



UNIVERSITI PUTRA MALAYSIA

**MOLECULAR CHARACTERIZATION AND FUNCTIONAL ANALYSIS
OF SELECTED EXPRESSED SEQUENCE TAGS FROM OIL PALM
CELL SUSPENSION CULTURE**

**LE VINH THUC
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SUSPENSION CULTURE**

By

LE VINH THUC

**Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia,
in fulfilment of the Requirements for the Degree of Doctor of Philosophy**

November 2009



This Thesis is Special Dedicated to

My Parents, Mr. Le Van Khoanh and Mrs. Tran Thi Kim Dang

And also to my beloved wife, Ngo Thi Thanh Truc

Their Sacrifice and Infinite Love Led Me to Present Achievements



Abstract of thesis presented to the senate of Universiti Putra Malaysia in fulfilment of the requirements for the degree of Doctor of Philosophy

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Faculty: Biotechnology and Biomolecular Sciences

A large quantity of Expressed sequence tags (ESTs) are available from various cDNA libraries of oil palm. The information from oil palm EST databases has been utilized to identify several interesting transcripts that are upregulated in cell suspension culture for molecular characterization and functional analysis. The first part of this study was to carry out the molecular characterization of selected ESTs of oil palm cell suspension culture which were Eg583 (Accession number: EU795363), Eg707 (Accession number: FJ196136) and EgHAD (Accession number: FJ196137).

The Eg583 sequence is highly similar to an unknown protein from rice. This predicted protein might be a transcription factor due to the presence of SIN3 domain and motifs of casein kinase II phosphorylation. The expression of this gene was not detectable in all tested tissues. This gene might be a member of a multigene family in the oil palm genome. The Eg707 sequence is highly similar to an unknown protein from *Arabidopsis* and might be a putative nuclear protein. Its amino acid sequence contains a Ald-Xan-dh-C2 domain that may be involved in ABA biosynthesis. *Eg707*



might be present as a single copy gene in the oil palm genome and its transcripts were highly expressed in tissue cultured materials compared to vegetative tissues. *Eg707* might have a role during oil palm somatic embryogenesis or at very early stage of embryo development. The *EgHAD* sequence is similar to a putative haloacid dehalogenase (HAD) superfamily hydrolase from monocots and phosphate hydrolase from dicots. However, the phylogenetic relationship of *EgHAD* is closer to monocots than dicots. *EgHAD* might be a member of a multigene family gene in the oil palm genome. It was highly expressed in leaves and meristem but lower expression was found in roots, female flowers, non-embryogenic and embryogenic calli in comparison to the oil palm cell suspension culture.

Functional analysis was carried out in rice by over-expressing *Eg707* and *EgHAD*, driven by a constitutive double Cauliflower Mosaic Virus 35S promoter. The constructs were made using the gateway technology with clonase (Invitrogen, USA). Transgenic plants over-expressing *Eg707* protein had small sized, rolled and erect leaves, less tillers, empty seeds and higher total chlorophyll content. The phenotypes of these and the presence of Xan-dh-C2 domain in *Eg707* protein, strongly suggest its involvement in ABA biosynthesis, particularly during somatic embryogenesis. Functional analysis of *Eg707* through RNAi-mediated gene silencing was unsuccessful since the T₁ seeds failed to germinate. Over-expression of *EgHAD* gene in rice produced more lateral roots and tillers than the wild type plants. However, it also reduced plant size, produced empty seeds and many tiny seeds which were not found in wild type plants. The suppression of the *EgHAD* orthologues in rice did not show any changes in the phenotype. *EgHAD* might be a metabolic protein involved in phosphate starvation mechanism and its expression might be necessary for seed germination.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

**PENCIRIAN MOLEKULAR DAN ANALISIS BERFUNGSI TAG JUJUKAN
TEREKSPRES TERPILIH DARIPADA KULTUR AMPAIAN SEL
KELAPA SAWIT**

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Sebilangan besar tag jujukan terekspres (ESTs) boleh didapati daripada pelbagai perpustakaan cDNA bagi kelapa sawit. Maklumat daripada pangkalan data EST kelapa sawit telah digunakan untuk mengenalpasti beberapa transkrip menarik yang telah ditingkat-kawalaturkan di dalam kultur ampaiian sel untuk pencirian molekul dan analisis fungsian. Bahagian pertama kajian ini adalah untuk menjalankan pencirian molekul pada EST yang terpilih daripada ampaiian kultur kelapa sawit iaitu Eg583 (Nombor kemasukan: EU795363), Eg707 (Nombor kemasukan: FJ196136) dan EgHAD (Nombor kemasukan: FJ196137).

Jujukan untuk klon Eg583 adalah amat seiras dengan protein yang belum dikenalpasti daripada padi. Protein yang diramalkan ini berkemungkinan merupakan faktor transkripsi disebabkan kewujudan domain SIN3 dan motif untuk pemfosforilan kasein kinase II. Ekspresi ungkapan gen ini tidak dapat dikesan dalam semua tisu yang diuji. Gen ini berkemungkinan merupakan ahli kepada keluarga gen berbilang di dalam genom kelapa sawit. Jujukan *Eg707* adalah sangat seiras dengan

protein yang belum dikenal pasti daripada *Arabidopsis* dan ia berkemungkinan merupakan putatif protein pada nukleus. Jujukan asid amino ini mengandungi domain Ald-Xan-dh-C2 yang mungkin terlibat dalam biosintesis ABA. *Eg707* berkemungkinan hadir sebagai salinan tunggal di dalam genom kelapa sawit dan transkripnya diekspres dengan tinggi dalam tisu yang dikulturkan berbanding dengan tisu vegetatif. *Eg707* berkemungkinan memainkan peranan semasa somatik embriogenesis atau pada tahap awal perkembangan embrio. Jujukan klon *EgHAD* adalah seiras dengan putatif haloasid dehalogenase (HAD) superfamili hidrolase daripada monokot atau hidrolase fosfat daripada dikot. Walau bagaimanapun, hubungan filogenetik *EgHAD* adalah lebih dekat dengan tumbuhan monokot daripada dikot. *EgHAD* berkemungkinan merupakan ahli keluarga gen berbilang di dalam genom kelapa sawit. Ia diekspres dengan tinggi dalam daun dan meristem tetapi pengekspresannya adalah rendah di dalam akar, bunga betina, kalus embriogenik dan tidak embriogenik berbanding dengan kultur ampai sel kelapa sawit.

Analisis berfungsi telah dijalankan di dalam padi melalui pengekspresan terlampau *Eg707* dan *EgHAD*, dibawa oleh gandaan konstitutif pendorong Cauliflower Mosaic Virus 35S. Konstruk telah dibuat melalui teknologi gateway dengan klonase (Invitrogen, USA). Tumbuhan transgenik yang mengekspreskan protein *Eg707* secara terkebih mempunyai daun yang kecil, bergulung dan tegang, kurang tangkai dan berbiji benih kosong dan mengandungi kandungan klorofil keseluruhan yang tinggi. Fenotip ini dan kehadiran domain Xan-dh-C2 di protein *Eg707* ini mencadangkan dengan kukuh penglibatannya dalam biosintesis ABA, terutamanya semasa somatik embriogenesis. Analisis berfungsi *Eg707* melalui kaedah gen

penyenyap berperantara-RNAi tidak berjaya kerana biji benih generasi T₁ gagal bercambah. Pengekspresan terlampau gen *EgHAD* dalam padi menghasilkan banyak akar lateral dan lebih tiler daripada padi jenis liar. Walau bagaimanapun, ia juga mengecilkan saiz tumbuhan, menghasilkan biji kosong dan banyak biji kecil yang tidak terdapat dalam pokok padi jenis liar. Penindasan ortolog *EgHAD* dalam padi tidak menunjukkan sebarang perubahan fenotip. *EgHAD* berkemungkinan merupakan protein metabolik yang terlibat dalam mekanisme kebuluran fosfat dan ekspresinya ini adalah penting untuk percambahan biji benih.

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DECLARATION

I hereby declare that the thesis is based on my original work except for equations and citations, which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at UPM or other institutions.

LE VINH THUC

Date: 2 December 2009

TABLE OF CONTENT

	Page
ABSTRACT	iii
ABSTRAK	v
ACKNOWLEDGEMENTS	viii
APPROVAL	x
DECLARATION	xii
LIST OF TABLES	xvi
LIST OF FIGURES	xviii
LIST OF ABBREVIATIONS	xxii
CHAPTER	
1 INTRODUCTION	1
2 LITERATURE REVIEW	5
2.1 Oil palm	5
2.2 Bioinformatics in molecular biological study	9
2.3 Rice as a monocotyledous model plant for gene functional study	12
2.4 Transformation of transgenes into monocots using <i>Agrobacterium tumefaciens</i>	13
2.5 Vector construction for gene transformation using Gateway technology	15
2.6 Gene over-expression as a tool to study gene function	18
2.7 Gene knockdown/knockout as a tool to study gene function	19
2.8 Real time PCR in molecular biology	23
2.8.1 Calculation of copy of transgenes in transgenic plants by using real-time PCR	25
2.8.2 Expression of transgenes in transgenic plants	26
3 CHARACTERIZATION OF SELECTED GENES FROM EST COLLECTION OF OIL PALM CELL SUSPENSION CULTURE	29
3.1 Introduction	29
3.2 Materials and methods	31
3.2.1 Materials	31
3.2.2 Sequence analysis	31
3.2.3 Extraction of genomic DNA	32
3.2.4 Isolation of total RNA	33
3.2.5 Southern blot hybridisation	36
3.2.6 Reverse Transcription PCR (RT-PCR)	41
3.2.7 Real time RT-PCR	43
3.3 Results and discussion	47
3.3.1 Total RNA isolation	47
3.3.2 Genomic DNA isolation	47



3.3.3	Clone Eg583 (Accession number: EU795363)	48
3.3.4	Clone Eg707 (Accession number: FJ196136)	58
3.3.5	Clone EgHAD (Accession number: FJ196137)	71
4	FUNCTIONAL ANALYSIS OF A GENE ENCODING AN UNKNOWN PROTEIN Eg707	82
4.1	Introduction	82
4.2	Materials and Methods	85
4.2.1	Materials	85
4.2.2	Vector construction for over-expression of Eg707	85
4.2.3	Construction of pMDC32 without <i>ccdB</i> gene	93
4.2.4	Vector construction for suppression studies for Eg707	97
4.2.5	Transformation of pMDC-Eg707, pMDC(-32) and pOpOff-Eg707 plasmids into <i>Agrobacterium tumefaciens</i> strain LBA4404 by electroporation	101
4.2.6	<i>Agrobacterium</i> -mediated transformation into rice	103
4.2.7	Germination of T1 seeds	107
4.2.8	Verification of transgenic plants by PCR	107
4.2.9	Phenotypic analyses of transgenic rice	109
4.2.10	Estimating the copy number of the T-DNA in transgenic rice by real-time quantitative PCR	110
4.2.11	Gene expression analysis of Eg707 by real-time PCR	112
4.2.12	Histological analysis	116
4.2.13	Data analysis	116
4.3	Results	117
4.3.1	Over-expression of Eg707 in rice	117
4.3.2	RNAi-mediated gene silencing study for Eg707	144
4.4	Discussion	149
4.5	Summary	157
5	FUNCTIONAL ANALYSIS OF EgHAD, A HALOACID DEHALOGENASE SUPERFAMILY HYDROLASE IN OIL PALM	159
5.1	Introduction	159
5.2	Materials and Methods	162
5.2.1	Materials	162
5.2.2	Vector construction for over-expression of EgHAD	162
5.2.3	Vector construction for suppression of EgHAD orthologues in rice	166
5.2.4	Transformation of pMDC-EgHAD, pMDC(-32) and pANDA-EgHAD plasmids into <i>A. tumefaciens</i> strain LBA4404 by electroporation	170
5.2.5	<i>Agrobacterium</i> -mediated transformation in rice	171
5.2.6	T1 seeds germination	171
5.2.7	Verification of transgenic plants by PCR	171
5.2.8	Phenotypic analyses of transgenic rice	172
5.2.9	Estimating the copy number of the T-DNA in transgenic rice by real-time quantitative PCR	172

5.2.10	Gene expression analysis of EgHAD by real-time PCR	172
5.2.11	Histological analysis	173
5.2.12	Data analysis	173
5.3	Results	174
5.3.1	Over-expression of EgHAD in rice	174
5.3.2	Suppression study for EgHAD	194
5.4	Discussion	207
5.5	Summary	213
6	CONCLUSIONS AND RECOMMENDATIONS FOR FUTURE RESEARCH	214
6.1	Conclusions	214
6.2	Future research	217
	REFERENCES	218
	APPENDICES	249
	BIODATA OF STUDENT	273
	LIST OF PUBLICATIONS	274

LIST OF TABLES

Table	Page
3.1 The primer sequences of Eg583, Eg707, EgHAD and GAPDH gene	42
3.2 Oligonucleotide primers and probe sequences for real time RT-PCR	44
3.3 C _T values of GAPDH and target Eg583 gene on different tested tissues of oil palm	58
4.1 The <i>attB</i> -specific primer sequences and expected size of the <i>attB</i> -specific PCR product	88
4.2 The forward and reverse primer sequences of CaMV 35S promoter and NOS terminator	98
4.3 The forward and reverse primer sequences of PDK intron	100
4.4 Primers and probe sequences for real-time quantitative PCR of SPS and HPT gene	110
4.5 Primer sequences of Eg707 and eFF-11 α for real-time PCR	114
4.6 The estimated copy numbers of Eg707 gene in pMDC-Eg707 plants	132
4.7 The estimated copy number of HPT gene in pMDC(-32) plants	133
4.8 The different physiological parameters of the leaves in wild type, pMDC(-32) and pMDC-Eg707 plants	141
4.9 Effect of over-expression of Eg707 on chlorophyll a, b, ratio chlorophyll a/b and total chlorophyll in the fourth leaf of plants	142
4.10 Phenotypic measurements of the panicle and seeds from wild type, pMDC(-32) and pMDC-Eg707 plants	144
4.11 The estimated copy numbers of HPT gene in T ₀ generation of pOpOff-Eg707 plants	147
4.12 Number of T1 seeds germinated from the pOpOff-Eg707 plants	148
5.1 The <i>attB</i> specific primer sequences and expected size of the <i>attB</i> -specific PCR product	164
5.2 The primer sequences and expected size of the EgHAD amplicon	166
5.3 The forward and reverse primer sequences and the expected sizes of the amplicons of <i>attB</i> 1-PCR EgHAD- <i>attB</i> 2 product and GUS gene	170
5.4 Oligonucleotide primers for real time RT-PCR of EgHAD	173
5.5 The estimated copy number of EgHAD gene in T ₀ plants of pMDC-EgHAD	181



5.6	Effect of over-expression of EgHAD on chlorophyll content in the flag leaf of pMDC-EgHAD plants	188
5.7	Length of panicle, number of seeds and number of full seeds in panicle from wild type, pMDC(-32) and pMDC-EgHAD plants	192
5.8	The estimated copy number of HPT gene in pANDA-EgHAD plants	198
5.9	Length, width and ratio between length and width of flag leaf blade of wild type and pANDA-EgHAD plants	204
5.10	Length of panicle, number of seeds, number of full seeds and empty seeds in the panicle of wild type and pANDA-EgHAD plants	205
5.11	Number of fully set seeds and number of germinated seeds from pANDA-EgHAD plants	206

LIST OF FIGURES

Figure		Page
2.1	BP and LR reactions of Gateway cloning technology (Invitrogen, CA, USA)	17
2.2	Processing of dsRNAs, cleavage of target RNAs and transitive RNA silencing	22
3.1	Formaldehyde agarose gel electrophoresis of RNA extracted from oil palm	47
3.2	DNA extracted from oil palm leaves on 1% (w/v) electrophoresed agarose gel	48
3.3	The nucleotide and deduced amino acid sequences of clone Eg583 (Acc. No. EU795363)	50
3.4	Amino acid sequence alignment of clone Eg583 (EU795363) with an unknown protein (BAD25663), a cold induced protein-like (BAB55503) from <i>O. sativa</i> and an unknown cold induced protein (AAM11916) from <i>D. antarctica</i>	51
3.5	Location of SIN3 domain (from amino acids 43 to 70) that was recognized based on deduced amino acid sequence of clone Eg583 using NCBI-CDD program	52
3.6	A neighbour-joining tree displaying the phylogenetic relationship of predicted protein of clone Eg583 (marked) to hypothetical protein OsI006314 (EAY85081), cold induced protein-like (BAB55503) from <i>O. sativa</i> and an unknown cold induced protein (AAM11916) from <i>D. antarctica</i>	53
3.7	Southern blot analysis with 3'UTR region of clone Eg583 as probe on oil palm genomic DNA digested with <i>TaqI</i> (1), <i>NotI</i> (2), <i>HindIII</i> (3) and <i>EcoRI</i> (4) (a), PCR analysis of 3'UTR of clone Eg583 (b) using cDNA (lane P) and oil palm genomic DNA (lane O) as templates	55
3.8	Analysis of RT-PCR products of clone Eg583 on a 2% (w/v) agarose gel	56
3.9	The nucleotide and deduced amino acid sequences of clone Eg707	60
3.10	Amino acid sequence alignment of clone Eg707 with an unknown protein from <i>A. thaliana</i> (NP-001031128), TPP1 protein from <i>A. thaliana</i> (NP-974612), an expressed protein (ABB47038) from <i>O. sativa</i> and a hypothetical protein MtrDRAFT-AC151964g13v2 (ABN07969) from <i>M. truncatula</i>	61
3.11	Predicted nuclear export signal (a) and aldehyde oxidase, xanthine dehydrogenase and molybdopterin binding domain (from amino acids 29 to 89) (b) in the deduced amino acid sequence of clone Eg707 using NES and NCBI-CDD programs, respectively	63



3.12	Schematic diagram of abscisic acid (ABA) biosynthetic pathways	64
3.13	A neighbour-joining tree displaying the phylogenetic relationship of predicted protein of clone Eg707 (marked) to hypothetical protein <i>TPDI</i> (NP-974612) from <i>A. thaliana</i> , expressed protein (ABB47038) from <i>O. sativa</i> and hypothetical protein MtrDRAFT-AC152185g23v2 (ABN07969) from <i>M. truncatula</i>	65
3.14	Southern blot of oil palm genomic DNA hybridised with full length cDNA of clone Eg707 washed under high (a) and low (b) stringency conditions	66
3.15	Analysis of RT-PCR products of clone Eg707 on a 2% (w/v) agarose gel	67
3.16	Relative expression levels of clone Eg707 in various tissues	68
3.17	The nucleotide and deduced amino acid sequence of clone EgHAD	72
3.18	Amino acid sequence alignment of clone EgHAD with putative HAD-superfamily hydrolase from <i>O. sativa</i> , haloacid dehalogenase-like hydrolase domain-containing protein 1A from <i>Z. mays</i> , GS1 - hydrolase from <i>A. thaliana</i> and GPP1 (glycerol-3-phosphatase 1) - hydrolase from <i>A. thaliana</i>	74
3.19	The haloacid dehalogenase-like (HAD) superfamily domain was recognized based on deduced amino acid sequence of clone EgHAD by NCBI-CDD program	75
3.20	A neighbour-joining tree displaying the phylogenetic relationship of predicted protein of clone EgHAD (marked) to hydrolase protein form other plants	77
3.21	Southern analysis for EgHAD clone in oil palm	79
3.22	Relative expression levels of clone EgHAD in different tissues	81
4.1	Schematic diagram showing the vector construction of pMDC-Eg707 expression clone	86
4.2	Schematic diagram of vector construction for pMDC(-32) plasmid	94
4.3	Schematic diagram of pOpOff-Eg707 suppression vector construction	99
4.4	Schematic diagram showing vector construction of pMDC-Eg707	119
4.5	Verification of pMDC-Eg707 binary plasmid after transformation of this construct into <i>A. tumefaciens</i> LBA4404 by using PCR analysis with <i>attB</i> specific primers (a), and by using restriction analysis with <i>HindIII</i> and <i>AfIII</i> restriction enzymes (b)	120
4.6	Verification of pMDC(-32) vector after transformation into <i>A. tumefaciens</i> LBA4404 by using PCR analysis with CaMV 35S promoter primers (a) and by using <i>XbaI</i> restriction enzymes (b)	121
4.7	Putative transformants of pMDC-Eg707 in rice after <i>Agrobacterium</i> -mediated transformation	123

4.8	Calli on regeneration medium after 25 days: wild type (a), pMDC(-32) (b), pMDC-Eg707 (c), percentage of induced green and brownish calli (d)	125
4.9	DNA extracted from 16 rice leaf samples on 1% (w/v) electrophoresed agarose gel	126
4.10	Putative transgenic plants with Eg707 were verified by PCR using NOS reverse primer and <i>attB</i> -Eg707 forward primer (a), CaMV 35S promoter primers (b) and NOS terminator primers (c)	128
4.11	PCR products from rice plants transformed with pMDC(-32) using CaMV 35S promoter primers (a) and NOS terminator primers (b)	129
4.12	Formaldehyde agarose gel electrophoresis of RNA extracted from rice leaves	134
4.13	The expression of Eg707 and copy number of transgene T ₀ transformed plants	136
4.14	Rice plant at flowering stage: wild type (a), transformed rice with CaMV 35S promoter (b), transformed plants with Eg707 gene (c), height and number of tillers at flowering stage (d), the correlation between the copy numbers of Eg707 gene and plant height of pMDC-Eg707 plants (e)	138
4.15	Histological analysis of wild type plants (a, b), pMDC(-32) plants (c, d) and pMDC-Eg707 plants (e, f); number of cells between two vascular bundles, length and width of parenchyma cell at the fourth layer from inside (g)	140
4.16	The phenotypes of seeds on the panicle of wild type, pMDC(-32) and pMDC-Eg707 plants after 20 days of flowering and mature seeds	143
4.17	Verification of pOpOff-Eg707 vector after transformation into <i>A. tumefaciens</i> LBA4404 by using PCR analysis with PDK intron forward and reverse primers (a) and by using with <i>attB</i> primers (b)	145
4.18	Putative transgenic plants with pOpOff-Eg707 T-DNA insertion were verified by PCR with CaMV 35S promoter primer pairs	146
5.1	Schematic diagram of the vector construction of pMDC-EgHAD expression clone	163
5.2	Schematic diagram showing vector construction of pANDA-EgHAD suppression clone	167
5.3	Schematic diagram of vector construction for pMDC-EgHAD	175
5.4	Verification of pMDC-Eg HAD binary plasmid by using PCR analysis of with <i>attB</i> specific primers (a) and restriction analysis with <i>Pst</i> I restriction enzymes (b) after transformation of this construct into <i>A. tumefaciens</i> LBA4404	176
5.5	Putative transformants of pMDC-EgHAD T-DNA in rice after <i>Agrobacterium</i> mediated transformation	178

5.6	PCR verification of putative pMDC-HAD plants by using NOS reverse primer and EgHAD forward primer (a,b), by using CaMV 35S primers (c) and by using NOS terminator primers (d)	180
5.7	The expression and copy number of EgHAD in transgenic plants	184
5.8	Effect of over-expression of EgHAD on height and number of tillers of pMDC-EgHAD plants at flowering stage	185
5.9	Histological analysis of wild type plants (a, b), pMDC(-32) plants (c, d) and pMDC-EgHAD plants (e, f); number of cells between two vascular bundles, length and width of parenchyma cell at the fourth layer from inside (g)	187
5.10	Flag leaf shape of pMDC(-32), wild type and pMDC-EgHAD plants (a), flag leaf area and number of leaves of pMDC-EgHAD, pMDC(-32) and wild type plants (b)	189
5.11	The flowers of wild type and pMDC-EgHAD plants	191
5.12	Tiny seeds on the panicle of pMDC-EgHAD plant	192
5.13	The shape of mature seeds from wild type (a), negative control (b) and pMDC-EgHAD (c) plants; the length, width and ratio between length and width of seed (d)	193
5.14	PCR product of <i>attB1</i> -PCR EgHAD- <i>attB2</i> from <i>Agrobacterium</i> harboring pANDA-EgHAD (a) and PCR product with GUS primers (b)	195
5.15	Resistance calli on selection medium (a), shoot induction (b), root induction (c), verified putative pANDA-EgHAD plants with GUS forward and reverse primer (d)	197
5.16	The copy number of transgene in the T ₀ pANDA-EgHAD plants and fold difference suppression of OsHAD gene relative to the wild type plant (a), the correlation of the expression level of OsHAD gene and the transgenes copy number in pANDA-EgHAD plant (b)	201
5.17	Effect of OsHAD suppression on plant height and number of tiller at flowering stage of pANDA-EgHAD plants	202
5.18	Histological analysis of wild type plants (a,b) and pANDA-EgHAD plants (c,d); number of cells between two vascular bundles, length and width of parenchyma cell at the fourth layer from inside (e)	203

LIST OF ABBREVIATIONS

2,4-D	-	2,4-dichlorophenoxyacetic acid
%	-	Percentage
α	-	Alpha
<i>A. tumefaciens</i>	-	<i>Agrobacterium tumefaciens</i>
aa	-	Amino acid
ANOVA	-	Analysis of variance
AS	-	Acetosyringone
BLAST	-	Basic Local Alignment Search Tool
bp	-	Base pair
BSA	-	Bovine serum albumin
CDD	-	Conserved domain database
cDNA	-	Complementary DNA
CI	-	Chloroform: isoamyl alcohol
cm	-	Centimeter
C_T	-	Threshold cycle
CTAB	-	Hexadecyl (or cetyl) trimethyl ammonium bromide
mm	-	Milimeter
DEPC	-	Diethylpyrocarbonate
DNA	-	Deoxyribonucleic acid
DNase I	-	Deoxyribonuclease I
dNTPs	-	Deoxynucleotides
EDTA	-	Ethylene diamine tetracetate
EST	-	Expressed Sequence Tag
EtBr	-	Ethidium bromide
g/L	-	Gram per liter
<i>g</i>	-	Gravitational acceleration
GAPDH	-	Glyceraldehyde-3-phosphate dehydrogenase
GUS	-	β -Glucuronidase
HCl	-	Hydrochloric acid
HPT	-	Hygromycin phosphotransferase gene
Kinetin	-	6-Furfurylaminopurine
C-terminal	-	Carboxyl terminal
L	-	Liter
LB	-	Lauria-Bertani
M	-	Molar
mg	-	Milligram (s)
MgCl ₂	-	Magnesium chloride



MgSO ₄	-	Magnesium sulphate
min	-	Minute (s)
mL	-	Milliliter
mM	-	Millimolar
MOPS	-	3-(N-morpholino) propanesulfonic acid
MS	-	Murashige and Skoog
NaCl	-	Sodium chloride
kb	-	Kilobase
NAA	-	μ-Naphtalene acetic acid
NaOAc	-	Sodium acetate
NaOH	-	Sodium hydroxide
BSA	-	Bovine serum albumin
NCBI	-	National Center for Biotechnology Information
NH ₄ OAc	-	Ammonium acetate
Nos	-	Nopaline synthase gene terminator
°C	-	Degree centigrade
OD	-	Optical density
ABA	-	Abscisic acid
ORFs	-	Open reading frames
PCI	-	Phenol: chloroform: isoamyl alcohol
x	-	Times
PCR	-	Polymerase Chain Reaction
Pfu	-	Plaque forming unit
pI	-	Isoelectric point
Poly (A)	-	Polyadenylated (mRNA)
rpm	-	Revolution per minute
PVP	-	Polyvinylpyrrolidone
N-terminal	-	Amino-terminal
qPCR	-	Quantitative PCR
R ²	-	Correlation coefficient
RNA	-	Ribonucleic acid
RNase	-	Ribonuclease
TBE	-	Tris-borate EDTA
RT-PCR	-	Reverse transcription – PCR
Jacq.	-	Jacquin
SA-AP	-	Streptavidine alkaline-AP
SDS	-	Sodium dodecyl sulfate
sec	-	Second
TAE	-	Tris-acetate-EDTA



TBE	-	Tris-borate-EDTA
T-DNA	-	Transferred-DNA
TE	-	Tris-EDTA
T _m	-	melting temperature
Tris	-	Tris [hydroxymethyl] aminomethane
Tris-HCl	-	Tris-hydrochloride
U	-	Unit
UTR	-	Untranslated region
<i>Ubq</i>		Ubiquitin
UV	-	Ultraviolet
V	-	Volt
v/v	-	Volume per volume
w/v	-	Weight per volume
μl	-	Microliter
μM	-	Micromolar
CaMV 35S	-	Cauliflower Mosaic Virus 35S