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GENETIC ANALYSIS OF HEIGHT INCREMENT (HINC) IN ELMINA-ULU REMIS OIL PALM USING SNP MARKERS

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ABSTRACT

Dwarf or short palm is considered one of the desired traits in oil palm breeding, offering advantages of extending the economic viability and harvesting. This study aimed to identify single nucleotide polymorphism (SNP) markers associated with height increment (Hinc) for future breeding improvement programmes. A total of 537 SNPs and 107 Elmina x Ulu Remis (ELxUR) hybrid palm crosses were used in the marker-trait association study, with phenotypic data collected at age 8 and 16. Eight significant SNPs were detected associated with Hinc on chromosome 1, 2, 3, 4 and 10. Candidate gene identification within these significant SNPs was performed using the palm genome sequence and bioinformatics tools, revealing 13 potential candidate genes and transcription factors linked to phytohormone biosynthesis, signalling and plant growth. This study provides a groundwork for implementing marker-assisted breeding for the height trait within the ELxUR oil palm breeding materials.

Keywords: *Elaeis guineensis*, marker-trait association, single nucleotide polymorphism, slow height increment.

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INTRODUCTION

Oil palm (*Elaeis guineensis* Jacq.) stands as the foremost commodity crop in Malaysia's agricultural sector. Over the years, efforts to improve yields have been made without compromising on breeding standards for the industry's sustainability. Harvesting ripe bunches from old and taller palm trees is one of the challenges faced by the industry. The breeding of short palms is considered a desirable trait because of its potential to benefit oil palm operational management and ease harvesting, which in turn prolongs the economic lifespan of planted palms and the land use of the plantation.

Dwarf oil palms are known to occur in several 'populations' such as derivatives of the Dumpy E206 palm, palms prospected at Pobe in Ivory Coast by CIRAD (formerly IRHO), the La Me type (a good DxP bred by CIRAD at their La Me breeding station in Ivory Coast). Additionally, Malaysian Palm Oil Board (MPOB) extensive germplasm collection, particularly population 12 from their Nigerian collection was famous for the short trait (Rajanaidu *et al*., 2017). In Malaysia, the Nigerian Prospection Material (NPM) of the MPOB is known to contain palms of dwarf stature (Rajanaidu & Jalani, 1994). Genetic variability in the NPM population is still high, giving ample scope for further selection (Noh *et al*., 2014).

The oil palm's annual trunk height increment (Hinc) primarily occurs through the continuous and annual production of new fronds. These fronds, or nodes, are arranged in a spiral pattern of eight whorls, with the spacing between them, known as internodes determining the overall height of the palm (Corley & Gray, 1976). These variations in frond production and internode spacing contribute to the observable height discrepancies among palms,

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a factor of particular significance for individuals engaged in harvesting activities. Palm height (HT) serves as a fundamental metric in assessing the growth and development of oil palms. While height measurements can be taken at any stage of growth, the bulk of recorded data stems from what is known as 'vegetative measurements' (VM). These VM encapsulate a range of assessments focused on the palm's vegetative features and growth dynamics, providing valuable insights into its overall performance. Reducing the height of oil palm trees has consistently been a significant consideration for both oil palm breeders and growers. This focus aims to minimise the expenses associated with harvesting from tall palms, particularly after reaching the age of 25 years. Several researches have shown that the height of oil palm is heritable (Lee *et al*., 2015; Teh *et al*., 2020).

Polygenic or quantitative inheritance occurs when a trait is controlled by more than one gene. Usually, the traits are polygenic inheritance when there is a wide variation of a trait. Height is a polygenic trait, controlled not by a single gene, but rather, by multiple genes that each make a small contribution to the overall expression of the trait. When there are large numbers of genes involved, it becomes hard to distinguish the effect of each gene, and even harder to identify the gene variants (alleles) that are inherited according to Mendelian inheritance rules. Circumstantial evidence in various molecular publications suggests that HT is under polygenic control, as quantitative trait loci were identified (Babu *et al*., 2019; Pootakham *et al*., 2015; Seng *et al*., 2016; Ting *et al*., 2023). Despite height being one of the most heritable and easily measured traits in oil palm, mapping alleles explaining natural variation in oil HT remains a formidable challenge. The height character in general not only dependent on expression of the regulating genes, as well as environmental factors that impact the expression pattern, highlighting the complex interplay between gene expression and the environment in determining the phenotype.

The advancements in single nucleotide polymorphism (SNP) marker technology and its successful application in identifying height-related traits in crops such as soybean (Yang *et al*., 2023), wheat (Chen *et al*., 2017; Cheng *et al*., 2023), coffee (Da Silva *et al*., 2024) and barley (Bai *et al*., 2021), suggest its potential for improving crop breeding programs. In the present study, the Elmina x Ulu Remis (ELxUR) populations were genotyped with 600 height-related SNP markers by AgriSeq™ Targeted Genotyping-by-Sequencing (GBS) Solutions (Thermo Fisher Scientific) to identify significant SNP markers associated with Hinc. The information gathered from the present study is expected to develop a marker-assisted identification of dwarf palms, which may aid future oil palm breeding programmes. This marker-assisted selection may be applied at an early stage of palm growth, especially at pre-nursery level to identify short palms before commercial planting of palms in the field.

MATERIALS AND METHODS

Plant Materials and DNA Extraction

Three crosses were developed from the Deli Dumpy palm E206 Elmina (0.175/964) and Ulu Remis *tenera* (0.151/2,626), denoted as ELxUR, as part of the breeding effort. These crosses included one T-sibling (SDP2) and two T-self crosses (SDP1 and SDP3), utilising two parental palms (PKg494/188/1,876 and PKg494/188/1,880). The objective of the cross breeding was to establish progenies of the F_2 cross with segregation for Hinc trait for molecular markers analysis. The trial was designed in a Complete Randomised Design (CRD), with a total of 192 palms planted in June 2002 at field PM02B of Pamol Timur Estate at a density of 136 palms ha^{-1}. However, out of the total 192 palms planted initially, only 107 ELxUR palms were available for sampling from the experimental plot. An additional 13 palms were randomly selected to serve as duplicates. Hence, a total of 120 palms were genotyped in this study.

The number of palms evaluated in each cross is shown in *Table 1*. Leaves were collected from mature palms, cleaned, punched into small pieces and kept at 4°C. The leaves samples were sent to Thermo Fisher Scientific in the USA for DNA isolation. DNA was extracted using MagMAX™ CORE Nucleic Acid Purification Kit (Plant Module).

TABLE 1. NUMBER OF PALMS EVALUATED IN EACH CROSS

Programme code	Cross	Total
SDP1	Selfed	29
SDP2	Sib-crossing	39
SDP3	Selfed	39

Phenotypic Data

The height of the palm is measured from the base of the trunk, or ground level to the base of frond number 41 (Corley & Tinker, 2016). Corley *et al*. (1971) suggested that height measurements should be conducted once palms have attained stable growth. Height measurements are typically taken a few years after the canopy has closed. This timing ensures that the measurement reflects the height of a palm when it is in middle age and encompasses the

period during which competition for light between neighbouring palms gradually intensifies. In this study, two sets of height data (palm age eight and 16) were available to be used for the marker-trait association study.

HT was measured from the ground level to the base of the leaf 41. Hinc was calculated using the following Equation (1):

$$HT/yr\left(\frac{Height\ increase}{yr}\right)=\frac{(Height\ in\ yr\ t)}{(t\text{-}2)}, \qquad (1)$$

where "*t* " is the age of the palm expressed in years, from the date of planting to the moment of a given measurement (Corley & Tinker, 2016). The latter deduction is to take into account that the growth of the trunk was not fully exposed in the initial two years after planting but only growth in the girth of the palm to produce the bole.

SNP Panel Design

The SNPs utilised in this study were primarily identified through a combination of gene differential expression analysis, QTL associated with stem Hinc trait, and candidate gene approach. The SNP marker mining process involved sourcing these SNPs from both the Oil Palm Genome Projects (OPGP) as well as publicly available SNPs related to oil palm height.

Those SNPs sourced from OPGP were filtered based on five selected origins [Eg-Deli-59 (Deli Dumpy Serdang), Eg-Deli-Ulu (IOI Ulu Remis), Eg-Ekona, Eg-Nigeria and Eg-Angola]. A total of 727 SNPs were shortlisted for AgriSeq™ Targeted GBS SNP panel design, including 21 SNPs compiled from four publications (Babu *et al*., 2019; Ong *et al*., 2018; Teh *et al*., 2020; Yaakub *et al*., 2020). The selected SNPs passed through AgriSeq's design quality control process. The primer designs were *in-silico* checked for specificity and sensitivity of the target regions using the oil palm reference genome (accession: GCF_000442705.1) and OPGP's oil palm reference genome (data not published). After that, the SNPs were submitted for the SNP panel design. Finally, 600 SNPs were selected to constitute the custom SNP panel.

GBS and SNP Calling

The GBS was done by Thermo Fisher Scientific (USA) using AgriSeq™ Targeted GBS Solutions. The AgriSeq™ Targeted GBS procedure includes target region PCR amplification, amplicon sequencing and sequence alignment for variant calling. Details on the AgriSeq™ genotyping workflow are available in the report by Carrasco *et al*. (2018). Only SNPs with call rates >90% were retained for subsequent analysis.

Marker-trait Association Study

The marker-trait association study was performed using PLINK v1.90 (Purcell *et al*., 2007). For enhanced accuracy of the genetic data, quality control measures excluded subjects with an SNP call rate below 90%. SNPs with a minor allele frequency (MAF) below 1% or a Hardy-Weinberg equilibrium (HWE) *p*-value below 10^{-6} were also removed from the dataset. The principal component analysis (PCA) and the marker-trait association analysis were performed in PLINK. The two input files (ped and map) for PLINK were converted from Hapmap files using Trait Analysis by Association, Evolution and Linkage (TASSEL) software version 5.2.85 (Bradbury *et al*., 2007). Marker-trait associations were performed using linear regression, with Hinc phenotypes as the dependent variable and principal components as covariates. The linear option in PLINK performs a linear regression analysis with each SNP as a predictor.

Identifying the Potential Candidate Genes for Hinc

The significant Hinc SNP positions were mapped to the oil palm whole genome sequence EG5.1 (http://genomsawit.mpob.gov.my/genomsawit/) and OPGP's oil palm reference genome (unpublished data) using SNP flanking sequences to define the boundaries for gene mining. Sequence similarity analysis (BLAST) was employed with the SNP-containing sequences as the query against the predicted oil palm gene model database (Chan *et al*., 2017; Ting *et al*., 2023) and OPGP Reference Gene Annotation (data not published). Candidate genes and transcription factors related to Hinc and stem height were identified through a comprehensive review of their biological functions, as documented in the literature and public databases, including http://palmxplore.mpob.gov.my/palmXplore/palmxplore_res and https://www.uniprot.org/. The search focused on biological activities, including the biosynthesis and signalling of phytohormones, DELLA genes, transcription factors and cell morphogenesis, which can contribute to dwarfism during the growth and development of plants (Ting *et al*., 2023).

RESULTS AND DISCUSSION

Hinc Data

Height data measurements were conducted at eight and 16 years after planting. Two sets of height data were utilised in this study to identify SNPs consistently associated with Hinc at eight (Hinc8) and 16 (Hinc16) years. The

three ELxUR crosses (SDP 1, SDP 2 and SDP 3) exhibited mean Hinc of 16.42 and 35.58 cm/yr for Hinc8 and Hinc16, respectively. For Hinc8, the recorded Hinc ranged from a maximum of 25.00 to a minimum of 8.33 cm/yr. Similarly, at Hinc16, the maximum and minimum Hinc were 50.86 and 19.86 cm/yr, respectively (*Table 2*). The coefficients of variation (CV) calculated for the Hinc8 and Hinc16 datasets were 18.94% and 14.87%, respectively. These values indicate that the ELxUR germplasm exhibits relatively narrow genetic variation in Hinc when compared to previous studies by Ong *et al*. (2018) and Ting *et al*. (2023), which reported CVs of up to 32.5% and 26.9%, respectively. Analysis of variance (ANOVA) indicated significant differences in Hinc among the crosses at both Hinc8 and Hinc16 ($p<0.05$).

SNP Data

Genomic DNA from 120 individuals palms was used to prepare AgriSeq HTS libraries. The libraries were genotyped using the Ion 540 Chef kit and Ion 540 Chip, generating approximately 80 million reads. Overall, the results achieved a 95.1% call rate, indicating that all 107 samples attained above-average coverage. The call rate for a given SNP is defined as the proportion of individuals in the study for which the corresponding SNP information is not missing (Reed *et al*., 2015). The distribution of raw reads across the sequenced portion of the genome was notably uniform, achieving an average base coverage uniformity of 95.8% with a mean depth of 335X. Approximately 96.2% of on-target reads were successfully aligned to the unique locations on the reference genome.

The genotyped SNP data was first scrutinised by comparing the genotype calls of 13 duplicate samples to verify the precision of genotyping results. An average of 97.9% of the SNPs exhibited identical genotype calls, with 1.8% missing data in either one or both of the duplicated samples. A minimal discrepancy in genotype calls (0.3%) was observed among the 13 duplicate samples. These genotyping results show reproducible genotypes.

A total of 600 SNP markers were genotyped, of which 537 SNP markers had a call rate exceeding 90.0%. The low call rate observed in some markers may be attributed to factors such as low DNA concentration, suboptimal primer and probe design, or issues during sequencing. A total of 537 SNP markers were genotyped, of which 216 SNPs (40.2%) were polymorphic and 321 SNPs (59.8%) were monomorphic. Of the 216 polymorphic SNPs, 193 (89.4%) were polymorphic in the SDP1 cross, and 192 (88.9%) were polymorphic in the SDP3 cross. As expected, all SNPs were detected as polymorphic in the SDP2 cross, which involved the sib-crossing of two palms (PKg494/188/1,876 and PKg494/188/1,880). Most of the polymorphic SNPs detected were transitions (A/G or C/T) (61.6%) while 38.4% were transversion SNPs. The most frequently observed variation was A/G, accounting for 31.5%, while the least common type of change was C/G, representing only 4.6% of the total polymorphisms (*Table 3*).

Although the genotyped SNPs were selected by different approaches, the transition: transversion ratio is 1.60, which was similar to earlier reported for oil palm by Pootakham *et al*. (2013; 2015) and Ting *et al*. (2014) at 1.77, 1.67 and 1.55, respectively. The 63 remaining SNPs, with less than 90.0% call rate, were excluded from this study to improve the accuracy of the genetic information.

TABLE 2. DESCRIPTIVE PARAMETERS OF HEIGHT INCREMENT (HINC) IN EACH CROSS OF ELxUR AT PALM AGE EIGHT AND 16

Item	Height increment (cm)							
	Year 8 (Hinc8)				Year 16 (Hinc16)			
	SDP1	SDP2	SDP3	Overall	SDP1	SDP2	SDP3	Overall
N	29	39	39	107	29	39	39	107
Mean	15.28	16.12	17.57	16.42	32.69	34.89	38.44	35.58
Std. Deviation	3.11	2.79	3.09	3.11	5.85	4.61	4.06	5.29
Minimum	11.33	9.33	8.33	8.33	19.86	24.79	28.57	19.86
Maximum	23.33	21.83	25.00	25.00	50.86	44.79	46.29	50.86
CV (%)	20.35	17.31	17.59	18.94	17.90	13.21	10.56	14.87

Note: N - number of palms; CV - coefficient of variation.

TABLE 3. SUMMARY OF SNPS USED FOR GBS

Total number of SNP	**600**			
Call rate >90%	537		89.5%	
Call rate <90%	63		10.5%	
Total number of monomorphic SNP	321		59.8%	
Total number of polymorphic SNP	216		40.2%	
	Monomorphic		**Polymorphic**	
SDP1	23	10.6%	193	89.4%
SDP2	0	0.0%	216	100.0%
SDP3	24	11.1%	192	88.9%
Transition				
A/G	68		31.5%	
C/T	65		30.1%	
Transversion				
A/T	27		12.5%	
T/G	24		11.1%	
A/C	22		10.2%	
C/G	10		4.6%	

Marker-trait Association Study

A total of 587 SNP markers with a minor allele frequency (MAF) higher than >5% were used to estimate the genetic relationship and mapping of marker-trait associations (MTAs) related to Hinc. The study included three crosses derived from ELxUR comprising 107 palms along with the two sets of phenotypic data (palm age eight and 16) for height. Prior to analysis, quality control (QC) was carried out to eliminate samples with a missing genotype rate exceeding 10%. Following the quality control (QC) steps, a total of 107 palm samples and 537 SNP markers remained for further analysis.

The association analysis of trait-marker data resulted in the identification of 22 SNP markers linked to Hinc8 (using PC1) on five chromosomes in ELxUR. Among the 22 SNPs markers, nine were located on chromosomes 4 and 3, respectively. Two SNP markers at chromosome 2 and one SNP marker mapped to chromosomes 1 and 10. Only markers with $P \leq 0.05$ will be considered as significantly associated with Hinc. By using the palm height phenotypic data at age 16 (Hinc16) with the PC1 as the covariate, 69 SNP markers were detected linked with Hinc16. Interestingly, all 22 SNP markers associated with the Hinc8 were also found in the 69 SNP significant markers list. Most of the SNP markers are located on chromosomes 4 and 10. However, redundant markers were identified and removed from further analysis. Reducing redundant SNPs based on similar genotype calls at the same genomic region can help streamline the genetic analysis. Consequently, pattern comparison and analysis resulted in the retention of only eight SNPs following the deduplication process (*Table 4*).

Remarkably, two out of 21 SNP markers compiled from previous publications were found to be linked to Hinc in ELxUR too. Specifically, two markers (SNPM00563 and SNPM03201) from Yaakub *et al*. (2020) were named M011 and M013 in this study, respectively. These two significant markers were associated with Hinc and petiole cross-section (SNPM0563) and rachis length (SNPM03201) located at chromosome 2 (Yaakub *et al*., 2020). Interestingly, SNPM03201 associated with rachis length in Yaakub *et al*. (2020) study was linked with Hinc in this study. However, the palm material [backcross of T128 (0.151/128)] used in the Yaakub *et al*. (2020) study does not have the common bloodline as shown in this study which is Elmina (0.175/964) and Ulu Remis (0.151/2,626). It suggests some of the SNP markers might be able to transfer between the palms of different origin. However, 10 selected SNP markers from Teh *et al*. (2020) which were associated with trunk height and frond length in *Dumpy* AVROS *pisifera* (DAV) x *Gunung Melayu dura* (GM) cross did not show any significant association with Hinc in this study.

Compiling the findings from various studies (Bhagya *et al*., 2020; Billotte *et al*., 2010; Lee *et al*., 2015; Ong *et al*., 2018; Pootakham *et al*., 2015; Teh *et al*., 2020; Ting *et al*., 2023; Yaakub *et al*., 2020), QTLs for HT, Hinc and rachis length were identified across nearly all chromosomes, except chromosome 3 in various genetic backgrounds. In this study, there were three SNP markers (M020, M024 and M270) identified significantly associated with Hinc located in chromosome 3 (*Table 4*). Contrastingly,

in Ting *et al*. (2023) study, major QTL effects on HT and Hinc were detected on chromosomes 6 and 8 of the Nigerian *tenera* (T128). Meanwhile, it was reported that the major QTL for HT and frond length were co-located on chromosomes 11 (Bhagya *et al*., 2020; Teh *et al*., 2020), 10, 14 and 15 (Pootakham *et al*., 2015). In summary, height regulation is likely dependent on genetic background, and it is necessary to correct Hinc data across different studies due to observations made in varying environments. It is challenging to compare one study to another because of environmental influences.

Potential Candidate Genes Identification

The physical positions of the SNP markers were acquired from the Genomsawit website of the MPOB Oil Palm Genome Programme (MPOB-OPGP) (http://genomsawit.mpob.gov.my) and OPGP's oil palm reference genome (data not published). These positions, along with the SNP flanking regions, can help to specify the boundaries for gene mining.

The BLAST results of eight significant SNPs identified 13 candidate genes associated with Hinc in oil palm. There were four phytohormones-related candidate genes located in chromosome 3 associated with three SNP markers (*Table 5*). The shortlisted phytohormones-related candidate genes, *gibberellin 3-beta-dioxygenase 1* (*GA3ox1*) were located in between markers M020 and M024. *GA3ox1* is an enzyme involved in the biosynthesis of *gibberellins* (*GAs*), catalysing the conversion of inactive GA precursors into active forms. *GAs* are plant hormones crucial for various aspects of plant growth and development (Mitchum *et al*., 2006; Yamaguchi *et al*., 1998). Cheng *et al*. (2023) demonstrated by gene editing of *GA3-oxidase*, an alternative semi-dwarfing gene in barley that combines an optimal reduction in plant height without restricting coleoptile emergence and subsequent seedling growth. Remarkably, two auxin signalling genes, protein kinase *PINOID 2* and auxin (Aux)-responsive protein *indole-3-acetic-acid-inducible 8* (*IAA8*) were identified on the same chromosome. *PINOID* is a protein kinase found in plants, particularly in *Arabidopsis thaliana*, and it plays a crucial role in the regulation of polar auxin transport and regulation of auxin signalling (Morita & Kyozuka, 2007; Zourelidou *et al*., 2014). *PINOID phosphorylates* and regulates the activity of *PIN* proteins. *PIN* proteins are integral to polar auxin transport, which determines the direction of auxin flow within plant tissues. This transport is essential for processes such as organ development, tropisms and embryogenesis. The constitutive expression of *PINOID* results in a phenotype in both shoots and roots resembling that of auxin-insensitive plants. This suggests that *PINOID*, encoding a serine-threonine protein kinase, functions as a negative regulator of auxin signalling, leading toward stature (Christensen *et al*., 2000). Meanwhile, auxin-response protein *IAA8* is well-known for its role as an early responder to auxin, engaging in auxin signalling by interacting with auxin response factor (*ARF*) protein and functioning as transcriptional repressors (Lavy & Estelle, 2016; Li *et al*., 2016; Luo *et al*., 2018). In Song *et al*. (2013) study, overexpression of *OsIAA4* in rice leads to less sensitivity to exogenous auxin, dwarfism and more tiller angles in comparison with wild-type plants. Meanwhile, there is another auxin-response protein identified at chromosome 2 is associated with marker M013.

The gene *indole-3-pyruvate monooxygenase YUCCA6* was also identified on Chromosome 3 which is associated with marker M270. *YUCCA6* is part of the *YUCCA* family of enzymes, which are responsible for the conversion of *indole-3-pyruvate to indole-3-acetic acid* (*IAA*), a major form of auxin

TABLE 4. ALLELE POSITION AND VARIANT ALLELE OF MARKERS ASSOCIATED WITH HINC ON OIL PALM CHROMOSOME

Marker ID	EG5.1 chromosome	Physical position (bp)	Ref_ allele	Var_ allele	*p*-value (Hinc8)	*p*-value (Hinc16)
M346	1	34,012,126	G	T	0.013	0.0001
M011	2	3,522,890	A	C	0.018	0.000000565
M013	2	4,297,558	G	A	0.004	0.00000994
M020	3	1,307,898	A	G	0.007	0.001
M024	3	2,014,780	A	G	0.002	0.006
M270	3	13,279,910	T	C	0.006	0.001
M180	4	54,707,398	T	C	0.004	0.0002
M379	10	26,037,658	C	T	0.0025	0.021

Note: bp - base pair.

in plants. This conversion is a key step in the biosynthesis of auxin (Kim *et al*., 2007; Won *et al*., 2011).

Furthermore, *BAM1* encodes a leucine-rich repeat receptor-like serine/threonine-protein kinase (*LRR-RLKs*) located on chromosome 4 and associated with marker M180. *LRR-RLK*s are known to participate in cellular differentiation and various developmental processes. They play crucial roles in root development, shoot development and xylem differentiation (Soltabayeva *et al*., 2022). *LRR-RLKs* also influence hormone signalling pathways, including brassinosteroid (BR) and GA signalling pathways (Clouse, 2011). Previous studies have suggested this gene has the potential to influence oil palm rachis length (Yaakub *et al*., 2020).

The gene repressor of GA1-3-like 1 (*RGL1*) is linked to marker M346 on chromosome 1. It is a key component in the GA signalling pathway in plants. *RGL1* is a member of the *DELLA* family of proteins, which act as negative regulators in the GA signalling pathway. GA are plant hormones that play a crucial role in various aspects of plant growth and development, including seed germination, stem elongation and flowering (Swain & Olszewski, 1996; Wen & Chang, 2002). The GA-deficient mutant *ga1-3* is a non-germinating, extreme dwarf that flowers late and produces male-sterile flowers in *Arabidopsis* (Tyler *et al*., 2004).

Some of the transcription factors involved in growth and development were shortlisted, *e.g*., *grf1-interacting factor 1* (*GIF1*) and *ethylene-responsive transcription factors* (*ERFs*). *GIF1* is known to interact with *growth-regulating factor 1* (*GRF1*), which is a transcription factor involved in the regulation of plant growth and development. The interaction between *GIF1* and *GRF1* suggests a role in modulating gene expression related to growth processes (Lu *et al*., 2020). *ERFs* are plant transcription factors essential for regulating gene expression in response to ethylene, a plant hormone pivotal in numerous physiological processes such as growth, development and stress responses (Müller & Munné-Bosch, 2015). The identification of candidate genes associated with eight significant SNPs on chromosomes 1, 2, 3, 4 and 10 has been achieved through mining. These genes exhibit potential effects on Hinc in oil palms, and this is supported by their known roles in influencing comparable traits in other crop species. Although the candidate genes identified were interesting, it's essential to note that their participation and impact in regulating the Hinc in oil palm remain speculative. Further studies and research are needed to support the prediction.

CONCLUSION

Our study marks an important advance in understanding the genetic basis of Hinc in oil palm, with findings that hold substantial promise for improving oil palm breeding and agricultural productivity. By identifying eight SNPs associated with Hinc in the ELxUR oil palm population, we have developed genetic markers that enable marker-assisted selection (MAS) for height traits. This allows breeders to target specific height-related traits early in the growth cycle, even at the pre-nursery stage, which facilitates the selection of shorter palms for commercial planting, optimising land use and potentially reducing harvest costs over the lifespan of the crop.

Figure 1. Progenies of ELxUR hybrid palm crosses at the field, where a significant difference in HT was observed.

TABLE 5. BLAST RESULTS TO THE OIL PALM GENOME IDENTIFIED 13 CANDIDATE GENES WITHIN THE 8 SIGNIFICANT SNPS AFFECTING THE HEIGHT INCREMENT IN IOI ELxUR CROSSES

Marker ID	EG5.1 chromosome	Physical position (bp)	Nearby gene	Candidate gene position	SNP position relative to the candidate gene	Candidate gene annotation
M346	1	34,012,126	p5.00_sc00027_p0005	33,423,370...33,426,263	-588kb	Della protein rgl1
			p5.00_sc00027_p0020	338,05,511...33,811,810	-206kb	GRF1-interacting factor 1
M011	2	3,522,890	p5.00_sc00263_p0032	3,624,681...3,677,297	+101kb	Phospholipase A
			p5.00_sc00263_p0023	3,815,313...3,815,789	+292kb	Ethylene-responsive transcription factor
M013	2	4,297,558	p5.00_sc00051_p0113	4,610,179...4,614,551	+312kb	Auxin-responsive protein IAA25
M020	3	1,307,898	O24648|G3OX_PEA	1,614,924...1,616,002	+307kb	Gibberellin 3-beta-dioxygenase 1
			p5.00_sc00220_p0033	710,029...711,493	-597kb	Protein kinase PINOID 2
M024	3	2,014,780	O24648|G3OX_PEA	1,614,924...1,616,002	-400kb	Gibberellin 3-beta-dioxygenase 1
M270	3	13,279,910	p5.00_sc00001_p0431	13,988,128...13,993,162	+708kb	Auxin-responsive protein IAA8
			p5.00_sc00001_p0389	13,044,638..13,047,562	-235kb	Indole-3-pyruvate monooxygenase YUCCA6
M180	4	54,707,398	Q8VZG8|Y4885_ARATH	55,216,179...55,494,670	+508kb	Leucine-rich repeat receptor-like serine/threonine-protein kinase
M379	10	26,037,658	p5.00_sc00004_p0377	26,034,509...26,038,761	-3kb	Protein TITF 6b
			p5.00_sc00004_p0376	26,021,267...26,031,321	-16kb	TPR repeat-containing thioredoxin
			p5.00_sc00004_p0332	25,218,092...25,244,739	-819kb	Hydroxyproline-rich glycoprotein

Note: bp - base pair.

These SNPs are located within genomic regions harbouring genes that have known functions related to plant growth and development, particularly in influencing height. The discovery of these candidate genes provides valuable insights into the molecular mechanisms underlying Hinc in oil palm. These genes may regulate key pathways and processes involved in plant growth, such as phytohormone signalling, cell elongation and morphogenesis. Understanding their roles in Hinc can potentially lead to the development of targeted breeding strategies aimed at enhancing this trait in oil palm populations.

Moving forward, further investigations are warranted to delve deeper into the roles and functions of these candidate genes. Additional studies, including functional genomics experiments, expression analyses and genetic mapping studies, can provide a more comprehensive understanding of how these genes contribute to Hinc in oil palms. Ultimately, this knowledge will empower breeders to make informed decisions and implement targeted breeding strategies to improve Hinc and overall productivity in oil palm cultivation.

In summary, the findings from this study establish a solid foundation for the development of improved oil palm varieties, enabling the selection of shorter palms that align with industry needs and promoting more sustainable and economically viable oil palm production systems.

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