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Contributed Paper

Identifying a DELLA Gene as a Height Controlling Gene in Oil Palm

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ABSTRACT

The research in oil palm (*Elaeis guineensis* Jacq.) yield improvement is still being done to serve the consumption demand needed for cooking oil, cosmetics and biodiesel production. Moreover, a harvesting-facilitating trait such as stem height still needs more improvement to lower labor intensity and harvesting losses of oil palm bunches. This study aims to understand the genetic control for the stem height trait (HT) and its correlation with yield component traits such as bunch number (BN) and fresh fruit bunch yield (FFB). To identify height controlling genes, gene-based markers targeting three candidate genes including DELLA gene, gibberellin (GA) 2-oxidase and asparagine synthase were designed and identified the association of their potential polymorphic markers with HT in various oil palm populations. We identified the DELLA gene (renamed as *EgDELLA1*), a GA nuclear repressor, at chromosome 14, highly associated with height in the GT population at p value of 0.0261, and 0.0429 for the two of three times of height phenotype recorded. The strongest expressions of *EgDELLA1* were found in apical meristem and, to a lesser extent in leaf and fruit development while no expression was detected in male inflorescence. This suggested that *EgDELLA1* is a major component of stem elongation initiating at apical meristem. In addition, weak to moderate positive correlation was found between HT and BN (r = 0.215-0.395), and between HT and FFB (r = 0.254-0.499) from the KU and GT populations. Thus, improvement of semi-dwarf oil palm with higher yield is possible

but still challenging. Our study provides some information that would be useful for oil palm variety improvement in the near future.

Keywords: oil palm, DELLA, height, dwarf, bunch number, fresh fruit bunch yield

1. INTRODUCTION

African oil palm (*Elaeis guineensis* Jacq.) is a perennial monoecious species with a life-span of up to 25-30 years, reaching up to 15-18 meters in height. African oil palm plant height increased by 45-75 cm (centimeter) per year [1]. When oil palm ages and grows to heights more than 2-3 meters, the harvesting of fruit bunches becomes more challenging. Oil palm with short stem height is favorable for bunch harvesting. Therefore, short stem and high yield are more favorable characteristics for selection by oil palm breeders. Several oil palm breeding teams have been successful at making oil palm hybrids with slow trunk growth. For example, interspecific F1 hybrids with height increase of only 15-25 cm per year were made by crossing *E. guineensis* with *E. oleifera*, which has a height increase of about 5-10 cm annually [2]. Intraspecific hybrid with high yield and annual height increase of about 40 cm was also made as an oil palm genetic resource [3]. Currently, information about genes controlling height in oil palm is still limited.

To date, two published QTL studies of height were conducted in an F2 population created from self-pollination of *Tenera* clone A43/9 [4] and in two *Tenera* palm F1 populations, which resulted from crossing from *Dura* and *Pisifera* individuals [5]. Three QTLs were found on chromosome 10, 14 and 15, that contributed to stem height and explained 10-21% of phenotype variance expected (PVE) [4]. Two potential genes, DELLA gene (*GAI1*) and GA2 -oxidase (*GA2OX2*), which linked the QTL on chromosome 14, were proposed to play an important role in height control. In another QTL study [5], they found one major QTL (51% of PVE) on LG 5 (chromosome 16) responsible for stem height in the F1 populations. There are 8 genes in the QTL region and asparagine synthase related gene was thought to play an important role in height control. They found the significantly higher expression (qRT-PCR) of the asparagine synthase related gene in the trunk of dwarf trees, as compared with the expression in tall trees but they were unable to determine whether any other of the 8 genes in this QTL region also control height in these populations.

Here, we aim to determine the correlation between height (HT) and yield component traits such as bunch number (BN) and fresh fruit bunch yield (FFB) and identify genes controlling height, targeting three candidate genes explained above including the DELLA gene, GA2 -oxidase and asparagine synthase. Several oil palm populations differing in age and planting locations were used in this study. The correlation between HT, BN and FFB was first determined in two different populations with the available trait information. Next, to identify the genes controlling height, the height contribution of each polymorphic locus of each candidate gene was compared using ANOVA analysis and the association analysis. To perform the association by TASSEL analysis, the population structure was first performed using STRUCTURE. The RNA expression

of genes with significant association with height was investigated using RT-PCR in various tissues.

2. MATERIALS AND METHODS

2.1 Plant Materials

Four oil palm populations and resources were used in this study, coming from four different growing regions in Thailand, named as GT, SIT, KU populations and CPI resource. The GT population samples were kindly provided from the Golden Tenera Company Limited, Krabi, Thailand. This GT population resulted from 30 crosses of crossing between 6 female parents and 5 male parents. The female parents were *Dura* fruit type, consisting of A 43/9D, A 1/2D, R 15/14D, R 8/9D, R 10/1D and R 10/5D while the male parents were *Pisifera* fruit type, consisting of R 9/8P, R 5/21P, R 3/8P, KA 17/2P and R 16/7P. The GT population contained 180 individuals planted in 2008. The SIT population samples were kindly provided by the Sitthiporn Kridakorn Research Station, Kasetsart University, Prachuap Khirikhan, Thailand. This SIT population consisted of 6 Tenera-type commercial oil palm lines, including Univanich, Nigeria, Uti, PaoRong, Papua New Guinea and Ekona. The SIT population contains 132 individuals (22 individuals from each commercial line) and was planted in 2008. The KU population samples were kindly provided by the oil palm technology development for commercial bio diesel industry in newly planted area project (OPTD), Department of Agronomy, Faculty of Agriculture, Kasetsart University, Saraburi, Thailand. This KU population consisted of 8 Tenera-type commercial oil palm lines, including Golden Tenera, Surat Thani 2, Surat Thani 3, Surat Thani 4, Surat Thani 5, Surat Thani 6, Univanich and Uti. The KU population contains 144 individuals (18 individuals from each commercial line) that were planted in 2010. The fourth oil palm resource was kindly provided by CPI Agrotech Company Limited, Chumphon, Thailand. This CPI oil palm resource contained 20 oil palm individuals. All individuals were self-fertilized from an oil palm named S1-47 (Tenera-type). These oil palm individuals were planted in 2008.

2.2 Phenotype Collecting

Phenotypes used in this study include height (HT, cm/palm), bunch number (BN, bunch number/palm/year) and fresh fruit bunch yield (FFB, kg (kilogram)/palm/year). For the GT population, BN and FFB was recorded in three successive years, 2013 (5 year palm), 2014 (6 year palm) and 2015 (7 year palm) while HT was recorded from 8 year oil palm in March 2016, October 2016 and March 2017. For the KU population, BN and FFB was recorded in one year, 2015-2016 (6 year oil palm), while HT was recorded 3 times from 5-6 year palm, in September 2015, March 2016 and August 2016. For the SIT population, only HT was recorded over 2 successive six months from 8 year oil palm in February 2016 and September 2016. Also, HT of the CPI resource was recorded once on March 2016 in 8 year oil palm.

2.3 Primer Designing, and Genotyping of SSR and Gene-based Markers

Oil palm leaf samples were subjected to DNA extraction by using DNA extraction kits, including DNeasy Plant Mimi Kit (QIAGEN, Germany) and Invisorb Spin Plant Mini Kit (STRATEC Biomedical AG, Germany). Markers used in genotyping were from both previous studies and newly designed gene-based markers. The previous markers included some SSR markers [6], mEgSSRffb10-8 and mEgACCO-pr2 [7].

In addition, newly designed gene-based markers were designed from candidate genes controlling height, including putative DELLA protein *GAI1* and putative *GA2OX2* [4], and asparagine synthase related protein [5]. New gene-based primers were designed to have a DNA product of about 150-300 bp (base pairs) covering from the promoter region (5′ UTR) to the end of genomic sequences of above candidate genes using primer3 (http://bioinfo.ut.ee/primer3-0.4.0/primer3/).

To identify the potential polymorphic markers controlling height, PCR was conducted in 2 oil palm groups with the opposite height score using short and tall CPI and GT DNA pools as templates. For the CPI resource, the short oil palms were pooled from 10 individuals of *Dura*, *Tenera* and *Pisifera* types, named as CPI 2/8T, CPI 2/10T, CPI 2/21T, CPI 1/25P, CPI 1/26D, CPI 1/28T, CPI 2/33P, CPI2/31P, CPI2/30D and CPI 2/27T while the tall oil palms were pooled from 10 individuals of both *Dura* and *Tenera* types, named as CPI 1/6T, CPI 1/14D, CPI 1/30D, CPI 1/31 D, CPI 1/34P, CPI 1/38T, CPI 1/40D, CPI2/41T, CPI 1/41T and CPI 2/44T. For the GT resource, the short oil palms were pooled from 10 individuals of *Tenera* type, named as T3/28, T2/25, T3/29, T1/27, T2/26, T1/25, T2/27, T1/26, T2/32 and T6/16. The tall oil palms were pooled from 10 individuals of *Tenera* type, named as T5/10, T5/6, T5/8, T3/10, T5/4, T5/12, T6/7, T5/14, T2/20 and T5/13. These 20 individuals from the GT resource were selected from the shortest and tallest oil palms in the GT population. After the potential polymorphic markers controlling height were identified, they were used for genotyping in the GT, SIT, KU and CPI populations. The PCR reaction for the amplification was conducted as the same as our previous study [7].

2.4 Statistical, Population Structure and Association Analyses

SPSS 11.5 was used to analyze the phenotype data, including HT, BN and FFB in terms of descriptive statistics and the correlation between the three traits. This statistics package was also used to analyze preliminary relationships or association of polymorphic loci of targeted genes with HT, BN and FFB, by comparing mean height using Independent-Samples T Test or One-Way ANOVA. If significant relationships were found using the above analysis, the population structure and association analysis was then performed. STRUCTURE 2.3.4 (http://pritchardlab.stanford.edu/structure.html) and STRUCTURE harvester (http://taylor0.biology.ucla.edu/structureHarvester/) were used to analyze population structure and determine optimal population (K), respectively. Inferred ancestry of individuals of optimal K from STRUCTURE output was used as Q-matrix by setting its value as covariance in the association analysis of the targeted markers with HT, BN and FFB traits, using TASSEL 2.1 (http://www.maizegenetics.net/#!tassel/c17q9).

2.5 Reverse Transcription Polymerase Chain Reaction Analysis (RT-PCR) of Target Genes Controlling Height

To test the expression of various plant tissues, leaf, stigma, ovary, young and mature fruit, male flower, and meristem samples were used. These samples were the same samples used by our previous study [7] except for the meristem tissues, which were added for this study. The meristem was collected from the top of CPI 1/26D and CPI 1/34P oil palm trees. These oil palms were two of the 20 samples used in the CPI sources for identification of markers associated with height as mentioned above.

Each sample was ground under liquid

nitrogen to a fine powder using a mortar and pestle. Total RNA extraction was performed by using InviTrap® Spin Plant RNA Mini Kit, Stratec Molecular GmbH (Germany). The RNA integrity was analyzed on 1% agarose gel and stained by ethidium bromide before visualization. The RNA concentration was measured using NanoDrop®ND-1000. The total RNA was immediately kept on ice or at -80 °C for longer use. The RT-PCR reaction was performed in three replicates for both target and reference genes using QIAGEN OneStep RT-PCR Kit, (QIAGEN, Germany). *Cyclophilin 2* (renamed as *EgCyp2*) was chosen for the reference gene [8]. The RT-PCR reaction was followed from our previous study [7].

3. RESULTS

3.1 Details of Height Frequency of the KU, GT and SIT Populations

In this study, height was recorded 3 times, HT-1, HT-2 and HT-3 for the KU and GT populations while in SIT population, it was recorded 2 times, HT-1 and HT-2. The time interval between each height recording for all three populations was 6 months. The mean height of the KU population was the shortest about 114 cm for HT-1, 136 for HT-2 and 152 cm for HT-3, with a 19 cm in average increase within 6 months. The mean height of the GT population was the tallest of the 3 populations recorded. Its mean height was 192 cm for HT-1, 231 cm for HT-2 and 263 for HT-3, with a 35.5 cm in average increase within 6 months. The mean height of the SIT population was 159 cm for HT-1 and 175 cm for HT-2, with a 16 cm increase within 6 months. The height comparison of the 3 populations is shown in Figure 1.

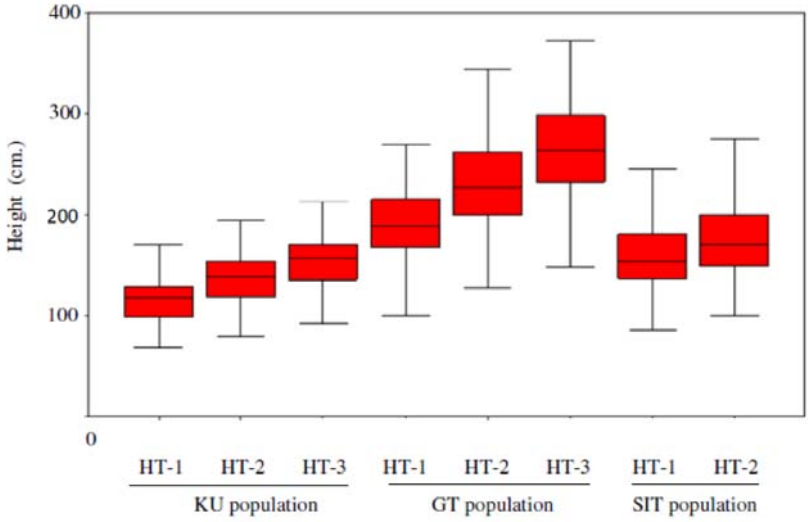

Figure 1. Comparison of height recorded over 6 month intervals (HT-1, HT-2 and HT-3) in years 2015-2016 for 6 year old oil palms of the KU population, in 2016 and 2017 for 8 year old oil palms of the GT population, and in 2016 for 8 year old oil palms of the SIT population.

3.2 Correlation Coefficient of Height with Bunch Number and Fresh Fruit Bunch Yield of the KU and GT Populations

The degree of correlation between height and yield components, including BN and FFB would help to determine a target height that could be selected by oil palm breeders. Only the KU and GT populations with available HT, BN and FFB trait data were used to determine the correlations between the traits. The result of correlation analysis of the KU population was illustrated in Table 1.

Table 1. Pearson Correlation of HT, BN and FFB of the 144 individuals from the KU population. Several levels of correlations were found from the three traits including very strong positive significant correlations (r = 0.893-0.940) for HT-1, HT-2 and HT-3, weak to moderate positive correlation (r = 0.335-405) between HT, BN and FFB, and very strong correlation (r = 0.828) between BN and FFB.

	HT-1	HT-2	HT-3	BN-2016	FFB-2016
HT-1		.940(**)	.893(**)	.405(**)	.359(**)
HT-2			.919(**)	.379(**)	.360(**)
HT-3				.337(**)	.335(**)
BN-2016					.828(**)
FFB-2016					

** Correlation is significant at the 0.01 level (2-tailed).

The height of all 3 records (HT-1, HT-2 and HT-3) was shown to have a weak to moderate positive correlation at r = 0.337-0.405 with BN and at r = 0.335-0.360 with FFB. However, BN was shown to have a very strong correlation with FFB at r = 0.828. For the correlation result of the GT population as shown in Table 2, height in all HT-1, HT-2 and HT-3 records was shown to have a weak positive correlation, at r = 0.215-0.395, with BN and a moderate correlation at r = 0.254-0.499, with FFB. In addition, BN was shown to have a strong correlation with FFB, at r = 0.733

Table 2. Pearson Correlation of HT, BN and FFB of the 180 individuals from the GT population. Several levels of correlations were found between the three traits including very strong positive significant correlation (r = 0.940-0.968) for HT-1, HT-2 and HT-3, weak positive correlation (r = 0.215-0.395) between HT and BN, moderate positive correlation (r = 0.254-0.499) between HT and FFB, and weak to strong positive correlation (r = 0.162-0.733) between BN and FFB.

	HT-1	HT-2	HT-3	BN-2013	FFB-2013	BN-2014	FFB-2014	BN-2015	FFB-2015
HT-1		.968(**)	.940(**)	.390(**)	.499(**)	.391(**)	.403(**)	.256(**)	.254(**)
HT-2			.953(**)	.392(**)	.480(**)	.387(**)	.404(**)	.282(**)	.288(**)
HT-3				.386(**)	.496(**)	.395(**)	.440(**)	.215(**)	.264(**)
BN-2013					.550(**)	.480(**)	.232(**)	.302(**)	.185(**)
FFB-2013						.376(**)	.675(**)	.062	.384(**)
BN-2014							.645(**)	.406(**)	.260(**)
FFB-2014								.162(**)	.470(**)
BN-2015									.733(**)
FFB-2015									

** Correlation is significant at the 0.01 level (2-tailed).

* Correlation is significant at the 0.05 level (2-tailed).

3.3 Marker Designing from DELLA, GA2 -oxidase (GA2OX2) and Asparagine Synthase Genes, and Identification of the Potential Markers Controlling Height

Sequences of DELLA gene (P5_sc00033.v1.gene762) and *GA2OX2* (P5_sc00033.v1.gene644) was obtained from a public database, Malaysian Palm Oil Board (MPOB) (http://genomsawit.mpob.gov.my/genomsawit/). The sequence length of the DELLA gene (renamed as *EgDELLA1*), which was 2695 bp containing 1000 bp of the 5′ UTR region and 1695 bp of expected transcribed gene, were used for marker development. Fourteen primers in the size length of 203-291 bp have been designed for covering the whole gene. Primers 1-4 (renamed mEgDELLA1-1 to mEgDELLA1-4) covered the promoter region while primers 5-14 (renamed mEgDELLA1-5 to mEgDELLA1-14) covered the gene region. For *GA2OX2* (P5_sc00033.v1.gene644), the sequence length, which was 2016 bp, containing 1000 bp of the expected promoter region and 1016 bp of the gene. Ten primer pairs in the size range of 213-299 bp have been designed. Primers 1-5 (mEgGA2OX2-1 to mEgGA2OX2-5) covered the 5′ UTR region and primers 6-10 (mEgGA2OX2-6 to mEgGA2OX2-10) covered the gene sequence. For Asparagine synthase gene, the genomic sequence (1745 bp) [5] were used to design 11 primer pairs (mEgAPG1-1 to mEgAPG1-11).

To identify potential polymorphic markers controlling height, separated pooled DNA of short and tall CPI resources and GT resources was used in PCR amplification. For the CPI resource, height of 10 short and 10 tall oil palm individuals was significantly different (p value = <0.001). The mean height of short individuals was 98.50 cm (range = 75-120 cm) while the mean height of tall individuals was 192 cm (range = 175-215 cm) in 8 year palms. For the GT resource, height of 10 short and 10 tall individuals was also significantly different (p value = <0.001). The mean height of the short individuals was 118.4 cm (range = 100-133 cm) while the mean height of the tall individuals was 268.8 cm (range = 256-296 cm) in 8 year palms. As a result, three polymorphic markers, including mEgDELLA1-1, mEgDELLA1-11 and mEgAPG1-4, have high possibility to control height. No polymorphic marker was found for *GA2OX2*. Then, the polymorphic markers were next PCR individually in the CPI and GT resources. The amplicons of mEgDELLA1-1 were located at the 5′ UTR of the gene sequence expected to be the promoter region while the amplicons of mEgDELLA1-11 and mEgAPG1-4 were located at the coding region of the genes. These three markers were next genotyped in the KU, GT and SIT population to determine their relationship or association with height.

3.4 Genotyping of mEgDELLA1-1, mEgDELLA1-11 and mEgAPG1-4, and Their Statistical Analyses

Genotyping of mEgDELLA1-1 (291 bp) (Forward primer, 5′ TTTTCGTACA TTCGGCTCTG 3′ and reverse primer, 5′ TATCAAATGGCACCGGCTAT 3′) was conducted in the GT and KU populations. Three alleles were amplified, including the top band (named as Allele A), the middle band (named as allele B), and the low band (named as allele C) (Figure 2A). Five genotypes were found in the GT population including genotypes, A/A, A/B, A/C, B/B and B/C while 4 genotypes were found in the KU population including A/A, A/B, A/C and B/B. The number of individuals in each genotype was different. Two genotypes,

A/A (61 individuals) and A/B (81 individuals) were found to make up the majority of the GT population while three genotypes, A/A (28 individuals), A/B (69 individuals) and A/C (39 individuals), make up the majority of the KU population. The sequencing of mEgDELLA1-1 amplicon products confirmed that they were parts of *EgDELLA1* with an expected of 291 bp. Next, ANOVA analysis was performed in the GT and KU populations. Only in the GT population, the mean height of individuals with genotype A/A was significantly shorter than that of individuals with genotype A/B, for HT-1, HT-2 and HT-3 at p value of 0.023, 0.013 and 0.024, respectively. The height difference between individuals containing genotype A/A and A/B was 19 cm for HT-1, 21 cm for HT-2, and 22 cm for HT-3 (Table 3). No significant height difference was found between genotypes A/C, B/B and B/C because the number of individuals were small as 5-18 palm individuals. This suggests that the promoter region (mEgDELLA1-1) which is about 630 bp upsteam from the *EgDELLA1* start codon is responsible for height difference in this GT oil palm population. In addition, no significance was found among genotypes amplified by mEgDELLA1-1 in the KU population even though the individuals with genotype A/A still displayed shorter (3-6 cm) height than individuals with genotype A/B in all 3 HT records as shown in Table 3.

Genotyping of mEgDELLA1-11 (262 bp) (Forward primer, 5′ TGTTTTCTTGC AAGCACTGG 3′ and reverse primer, 5′ CCTGACAATCAAGCTGCTCA 3′) was conducted in the GT and SIT populations. Two alleles were found including the top band (named as allele A) and the low band (named as allele B) (Figure 2B). Two genotypes were found in the GT and SIT populations, including genotype B/B and A/B. The 115 and 65 individuals of GT population contained genotypes B/B and A/B, respectively while 70 and 59 individuals of the SIT population contained genotypes B/B and A/B, respectively. After the ANOVA analysis was performed in the populations, no height difference was found between genotype B/B and A/B in the GT population but a significant height difference was found in the SIT population (p value = 0.026) only from the HT-1 record. The height difference between individuals of the SIT population containing genotype A/B with genotype B/B of mEgDELLA1-11 was 13 cm for HT-1 (Table 3).

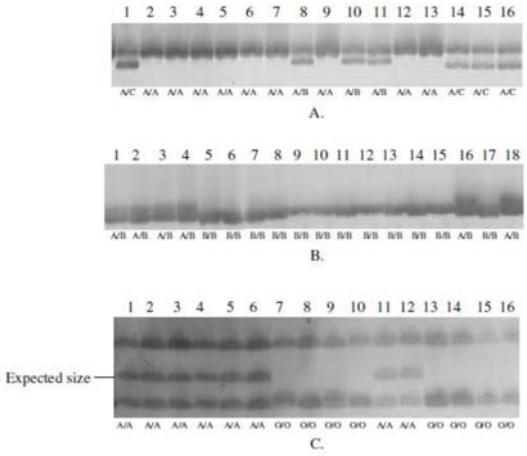

Figure 2. The pattern of PCR product on 4.5% acrylamide gels for three polymorphic markers, mEgDELLA1-1 amplified in the GT population (A.), mEgDELLA1-11 amplified in the SIT population (B.) and mEgAPG1-4 amplified in the KU population (C.).

Genotyping of mEgAPG1-4 (Forward primer, 5′ CGGCTTCGTGCTCTACGA TA 3′ and reverse primer, 5′ ACAGCGGGA TCTTTCCATC 3′) was conducted only in the KU population. The presence of one allele with the expected size of 191 bp was found from the genotyping as shown in Figure 2C. Presence and absence of amplicon

product were both detected, which named as genotype A/A and O/O, respectively. The sequencing of mEgAPG1-4 amplicon product confirmed that it was part of the asparagine synthase gene sequence with an expected size of 191 bp. ANOVA analysis was conducted in only 3 commercial lines, Surat Thani 2, Surat Thani 5 and Surat Thani 6 because they were shown to represent the majority of individuals to have this allele present. Significant height difference was found between the allele that resulted in amplicon product and the allele that resulted in no product, at p value of 0.005 for HT-1, 0.007 for HT-2 and 0.015 for HT-3. The height difference between individuals with band producing alleles and individuals with alleles that do not produce bands was 18 cm for HT-1, 20 cm for HT-2 and 18 cm for HT-3 (Table 3).

Table 3. Genotyping results of the 3 markers (mEgDELLA1-1, mEgDELLA1-11 and mEgAPG1-4, mean height details in each allele locus and statistical analysis of the mean height difference between each genotype in the KU, GT and SIT populations. The regions amplified by mEgDELLA1-1 are shown to control height in the GT population, amplified by mEgDELLA1-11 are shown to control height in the SIT population. Regions amplified by mEgAPG1-4 are shown to control height in some commercial lines (Surat Thani 5, Surat Thani 5 and Surat Thani 6) of the KU population.

Markers	Population	Allele loci	Number of individuals in each locus	Mean height (cm±SD) of each record			Statistical analysis using one-way ANOVA		
				HT-1	HT-2	HT-3	HT-1	HT-2	HT-3
mEgDELLA1-1	GT population	A/A	61	181±35	220±40	250±44	0.023*	0.013*	0.024*
		A/B	81	200±36	241±41	272±44			
		A/C	12	194±35	234±43	272±51			
		B/B	18	187±28	223±29	254±35			
		B/C	5	206±31	247±28	274±29			
		total	177	192±35	231±40	263±44			
	KU population	A/A	28	110±19	129±24	120±21	N/A	N/A	N/A
		A/B	69	116±23	132±35	124±28			
		A/C	39	113±22	139±30	126±26			
		B/B	5	129±16	151±19	140±17			
		total	141	114±22	134±31	124±26			
mEgDELLA1-11	GT-population	B/B	115	191±34	231±40	263±45	N/A	N/A	N/A
		A/B	65	194±36	233±41	263±43			
		Total	180	192±35	232±40	263±44			
	SIT population	B/B	70	165±41	181±43		0.026*	N/A	
		A/B	59	152±27	170±29				
		Total	159	159±36	175±37				
mEgAPG1-4	KU population (in Surat Thani 2, Surat Thani 5 and Surat Thani 6 lines)	A/A (present)	18	105±22	126±24	142±30	0.005*	0.007**	0.015*
		O/O (absent)	36	123±20	146±24	160±21			
		Total	54	117±22	139±25	154±26			

** Highly significant level at p value < 0.01

* Significant level at p value = 0.01-0.05

N/A = non significance, cm = centimeter, SD = standard deviation

3.5 Association of mEgDELLA1-1 with Height, Bunch Number and Fresh Fruit Bunch Yield of the GT Population

Three types of data were used for association analysis using TASSEL including genotyping data information from targeted gene-based markers, phenotype data and the Q-matrix of the optimal K, analyzed by STRUCTURE. To analyze the GT population structure (180 individuals), 40 random SSR markers [6] were used in the analysis. STRUCTURE was run 3 times at this setting: Length of Burnin Period = 50,000, number of MCMC Reps after Burnin = 50,000 and K setting = 1-10. The optimal K was determined by using STRUCTURE Harvester [9]. The optimal K calculated using the Evanno method [10] was 3, because the highest Delta K = 2658 was found for this value. The gene-based markers included mEgDELLA1-1, which was shown to associate with height using ANOVA analysis, mEgDELLA1-11, which was not associated with height in the GT population by ANOVA analysis and mEgACCO-pr2 on chromosome 10, which was reported to control FFB in oil palm [7]. In addition, mEgSSRffb10-8, the SSR marker, which was reported to link with BN and FFB QTLs on chromosome 6 was also included in the genotyping data [7]. The phenotypes of this population included HT-1, HT-2, HT-3, BN-2013, FFB-2013, BN-2014, FFB-2014, BN-2015 and FFB-2015. After performing TASSEL analysis using the GLM model and 10,000 permutations, mEgDELLA1-1 was found to associate significantly with HT-1 and HT-2 with a p value of 0.0429 and 0.0261, respectively. This marker was found to nearly associate with HT-3 with a p value of 0.0571. In addition, mEgACCO-pr2 was found to significantly associate with BN-2015 with a p value of 0.0384. Markers, mEgDELLA1-11 and mEgSSRffb10-8 did not associate significantly with any traits.

3.6 The RT-PCR Expression of EgDELLA1 in Various Tissues

The RNA expression of *EgDELLA1* was examined via RT-PCR from various tissues, including leaf, stigma, ovary, young and mature fruit, and male inflorescence and meristem tissues. The primer chosen for RT-PCR was mEgDELLA1-11 (Forward primer, 5′ TGTTTTCTTGCAAGCACTGG 3′ and reverse primer, 5′ CCTGACAATCAA GCTGCTCA 3′) with an expected size of 262 bp. *EgCyp2* was used as a reference control. Expression of *EgDELLA1* was found in almost all tissues, except for in male inflorescence (Figure 3). The highest expression of this gene was found in meristem tissues, and to a lesser extent in leaves and developing fruits. Results suggest that the gene contributes to height by controlling stem elongation.

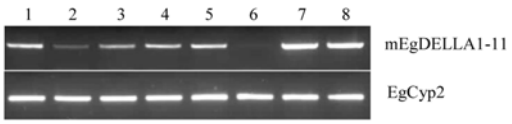

Figure 3. An RT-PCR for expression of *EgDELLA1* in various tissues was amplified by primer mEgDELLA1-11 (262 bp), at 35 PCR cycles. *EgCyp2* (163 bp) was used as a reference control. Lanes 1= leaf from clone B, 2 = 1 day opened stigma from B15/9D, 3 = ovary from B15/9D, 4 = young fruit from clone B, 5 = mature fruit from clone B, 6 = male inflorescence from clone B, 7 = meristem from CPI 1/26 D, 8 = meristem from CPI 1/34 P.

4. DISCUSSION

4.1 Correlations Between Height, Vegetative and Yield Component Traits

Height increment in oil palm depends on several factors, including genotypes, age, planting area, environment, seasoning,

and management practices. This study showed that age, planting area and seasoning affected height increment in oil palm. We found that height increment was low in young age palm and dry seasons, and was rapidly increasing during a raining season. In terms of genotypes, several oil palm breeding teams, such as ASD Costa Rica Company, Costa Rica, have successfully transferred the dwarf gene from *E. oleifera* to *E. guineensis* by several backcrosses of *E. guineensis* to the interspecific hybrid and named the resulting offspring COMPACT lines. The COMPACT lines contained several characters such as high yield, disease resistance and dwarf character and short leaf from *E. Oleifera* [11] .

A strong positive correlation was also found between height and other vegetative traits, including total plant dry weight (r = 0.71), crop growth rate (r = 0.65), leaf area (r = 0.90) and leaf number (r = 0.73) [12]. However, the height correlation with FFB was weak to moderately positive correlation in both the GT and KU populations. The positive correlation between seed yield and plant height was reported in many plant species such as wheat [13, 14], maize [15] and chick pea [16]. However, plant height also had a negative correlation with grain yield in wheat [17], mungbean [18] and basmati rice [19] .

For intraspecific palm varieties, too short or tall oil palms are not selected for the commercial lines of *E. guineensis*. The reasons may include the tree architecture of oil palms, which affect the amount of photosynthetic reserves, which are stored as glucose and starch in the tree trunk [20]. This suggests that dwarf oil palms with thick trunks would have higher yield than the dwarf palm with thinner trunk, due to more area for storing food reserves. In the same way, tall oil palms have much trunk volume for food reserves but their harvesting difficulty prohibit from becoming preferable commercial lines. Consequently, improvement of a dwarf oil palm with yield component traits is challenging for oil palm breeders.

4.2 Role of a Stem Elongation Controlled by DELLA Genes

Using the BlastX tool (http://blast.ncbi.nlm.nih.gov/), *EgDELLA1* was predicted as a putative DELLA protein RGL1-like of both *E. guineensis* and *Phoenix dactylifera* (E = 0). It also is highly matched with other DELLA protein RGL1-likes of other species such as *Citrus clementina* (E = 5e-128), *Jatropha curcas* (E = 7e-118), *Glycine max* (E = 5e-116), *Vigna angularis* (E = 9e-115), and *Solanum lycopersicum* (E = 4 e-119), DELLA protein *GAI1* of *Glycine soja* (E =1e-110), and GRAS transcription factor of *Medicago truncatula* (E = 2e-109). DELLA proteins are members of the GRAS protein family, which plays an important role in diverse processes such as signal transduction, meristem maintenance and development [21]. Examples of DELLA genes include *GAI*, *RGA*, *RGA-LIKE1* (*RGL1*), *RGL2* and *RGL3* in *Arabodopsis*, *SLR1* in rice and *SLN1* in barley. DELLA proteins take part in GA signaling by acting as GA nuclear negative regulators. They can inhibit GA-promoted processes by modulating GA and ABA pathways [22]. Gain-of-function mutations of DELLA genes reduced GA signaling, resulting in dwarfing phenotype while loss-of-functions of DELLA genes increased GA signaling, resulting in tall and slender phenotypes [23]. GA promotes growth by repressing the DELLA protein via proteosomal degradation. GA-dependent GID1-DELLA complex formation, resulting in DELLA recognition and ubiquitylation by SCF^{SLY1}, leads to DELLA proteolysis via the 26S

proteosome. Mutations that occur in any proteins in the complex formation can affect the DELLA degradation, such as a mutation in *SLY* protein (one F-box subunit of SCF that catalyze polyubiquitylation of DELLA protein) that leads to DELLA not be degraded, resulting in inhibited GA response [24]. In addition, mutations within the N-terminal DELLA domain, which is essential for GA-dependent ribosomal degradation, or mutations out of the domain lead to GA insensitivity [25].

Stem growth of palms is involved the formation of wide stem base without internodal elongation like in cereal species such as rice and wheat [26]. Palms have one terminal growing point at the apex of the stem, containing apical meristem where the primary stem growth begins. The highest expression of *EgDELLA1* was found in apical meristem tissues, and to a lesser extent, leaves and fruit development. No expression was found in male inflorescence, suggesting that the gene is highly expressed in the active tissues, meristem, which is important for stem elongation and leaf initiation in oil palm. This expression corresponds to the expression of dwarfing genes in wheat, *Rht A1*, *Rht B1* and *Rht D1* [27] and the expression of *OsGAI* in rice [28] where they are highly expressed in stem internodes, which are important for stem elongation, compared to expression in other tissues such as peduncle and peduncle node in wheat or root and leaf blade in rice. In addition, DELLA genes were also involved in modulating flower development and seed germination.

The polymorphic promoter region of *EgDELLA1* was found to be associated with variation in stem height in the GT population. This suggests that this region may affect the expression of the *EgDELLA1* gene. High expression of *EgDELLA1* is linked to reduced height while low expression of *EgDELLA1* results in an increase in GA response, which leads to tall height. Some evidence has shown that high expression of DELLA genes reduced height. For example, over expression of Rose DELLA gene, *RøDELLA* in transgenic polargonium led to reduced growth, associated with an increase in node and branch number and also a delay in flower and root formation [29]. Moreover, overexpression of rice *SLR1-like 1* (*SLRL1*) in normal rice plants induced a dwarf phenotype [30].

In conclusion, we identified *EgDELLA1* as controlling some height variation in some oil palm genotypes. We propose that the DELLA genes are GA-negative regulators which play a crucial role in controlling height in some oil palm genotypes but GA-biosynthesis enzymes may play more crucial roles in other oil palm genotypes as well. It is possible that several genes in both GA signaling, GA biosynthesis and other pathways such as reserve assimilation, may contribute to height variation in some genotypes. Consequently, improvement of dwarfing oil palm with higher yield is still challenging because both traits are complex with several genes and environmental factors controlling them.

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