genetic blueprint and identifying key markers for desired agronomic traits, this data can inform breeding strategies that enhance the resilience, yield, and nutritional quality of pigmented rice. Specifically, the preliminary data will guide future breeding efforts making use of genetic markers related to yield and environmental stress tolerance. Genomic data can be used to inform and train farmers in adopting improved rice varieties that are adapted to local conditions, thereby promoting long-term agricultural sustainability. The tools developed for the metabolic characterisation of pigmented rice seeds could be used to assess the quality of downstream products.



Figure 14. Coordination of activities and exchange of knowledge between different research organisations.

We have carried out different activities aimed at promoting scientific knowledge fostering collaborations and disseminating knowledge. The project placed significant emphasis on fostering international collaborations and promoting knowledge exchange through regular online meetings and the exchange of researchers (Fig. 14).

Dissemination activities included seminars and focused discussions designed to engage various stakeholders, such as farmers, students, and community members. These engagements aimed to bridge the gap between research findings and practical applications, ensuring that advancements in rice genomics reached those directly impacted by the outcomes. We initiated informal consultations with local farming communities, these consultations provided an opportunity to gather insights from farmers about their experiences and challenges with pigmented rice cultivation. The dialogue ensured that the project's outputs, such as the development of new rice varieties, were aligned with the needs and expectations of the end-users (Fig 15). Educational seminars were held with Thai students to raise awareness about the scientific and agronomic significance of pigmented rice. These seminars aimed to inspire the next generation of researchers and practitioners by showcasing the innovative genomic technologies and agricultural strategies developed through the project (Fig 15).



Figure 15. Activities implemented to disseminate information from the project to the stakeholders and rural communities.

In addition to the scientific activities, the project also examined the economic dimensions of pigmented rice. Efforts were directed towards understanding and enhancing the market value and stability of these rice varieties. This aspect of the project was critical for ensuring the long-term viability and adoption of the improved varieties. A core activity of the project was taking the initial steps for development of new pigmented rice varieties with improved

agronomic traits, nutritional quality, and resilience. This involved not only advanced genomic research and breeding strategies but also the integration of feedback from field trials and community engagements to produce varieties that are both high-performing and acceptable to farmers and consumers (Fig 15).

The project has also supported the academic partnership between the University of Warwick and Universitas Gadjah Mada by creating a joint MSc program that will enable the exchange of post-graduate students (Fig 16). In addition, the principal investigator has strengthened ties with Universitas Gadjah Mada department of Biotechnology acting as supervisor of two PhD students.



Figure 16. Strengthening the academic partnership between UK and Indonesia leading academic institutions.

These activities illustrate the project's commitment to a comprehensive approach that combined cutting-edge science with real-world application, ensuring that the findings were impactful both locally and globally.

Future Development Plan: We will focus on consolidating the preliminary data and translating it into practical outcomes that ensure the long-term sustainability of the characterised pigmented rice varieties. The following steps outline the proposed timeline:

Year 1: Data Validation and Pilot Trials. Complete the assembly and validation of the genomes of ten high-performing pigmented rice landraces using Oxford Nanopore Technologies. Conduct pilot field trials to assess the agronomic performance of these varieties under varying environmental conditions. This will provide critical data on yield stability and environmental resilience. Implement training sessions for local farmers,

breeders, and researchers in genomic-assisted breeding and sustainable agricultural practices.

Year 2: Scaling and Broader Application. Scale up the field trials to more regions within Indonesia and Thailand to test a wider variety of environmental conditions and farming practices. Develop a robust breeding program in collaboration with other institutions using the genomic data and identified molecular markers to produce high-yielding, climate-resilient rice varieties. Identify local and international stakeholders to promote the marketability of pigmented rice as a high-value, nutritionally superior product. This will involve the establishment of a more developed value chain, ensuring that pigmented rice reaches both local and international markets.

Sustaining the project will require strong financial backing, especially for scaling up research and agricultural development. A key component of the sustainability strategy will involve securing long-term funding from a combination of UK-based and international funding bodies, with a focus on collaboration with Indonesian and Thai partners. Some potential funding sources include: UKRI offers multiple funding streams, including through the Global Challenges Research Fund (GCRF) and the Biotechnology and Biological Sciences Research Council (BBSRC). These sources can provide financial research support that tackles global development challenges, particularly in agriculture and food security. Bill and Melinda Gates Foundation supports agricultural development projects worldwide, focusing on increasing productivity and ensuring food security for smallholder farmers. The Indonesian and Thai governments, through their agricultural and science ministries, may provide grants and subsidies aimed at enhancing agricultural innovation and food security. Collaboration with local agribusinesses and NGOs will be pursued to secure investment for the development and distribution of improved rice varieties.

13. Conclusions.

The discussion highlights the multifaceted significance of the project aimed at improving the agronomical and nutritional characteristics of pigmented rice varieties grown by farmers in Southeast Asia. The project's outcomes hold immense promise for enhancing the livelihoods of rural communities, promoting sector-relevant innovation, and fostering regional cooperation in agricultural development. By harnessing cutting-edge genomic technologies and integrating them with traditional breeding practices, the project paves the way for a more sustainable and resilient agricultural future. The broader implications of the project extend to global food security efforts, offering a model for similar initiatives worldwide. The successful implementation of genomic-assisted breeding programs can address pressing challenges related to climate change, population growth, and food scarcity. As the world navigates the complexities of modern agriculture, projects like this underscore the importance of innovation, collaboration, and sustainability in ensuring a stable and nutritious food supply for all. In summary, the project's achievements in genomic-assisted breeding of pigmented rice varieties represent a significant advancement in agricultural science and practice. The potential scalability of these innovations at the ASEAN level and beyond highlights the transformative impact of genomic technologies on global food systems. Continued investment in research, infrastructure, and capacity-building is essential for realizing the full potential of these innovations and achieving a more food-secure and resilient world.

Building on our current progress and preliminary results of the genomic-assisted breeding project, this plan outlines the remaining steps necessary to achieve the project's objectives. We have completed genome assemblies, validated molecular markers, conducted field trials, and ensured effective dissemination and application of the research outcomes. To date, the project has achieved significant milestones: The high-quality genomes of ten pigmented rice landraces have been assembled using long-read sequencing technologies provided by Oxford Nanopore Technologies. We have included additional sequencing to fill any remaining gaps and ensure the highest possible accuracy. The team has used advanced bioinformatics tools to refine the assemblies, ensuring that all genetic variants are accurately identified and annotated. Additionally, Diversity Arrays Technology (DART) assays have been developed to identify and validate over 0.2M genetic markers associated with desirable traits. This step is crucial for ensuring the reliability and reproducibility of the markers, which will be used in subsequent breeding programs. Preliminary metabolic characterisations of the selected rice varieties have been completed, providing a comprehensive understanding of their nutritional profiles. Initial field trials in Thailand and Indonesia have shown promising results (Fig 15), with improved varieties demonstrating enhanced yield and resilience. We

expect the data to be available for analysis in mid-2025, which will allow us to narrow down the selection of suitable varieties for further work. However, for robust assessment, these varieties should be evaluated over several periods using multiple geographical (costal or inland) locations.

With molecular markers at hand, we have started to integrate genomic and field data experiments conducted across multiple locations in Thailand and Indonesia, to account for different environmental conditions and stress factors such as drought and salinity. These materials have been evaluated for their agronomic performance, including yield, growth rate, and resistance to pests and diseases. Data from these trials has been meticulously collected and analysed to assess the effectiveness of the genomic-assisted breeding approach. Future field trials will also involve collaboration with local farmers and agricultural extension services to ensure the practical applicability and scalability of the improved rice varieties. We expect these tasks to be continued in the future by small breeding companies and other stakeholders. A critical aspect of the project's success will be the dissemination of findings and training of stakeholders. We have already presented the outline of the project, focusing on demonstrating the benefits of the improved pigmented rice varieties and providing hands-on training in genomic-assisted breeding techniques. We have liaised with local agricultural departments in Indonesia and Thailand to disseminate knowledge and skills necessary to adopt and sustain the innovations developed through the project.

To ensure the long-term impact of the project, we must engage with policymakers and provide recommendations for supportive agricultural policies. We have had close interactions with government agencies in Indonesia and Thailand to explore the draft of policies to promote the adoption of genomic-assisted breeding practices. These policies may include incentives for farmers to grow improved pigmented rice varieties, funding for continued research and development, and measures to protect intellectual property rights associated with the new breeding technologies. Effective policy support will help create an enabling environment for the widespread adoption and sustainability of the project outcomes.

The completion of this project will mark a significant advancement in the field of genomic-assisted breeding for pigmented rice landraces. The enhanced varieties developed through this research have the potential to improve food security, economic stability, and public health not only in Thailand and Indonesia but also in other countries in Southeast Asia. The final report will not only document the project's achievements but also provide a roadmap for future research and development. Recommendations for scaling the innovations to other crops and regions will be included, along with proposals for continued collaboration among member states. By building on the successes of this project, future initiatives can further advance agricultural productivity and sustainability, contributing to a more food-secure world.

In summary, completing a pilot assessment of the value of a genomic-assisted breeding project will be critical to improving pigmented rice landraces grown by rural farmers. Through a combination of advanced molecular techniques, field trials, effective dissemination, and supportive policies, we hope to build resources that in the future will deliver lasting benefits to rural communities in Southeast Asia.

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