Sains Malaysiana 52(11)(2023): 3045-3059
http://doi.org/10.17576/jsm-2023-5211-03

Advances in Sago Palm Research: A Comprehensive Review of Recent Findings

(Kemajuan dalam Penyelidikan Pokok Sagu: Suatu Kajian Komprehensif Penemuan Terkini)

Fifi Hafizzah Pendi[1], Wei-Jie Yan[1], Hasnain Hussain[1,*], Hairul Azman Roslan[1] & Norzainizul Julaihi[2]

[1]Centre for Sago Research, Faculty of Resource Science and Technology, Universiti Malaysia Sarawak, 94300 Kota Samarahan, Sarawak, Malaysia
[2]Land Custody and Development Authority, Level 4, 8 & 12, Wisma Satok, Jalan Satok, 93400 Kuching, Sarawak, Malaysia

Received: 3 July 2023/Accepted: 16 October 2023

ABSTRACT

The sago palm (*Metroxylon sagu* Rottb.) is an indigenous plant in Papua New Guinea, Indonesia, Malaysia, and Thailand. It provides substantial needs to the locals, especially through the starch it produces in its trunk. Exhibiting remarkable adaptability, this palm can thrive in challenging environments like swamps and peat soils. This paper provides an overview of the molecular methods previously utilised to decipher the genes responsible conferring the characteristics of sago palm molecular techniques employed to investigate *M. sagu* molecular properties include molecular markers, genome walking, rapid amplification of cDNA ends (RACE), polymerase chain reaction (PCR) and sequencing. Additionally, this article presents other molecular techniques that can be applied to *M. sagu* for future crop breeding.

Keywords: Gene expression; genome assembly; *Metroxylon sagu*; non-trunking; trunking

ABSTRAK

Pokok sagu (*Metroxylon sagu* Rottb.) adalah tumbuhan asli di Papua New Guinea, Indonesia, Malaysia dan Thailand. Pokok sagu ini sangat berguna kepada masyarakat tempatan dengan menyediakan keperluan hidup terutamanya tepung sagu yang dihasilkan dalam batang tumbuhan unik ini. Memiliki daya penyesuaian yang tinggi, pokok sagu boleh hidup pada persekitaran yang mencabar seperti tanah paya dan tanah gambut. Kertas ini merumuskan teknik molekul yang pernah digunakan untuk mempelajari dan mentafsir gen yang bertanggungjawab terhadap ciri pokok sagu. Antara teknik molekul diaplikasikan ialah penitian *genom*; *kandungan* cDNA *amplifikasi pantas (RACE)*; tindak balas berantai polimerase dan teknik penjujukan.

Kata kunci: Berbatang; himpunan genom; *Metroxylon sagu*; pengekspresan gen; tak berbatang

Introduction

The sago palm (*Metroxylon sagu*) is a monocot, suckers-producing, and hapaxanthic plant in the Arecaceae family (Flach 1997). Distributed globally across Southeast Asia, Oceania, and India, this palm is an important socioeconomic crop in Southeast Asia by providing essential needs such as food security, building materials, and farm feeds to the locals. This palm's robustness makes it thrive in tough environments such as peat soils and swamps (Bintoro et al. 2018). Global distribution estimated at 6.5 million hectares, Papua New Guinea (PNG) and Seram Island of Maluku, Indonesia, are acknowledged as the centre of palm diversity (Ellen 2006; Flach 1997). The sago palm is known for its remarkable starch productivity, is highly valued as a cash crop in countries where it thrives. The palm stores starch in its trunk and can yield up to 24 metric tonnes (MT) of starch per hectare annually, surpassing other significant starch-producing crops like rice (6 MT/ha/year), maize (5.5 MT/ha/year), wheat (5 MT/ha/year), and potato (2.5 MT/ha/year) (Ahmad et al. 2022). In the past, sago starch was significant as a staple food and a valuable trade commodity in the Malay Archipelago (Tan 1983). People commonly consume sago starch in the form of a glue-like mass or modified into various confectionary products (Hirao et al. 2018; Nishimura 2018). Notably, sago starch is also found

beyond Southeast Asia in countries like Japan and Taiwan as the main ingredient to prepare noodles and dumplings (Hirao et al. 2018), and sago-based sugar product as an alternative to sugar cane (Bujang 2018).

In the event of crop failure brought on by disease or climatic change, *M. sagu* is a promising crop to supplement other starch-producing crops because of its adaptability, minimal maintenance needs and high starch yield (Ellen 2006). Presently, Sarawak, a state in Malaysia, is the largest sago starch producer of the country (Lim, Chung & Hussain 2020b). However, large-scale cultivation of this unique palm is hindered by the prolonged maturation period and the occurrence of non-trunking palms, a condition where the affected palm appear stunted and no trunk development (Novero 2012; Yong et al. 2018). Recognising the importance of *M. sagu* in combating hunger, the Food and Agriculture Organisation (FAO) has urged extensive research on this crop to facilitate further development of this often-overlooked plant. Compared to other palm families, *M. sagu* has received less attention in scientific research. However, it is essential to include *M. sagu* in the global food security plan to meet future food security needs while discovering more about this distinctive palm species. This can be done by educating people about the value of *M. sagu* and supporting the government's initiatives to encourage its cultivation and use. Hence, the information gained through active molecular studies of this palm will allow researchers to identify the key genes that result in higher-yielding sago palm, shorter maturation periods, better land adaptability, and developing biomarkers to be used for selection and investigating other physiological issues such as the non-trunking phenomenon in sago palm. This article discusses previous molecular studies conducted on *M. sagu* and presents future perspectives on utilising molecular techniques and omics technologies for knowledge discovery and to enhance breeding strategies for this palm species.

PHYLOGENETIC STUDIES

The earliest study conducted on the sago palm focused on its phylogeny that aimed to establish the evolutionary relationships among different varieties based on their physical characteristics (Boonsermsuk et al. 1997; Kjær et al. 2004). However, relying on morphological features introduced uncertainty and variations to the local taxonomy. Therefore, it became crucial to accurately identify specific sago palm varieties, particularly for breeding purposes, to ensure the sustainable future production of sago starch. Genotyping studies were conducted on sago palms to gather data on their biological diversity and genetic classification, to support germplasm collection and conservation for future crop breeding efforts (Pati et al. 2021). The findings of various phylogenetic studies on sago palms are summarised in Table 1.

The studies on different morphology presented by the presence and absence of spines observed on sago palm trunks and leaf fronds supported Beccari (1918) to resolve *M. rumphii* variety. According to Enguito and Novero (2018), the presence of the spininess on the sago palms were attributed by cytosine methylation resulting in mutability of the GC base pair which may be triggered by environmental stress experienced by these palms. Meanwhile, regardless of the markers used, studies in Indonesia reported high levels of variation at the individual level. The high genetic variation recorded among wild sago progenies results from self-fertilisation that occurs in the wild (Abbas et al. 2017). Generally, these studies supported Flach's assertion that Papua is the centre of sago palm diversity and origin, whilst strongly presented high genetic variation among Papuan sago palm accession (Abbas et al. 2010). However, the variety of the sago accessions in these studies was not mentioned. As for sago palms in Sarawak, they were suggested to belong to the same variety, *M. sagu* as the genotypes were identical (Nisar & Hussain 2022).

GENE CHARACTERISATION STUDY

Gene characterisation studies provide information into gene structure, function, and unravel their contributions to biological processes. For gene characterisation studies for sago palms researchers used several techniques, such as Rapid Amplification of cDNA Ends (RACE) and genome walking, to investigate the GA 20-oxidase gene (GA20ox) and alcohol dehydrogenase gene (Adh), respectively (Jamel et al. 2011; Wee & Roslan 2012).

The gibberellins (GAs) are diterpene plant hormones. The GA biosynthetic pathway has been elucidated to regulate higher plant growth and development by stimulating cell elongation and cell division. To properly regulate plant growth, it is necessary to identify the genes that encode enzymes that synthesize GAs (Fukazawa et al. 2021; Zhang et al. 2022). There are three key enzymes in GA biosynthesis, namely the GA2-oxidase, GA3-oxidase, and GA20ox. GA2ox is the key enzymes in GA degradation process meanwhile both GA3ox and GA20ox are key enzymes in the synthesis process of GA, loss of function in these key enzymes result in dwarfism in plants (Withanage et al. 2015). In the study by Jamel et al. (2011), characterisation of GA20ox from sago palm reported the size of the GA20ox is small, measuring 1332 base pairs (bp) including introns and its corresponding complementary DNA (cDNA) having a length of 1161 bp. BLAST analysis against the *MsGenome20ox* from sago palm showed that it shared 60% similarity with GA20ox gene from *Arabidopsis*, wheat, and maize (Figure 2).

TABLE 1. Phylogeny studies conducted on *M. sagu* in Thailand, Indonesia, and Malaysia via molecular markers

Molecular markers	Location	Target concern	Discovery	References
RAPD	Southern Thailand		Limited genetic variation between the spiny and non-spiny sago palms	(Boonsermsuk et al. 1997)
AFLP	Papua New Guinea	Spiny vs Non-spiny sago palms	Unrooted neigbour joining network do not separate the morphologically different palms	(Kjær et al. 2004)
ITS	Mindanao, Philippines		Spininess of the palm is caused by DNA methylation due to environmental stress	(Enguito & Novero 2018)
RAPD	Indonesia	Genetic diversity in *M. sagu* accessions from Papua, Maluku, Sulawesi, Kalimantan, Java, and Sumatra islands	Genetics of the sago palms were inclined to mix regardless of localities and populations	(Abbas et al. 2009)
cpDNA		Genetic diversity in *M. sagu* accessions from Indonesia	Genetic variation was the highest among sago palm individuals followed by population level and island level	(Abbas et al. 2010)
Starch biosynthesis waxy (Wx)			Genetic variation was the highest among sago palm individuals followed by population level and island level	(Abbas & Ehara 2012)
Mitochondrial nad2 gene marker			Genetic diversity among sago palm populations are low despite morphological differences. UPGMA grouped *M. sagu* to be closely related to *Cocos nucifera* and *Pheonix dactylifera*	(Abbas et al. 2019)
cpDNA associated with maturase K (matK)		Genetic diversity in *M. sagu* accessions from Indonesia	Genetic diversity among sago palm populations are low despite morphological differences	(Abbas Tjolli & Munarti 2019)
Microsatellite SSR marker			SSR markers developed from partial sago genome clustered sago palms into groups mentioned in Abbas, Tjolli and Munarti (2009)	(Purwoko et al. 2019)
AFLP	Sarawak, Malaysia	Genetic variation and sago palm identification in Mukah, Pusa and Samarahan	Genetic variation was found the highest (99%) among individual sago palms, 1% genetic variation among sago palms of various regions and no genetic varoiation within the sago palm population UPGMA dendrogram clustered population together	(Nisar & Hussain 2022)
5S nuclear ribosomal DNA (nrDNA)	Sago palms throughout Southeast Asia and Oceania	Phylogenetic relationships between sections in *Metroxylon* genus	Cladistic analysis of neighbour-joining (NJ) dendrogram showed clade section *Metroxylon* (*M. sagu*) was distant from *Coelococcus* (*M. amicarum*, *M. solomonense*, *M. warburgii, M. vitiense,* and *M. paulcoxii*). Figure 1 shows NJ dendrodram based on 5S nrDNA	(Ehara et al. 2019)

AFLP: Amplified Fragment Length Polymorphism, ITS: Internal transcribed spacer, RAPD: Random amplification of polymorphic DNA, cpDNA: Plastid chloroplast

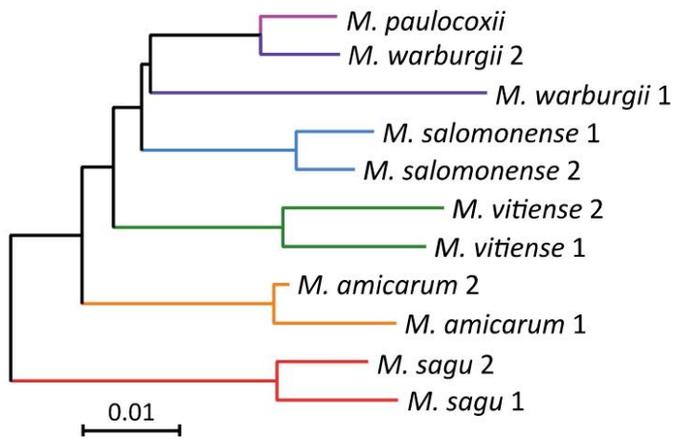

FIGURE 1. Cladistic analysis (NJ dendrogram) of *Metroxylon* genus based on 5S nuclear ribosomal DNA (nrDNA)

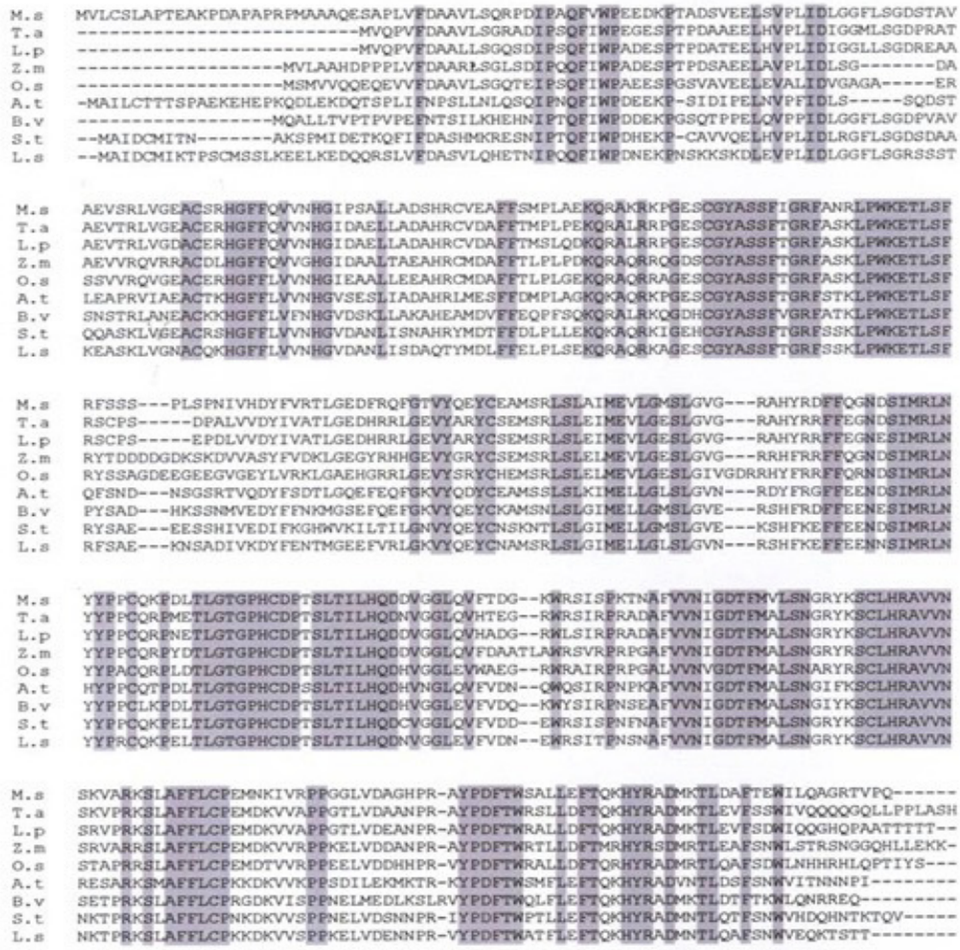

FIGURE 2. Alignment of the deduced amino acid sequence for sago *Ms20ox* cDNA with the GA 20-oxidases from other plant species. Shaded regions represent all GA20-oxidases. Dashes are used to ensure maximum sequence homology. M. s (Ms*20*ox), T. a (*Triticum aestivum*), L. p (*Lolium perenne*), Z. m (*Zea mays*), O. s (*Oryza sativa*), A. t (*Arabidopsis thaliana*), B. v (*Beta vulgaris*), S. t (*Solanum tubersosum*) and L. s (*Lactuca sativa*)

Alignment of *Ms20ox* with GA20ox protein sequences of other plants; *Triticum aestivum*, *Lolium perenne*, *Zea mays*, *Oryza sativa*, *Arabidopsis thaliana*, *Beta vulgaris*, *Solanum tuberosum*, and *Lactuca sativa* identified several highly conserved motifs, LPWKETLSF, SLTILHQ, CGYASSF, AFFLCP, YPDFTW, SCLHRAVVN, TLGTGPHCDP, and HGFF. Motifs such as LPWKETLSF and NYYPXCQKP are known to be involved in the binding of GA substrate and the binding of 2-oxoglutarate cofactor in GA biosynthesis pathway, respectively (Zhou & Underhill 2015). However, the function of other identified motifs in sago palm GA20ox is unknown. Presently, the herbicides usage is heavily relied in agriculture to boost plant growth and yield products. However, continuous use of herbicides can lead to detrimental effects to the environment including herbicide-resistant weeds and contamination of waterbodies (Abbas et al. 2018). Meanwhile, bioherbicides, nature-based herbicides deemed to be the absolute solution to chemical-based herbicides posed other disadvantages such as high susceptibility to environmental degradation by temperature, light, humidity, and microbial activity. Apart from that, the formulation for natural-based herbicides is expensive due to complexity to synthesis by chemical methods (Campos et al. 2023). GA20ox is responsible in catalysing the formation of bioactive GA precursors GA_9 via the oxidation of GA_{12} at C-20 (Phillips et al. 1995). The level of active GAs in plants is controlled by GA20ox, which affects plant development by changing the amount of GA20ox mRNA in *Arabidopsis* (Miao et al. 2010). A common sequence of amino acids is essential for proper GA biosynthesis. For instance, CsGA1ox/ds from *Cucumis sativa* has different amino acids, Phe_{93}, Pro_{106}, and Ser_{202}, than the usual ones in GA3ox, Tyr_{93}, Met_{106}, and Thr_{202}. Changing these amino acids to the normal ones in GA3ox gave CsGA1ox/ds the ability to make active GAs. Also, when the altered CsGA1ox/ds was overexpressed in *Arabidopsis*, made the plants grow faster. Moreover, a modified version of GA3ox5 that had the same amino acids as CsGA1ox/ds could not turn GA_9 into GA_4, showing how important these three amino acids are for GA 3-oxidase activity (Miao et al. 2010). Therefore, through the determination of the motif sequence in sago palm may help to accelerate the growth and increase the yield of the palm as well as the palm maturity. Another gene isolated and characterised in sago palm is the alcohol dehydrogenase (*Adh*) gene (Wee & Roslan 2012). Adh is an enzyme responsible for various biological activities, from germination to encountering plant abiotic stress (Benz, Rhode & Cruzan 2007). Wee and Roslan (2012) reported the identification of conserved amino acid regions located at Cys-48/His-70/Cys-178 and Cys-100/ Cys-103/Cys-106/Cys-114 in sago palm Adh1 (*msAdh1*). The conserved regions are two zinc-binding domains were the same as those found in oil palm (*Elaeis guineensis*) and Mexican fan palm (*Washingtonia robusta*). Additionally, other motifs involved in substrate binding were also found conserved in *msAdh1* such as the amino acid Asp-227 that binds to the adenosine ribose of coenzyme and amino acids, whereas Phe-93, Leu-57, and Leu-116 motifs bind to the alcohol substrate. Sequence homology search of *msAdh1* using NCBI BLASTX also show high degree of similarity to other Adh such as oil palm Adh (91%), followed by maize and rice Adh (87%), *W. robusta* AdhB (85%), and *Arabidopsis* Adh (82%). Furthermore, MEGALIGN analysis indicated *msAdh*1 belongs to monocot and is closely related to oil palm compared to rice and maize (Figure 3) (Wee & Roslan 2012). Likewise, GAs Adh has many roles in plant development, such as seed and fruit formation. It also helps plants to confer environmental stresses, such as salinity, drought, and microbial attack. One of the most important members of the *Adh* gene family is *Adh1*. Study in various plants such as *Vitis vinifera* (grapes), *Zea mays* (maize), *Elaeis guineensis* (oil palm), and *Arabidopsis* found *Adh1* enables plants to survive hypoxia during flooding or waterlogging by switching to anaerobic respiration (Lim et al. 2023; Shi et al. 2017; Ventura et al. 2020). *Adh1* also enhance plant immunity response against pathogen attacks. A study reported overexpression of *AtAdh1* in *Arabidopsis* increased the transcript levels of multiple stress-related genes, accumulates soluble sugars and callose deposition (Shi et al. 2017). Hence, these studies indicate that *Adh1* is important by providing resistance to plants against both abiotic and biotic stress. Another gene that has been characterised in sago palm is fructose-1,6-bisphosphate aldolase (FBAld), which is an enzyme that catalyses the conversion of D-fructose-1,6-phosphate (FBP) to D-glyceraldehyde-3-phosphate (G3P) and dihydroxyacetone phosphate (DHAP) and essential for glycolysis and gluconeogenesis. Roslan, Hossain and Gerunsin (2017) isolated and characterises sago palm FBAld (msFBAld) cDNA. The isolated msFBAld cDNA has an open reading frame of 1020 bp with 340 predicted amino acid sequence with a high degree of homology with ClassI FBAld from other plants. Additional genomic sequence analysis of msFBAld having a length of 2322 bp, it was determined that the sequence consists of five exons. Meanwhile, conserved domain analysis of the active site showed the presence of fifteen amino acids that are crucial for catalytic activity and phylogenetic analysis localised msFBAld in the chloroplast (Wee & Roslan

2012). Homology modelling and Ramachandran plot generated a molecular 3D structure of msFBAldshowing a homotetramer structure with a central alpha/beta-TIM-barrel. The model also shows the presence of a catalytic dyad, Lys209-Glu167, that could be involved in Schiff's base formation and aldol condensation (Figure 4).

COPY NUMBER VARIATION (CNV)

Gene copy number variation (CNV) analysis is another work that has been undertaken in sago palm. The CNVs, like single nucleotide polymorphisms (SNPs), are significant for understanding genomic variability and are thought to be involved in the long-term evolution of plant adaptation to its environment (Dolatabadian et al. 2017), stress tolerance (Prunier et al. 2019; Sieber et al. 2016), and disease resistance (Boocock et al. 2015; Wei, Chen & Kuang 2016). Lim, Chung and Hussain (2020b) studied the organellar genome copy number using using combinatorial approach of the long-PCR and qPCR across different organs, growth stages, phenotypes, and localities in Sarawak. By emulating combinatorial approached utilised by Kumar, Oldenburg andBendich (2014) to overcome miscalculation pose by long-PCR and qPCR, the higher ratio of long-PCR/qPCR provides higher levels of unimpeded DNA. Lim, Chung and Hussain (2020b) found mature leaf attained the highest unimpeded plastome and mitogenome copy number compared to other organs such as young and mature roots, aged leaf, and pneumatophore. Across the growth stages, namely *Plawei*, *Plawei Manit*, *Bubul*, *Angau Muda*, and *Angau Tua*, the second growth stage, *Plawei Manit* scored the highest unimpeded plastome and mitogenome copy number. As for the phenotypic study, the non-trunking and spiny sago palms recorded low organellar genome copy

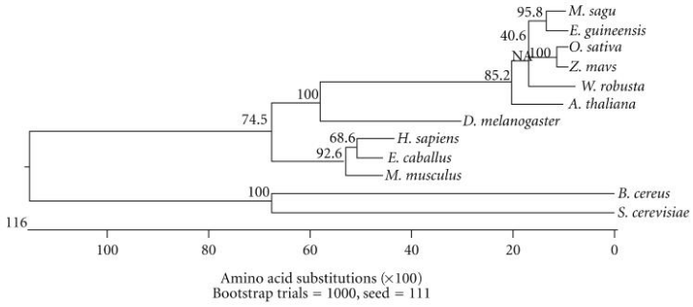

FIGURE 3. Phylogenetic tree of *msAdh1* of sago palm constructed using clustal method based on amino acid similarities

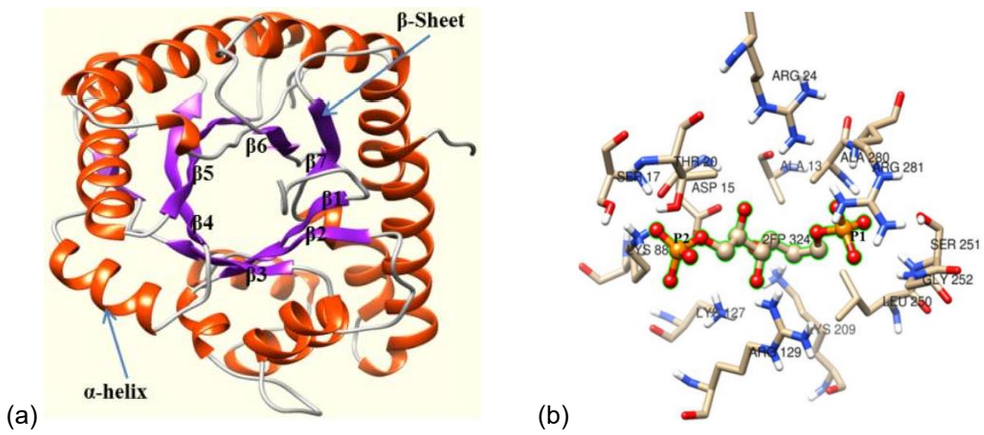

FIGURE 4. Molecular modeled structure of msFBAld generated via SWISS-MODEL web server (a) Monomeric structure of msFBAld containing β/α TIM-barrel, and (b) Predicted binding residues at the active site of msFBAld identified by COFACTOR

numbers in both long-PCR and qPCR reactions. However, no significant differences in genome copy number were identified in sago palms from different localities. The study on organellar genomes helps to elucidate the role of both mitogenomes and plastome in influencing characteristics of an organism. This present study has further strengthened the Beccari's documentation of one species of sago palm distributed across Papua New Guinea, Indonesia, Thailand, and the Philippines despite conferring different phenotype (spiny). The spiny is caused by extra copies of the mitogenome to cater extra ATP required for spine formation that offers protection to sago palms against sago beetle (*Rhynchophorus ferrugineus*) larvae. Therefore, the information obtained from this study lay the foundation to develop molecular markers to distinguish lineages and evolutionary genetics in sago palms, and as selection index for future breeding program as well as the detection of the non-profitable, non-trunking sago palm.

GENOME STUDY

Advances in sequencing technologies and lower costs have accelerated the interest in exploring genome structure and expression patterns. To date, three genome assembly studies and one chloroplast genome sequence were conducted on *M. sagu*. All the studies utilised sago palm leaf samples to extract genomic DNA. Table 2 tabulates different strategies employed to decipher the sago palm genome size.

Using qPCR analysis, the genome of *M. sagu* was estimated to be approximately 1.87 Gbp. This is comparable to the size of the oil palm genome, which is about 1.8 Gbp (Roslan et al. 2020). Another sago palm genome estimation study using k-mer statistical approach, reported by Lim et al. (2021), found that it ranged from 464 to 616 Mbp, which is comparable to the date palm genome size of 605.4 Mbp. On the other hand, hybrid genome assembly approach by combining long reads from Nanopore sequencing and short reads from Illumina sequencing platforms yielded genome estimation of *M. sagu* to be 510 Mbp with a BUSCO completeness of 97.9% (Lim et al. 2022). Meanwhile, the work on the chloroplast genome of *M. sagu* has been reported to have a singular conformation with a quadripartite structure, spanning a total length of 157,300 bp, including LSC, SSC, and IR regions (Lim, Chung & Hussain 2020a).

TABLE 2. Strategies employed and the findings of *M. sagu* genome size

Genome	Strategy	Findings	References
Whole genome size estimation	Real-time quantitative polymerase chain reaction (qPCR) *Pichia pastoris* as control	*M. sagu* genome size estimated to be 1.87 Gbp and is comparable to oil palm genome size of 1.8 Gbp (Mahmoud 2021)	(Roslan et al. 2020)
	K-mer statistical prediction	*M. sagu* genome size is estimated to range from 464 Mbp to 616 Mbp, similar to date palm genome size of 605.4 Mbp (Al-Mssallem et al. 2013)	(Lim et al. 2021)
	Hybrid high throughput sequencing (short-reads Illumina sequencing + long-reads Nanopore) with *de novo* assembly	Genome size estimated to be 510 million base pairs (509,812,790 bp) with BUSCO completeness of 97.9%	(Lim et al. 2022)
Chloroplast genome (cp)	Illumina high-throughput sequencing with *de novo* assembly	*M. sagu* cp genome reported to be of singular conformation with quadripartite structure spanning a total length of 157,300 bp encompassing 85,257 bp large single-copy region (LSC) and 17,533 bp small single-copy region (SSC), which are separated by a pair of inverted repeats (IRs) of 27,245 bp each	(Lim, Chung & Hussain 2020a, 2020b)

In summary, the genome assembly studies reported that this palm belongs to the Arecales order and is closely related to oil palm (*E. guineensis*) and date palm (*P. dactylifera*) (Lim et al. 2022). Meanwhile, the maximum likelihood phylogenetic tree on the chloroplast genome showed a close relationship of *M. sagu* with its congeneric species *M. warburgii* and other Calameae members *Calamus carytoides*, *Pigafetta elata,* and *Salacca ramosiana* (Lim, Chung & Hussain 2020a). The annotation of protein-coding genes from *M. sagu* shows that starch production is the main highlight compared to other pathways involved in carbohydrate metabolism (Lim et al. 2022). The genome study of this plant species is important because there was little known, molecularly, of this plant species. With the new knowledge of the genome, it will further provide caluable insight on the evolutionary relationships of this species compared to other Arecales, especially related to diversification of the *M. sagu* into starch-producing species compared to other species of the same order.

GENE EXPRESSION STUDIES

Among the early methods used in sago palm research includes Expressed sequence tags (EST), differential reverse transcription PCR (DDRT-PCR), and Differential Reverse Transcription PCR (DDRT-PCR), and RACE was commonly used to investigate the genes responsible for the development of *M. sagu*. (Roslan & Anji 2011). In their study, Roslan and Anji (2011) examined the expression of *chitinase* in different parts of the sago palm, including the leaf, meristem, and inflorescence, using DRT-PCR and RACE techniques. The results of DDRT-PCR showed that *chitinase* was expressed in all tissues except the leaves of *M. sagu*. By analysing the sequence of the sago palm *chitinase*, they identified a high similarity to the chitinase domain family 19 and class I *chitinase*.

Another study conducted by Wee and Roslan (2012a) utilised EST analysis to investigate the transcriptome of *M. sagu*. They identified 372 tentative unique genes (TUGs), consisting of 340 singletons and 32 contigs. Through BLASTX analysis they found that the majority of the TUGs (86.6%) showed similarity to known proteins, while 20 TUGs were novel to *M. sagu*. Using BLAST2GO program, they discovered that these transcripts were involved with primary metabolic processes such as glycolysis pathway, as well as stress tolerance mechanisms against both abiotic and biotic factors.

TRUNKING AND NON-TRUNKING SAGO PALM STUDIES

Efforts to decode the responsible genes towards two physically different *M. sagu*. Several omics approaches were conducted to show the putative genes towards the occurrences of trunking and non-trunking sago palm. Sago palm is a unique plant that stores starch in its trunk. The taller and bigger the trunk size, more sago starch can be obtained even from one palm. This makes sago palm a valuable food source of food and income to the locals. However, the occurrences of some sago palms failed to develop trunks, a condition known as non-trunking palms, even years after planting. Non-trunking palms pose exorbitant economic losses to the sago farmers. In addition, as the plant is known to be the highest starch-producing species per hactare, understanding the molecular and physiological characteristic of this species is important for the plant to used as potential source as food security.

Table 3 presents the omics strategies to study the differentially expressed genes (DEGs) of the two different phenotypic palms. Several omics approaches used were metabolomics which utilised the nuclear magnetic resonance (NMR) spectroscopy and gas chromatography-mass spectrometry (GC-MS), proteomics approach which utilised the combination of two-dimensional gel electrophoresis (2-DE) and matrix-assisted laser desorption/ionisation time of flight (MALDI-TOF) mass spectrometry (MS), and genomics approach through gene fishing and Representational Difference Analysis (RDA).

ALTERNATIVE BREEDING STRATEGIES FOR SAGO PALM

The increasing demand for non-traditional food and fuel sources has raised interest in sago palm research. However, due to the sago palm's lengthy life cycle, long flowering time, and poor seed germination rate, no breeding programmes for sago palms have been reported (Novero 2012). *Agrobacterium*-mediated and direct particle bombardment are two commonly used methods for plant genetic transformation. Since monocotyledonous plants are recalcitrant towards gene transfer using *Agrobacterium*, sago palm embryonic calli were the chosen tissue to undergo transformation. In a study by Ibrahim, Hossain and Roslan (2014), successful genetic transformation of *M. sagu* embryonic callus was achieved, and the researchers compared the effectiveness of *Agrobacterium* and particle bombardment methods.

TABLE 3. Different omics technologies to study the genes responsible towards trunking and non-trunking sago palms

Omics approach	Profiling technology	Findings	Remarks	References
Metabolomics	NMR and GC-MS	Oils and waxes, haloalkanes, sulfite esters, phosphonates, phosphoric acid, thiophene ester, terpenes, and tocopherols were differentially expressed between trunking and non-trunking	Highlight metabolites like acetyl-CoA, thiopenes, and tocopherol presence were due to environmental stress	(Hussain et al. 2020b)
Proteomics	2-DE with MALDI-ToF/ ToF MS/MS	Found 865 protein spot from trunking and non-trunking Nineteen significantly proteins were found upregulated in the non-trunking RubisCo, phosphoglycerate kinase (PGK), oxygen-evolving enhancer (OEE) proteins, carbonic anhydrase (CA), glutamine synthetase (GS), phosphoribulokinase, chloroplastic-like protein, malate dehydrogenase (MDH), and ascorbate peroxidase were among the proteins found significantly upregulated Bioinformatics analysis described the upregulated proteins involved in photosynthesis, tricarboxylic acid cycle, glycolysis, carbon utilisation and oxidative stress metabolic pathways	Non-trunking phenotype was caused by environmental stress and the upregulated proteins played crucial roles in the metabolism processes for palm survival	(Hussain et al. 2020a)

Genomics	GeneFishing and RDA	*Gene Fishing* Nutrient-related regulation such as asparagine synthetase C (AsnC) and nonsymbiotic hemoglobin 1 (nsHb) were expressed higher in trunking and non-trunking, respectively Transport-related proteins (ER-Golgi Soluble N-ethylmale-imide-sensitive factor-attachment Protein Receptors (SNARE) complex, TRX-m3, and peptide-2 ATP binding cassette (ABC)) were expressed more in non-trunking whereas transport proteins sorting nexin (SNX), intracellular functions glucosyltransferase 2, inorganic phosphate transporter 3 (PHT3) and cobalt transport protein (CbiO) were found highly expressed in trunking palms Defence mechanism proteins were also expressed in both palms. Enzymes O-antigen ligase and beta-glucosidase expressed higher in trunking whereas UDP-N-acetylmuramoyl-L-alanyl-D-glutamate lysine ligase (MurE) enzyme and mitogen-activated protein kinase (MAPK4), highly expressed in non-trunking Eukaryotic translational initiator factor 5A (eIF-5A) complex expressed higher by 80% in trunking palms *RDA* Eight and 16 DETs expressed differently in trunking and non-trunking, respectively DETs found in non-trunking are responsible in chemical defence, plant metabolism, signalling and salinity stress DETs found in trunking responsible for plant growth, and stress tolerance with genes encode for neutralising imbalanced plant stress	Trunking sago palms has stronger defence against pathogen attacks and environmental stress as shown through higher expression of defence related and regulative proteins like beta-glucosidase and eIF-5A, respectively Non-trunking palms are nutritional challenged as denoted by expression of nsHb transcript	(Hussain et al. 2022)

The study showed that the transformation rate by particle bombardment was estimated to be 1.4%, whereas the rate caused by bombardment caused by *Agrobacterium* was substantially lower at 0.5%. Dot blot analysis was used to detect the incorporation of foreign DNA, the *gus* and *bar* genes were both integrated and functionally viable in the genome of the sago palm. Through this comparative transformation study, it is hoped to inspire more interest in transformation studies in sago palm and later result in producing new and better sago palm varieties. Figure 5 shows the *gus* histochemical staining of the transformed callus at nine months after transformation with *Agrobacterium* and particle bombardment.

FUTURE PERSPECTIVE

MOLECULAR AND OMICS STUDIES FUELLING FUTURE CROP IMPROVEMENT

Advancements in sequencing technologies, cheaper sequencing costs, and user-friendly pipelines and bioinformatics tools have increased the possibilities to sequence any organisms. An array of approaches with omics suffixes like genomics, transcriptomics, proteomics, metabolomics, and more provide one with the opportunity to understand the underlying biological traits of an organism. In recent years, there has been an increase in the publication of high-quality genome assemblies in crops due to the use of well-established methods. One of the key contributions of genome sequencing lies in fathoming the interaction of genes in coordinating the organism's growth, development, and interaction/adaptation to the environment. The genome sequence studies enable scientists to discover genetic markers across the whole genome through the identification and properties of the genes leading to a valuable source of novel genes to improve crop varieties. Several research projects have been undertaken to enhance crop improvement in various plant species. For example, the Rice Genome Project (Wang et al. 2018), that focused on rice, while maize improvement was the objective of the study conducted by Jiao et al. (2012). Similarly, sorghum improvement efforts were documented in the research by Mace et al. (2013), pearl millet enhancement by Varshney et al. (2017), and work on soybean by Lam et al. (2010). These studies highlighted vast genetic diversity in different lines of cultivars and demonstrated the use of identified SNPs, indels and copy number variation that can potentially be used as a selection index for future crop breeding programs (Jiao et al. 2012; Varshney et al. 2017; Wang et al. 2018). By taking into considerations of past omics studies from other crops inspires researchers to take into account developing superior better sago palm varieties.

The publication by Lim et al. (2021) and Roslan et al. (2020) along with the genome sequence development described by Lim, Chung and Hussain (2020a), has laid the foundation for advancing our understanding of the sago palm genome. These studies have the potential to provide valuable insights into the agronomic traits of this palm species. The recent release of a high-quality *M. sagu* genome sequence marks a significant milestone and paves the way for a new era in molecular biology

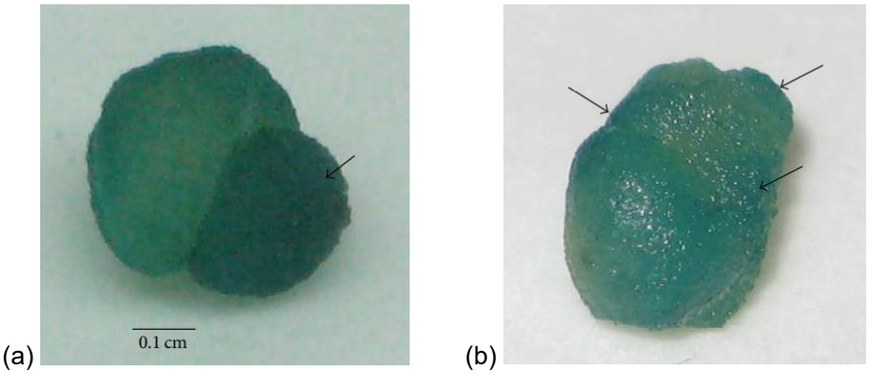

FIGURE 5. *Gus* histochemical staining of transformed callus at 9 months after transformation with (a) Agrobacterium and (b) gene gun

research on *M. sagu*. It also has the potential to expedite the breeding program for this non-model plant, similar to the active efforts being made in oil palm research. Furthermore, it is also beneficial to conduct sequencing and phenotyping on different landraces of sago palms differing in geographic location, wild and domesticated sago palms. However, the current omics studies conducted on *M. sagu* are still in the early stages. Post-genomics tools such as transcriptomics can be implemented on *M. sagu* to exploit knowledge of underlying functional genes and regulatory mechanisms concerning plant response to any stimuli. Studying differentially expressed genes can provide valuable knowledge about the genes responsible for desired features. In order to control gene expression and ultimately improve the sago palm, the availability of the whole genome sequence and transcriptome sequence could be very beneficial.

ACKNOWLEDGEMENTS

Sarawak Research and Development Council (SRDC) Grant RDCRG/CAT/2019/23 and UNIMAS F07/GRADUATES/85105/2022 awarded to HH supported the research study related to this review.

REFERENCES

Al-Mssallem, I. S., Hu, S., Zhang, X., Lin, Q., Liu, W., Tan, J., Yu, X., Liu, J., Pan, L., Zhang, T., Yin, Y., Xin, C., Wu, H., Zhang, G., Ba Abdullah, M. M., Huang, D., Fang, Y., Alnakhli, Y. O., Jia, S., Yin, A., Alhuzimi, E., Alsaihati, B., Al-Owayyed, S., Zhao, D., Zhang, S., Al-Otaibi, N., Sun, G., Majrashi, M., Li, F., Tala, Wang, J., Yun, Q., Alnassar, N. A., Wang, L., Yang, M., Al-Jelaify, R. F., Liu, K., Gao, S., Chen, K., Alkhaldi, S. R., Liu, G., Zhang, M., Guo, H. & Yu, J. 2013. Genome sequence of the date palm Phoenix dactylifera L. *Nature Communications* 4(1): 1-9. https://doi.org/10.1038/ncomms3274

Abbas, B. & Ehara, H. 2012. Assessment genetic variation and relationship of sago palm (*Metroxylon sagu* Rottb.) in Indonesia based on specific expression gene (Wx Genes) markers. *African Journal of Plant Science* 6(12): 314-320. https://doi.org/10.5897/AJPS12.015

Abbas, B., Renwarin, Y., Bintoro, M.H., Sudarsono, S., Surahman, M. & Ehara, H. 2010. Genetic diversity of sago palm in Indonesia based on chloroplast DNA (CpDNA) Markers. *Biodiversitas Journal of Biological Diversity* 11(3): 112-117. https://doi.org/10.13057/biodiv/d110302

Abbas, B., Bintoro, M.H., Sudarsono, Surahman, M. & Ehara, H. 2009. Genetic relationship of sago palm (*Metroxylon sagu* Rottb.) in Indonesia based on RAPD markers. *Biodiversitas Journal of Biological Diversity* 10(4): 168-174. https://doi.org/10.13057/biodiv/d100402

Abbas, B., Dailami, M., Santoso, B. & Munarti. 2017. Genetic variation of sago palm (*Metroxylonsagu* Rottb.) progenies with natural pollination by using RAPD markers. *Natural Science* 9(4): 104-109. https://doi.org/10.4236/ns.2017.94010

Abbas, B., Tjolli, I. & Munarti. 2019. Genetic diversity of sago palm (*Metroxylon sagu*) accessions based on plastid CpDNA *Mat*K gene as DNA barcoding. *Biodiversitas Journal of Biological Diversity* 21(1): 219-225. https://doi.org/10.13057/biodiv/d210128

Abbas, B., Tjolli, I., Dailami, M. & Munarti. 2019. Phylogenetic of sago palm (*Metroxylon sagu*) and others monocotyledon based on mitochondrial Nad2 gene markers. *Biodiversitas Journal of Biological Diversity* 20(8): 2249-2256. https://doi.org/10.13057/biodiv/d200820

Abbas, T., Zahir, Z.A., Naveed, M. & Kremer, R.J. 2018. Limitations of existing weed control practices necessitate development of alternative techniques based on biological approaches. *Advances in Agronomy* 147: 239-280. https://doi.org/10.1016/bs.agron.2017.10.005

Ahmad, M.N., Awang Adeni, D.S., Suhaili, N. & Bujang, K. 2022. Optimisation of pre-harvest sago frond sap for the production of L-lactic acid using *Lactococcus lactis* IO-1. *Biocatalysis and Agricultural Biotechnology* 43: 102435. https://doi.org/10.1016/j.bcab.2022.102435

Beccari, O. 1918. Asiatic Palms-Lepidocaryeae: part 3: The species of the genera Ceratolobus, Calospatha, Plectocomia, Plectocomiopsis, Myrialepis, Zalacca, Pigafettia, Korthalsia, Metroxylon and Eugeissona. A*nnals of the Royal Botanic Garden, Calcutta* 12: 156-195.

Benz, B.R., Rhode, J.M. & Cruzan, M.B. 2007. Aerenchyma development and elevated alcohol dehyrogenase activity as alternative responses to hypoxic soils in *Piriqueta caroliniana* complex. *American Journal of Botany* 94(4): 542-550.

Bintoro, M.H., Iqbal Nurulhaq, M., Pratama, A.J., Ahmad, F. & Ayulia, L. 2018. Growing area of sago palm and its environment. In *Sago Palm Multiple Contributions to Food Security and Sustainable Livelihoods*, edited by Ehara, H., Toyoda, Y. & Johnson, D.V. Singapore: Springer Nature. pp. 17-30.

Boocock, J., Chagné, D., Merriman, T.R. & Black, M.A. 2015. The distribution and impact of common copy-number variation in the genome of the domesticated apple, Malus x domestica Borkh. *BMC Genomics* 16: 848. https://doi.org/10.1186/s12864-015-2096-x

Boonsermsuk, S., Anai, T., Hasegawa, K. & Hisajima, S. 1997. Variation in random polymorphic DNA of sago palm (*Metroxylon* Spp.) in Thailand. *Japanese Journal of Tropical Agriculture* 41(2): 89-92.

Bujang, K. 2018. Production, purification, and health benefits of sago sugar. In *Sago Palm: Multiple Contributions to Food Security and Sustainable Livelihoods*, 1st ed., edited by Ehara, H. Johnson, D.V. & Toyoda, Y. Singapore: Springer Nature. 1: 229-308.

Campos, E.V.R., Ratko, J., Bidyarani, N., Takeshita, V. & Fraceto, L.F. 2023. Nature-based herbicides and micro-/nanotechnology fostering sustainable agriculture. *ACS Sustainable Chemistry & Engineering* 11(27): 9900-9917. https://doi.org/10.1021/acssuschemeng.3c02282

Dolatabadian, A., Patel, D.A., Edwards, D. & Batley, J. 2017. Copy number variation and disease resistance in plants. *Theoretical and Applied Genetics* 130(1): 2479-2490.

Ehara, H., Yamamoto, T., Tsuchiya, T., Hitoshi, N., Dowe, J.L., McClatchey, W.C., Mishima, T., Itaya, A., Mizota, C., Pasolon, Y.B., Ala, P., Tuiwawa, M., Naikatini, A., Rounds, I.A., Foliga, T., Lui, S. & Kwan, S. 2019. Phylogenetic study of metroxylon palms in Southeast Asia and Oceania based on 5S NrDNA spacer sequence data. *Sago Palm* 26(2): 37-43.

Ellen, R. 2006. Local knowledge and management of sago palm (*Metroxylon sagu* Rottboel) diversity in South Central Seram, Maluku, Eastern Indonesia. *Journal of Ethnobiology* 26(2): 258-298.

Enguito, R.Z.C. & Novero, A.U. 2018. Genetic variation of sago palm (*Metroxylon sagu* Rottb.) samples from Mindanao, Philippines using internal transcribed spacer analysis. *Malaysian Applied Biology* 47(3): 39-48.

Flach, M. 1997. *Sago Palm: Metroxylon sagu Rottb.: Promoting the Conservation and Use of Underutilized and Neglected Crops*. 1st ed. Vol. 13. Rome: International Plant Genetic Resources Institute.

Fukazawa, J., Miyamoto, C., Ando, H., Mori, K. & Takahashi, Y. 2021. DELLA-GAF1 complex is involved in tissue-specific expression and gibberellin feedback regulation of GA20ox1 in Arabidopsis. *Plant Molecular Biology* 107(3): 147-158.

Hirao, K., Kondo, T., Kainuma, K. & Takahashi, S. 2018. Starch properties and uses as food for human heatlh and welfare. In *Sago Palm: Multiple Contributions to Food Security and Sustainable Livelihoods*, 1st ed., edited by Ehara, H., Johnson, D.V. & Toyoda, Y. 1: 285-288. Singapore: Springer Nature.

Hussain, H., Edward-Atit, A.S., Julaihi, N., Tommy, R., Nisar, M., Hamdan, N. & Ehara, H. 2022. Identification of differentially expressed transcripts for trunk formation in sago palm using annealing control primer genefishing technique. *Journal Pf Applied Biology and Biotechnology* 10(2): 2-4.

Hussain, H., Mustafa Kamal, M., Al-Obaidi, J.R., Hamdin, N.E., Ngaini, Z. & Mohd-Yusuf, Y. 2020. Proteomics of sago palm towards identifying contributory proteins in stress-tolerant cultivar. *Protein Journal* 39(1): 62-72. https://doi.org/10.1007/s10930-019-09878-9

Hussain, H., Yan, W-J., Ngaini, Z., Julaihi, N., Tommy, R. & Ahmad Bhawani, S. 2020. Differential metabolites markers from trunking and stressed non-trunking sago palm (*Metroxylon sagu* Rottb.). *Current Chemical Biology* 14(4): 262-278. https://doi.org/10.2174/2212796814999200930120925

Ibrahim, E.R., Hossain, M.A. & Roslan, H.A. 2014. Genetic transformation of *Metroxylon sagu* (Rottb.) cultures via *Agrobacterium* -Mediated and particle bombardment. *BioMed Research International* 2014: 348140. https://doi.org/10.1155/2014/348140

Jamel, B., Hussain, M.H., Salleh, M.A. & Busri, N. 2011. Isolation and characterization of the GA 20-Oxidase CDNA from sago palm (*Metroxylon sagu* Rottb.). *Asia-Pacific Journal of Molecular Biology and Biotechnology* 19(2): 83-93.

Jiao, Y., Zhao, H., Ren, L., Song, W., Zeng, B., Guo, J., Wang, B., Liu, Z., Chen, J., Li, W., Zhang, M., Xie, S. & Lai, J. 2012. Genome-wide genetic changes during modern breeding of maize. *Nature Genetics* 44(7): 812-815. https://doi.org/10.1038/ng.2312

Kjær, A., Barfod, A.S., Asmussen, C.B. & Seberg, O. 2004. Investigation of genetic and morphological variation in the sago palm (*Metroxylon sagu*; Arecaceae) in Papua New Guinea. *Annals of Botany* 94(1): 109-117. https://doi.org/10.1093/aob/mch112

Kumar, R.A., Oldenburg, D.J. & Bendich, A.J. 2014. Changes in DNA damage, molecular integrity, and copy number for plastid DNA and mitochondrial DNA during maize development. *Journal of Experimental Botany* 65(22): 6425-6439. https://doi.org/10.1093/jxb/eru359

Lam, H-M., Xu, X., Liu, X., Chen, W., Yang, G., Wong, F-L., Li, M-W., He, W., Qin, N., Wang, B., Li, J., Jian, M., Wang, J., Shao, G., Wang, J., Sun, S.S.M. & Zhang, G. 2010. Resequencing of 31 wild and cultivated soybean genomes identifies patterns of genetic diversity and selection. *Nature Genetics* 42(12): 1053-1059. https://doi.org/10.1038/ng.715

Lim, H., Kobayashi, M.J., Marsoem, S.N., Irawati, D., Kosugi, A., Kondo, T. & Tani, N. 2023. Transcriptomic responses of oil palm (*Elaeis guineensis*) stem to waterlogging at plantation in relation to precipitation seasonality. *Frontiers in Plant Science* 14: 1213496. https://doi.org/10.3389/fpls.2023.1213496

Lim, L.W.K., Chung, H.H. & Hussain, H. 2020a. Complete chloroplast genome sequencing of sago palm (*Metroxylon sagu* Rottb.): Molecular structures, comparative analysis and evolutionary significance. *Gene Reports* 19: 100662. https://doi.org/10.1016/j.genrep.2020.100662

Lim, L.W.K., Chung, H.H. & Hussain, H. 2020b. Organellar genome copy number variations and integrity across different organs, growth stages, phenotypes and main localities of sago palm (*Metroxylon sagu* Rottboll) in Sarawak, Malaysia. *Gene Reports* 21: 100808. https://doi.org/10.1016/j.genrep.2020.100808

Lim, L.W.K., Chung, H.H., Hussain, H. & Gan, H.M. 2021. Genome survey of sago palm (*Metroxylon sagu* Rottboll). *Plant Gene* 28: 100341. https://doi.org/10.1016/j.plgene.2021.100341

Lim, L.W.K., Lau, M.M.L., Chung, H.H., Hussain, H. & Gan, H.M. 2022. First high-quality genome assembly data of sago palm (*Metroxylon sagu* Rottboll). *Data in Brief* 40: 107800. https://doi.org/10.1016/j.dib.2022.107800

Mace, E.S., Tai, S., Gilding, E.K., Li, Y., Prentis, P.J., Bian, L., Campbell, B.C., Hu, W., Innes, D.J., Han, X., Cruickshank, A., Dai, C., Frère, C., Zhang, H., Hunt, C.H., Wang, X., Shatte, T., Wang, M., Su, Z., Li, J., Lin, X., Godwin, I.D., Jordan, D.R. & Wang, J. 2013. Whole-genome sequencing reveals untapped genetic potential in Africa's indigenous cereal crop sorghum. *Nature Communications* 4(1): 2320. https://doi.org/10.1038/ncomms3320

Mahmoud, A. 2021. *Genome Sequence of Oil Palm*. In Oil Crop Genomics edited by Tombuloglu, H., Unver, T., Tombuloglu, G., Hakeem, K.R. Springer, Cham. https://doi.org/10.1007/978-3-030-70420-9_6

Miao, H., Jiang, B., Chen, S., Zhang, S., Chen, F., Fang, W., Teng, N. & Guan, Z. 2010. Isolation of a Gibberellin 20-Oxidase CDNA from and characterization of its expression in chrysanthemum. *Plant Breeding* 129(6): 707-714. https://doi.org/10.1111/j.1439-0523.2009.01736.x

Nisar, M. & Hussain, H. 2022. Assessment of the genetic variations of sago palm *Metroxylon Sagu* in three regions of Sarawak, Malaysia using amplified fragment length polymorphism (AFLP) marker. *Chemical and Biological Technologies in Agriculture* 9: 46. https://doi.org/10.1186/s40538-022-00315-1

Nishimura, Y. 2018. Sago Starch: Transformation of extraction and consumption processes in traditional Indonesian societies. In *Sago Palm: Multiple Contributions to Food Security and Sustainable Livelihoods*, 1st ed., edited by Ehara, H., Johnson, D.V. & Toyoda, Y. Singapore: Spinger Nature. 1: 221-230.

Novero, A. 2012. Recent advances in sago palm (*Metroxylon sagu* Rottboell) micropropagation. In *Frontiers on Recent Developments in Plant Science*, edited by Goyal, A. & Maheshwari, P. Lethbridge, Canada: Bentham Science Publishers. pp. 60-66. https://doi.org/10.2174/978160805403911201010060

Pati, P., Gupta, M.K., Gouda, G. & Rathore, S.K. 2021. Conservation of rice germplasm by bioinformatics strategy. In *Applications of Bioinformatics in Rice Research*, edited by Gupta, M.K. & Behera, L. Singapore: Springer. pp. 315-332. https://doi.org/10.1007/978-981-16-3997-5_15

Phillips, A.L., Ward, D.A., Uknes, S., Appleford, N.E.J., Lange, T., Huttly, A.K., Gaskin, P., Graebe, J.E. & Hedden, P. 1995. Isolation and expression of three Gibberellin 20-Oxidase CDNA clones from Arabidopsis. *Plant Physiology* 108(3): 1049-1057. https://doi.org/10.1104/pp.108.3.1049

Prunier, J., Giguère, I., Ryan, N., Guy, R., Soolanayakanahally, R., Isabel, N., MacKay, J. & Porth, I. 2019. Gene copy number variations involved in balsam poplar (*Populus balsamifera* L.) adaptive variations. *Molecular Ecology* 28(6): 1476-1490. https://doi.org/10.1111/mec.14836

Purwoko, D., Cartealy, I.C., Tajuddin, T., Dinarti, D. & Sudarsono. 2019. SSR Identification and marker development for sago palm based on NGS genome data. *Breeding Science* 69(1): 1-10. https://doi.org/10.1270/jsbbs.18061

Roslan, H.A. & Anji, S.B. 2011. Characterization of inflorescence-predominant chitinase gene in *Metroxylon sagu* via differential display. *3 Biotech* 1(1): 27-33. https://doi.org/10.1007/s13205-011-0004-x

Roslan, H.A., Hossain, M.A. & Gerunsin, J. 2017. Molecular and 3D-structural characterization of fructose-1,6-bisphosphate aldolase derived from *Metroxylon sagu*. *Brazilian Archives of Biology and Technology* 60(0): e17160108. https://doi.org/10.1590/1678-4324-2017160108

Roslan, H.A., Hossain, M.A., Ngieng, N.S. & Hussaini A. 2020. Sago palm genome size estimation via real-time quantitative PCR. *Current Applied Science and Technology* 20(2): 208-216.

Shi, H., Liu, W., Yao, Y., Wei, Y. & Chan, Z. 2017. *Alcohol dehydrogenase 1 (ADH1)* confers both abiotic and biotic stress resistance in *Arabidopsis*. *Plant Science* 262: 24-31. https://doi.org/10.1016/j.plantsci.2017.05.013

Sieber, A.N., Longin, C.F.H., Leiser, W.L. & Würschum, T. 2016. Copy number variation of CBF-A14 at the Fr-A2 locus determines frost tolerance in winter durum wheat. *Theoretical and Applied Genetics* 129(6): 1087-1097.

Tan, K. 1983. *The Swamp-Sago Industry in West Malaysia: A Study of the Sungei Batu Pahat Floodplain*. Pasir Panjang, Singapore: Institute of Southeast Asian Studies.

Varshney, R.K., Shi, C., Thudi, M., Mariac, C., Wallace, J., Qi, P., Zhang, H., Zhao, Y., Wang, X., Rathore, A., Srivastava, R.K., Chitikineni, A., Fan, G., Bajaj, P., Punnuri, S., Gupta, S.K., Wang, H., Jiang, Y., Couderc, M., Katta, M.A.V.S.K., Paudel, D.R., Mungra, K.D., Chen, W., Harris-Shultz, K.R., Garg, V., Desai, N., Doddamani, D., Kane, N.A., Conner, J.A., Ghatak, A., Chaturvedi, P., Subramaniam, S., Yadav, O.P., Berthouly-Salazar, C., Hamidou, F., Wang, J., Liang, X., Clotault, J., Upadhyaya, H.D., Cubry, P., Rhoné, B., Gueye, M.C., Sunkar, R., Dupuy, C., Sparvoli, F., Cheng, S., Mahala, R.S., Singh, B., Yadav, R.S., Lyons, E., Datta, S.K., Hash, C.T., Devos, K.M., Buckler, E., Bennetzen, J.L., Paterson, A.H., Ozias-Akins, P., Grando, S., Wang, J., Mohapatra, T., Weckwerth, W., Reif, J.C., Liu, X., Vigouroux, Y. & Xu, X. 2017. Pearl millet genome sequence provides a resource to improve agronomic traits in arid environments. *Nature Biotechnology* 35: 969-976. https://doi.org/10.1038/nbt.3943

Ventura, I., Brunello, L., Iacopino, S., Valeri, M.C., Novi, G., Dornbusch, T., Perata, P. & Loreti, E. 2020. Arabidopsis phenotyping reveals the importance of alcohol dehydrogenase and pyruvate decarboxylase for aerobic plant growth. *Scientific Reports* 10: 16669. https://doi.org/10.1038/s41598-020-73704-x

Wang, Wensheng, Ramil Mauleon, Zhiqiang Hu, Dmytro Chebotarov, Shuaishuai Tai, Zhichao Wu, Min Li, et al. 2018. "Genomic Variation in 3,010 Diverse Accessions of Asian Cultivated Rice." *Nature* 557 (7703): 43–49. https://doi.org/10.1038/s41586-018-0063-9.

Wee, C.C. & Roslan, H.A. 2012. Isolation of alcohol dehydrogenase CDNA and basal regulatory region from *Metroxylon sagu*. *International Scholarly Research Network Molecular Biology* 2012: 839427. https://doi.org/10.5402/2012/839427

Wei, C., Chen, J. & Kuang, H. 2016. Dramatic number variation of R genes in solanaceae species accounted for by a few R gene subfamilies. *PLoS ONE* 11(2): e0148708. https://doi.org/10.1371/journal.pone.0148708

Withanage, S.P., Hossain, M.A., Kumar, M.S., Roslan, H.A., Abdullah, M.P., Napis, S.B. & Ab Shukor, N.A. 2015. Overexpression of *Arabidopsis thaliana* gibberellic acid 20 oxidase (AtGa20ox) gene enhance the vegetative growth and fiber quality in kenaf (*Hibiscus cannabinus* L.) plants. *Breeding Science* 65(3): 177-191. https://doi.org/10.1270/jsbbs.65.177

Yong, C.M.R., Sobeng, Y., Zaini, F. & Busri, N. 2018. Suitability of peat swamps areas for commercial production of sago palms: The Sarawak experience. In *Sago Palm: Multiple Contributions to Food Security and Sustainable Livelihoods*, edited by Ehara, H., Toyoda, Y. & Johnson, D.V. Singapore: Springer Nature. pp. 91-108.

Zhang, M., Ma, Y., Zheng, X., Tan, B., Ye, X., Wang, W., Zhang, L., Li, J., Li, Z., Cheng, J. & Feng, J. 2022. The distribution of bioactive gibberellins along peach annual shoots is closely associated with PpGA20ox and PpGA2ox expression profiles. *BMC Genomics* 23(1): 730. https://doi.org/10.1186/s12864-022-08943-5

Zhou, Y. & Underhill, S.J.R. 2015. Breadfruit (*Artocarpus altilis*) gibberellin 20-oxidase genes: Sequence variants, stem elongation and abiotic stress response. *Tree Genetics and Genomes* 11: 84. https://doi.org/10.1007/s11295-015-0909-3

*Corresponding author; email: hhasnain@unimas.my