International Journal of Agricultural Technology 2019 Vol. 15(6):947-958
Available online http://www.ijat-aatsea.com
ISSN 2630-0192 (Online)

Lignin biosynthesis genes (*OsPAL* and *Os4CL3*) sequencing of native upland rice varieties from Pala U Village, Thailand

Laosutthipong, C.[*], Seritrakul, P. and Na Chiangmai, P.

Faculty of Animal Sciences and Agricultural Technology, Silpakorn University, Phetchaburi IT Campus, Cha-am, Phetchaburi, 76120, Thailand.

Laosutthipong, C., Seritrakul, P. and Na Chiangmai, P. **(**2019**).** Lignin Biosynthesis Genes **(***OsPAL* and *Os4CL3***)** Sequencing of Native Upland Rice Varieties from Pala U Village, Thailand. International Journal of Agricultural Technology 15(6):947-958.

Abstract The results indicated that all seven *OsPAL* gene sequences showed higher similarity to *Oryza sativa* Japonica (99.4-100%) than *Oryza sativa* Indica (98.7-99.4%). For *Os4CL3* gene, all seven sequences revealed 100% sequence identity to both *Oryza sativa* Japonica and *Oryza sativa* Indica. These lignin biosynthesis genes information from native upland rice varieties in Thailand were established as the basic information for further genetic conservation and breeding improvement.

Keywords: upland rice, *OsPAL*, *4CL3*

Introduction

Rice (*Oryza sativa*) is the main food crop of the world and the most cereal consumed and produced by human. Historical rice farming has been reported that the first rice cultivation originated in Asia and later widely spread to other parts of Europe, America and Africa (David *et al.*, 2010). Rice production systems can be divided into lowland and upland rice. Upland rice is normally grown on slop level and unbounded land. Most of upland farmers cultivated local rice, which consists of various varieties and still need productivity improvement. Enhancing rice yield in upland rice has progressed in many countries such as Asia, Africa and Latin America (Saito *et al.*, 2018).

In Thailand, the landrace upland rice is cultivated not only in the north (Karladee *et al.*, 2012) but also in the south, including Prachuap Khiri Khan Province by ethnic minority farmers in the highland such as Pala U village, Hua Hin district. In this area, the Paganyaw (a sub-group Karen people) farmers grown upland rice using their local wisdom, primarily for consumption in family, ritual and ceremonies (Vechpong *et al.*, 2015). The productivity of upland rice in northern Thailand was estimated to be about 0.9 tons per hectare (t ha^{-1}), which is lower than the average of the rough rice yield (2.80 t ha^{-1}) (Michael, 2017). The reasons for this low yield is due to various factors including fluctuated environment, low-water support, and

[*] **Corresponding Author:** Laosutthipong, C.; **Email:** laosutthipong_c@silpakorn.edu

genetic character in each rice varieties although most of varieties are thought to be tolerant species.

Lodging is one of a serious problem in upland rice before harvesting, since many varieties have the tall plant stature in mature state (Gupta and O'Toole, 1986). To detect lodging in the field, monitoring of rice lodging and estimation of the lodging rate has been developed using thermal infrared remote sensing technology (Liu *et al.*, 2018). Recently, rice genetic improvement research works focused on the increasing rice lodging resistance varieties to facilitates high gain yield (Ookawa *et al.*, 2010; Plaza-Wüthrich *et al.*, 2016). Lodging resistant traits were developed to prevent plant stem bending or breaking, which can affect rice growth and grain yield (Okuno *et al.*, 2014). Lodging resistance is related to plant height, biomass, stem diameter and stem cell wall properties (Islam *et al.*, 2007; Tanaka *et al.*, 2003). Several studies have revealed the impact of cell wall components on culm strength such as lignin and cellulose accumulation, which were lower than that in lodging-resistant varieties (Zhang *et al.*, 2016). Light factor affected on lodging-related gene expression by which lignin and cellulose reduction in culms during shading and inducing defective cell wall development and higher risk to lodging (Wu *et al.*, 2017).

Lignin is one of the major components of plant secondary cell wall, which is produced as secondary metabolite into cross-linked phenolic polymers. Lignin biosynthesis consists of three main processes: (i) lignin monomers biosynthesis in cytosol (G, H, and S subunit), (ii) transportation to the apoplast, and (iii) polymerization in secondary cell wall (Liu *et al.*, 2018). Overall steps in lignin biosynthesis require several enzymatic genes expression (Yoon *et al.*, 2015). The first step is the phenylpropanoid pathway, which utilize Phenylalanine ammonia-lyase (PAL) enzyme to start changing phenylalanine to cinnamic acid. *PAL* gene is reported in 4 types in *Arabidopsis* which differently expressed in each tissues (Raes *et al.*, 2003) and mutant plant of all 4 genes showed growth stunt and less lignin accumulation (Huang *et al.*, 2010). The third enzyme in the general phenylpropanoid pathway is 4-Coumarate-coenzyme A ligase (4CL), which produce the monolignol precursor *p*-coumaroyl-CoA. The *4CL* gene suppression in *Pinus radiata* (coniferous gymnosperm) revealed a dwarf phenotype (Wagner *et al.*, 2009). Moreover, there are five *4CL* genes in rice (*Oryza sativa*) and all genes are expressed in stem tissue. The highest expression of *Os4CL3* was found in the thickening vascular cell and around parenchyma cell (Gui *et al.*, 2011). Suppression of *Os4CL3* expression affected on lignin content, plant growth reduction, and other morphologies (Gui *et al.*, 2011).

The relationship between lignin and plant lodging has been studied in buckwheat (*Fagopyrum esculentum*) that revealed the important roles of lignin biosynthesis enzymes (PAL, 4CL, CAD, and PAD) related to lodging resistance (Hu *et al.*, 2017). Several studies have shown that lignin

accumulation in plant cell wall enhances the mechanical strength of plant stem and reduces risk of lodging in rice (Islam *et al.*, 2007; Tanaka *et al.*, 2003). However, the lignin biosynthesis genes in the native upland rice, which has high risk to lodging problem, are still unclear, especially in Thailand. This study aimed to characterize the two important lignin biosynthesis genes (*PAL* and *4CL3*) in seven native upland rice varieties which grown in Pala U village, Prachuap Khiri Khan Province, Thailand.

Materials and Methods

Plant material

Seven upland rice (*Oryza sativa* L.) varieties were used in this study (var. *Aung Jerng Yai* (R1), *Nah San* (R2), *Bue Soo Sue La* (R3), *Kao Niew Pala U* (R4), *Bue Ke* (R5), *Bee Kor Bi* (R7), and *Rao Soo Ya* (R8). All seed varieties were collected from the ethnic minority farmers at Pala U village, Hua Hin district, Prachuap Khiri Khan province, Thailand. The rice seeds were overnight soaked in sterile water and grown in nursery boxes for 14 days. Whole seedlings were collected for RNA extraction.

RNA extraction

Total RNA from 50 mg fresh rice seedlings were extracted using the Plant Total RNA Mini Kit (Geneaid Biotech Ltd., Taiwan) according to the manufacturer's protocol. Rice samples were homogenized by grinding with micropestle, added 500 µl RB Buffer and 5 µl of ß-mercaptoethanol. The sample mixtures were incubated at 60 ℃ for 5 min and transferred to the Filter Column. Then, column was centrifuged and the clarified filtrate was collected to a new 1.5 ml centrifuge tube. Next, 250 µl absolute ethanol was applied to filtrate, followed by vigorous shaking. The mixture was transferred to RB column and centrifuged. The flow-through was discarded, and 500 µl W1 buffer was added to the RB column . After centrifuge, the RB column was washed twice with 600 µl of Wash Buffer and eluted using 50 µl of RNase-free Water. The total extracted RNA was quantified with a Nanodrop spectrophotometer (OD260/280) prior cDNA synthesis.

RT-PCR and sequencing

The rice cDNA was synthesized from 1 µg of total RNA using iScript™ cDNA Synthesis Kit (Bio-Rad Laboratories, USA). The reaction consists of 5x iScript Reaction Mix, iScript Reverse Transcriptase (RT), Nuclease-free water, and RNA template. After incubation, the cDNA was amplified by PCR (polymerase chain reaction). This study used two sets primers specific to genes encoding enzymes involved in the lignin biosynthetic pathway of *Oryza sativa*, phenylalanine ammonia-lyase

(*OsPAL)* and *Oryza sativa* 4-coumarate-CoA ligase 3 (*Os4CL3*). *OsPAL* primers compose of forward primer (*OsPAL*_F:5'-CGAACCGCTTCGTGTATCTTC-3') and reverse primer (*OsPAL*_R:5'-GGATGGAATCGAGTAGCAATAC-3'). *Os4CL3* primers were designed using sequence 4CL3 gene XM_015770230 at location LOC4328485 (*Os4CL3*_F:5'- CGCAAGCACAACATCACCAT-3' and *Os4CL3*_R:5'-TACCGTAACCCTGTCCGAGG-3').

The PCR reaction mixtures consist of 1x Ultra-pure *Taq* PCR master mix (1 U of Ultra-pure *Taq* polymerase, 2 mM $MgCl_2$ and 200 µM of each dNTPs) (Geneaid Biotech Ltd., Taiwan), 0.8 µM of each primer, and 1 µl of cDNA template. The PCR cycle conditions were performed in the thermocycler (Biometra® T-gradient Thermoblock Thermal Cycler, Germany) with the initial denaturation at 94°C for 5 min, followed by 30 cycles of denaturation at 94°C for 30 s, annealing at 55°C for 30 s, and extension at 72°C for 1 min. After final extension at 72°C for 7 min, the PCR products were cooled down to 20°C. The PCR products were determined on 1.5% agarose gel electrophoresis. The single DNA band was excised under UV-light and purified using the GenepHlow™ Gel/PCR Kit (Geneaid Biotech Ltd., Taiwan). Then, the purified PCR products were sent to DNA sequencing service which was performed in ABI Prism 3730XL DNA sequencer (U2Bio, Korea).

Data analysis

DNA sequences from *OsPAL* and *Os4Cl3* genes were edited using MEGA 7.0 software (Kumar *et al.*, 2016) and aligned with sequences in GenBank database at website: http://www.ncbi.nlm.nih.gov/ using BLASTn tool for the percentage similarity determination. All sequences were submitted to GenBank database by using online BankIt program. Multiple sequence alignments were then analyzed by ClustalW with reference rice sequences from GenBank database running in BioEdit program (Hall, 1999). *OsPAL* reference sequence was *Oryza sativa* Japonica Group phenylalanine ammonia-lyase-like (LOC4330034), mRNA (accession no.XM015769634.1), while *Os4Cl3* reference sequences were *Oryza sativa* Japonica Group 4CL-3 mRNA for 4-coumarate: CoA ligase-3, complete cds (AB234050.1) and *Oryza sativa* Japonica Group probable 4-coumarate-CoA ligase 3, mRNA (accession no.XM015770230).

Phylogenetic tree was constructed and analyzed using MEGA 7.0 program. The seven sequences of *OsPAL* from native upland rice were compared and grouped with eight reference sequences (XM015769634.1 *(Oryza sativa,* Japonica), EF576408.1 (*Oryza sativa*, Indica), EF576267.1 (*Oryza sativa*, *Indica*), XM006647481 (*Oryza brachyantha*), X16099.1 *(Oryza sativa,* Japonica), AB565487.1 (*Sorghum bicolor*), M95077.1 (*Zea mays*), Z49147.1 (*Hordeum vulgare*), and JQ005112.1 *(Triticum aestivum).*

The seven sequences of *Os4CL3* from native upland rice were analyzed in MEGA 7.0 program for phylogenetic tree construction with eight *4CL* reference sequences; AB234050 (*Oryza sativa*, Japonica), XM015770230.2 (*Oryza sativa*, Japonica), CP018158 (*Oryza sativa*, Indica), XM006646898.2 (*Oryza brachyantha*), XM002451602.2 (*Sorghum bicolor*), XM023301294.1 (*Zea mays*), XM004951660.4 (*Setaria italica*), and XM020298358.1 (*Aegilops tauschii*).

Results

The lignin synthesis enzyme sequence amplification

Total RNA from 7 varieties of native upland rice were extracted and reverse transcribed into cDNA. The *OsPAL* and *Os4CL3* primers were used to amplified by conventional PCR. The gel electrophoresis results are shown in figure 1 and 2 (Figure 1 and 2). PCR products were 203 base pairs (*OsPAL*) and 193 base pairs (*Os4CL3*).

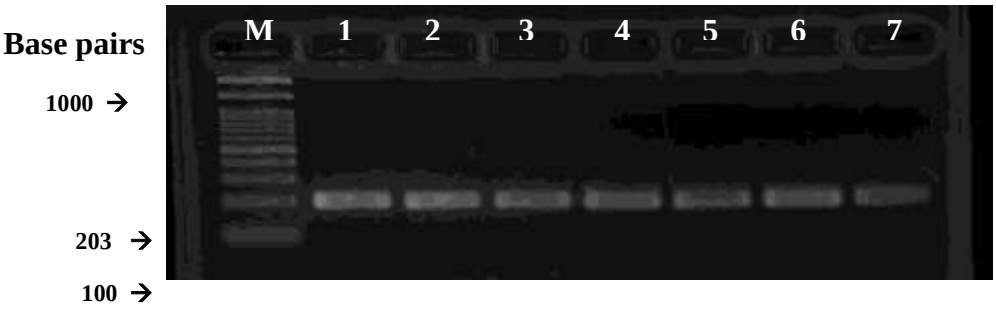

Figure 1. Gel electrophoresis of *OsPAL* PCR products **(**203 bp**)**
(Lane M = 100 bp DNA marker, Lane number 1-7 = PCR products from 7 native rice varieties)

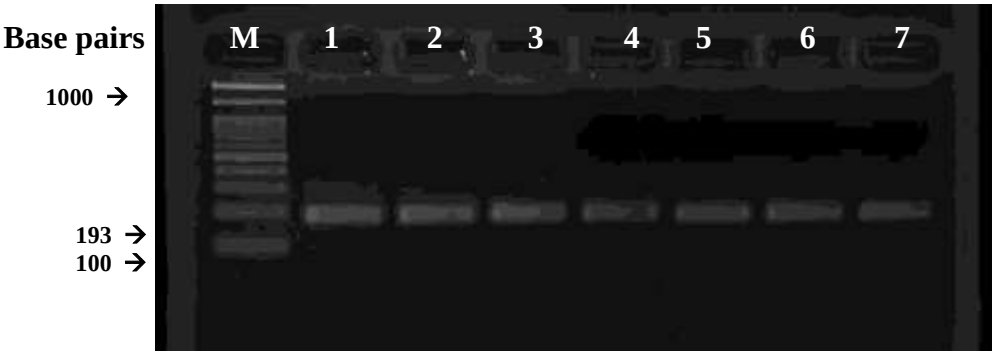

Figure 2. Gel electrophoresis of *Os4CL3* PCR products **(**193 bp**)**
(Lane M = 100 bp DNA marker, Lane number 1-7 = PCR products from 7 native rice varieties)

Sequence analysis

After sequencing, nucleotide sequences were analyzed using MEGA 7.0 software and aligned on NCBI GenBank database (http://www.ncbi.nlm.nih.gov) using BLASTn tool. The edited 169 bp of *OsPAL* sequence alignment results revealed 99.4-100% identity to *Oryza sativa* Japonica Group phenylalanine ammonia-lyase-like (LOC4330034), mRNA sequence (accession no. XM015769634) whereas 98-99% identity to *Oryza sativa* (Indica cultivar-group) clone OSR-97-192-E1 phenylalanine ammonia-lyase mRNA, partial cds (accession no. EF576230.1). For *Os4CL3*, all edited seven 163 bp.-length nucleotide sequences from native upland rice showed 100% similar to *Oryza sativa* Japonica Group probable 4-coumarate-CoA ligase 3 (LOC4328485), mRNA (accession no. XM015770230.2) while 93-94% similar to *Oryza sativa* Indica Group cultivar Shuhui498 chromosome 6 sequence (accession no. CP018162.1). All seven *OsPAL* and *Os4CL3* sequences were submitted to GenBank NCBI database using BankIt online submission platform. The accession number for all sequences were assigned in MN215519 to MN215525 for *OsPAL* and MN215526 to MN215532 for *Os4CL3.*

Multiple sequence alignment was run using ClustalW from BioEdit program. The result showed 100% nucleotide sequence similarity for all seven *OsPAL* sequences from native upland rice with reference sequence XM015769634.1 for 163 bp in length (Figure 3).

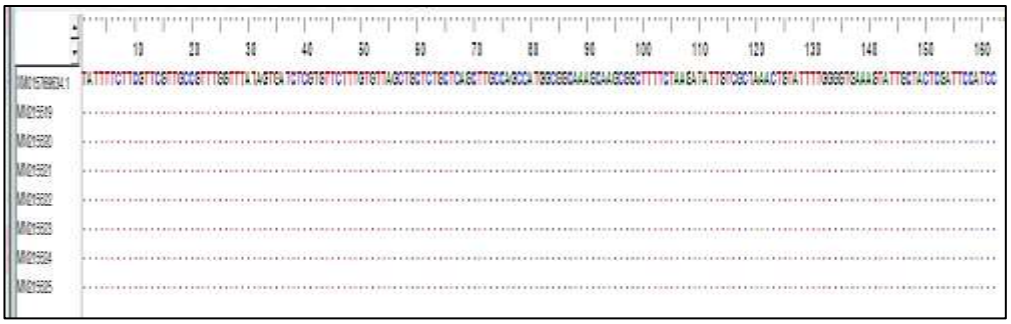

Figure 3. Multiple Sequence Alignment of *OsPAL* sequences
All seven *OsPAL* sequences (MN215519 to MN215525) and the *OsPAL* reference sequence XM015769634.1 from GenBank were aligned using BioEdit program. The identical nucleotide bases to XM015769634.1 were shown in dots (.).

For multiple sequence alignment of *Os4CL3* gene, all seven nucleotide sequences from native upland rice revealed 100% identity to two reference sequences XM015770230.2 and AB234050 for 156 bp in length (Figure 4).

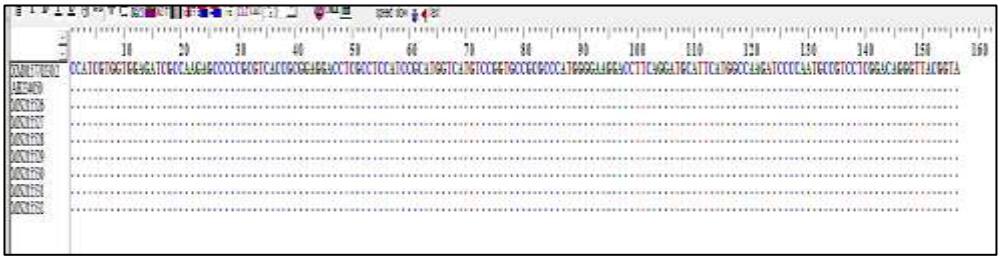

Figure 4. Multiple Sequence Alignment of *Os4CL3* sequences
All seven *Os4CL3* sequences (MN215526 to MN215532) and the *Os4CL3* reference sequence XM015770230.2 and AB234050 from GenBank were aligned using BioEdit program. The similar nucleotide base to XM015770230.2 were showed in dots (.).

Phylogenetic analysis of all seven nucleotide sequences from the *OsPAL* gene were performed in MEGA 7.0 using the Maximum Likelihood method based on the Kimura 2-parameter model. All seven *OsPAL* sequences (MN215519 to MN215525) were grouped in the same clade with *Oryza sativa* Japonica rice (XM015769634.1 and X16099.1) (Figure 5). For *Os4CL3* gene, the nucleotide sequence from seven native upland rice varieties were analyzed and the phylogenetic tree constructed. The result showed that all seven *Os4CL3* sequences (MN215526 to MN215532) were grouped in the same branch of other rice *4CL3* sequences; AB234050 (*Oryza sativa*, Japonica), XM015770230.2 (*Oryza sativa*, Japonica), and CP018158 (*Oryza sativa*, Indica) (Figure 6).

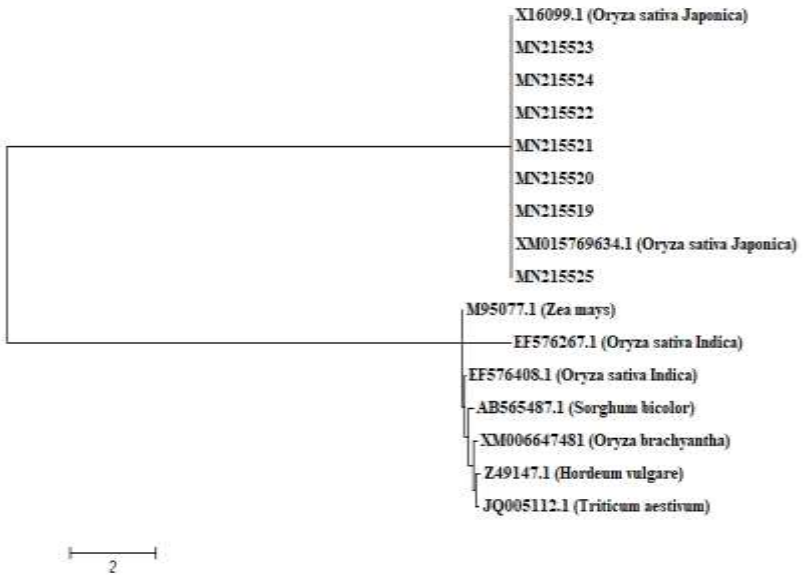

Figure 5. The *OsPAL* Molecular Phylogenetic analysis by Maximum Likelihood method
All seven *OsPAL* sequences (MN215519 to MN215525) from native upland rice were analyzed with eight reference sequences from related species. The evolutionary history was

inferred by using the Maximum Likelihood method based on the Kimura 2-parameter model (Kimura, 1980) and were conducted in MEGA7.0 (Kumar *et al.*, 2016).

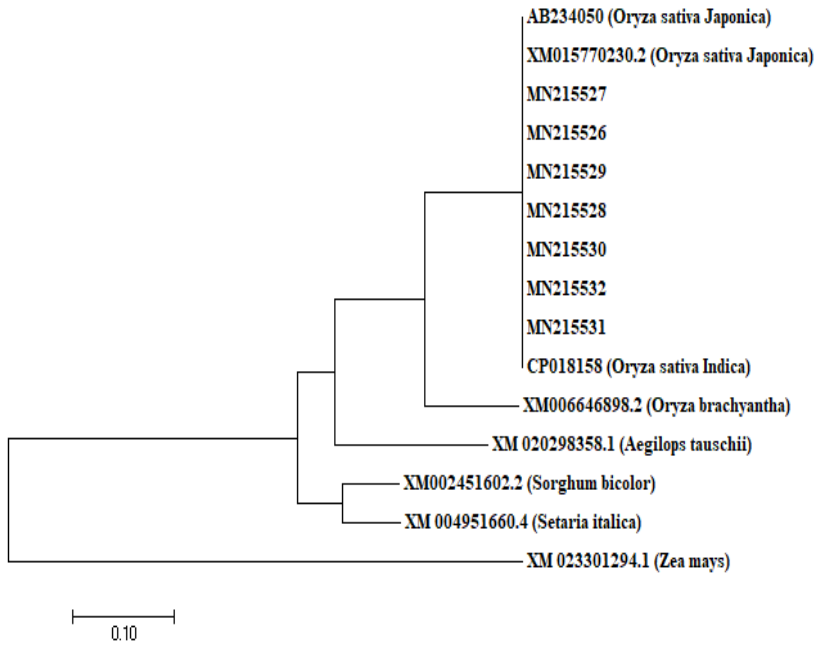

Figure 6. The *Os4CL3* Molecular Phylogenetic analysis by Maximum Likelihood method

All seven *Os4CL3* sequences (MN215526 to MN215532) from native upland rice were analyzed with eight reference sequences. The evolutionary history was inferred by using the Maximum Likelihood method based on the Kimura 2-parameter model (Kimura, 1980) and were conducted in MEGA7.0 (Kumar *et al.*, 2016).

Discussion

Genetic diversity in the native upland rice has been investigated in various country such as Indonesia (Muhidin *et al.*, 2017), North East India (Vanlalsanga *et al.*, 2019), Brazil (Coelho *et al.*, 2017), and Malaysia (Tuhina-Khatun *et al.*, 2015). In Thailand, the native upland rice is grown at Pala U village, Hua Hin district, Prachuap Khiri Khan Province, among other places. In this area, lodging problem was still studied within the individual varieties. This study aimed to utilize the lignin synthesis enzyme genes for genetic sequence characterization and diversity. The Phenylalanine ammonia-lyase (*PAL*) and 4-Coumarate-coenzyme A ligase 3 (*4CL3*) were selected for sequence analysis.

All seven varieties of native upland rice were extracted mRNA and RT-PCR, then the amplified PCR product were analyzed. For the *OsPAL* gene in rice, its nucleotide sequence has been reported in 1989 since it is the gene that encode the first enzyme (Phenylalanine ammonia-lyase (EC

4.3.1.5)) in lignin synthesis pathway (Minami *et al.*, 1989). This study, 169 bp nucleotide sequence from seven varieties were aligned in NCBI GenBank database using BLASTn tool and showed more similarity to *Oryza sativa* Japonica (accession no. XM015769634) (99.4-100%) than *Oryza sativa* Indica (98-99%) (accession no. EF576230.1). Phylogenetic tree analysis also revealed that all nucleotide sequences (accession no .MN215219 to MN215525) were grouped in the same clade of *Oryza sativa* Japonica rice (XM015769634.1 and X16099.1) when compared with other monocot plant sequences. Moreover, all sequences were 100% identical to XM015769634.1 in multiple sequence alignment suggesting that this region in mRNA of *OsPAL* from native upland rice was conserved and normally lignin synthesis gene expressed in these plant. In Arabidopsis (*Arabidopsis thaliana*), the quadrupole *pal* genes mutant plant showed less lignin accumulation and growth stunt (Huang *et al.*, 2010). Abnormal plant developments such as stunted growth, altered leaf, and root formation were observed in *Pal* mutant Tobacco (*Nicotiana tabacum*) (Elkind *et al.*, 1990) and the medicinal *Salvia miltiorrhiza* (Song *et al.*, 2011).

In case of the 4-Coumarate-coenzyme A ligase 3 (*4CL3*) gene, all nucleotide sequences were analyzed and aligned on BLASTn program. The result showed sequence identity to *Oryza sativa* Japonica (accession no. XM015770230.2) in 100% while less similar to *Oryza sativa* Indica (93-94%). Phylogenetic tree analysis revealed that all sequences (MN215526 to MN215532) were grouped in the same branch of other rice *4CL3* sequences; AB234050 (*Oryza sativa* Japonica), XM015770230.2 (*Oryza sativa* Japonica), and CP018158 (*Oryza sativa* Indica). For multiple sequence alignment of *Os4CL3* gene, all seven nucleotide sequences from native upland rice revealed 100% identity to two reference sequences XM015770230.2 and AB234050 for 156 bp in length, indicating that these varieties have the normal feature in nucleotide sequence and lignin biosynthesis. Previously, suppression of *Os4CL3* expression in rice resulted in significant lignin reduction, shorter plant growth, and other morphological changes (Gui *et al.*, 2011) and the dwarfism phenotype was found in the coniferous gymnosperm *Pinus radiata 4CL* mutant (Wagner *et al.*, 2009).

In summary, this study characterized DNA sequences of genes encoding two key enzyme in the lignin biosynthesis pathway (*OsPAL* and *Os4CL3*) from seven varieties of the native upland rice which were cultivated at Pala U village, Thailand. Our result found that all sequences from both genes were identical to *Oryza sativa* Japonica cultivar and had no sequence variations. For further investigation, gene expression level of all varieties will be quantified, which may shed light onto the relationship between lignin biosynthesis gene expression and the lodging phenotype in upland rice.

Acknowledgement

The auther would like to thank to Faculty of Animal Sciences and Agricultural Technology, Silpakorn University, Phetchaburi IT campus for laboratory facility. We also acknowledge to the Editor and reviewers for their valuable suggestions to improve the manuscript.

References

Coelho, G. R. C., Brondani, C., Hoffmann, L. V., Valdisser, P., Borba, T. C. O., Mendonca, J. A., Rodrigues, L. A. and de Menezes, I. P. P. (2017). Genetic diversity of high performance cultivars of upland and irrigated Brazilian rice. Genetics and Molecular Research, 16:1-11.

David, D., Pandey, S. and Nelson, A. (2010). Emerging trends and spatial patterns of rice production. In: Rice: Emerging Trends and Spatial Pattern of Rice Production, International Rice Research Institute, pp. 15-35.

Elkind, Y., Edwards, R., Mavandad, M., Hedrick, S. A., Ribak, O., Dixon, R. A. and Lamb, C. J. (1990). Abnormal plant development and down-regulation of phenylpropanoid biosynthesis in transgenic tobacco containing a heterologous phenylalanine ammonia-lyase gene. Proceedings of the National Academy of Sciences of the United States of America, 87:9057-9061.

Gui, J., Shen, J. and Li, L. (2011). Functional characterization of evolutionarily divergent coumarate: coenzyme A ligases in rice. Plant Physiology, 157:574-586.

Gupta, P. C. and O'Toole, J. C. (1986). Characteristics of upland rices. In: Upland Rice: A Global Perspective, International Rice Research Institute, pp. 105-107.

Hall, T. A. (1999). BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic acids symposium series, 41:95-98.

Hu, D., Liu, X. B., She, H. Z., Gao, Z., Ruan, R. W., Wu, D. Q. and Yi, Z. L. (2017). The lignin synthesis related genes and lodging resistance of Fagopyrum esculentum. Biologia Plantarum, 61:138-146.

Huang, J., Gu, M., Lai, Z., Fan, B., Shi, K., Zhou, Y.-H., Yu, J. Q. and Chen, Z. (2010). Functional analysis of the Arabidopsis *PAL* gene family in plant growth, development, and response to environmental stress. Plant Physiology, 153:1526-1538.

Islam, M. S., Peng, S., Visperas, R. M., Ereful, N., Bhuiya, M. S. U. and Julfiquar, A. J. F. c. r. (2007). Lodging-related morphological traits of hybrid rice in a tropical irrigated ecosystem. Field Crops Research, 101:240-248.

Karladee, D., Boonsit, P., Suriyong, S. and Jamjod, S. (2012). Population heterogeneity of upland rice in northern Thailand. Thai Journal of Agricultural Science, 45:99-104.

Kimura, M. (1980). A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. Journal of Molecular Evolution, 16:111-120.

Kumar, S., Stecher, G. and Tamura, K. (2016). MEGA7: Molecular Evolutionary Genetics Analysis Version 7.0 for Bigger Datasets. Molecular Biology and Evolution, 33:1870-1874.

Liu, T., Li, R., Zhong, X., Jiang, M., Jin, X., Zhou, P., Liu, S., Sun, C. and Guo, W. (2018). Estimates of rice lodging using indices derived from UAV visible and thermal infrared images. Agricultural and Forest Meteorology, 252:144-154.

Liu, Q., Luo, L. and Zheng, L. (2018). Lignins: Biosynthesis and Biological Functions in Plants. International Journal of Molecular Sciences, 19:335.

Michael, S. (2017). THAILAND: Rice Production Rebounds Following El Nino. Retrieved from https://reliefweb.int/report/thailand/thailand-rice-production-rebounds-following-el-nino.

Minami, E. I., Ozeki, Y., Matsuoka, M., Koizuka, N. and Tanaka, Y. J. E. J. o. B. (1989). Structure and some characterization of the gene for phenylalanine ammonia-lyase from rice plants. European Journal of Biochemistry, 185:19-25.

Muhidin, Kadidaa, B., Sadimantar, G. R., Suaib and Safuan, L. O. (2017). Genetic diversity of local upland rice (*Oryza sativa* L.) genotypes based on agronomic traits and yield potential in north Buton, Indonesia. Asian Journal of Crop Science, 9:109-117.

Okuno, A., Hirano, K., Asano, K., Takase, W., Masuda, R., Morinaka, Y., Ueguchi-Tanaka, M., Kitano, H. and Matsuoka, M. (2014). New approach to increasing rice lodging resistance and biomass yield through the use of high gibberellin producing varieties. PLoS One, 9:e86870.

Ookawa, T., Hobo, T., Yano, M., Murata, K., Ando, T., Miura, H., Asano, K., Ochiai, Y., Ikeda, M. and Nishitani, R. (2010). New approach for rice improvement using a pleiotropic QTL gene for lodging resistance and yield. Nature Communications, 1:132.

Plaza-Wüthrich, S., Blösch, R., Rindisbacher, A., Cannarozzi, G. and Tadele, Z. (2016). Gibberellin deficiency confers both lodging and drought tolerance in small cereals. Frontiers in Plant Science, 7:643.

Raes, J., Rohde, A., Christensen, J. H., Van de Peer, Y. and Boerjan, W. (2003). Genome-wide characterization of the lignification toolbox in Arabidopsis. Plant Physiology, 133:1051.

Saito, K., Asai, H., Zhao, D., Laborte, A. G. and Grenier, C. (2018). Progress in varietal improvement for increasing upland rice productivity in the tropics. Plant Production Science, 21:145-158.

Song, J. and Wang, Z. (2011). RNAi-mediated suppression of the phenylalanine ammonia-lyase gene in *Salvia miltiorrhiza* causes abnormal phenotypes and a reduction in rosmarinic acid biosynthesis. Journal of Plant Research, 124:183-192.

Tanaka, K., Murata, K., Yamazaki, M., Onosato, K., Miyao, A. and Hirochika, H. (2003). Three distinct rice cellulose synthase catalytic subunit genes required for cellulose synthesis in the secondary wall. Plant Physiology, 133:73-83.

Tuhina-Khatun, M., Hanafi, M. M., Rafii Yusop, M., Wong, M. Y., Salleh, F. M. and Ferdous, J. (2015). Genetic variation, heritability, and diversity analysis of upland rice (*Oryza sativa* L.) genotypes based on quantitative traits. BioMed Research International, 2015:290861.

Vanlalsanga and Singh, Y. T. (2019). Genetic diversity and population structure in upland rice (*Oryza sativa* L.) of Mizoram, north east India as revealed by morphological, biochemical and molecular markers. Biochemical Genetics, 57:421-442.

Vechpong, T., Chaingmai, P. N. and Pompranee, P. (2015). Participation of community in knowledge management: case study of Paganyaw way of upland rice cultivation in Prachuap Khiri Khan. International Journal of Behavioral Science, 10:13-20.

Wagner, A., Donaldson, L., Kim, H., Phillips, L., Flint, H., Steward, D., Torr, K., Koch, G., Schmitt, U. and Ralph, J. (2009). Suppression of 4-Coumarate-CoA ligase in the coniferous gymnosperm *Pinus radiata*. Plant Physiology, 149:370-383.

Wu, L., Zhang, W., Ding, Y., Zhang, J., Cambula, E. D., Weng, F., Liu, Z., Ding, C., Tang, S., Chen, L., Wang, S. and Li, G. (2017). Shading Contributes to the Reduction of Stem Mechanical Strength by Decreasing Cell Wall Synthesis in Japonica Rice (*Oryza sativa* L.). Frontiers in Plant Science, 8:1-16.

Yoon, J., Choi, H. and An, G. (2015). Roles of lignin biosynthesis and regulatory genes in plant development. Journal of Integrative Plant Biology, 57:902-912.

Zhang, W. J., Wu, L. M., Ding, Y. F., Fei, W., Wu, X. R., Li, G. H., Liu, Z. H., She, T., Ding, C. Q. and Wang, S. H. (2016). Top-dressing nitrogen fertilizer rate contributes to decrease culm physical strength by reducing structural carbohydrate content in japonica rice. Journal of Integrative Agriculture, 15:992-1004.

(Received: 22 July 2019, accepted: 30 October 2019)