

Resequencing And Nucleotide Variation Of Sucrose Synthase (Nmsusy1) Gene In A Tropical Timber Tree *Neolamarckia macrophylla*

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Abstract: Sucrose synthase (SuSy) is a key enzyme that catalyses the reversible synthesis and degradation of sucrose. It provides greater impact in regulating the photosynthetic processes and environmental stresses in plants. Thus, the nucleotide variation of partial NmSusy1 genomic DNAs (750 bp) generated through PCR amplification was examined in this study, and this followed by resequencing from 15 selected *Neolamarckia macrophylla* clones. The consensus sequences were aligned to detect the presence of single nucleotide polymorphisms (SNPs). In total, five SNPs were detected at nucleotide 1, 2, 34, 35 and 397. Of these, four SNPs were located at the predicted coding region while one SNP was located at the predicted non-coding region. Interestingly, one single base pair InDel polymorphism was also detected at nucleotide 17. On average, one SNP at every 150 bp was detected based on the 15 NmSusy1 sequences. There was one non-synonymous mutation detected, whereby amino acid glutamic acid (E) was replaced by arginine (R) in one of the 15 samples tested. This non-synonymous SNP might change the structural, functional or biochemical properties of the enzyme being produced and therefore possibly lead to changes in phenotypic characteristic of the trees. Overall, this study has demonstrated that resequencing is an effective technique for classifying molecular diversity or nucleotide variation in the Susy gene of *N. macrophylla*. Those SNPs, once validated, could potentially be used as a tool in marker-assisted selection (MAS) that enables more precise and accurate in the selection and prediction of yield or performance at the early developmental stages, such as at the seedling stage.

Index Terms: *Neolamarckia macrophylla*, red kelampayan; resequencing, sucrose synthase (SuSy); single nucleotide polymorphism (SNP), nucleotide variation, marker-assisted selection

1 INTRODUCTION

Sucrose is important to the plant growth and development. It is the main photosynthate and serves as the main transport sugar, nutrients and potential signal molecule in plants. Sucrose synthase (SuSy) is a key enzyme that catalyses the reversible synthesis and degradation of sucrose. Since sucrose cannot be utilized by plant directly, sucrose and uridine diphosphate (UDP) need to be cleaved by SuSy enzyme into fructose and UDP-glucose at pH 8.0 to pH 8.8 whereby UDP-glucose serves as the precursor for cellulose biosynthesis, which is crucial for wood formation [1]. Hypoxic or anoxic condition on the other hand, will cause the falling of pH in cells (pH 6.0 to pH 6.5), leading to degradation of sucrose by SuSy enzyme into UDP-glucose and fructose [2]. Hence, under reduced oxygen condition, plants are still able to survive as SuSy enzyme will increase degradation of sucrose. SuSy gene is regulated by the level of its own enzyme products.

It was reported that sucrose, glucose and D-mannose are able to up-regulate Sus1, which is a major SuSy gene in *Arabidopsis thaliana* [3]. It is proposed that SuSy enzymes consist of two forms, namely the soluble SuSy (S-SuSy) and particulate SuSy (P-SuSy). Soluble SuSy can be found in the cytoplasm while particulate SuSy is bound on the plasma membrane [4]. S-SuSy allocates carbon for metabolic work such as respiration, producing storage and building blocks molecules. It cleaves sucrose into UDP-glucose and fructose whereby fructose is used in glycolysis and starch formation [5]. On the other hand, P-SuSy supplies UDP-glucose from the degradation of sucrose for cellulose and callose synthesis. Polysaccharide is produced by using the high energy bond conserved during the process [6]. The activation of SuSy is thought to be related to adaptation to anaerobic condition. In maize roots, SuSy acts as anaerobic proteins under anaerobic conditions to degrade sucrose. It requires less ATP and thus more energy-efficient [5]. Phosphorylation enabled the conversion of P-SuSy into S-SuSy [1]. Apart from catalysing sucrose, SuSy is suggested to be present in mitochondrial of maize to regulate nutrient or sugar signalling through the opening of mitochondrial permeability transition pores. It is also present in chloroplasts whereby it helps in regulating the photosynthetic processes in plastids. Under abiotic stress conditions such as osmotic stress or draught, excessive synthesis of sucrose occurs in order to maintain membrane function and structure. Thus, Susy gene can be used as a potential biomarker and candidate gene for environmental stresses [7]. Despite the high economic value of tropical wood, there is no genetic information available in the public genomic databases about Susy gene in *Neolamarckia macrophylla*. In fact, the present study was the first molecular study to isolate the Susy genomic sequence and subsequently, to determine the nucleotide variation of Susy gene in *N. macrophylla*. *N. macrophylla* or commonly known as red kelampayan belongs to the Rubiaceae family. It is a fast growing tropical tree native to Eastern Indonesia. It has been cultivated widely in many countries such as in Malaysia, India and Philippines. It is able

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