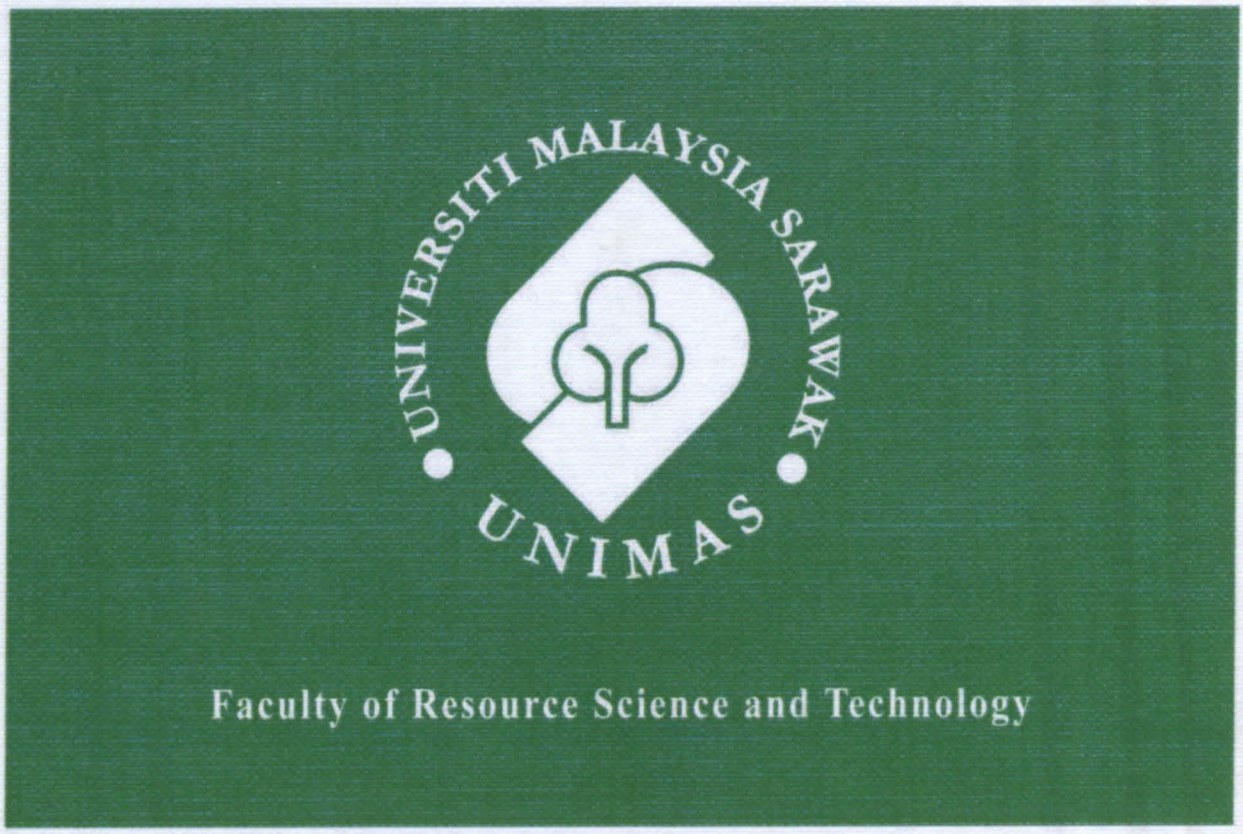


# Isolation and Sequence Analysis of LRR-RLK (Leucine-Rich Repeat Receptor-Like Protein Kinase) Gene of Kelampayan (*Neolamarckia Cadamba*)

**Phang Wei Kit (53584)**

**Bachelor of Science with Honours**
**(Resource Biotechnology)**
**2018**

# Isolation and Sequence Analysis of LRR-RLK (Leucine-Rich Repeat Receptor-Like Protein Kinase) Gene of Kelampayan (*Neolamarckia Cadamba*)

**Phang Wei Kit (53584)**

**This thesis is submitted in partial fulfilment of the requirement of the degree**

**Bachelor of Science with Honours**

**(Resource Biotechnology)**

**Faculty of Resource Science and Technology**

**Universiti Malaysia Sarawak**

2018

**UNIVERSITI MALAYSIA SARAWAK**

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Final Year Project Report ☑

Masters ☐

PhD ☐

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This declaration is made on the 11th day of June year 2018

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I PHANG WEI KIT (57584), Faculty of Resource Science and Technology

(PLEASE INDICATE NAME, MATRIC NO. AND FACULTY) hereby declare that the work entitled, Isolation and Sequence Analysis of LRR-RLK (Leucine-Rich Repeat Receptor-Like Protein Kinase) Gene in Kelompayan (Neolamarckia cadamba) is my original work. I have not copied from any other students' work or from any other sources with the exception where due reference or acknowledgement is made explicitly in the text, nor has any part of the work been written for me by another person.

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**Supervisor's Declaration:**

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# Isolation and Sequence Analysis of *LRR-RLK* (Leucine-Rich Repeat Receptor-Like Protein Kinase) Gene of Kelampayan (*Neolamarckia Cadamba*)

Phang Wei Kit

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## ABSTRACT

*Neolamarckia cadamba* or locally known as kelampayan, belongs to the Rubiaceae family. *N. cadamba* is a fast-growing tree species and has economic importance in timber industry. Leucine-rich repeat receptor-like protein kinase (LRR-RLK) genes are the largest group of receptor-like kinase (RLK) encoding genes. LRR-RLKs have been studied comprehensively for their essential roles in plant development and stress responses. This rises the needs to determine the gene sequence of *LRR-RLK* in *N. cadamba* and the identity of the gene sequence as compared to the similar gene in other plant species. Total genomic DNA was extracted from the leaves of *N. cadamba* using cetyltrimethylammonium bromide (CTAB) followed by DNA purification and integrity assessment. Four EST sequences were retrieved from *N. cadamba* EST database for *in silico* analysis. Primers were designed based on comparison of *LRR-RLK* EST sequences of *N. cadamba* with genomic sequence of another Rubiaceae plant. Gradient polymerase chain reaction (PCR) was utilized to amplify the *LRR-RLK* partial gene and amplicons with desired size of approximately 619 bp were obtained. The PCR products of *LRR-RLK* partial gene was sequenced and *in silico* analysis on these sequences was conducted. This effort could provide data for tissue-specific expression model, for selection of tree genetic properties and functional analysis of *LRR-RLK* genes in *N. cadamba*.

**Key words**: *Neolamarckia cadamba* (kelampayan), leucine-rich repeat receptor-like protein kinase (*LRR-RLK*), gene isolation, gene sequence analysis

## ABSTRAK

*Neolamarckia cadamba atau dikenali sebagai kelampayan, merupakan anggota famili Rubiaceae. N. cadamba ialah spesies pokok yang cepat tumbuh serta mempunyai kepentingan ekonomik dalam industri penghasilan kayu. Gen leucine-rich repeat receptor-like protein kinase (LRR-RLK) ialah kumpulan terbesar gen yang mengekod receptor-like kinase (RLK). LRR-RLK telah dikaji dengan komprehensif untuk mengetahui tugas penting mereka dalam tumbuhbesaran tumbuhan dan respon terhadap tekanan. Hal ini telah memberikan keperluan untuk mengetahui jujukan gen LRR-RLK dalam N. cadamba dan identiti gen tersebut berbanding dengan jujukan gen yang sama dalam spesies tumbuhan lain. DNA genomik penuh diekstrak daripada daun N. cadamba menggunakan cetyltrimethylammonium bromide (CTAB) diikuti dengan purifikasi DNA serta ujian kestabilan. Empat jujukan EST diperoleh daripada pangkalan data EST N. cadamba untuk mejalankan analisasi in silico. Primer direka berdasarkan perbandingan antara jujukan EST LRR-RLK N. cadamba dengan jujukan genomik tumbuhan Rubiaceae yang lain. Tindakbalas berantai polymerase (PCR) gradien digunakan untuk mengamplifikasikan gen separa LRR-RLK dan amplicon yang mempunyai saiz yang dikehendaki dengan anggaran 619 bp telah dihasilkan. Produk PCR gen separa LRR-RLK telah dijujukan dan analisasi in silico dijalankan ke atas jujukan ini. Usaha ini dapat menghasilkan maklumat yang boleh digunakan untuk model ekspresi tisu tertentu, pemilihan ciri genetik pokok dan analisasi fungsi gen LRR-RLK dalam N. cadamba.*

***Kata kunci**: Neolamarckia cadamba (kelampayan), leucine-rich repeat receptor-like protein kinase (LRR-RLK), pengasingan gen, analisasi jujukan gen*

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| | |
|---|---|
| % (v/v) | Percentage of volume of solute in total volume of solution |
| % (w/v) | Percentage of weight of solution in total volume of solution |
| °C | Degree Celcius |
| µL | Microliter |
| A | Adenine |
| A | Ampere |
| BAK1 | BR1-ASSOCIATED RECEPTOR KINASE1 |
| BLAST | NCBI Basic Local Alignment Search Tool |
| BR1 | BRASSINOSTEROID-INSENSITIVE1 |
| bp | Base pair |
| C | Cytosine |
| C-terminal | Carbon-terminal |
| C-terminus | Carbon-terminus |
| *C. canephora* | *Coffea canephora* |
| *C4H* | Cinnamate 4-hydroxylase |
| CC | Cysteine-containing |
| *CCoAOMT* | Caffeoyl-coenzyme A O-methyltransferase |
| CD | Conserved domain |
| cDNA | Complementary deoxyribonucleic acid |
| CDS | Coding DNA sequence |
| *CesA* | Cellulose synthase |

| | |
|---|---|
| CLV1 | CLAVATA1 |
| CORE | COLD SHOCK PROTEIN RECEPTOR |
| CSPR | CSP22 RESPONSIVENESS |
| CTAB | Cetyltrimethylammonium bromide |
| Cys | Cysteine |
| DAMP | Damage-associated molecular pattern |
| DNA | Deoxyribonucleic acid |
| EDTA | Ethylenediamine tetraacetic acid |
| EMS1 | EXCESS MICROSPOCYTES1 |
| EST | Expressed Sequence Tag |
| FLS2 | FLAGELLIN-SENSITIVE2 |
| G | Guanine |
| g | Gram |
| GO | Gene ontology |
| GSO1 | GASSHO1 |
| GSO2 | GASSHO2 |
| HCl | Hydrochloric acid |
| HCS | Highly conserved segment |
| HSP | High scoring segment pair |
| InDel | Insertion-deletion |
| kb | Kilobases |
| L | Liter |
| LRR | Leucine-rich repeat |

| | |
|---|---|
| LRR-RLK | Leucine-rich repeat receptor-like protein kinase |
| LRR-RLP | Leucine-rich repeat receptor-like protein |
| LSE | Lineage-specific expanded |
| M | Moles/liter |
| m | Meter |
| MAMP | Microbe-associate molecular pattern |
| mL | Milliliter |
| mM | Millimoles/liter |
| mRNA | Messenger ribonucleic acid |
| N-terminal | Nitrogen-terminal |
| N-terminus | Nitrogen-terminus |
| *N. cadamba* | *Neolamarckia cadamba* |
| NCBI | National Center for Biotechnology Information |
| ng | Nanogram |
| NaCl | Sodium chloride |
| NaOH | Sodium hydroxide |
| NBS-LRR | Nucleotide-binding site-leucine-rich repeat |
| NIK | NSP-INTERACTING KINASE |
| NSP | Nuclear shuttle protein |
| PCR | Polymerase chain reaction |
| PEPR | PEP RECEPTOR |
| PGIP | Polygalacturonase-inhibiting protein |
| pmol | Picomoles |

| | |
|---|---|
| PRR | Pattern recognition receptor |
| PS | Plant specific |
| PVP | Polyvinylpyrrolidone |
| PXY | PHLOEM INTERCALATED WITH XYLEM |
| RI-like | Ribonuclease inhibitor-like |
| RLK | Receptor-like protein kinase |
| RNA | Ribonucleic acid |
| rpm | Revolutions per minute |
| RUL1 | REDUCED IN LATERAL GROWTH1 |
| SERK1 | SOMATIC EMBRYOGENESIS RECEPTOR KINASE1 |
| SNP | Single nucleotide polymorphism |
| subsp | Subspecies |
| T | Thymine |
| TAE | Tris-acetate-EDTA |
| TE | Tris-EDTA |
| TpLRR | *Treponema pallidum* LRR |
| U | Enzyme unit |
| USA | United States of America |
| V | Volt |
| VH1 | VASCULAR HIGHWAY1 |
| VS | Variable segment |
| *XET* | Xyloglucan endotransglycosylate |

# 1.0 INTRODUCTION

Majority of terrestrial biomass production is represented by forest trees. A part of being the main biomass contributors, forest trees serve various benefits to the ecosystem such as habitat to wildlife, economical wood source, food source and others. However, rapid degradation of forest due to global demand creates a realization that there is a need for sustainability. Hence, establishment of forest plantations focusing on fast-growing species is needed to reduce pressure on natural forests extraction while able to produce high quality wood supply.

In Malaysia, the Sarawak state government has proposed to establish 1 million hectares of forest plantations within 15 years. *Neolamarckia cadamba* or locally known as kelampayan, is a one of the prominent timber species which has been selected for large-scale planted forest development in Sarawak for its short rotation period (Tiong *et al.*, 2014a). Under optimal conditions, this tree species is expected to achieve a height of 17 m and the diameter length could reach 25 cm at breast length within 9 years. Hence, *N. cadamba* could secure early economic return within 8 to 10 years as it can be utilized as the sources of raw material for plywood, pulp and paper manufacturing.

*N. cadamba* pharmaceutical studies have been conducted on antioxidant, antifungal, antibacterial activity of fruit, antidiabetic characteristic, hypoglycemic activity of leaf and phytochemical analysis property (DonPaul, 2016). Moreover, *N. cadamba* is reported in studies by Dubey *et al.* (2011) for its role as an important ayurvedic medicine in treatments for ever, dysentery, uterine diseases, and diabetes. Despite kelampayan is prominent for its economical

and pharmaceutical importance, the genetic information of this is still far to be fully documented and understood. Hence, genetic analysis of this indigenous species is crucial for molecular characterization and to preserve its high quality.

Plant physiological growth is regulated by various genes. This includes the formation of plant woody tissue from meristems which is responsible for majority of plant biomass. One group of genes that is known to control plant development is the leucine-rich repeat receptor-like protein kinase (LRR-RLK) genes family. LRR-RLKs are membrane bound signaling proteins with extracellular receptor domains that can perceive and respond to endogenous and exogenous signals (Tör *et al.*, 2009). *LRR-LRK* genes are extensively studied and these studies have demonstrated that *LRR-RLK* genes involve in various plant pathways such as cell differentiation, cellular morphogenesis, hormone signaling, disease resistance and stress response. An example of *LRR-RLK* genes is PHLOEM INTERCALATED WITH XYLEM (PXY) gene in which it regulates proper orientation of procambial cell division and wood formation (Fisher & Turner, 2007; Sieburth, 2007). However, functions of many discovered and predicted LRR-RLK genes remain unknown.

Before 1970, DNA could only be analyzed indirectly by protein or RNA sequencing or by genetic analysis which make it difficult for cellular molecule analysis (Alberts *et al.*, 2002). Advancement in recombinant DNA technology eases DNA analysis by allowing specific region of genome to be isolated, determination of nucleotide sequence as well as amplifying unlimited DNA copies. DNA isolation and sequencing has become a fundamental skill which provide biologists the ability to determine functions and structures of various classes of genes and

proteins (Alberts *et al.*, 2002). In plant genomic studies, one common DNA extraction technique is cetyltrimethylammonium bromide (CTAB) extraction method which is deemed as cost-effective and productive (Doyle & Doyle, 1990). Other essential skills include DNA sequencing which is practiced for a direct identification of the specific nucleotide bases sequence and polymerase chain reaction (PCR) which is applied for multiplication of specific DNA fragments (Saiki *et al.*, 1988).

Discovery of *LRR-RLK* in Arabidopsis (Gou *et al.*, 2010), rice (Shiu *et al.*, 2004), poplar (Zan *et al.*, 2013) and tomato (Wei *et al.*, 2015) have shed a light that this gene could also be isolated out from other plants. Tör *et al.* (2009) stated that characterizing ligands for LRR-RLK is essential to further understand various processes regarding plant differentiation, perception of beneficial and harmful stimuli, and functional coordination between different types of receptors under varying environmental circumstances. Hence, there is a need to determine the sequence of *LRR-RLK* gene which is associated with plant growth and wood development of *N. cadamba*. Besides, there is no valid documentation of this gene in *N. cadamba*. Hence, this study is expected to provide valid data of *LRR-RLK* partial gene which could be contributed into the database. In future direction, this information could be used for tissue-specific expression model, for selection of tree genetic properties and for studying the relationship between *LRR-RLK* gene, growth rate and wood quality of *N. cadamba*. Therefore, the objectives of this study were to isolate and sequence *LRR-RLK* partial gene from *N. cadamba* as well as to characterize the homology, structural and functional properties of *LRR-RLK* partial gene sequence.

# 2.0 LITERATURE REVIEW

## 2.1 Kelampayan (*Neolamarckia cadamba*)

### 2.1.1 Growth Performance and Physical Characteristics

*Neolamarckia cadamba*, locally known as kelampayan, is a fast-growing evergreen tree species which grows well in Southeast Asia and India (Dubey *et al.*, 2011). In Malaysia, this tree is majorly planted in Sarawak, Sabah, Perak and Pahang (Nordahlia *et al.*, 2014). *N. cadamba* is characterized as medium-sized to large-sized tree which can grow up to 40 m tall and without branches for more than 25 m (Bong, 2011). This tree has an umbrella-shaped crown and the branches are arranged in tiers. The bark of this tree is gray and it is distinguishable by its smooth surface in young tree and rough or longitudinally fissured in old tree. The fruits are small and contain yellow-orange infructescence (Dwevedi *et al.*, 2015). The leaves have a shape of elliptical-oblong or ovate and they are slightly aromatic with unpleasant taste (Dubey *et al.*, 2011). According to Joker (2000), *N. cadamba* grown best on the deep, moist and alluvial sites which are typically found in riverbanks secondary forests and in the transitional zone between swampy, permanently or periodically flooded area.

### 2.1.2 Taxonomy of *N. cadamba*

*Neolamarckia cadamba* has been known with varying nomenclatures including *Nauclea cadamba*, *Anthocephalus cadamba*, *Anthocephalus chinensis*, *Neonauclea megaphylla*, and others (Dwedvedi *et al.*, 2015). According to Integrate Taxonomic Information System (ITIS) (2011), taxonomy of *N. cadamba* is listed as below:

Kingdom : Plantae

Subkingdom : Viridiplantae

Infrakingdom : Streptophyta

Superdivision : Embryophyta

Division : Tracheophyta

Subdivision : Spermatophytina

Class : Magnoliopsida

Superorder : Asteranae

Order : Gentianales

Family : Rubiaceae

Genus : *Neolamarckia*

Species : *Cadamba*

### 2.1.3 Beneficial Values of *N. cadamba*

*Neolamarckia cadamba* is one of the multipurpose plants which are important in various areas including religious, environmental, commercial and medicinal. In India, it traditionally serves as an ornamental tree with religious significance (Dwevedi *et al.*, 2015). In term of environmental role, *N. cadamba* is a suitable tree for reforestation due to its fast-growing properties. Its umbrella-shaped crown could provide shade to lower dipterocarp plants (Joker, 2000). The fruits and inflorescenses are thought to be edible to humans and the leaves of *N. cadamba* are usually used as cow feeds (DonPaul 2016). *N. cadamba* provides timber sources for commercial productions such as plywood, wooden sandals, chopsticks, less expensive furniture, paper pulp, canoe and various types of wooden board (Lim & Chung, 2002). Ahmed *et al.* (2011) demonstrated the antidiabetic properties of *N. cadamba* by using methanol extracted from leaves to reduce blood glucose level in hyperglycemic mice. Various phytochemicals and secondary metabolites can be extracted from *N. cadamba* including cadamine, quinoline, quinovic acid, β-sitosterol, triterpenes, saponins and secoiridoids (Dwevedi *et al.*, 2015; Khandelwal *et al.*, 2016).

### 2.1.4 Genetic Constituents of *N. cadamba*

Up to date, various genomics and genetics studies have been conducted to understand the potential genetic factors that involve regulation of wood quality of *N. cadamba*. DNA variations discovered in *N. cadamba* originated from different areas in Sarawak have provided an idea that genetic relatedness and diversity between different populations could contribute to cultivation of superior traits plant based on marker selection (Tan *et al.*, 2007; Tiong *et al.*, 2014a). A study by Ho *et al.* (2014) successfully generated 6 622 high quality expressed sequence tags (ESTs)

from a developing xylem cDNA library and found out that this EST database contains many genes involved in lignin and cell wall biosynthesis. Some of these documented important genes are tubulin genes, *cellulose synthase* (*CesA*), *xyloglucan endotransglycosylate* (*XET*), *cinnamate 4-hydroxylase* (*C4H*), *caffeoyl-coenzyme A O-methyltransferase* (*CCoAOMT*) (Pang *et al.*, 2015). Besides, Bong (2011) had determined the polymorphism in *COBRA* genes of *N. cadamba* which could be useful for genetic marker development to improve wood formation of the tree. Nevertheless, there are still a lot of genes yet to be discovered in *N. cadamba*.

## 2.2 Plants Defense Mechanism and Stress Response

Like many organisms, plants are hosts to diverse array of pathogens. Afzal *et al.* (2008) stated that many pathogen-resistant proteins in plants share highly identical structures, motifs and domains throughout evolutionary conservation and convergence. This suggests that various plant defense responses may be based on common signaling pathways among different plant species. Plant resistance or susceptibility to pathogen attack depends on intertwined defensive layers which include preformed barriers, inducible innate immunity and gene-mediated resistance (Thordal-Christensen, 2003). Although plants lack antibodies and specific immune response cells, they possess remarkable defense strategies to adapt to environmental stresses as well as detecting molecular signals through the utilization of transmembrane receptors. Generally, defensive cascade responses can be initiated when foreign or pathogen-derived elicitors are perceived by receptors and followed by transmission of secondary signals through the plasma membrane. As intracellular signaling molecules accumulate, a series of phosphorylation and dephosphorylation activity will occur. In combination with these cascade reactions, changes in metabolic activities will lead to downstream defense mechanisms such as

activation of genes encoding for defense proteins, hypersensitive response, and programmed cell death (Gachomo *et al.*, 2003; Montesano *et al.*, 2003).

## 2.3 Leucine-Rich Repeat (LRR)

Matsushima and Miyashita (2012) described that leucine-rich repeat (LRR) sites are discovered in more than 14 000 proteins. LRR genes are widely known to have functional roles in protein-ligand interactions, protein-protein interactions and innate immune response. Majority of known LRR domains contain 2 to 45 leucine-rich repeats and the length of each repeat ranges about 20 to 30 amino acid residues (Ng & Xavier, 2011). All LRR units are composed of 2 distinct amino acid residues stretches known as highly conserved segment (HCS) and variable segment (VS). The HCS is characterized by the conserved patterns of either LxxLxLxxNxL or LxxLxLxxCxxL in which 'x' can be any amino acid, 'L' is leucine, valine, isoleucine or phenylalanine, 'N' is asparagine, threonine, serine, or cysteine, and 'C' is cystein, serine or asparagine (Kobe & Kajava, 2001). Eight classes of LRR have been categorized based on lengths and consensus sequences differences in the VS region of LRR repeating units. These classes are known as ribonuclease inhibitor-like (RI-like), cysteine-containing (CC), Bacterial, SDS22-like, plant specific (PS), Typical, *Treponema pallidum* LRR (TpLRR), and IRREKO (Matsushima & Miyashita, 2012).

LRR is an active motif that it usually found associated with other motifs. This further distinguish LRR-encoding genes into three major groups identified in plants known as leucine-rich repeat receptor-like protein kinase (LRR-RLK), nucleotide-binding site-leucine-rich repeat (NBS-LRR) and, LRR-only (Tang *et al.*, 2010; Wang *et al.*, 2011). LRR-only group includes