

CLOWING AND CHARACTERIZATION OF α -GLOBULIN PROMOTER INTO
AN EXPRESSION VECTOR

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ABSTRACT

The world population is expected to rise by an addition of 2 billion by 2030 and rice consumers are projected to increase by 1.8% annually. Hence, rice production must be increased between 25-45% to match-up the growing population since it is a staple food to more than half of the world's population. The manipulation of targeted gene in endosperm is a reliable tissue for the production of recombinant proteins over other tissues because it is more cost-effective, it is easier to scale-up agricultural yield, provides a larger storage ability and safe long-term storage. However only a few endosperm-specific promoters have been identified. The present research, successfully constructed the recombinant plasmid, pCAMGpro from the expression vector, pCAMBIA1305.2 containing the strong endosperm-specific α -globulin promoter (AsGpro). The AsGpro was successfully amplified from pmCACA:GFP using the forward primer, AsGproF_HindIII (5' CACAAACGTGCAAA**AAGCTT**AATTCG 3') and the reverse primer, AsGproR_BamHI (5' GAC**GGATCCC**GAGATTGTAGAAGG 3') at 55°C. The size of the promoter fragment was approximately 848 bp. Sequencing and subsequent bioinformatics analysis, confirmed 98% homology of nucleotides to *A. sativa* (Glo1) gene, promoter region (Accession number: AY795082.1). This fragment was then cloned into pMR104a to generate the recombinant plasmid pMRGpro. Subsequent cloning of the recombinant cassette into the expression vector, pCAMBIA1305.2 to create the new recombinant plasmid, pCAMGpro was achieved. These finding can be used to genetically modify rice to express high levels of endosperm specific nutritional proteins of interest that would increase food production and help in alleviating the food crisis facing the world.

ABSTRAK

Endosperm merupakan 60% pembekal terbesar kepada organ penyimpanan untuk protein dan kanji. Populasi dunia dijangka akan meningkat kepada 2 bilion pada kadar 1.8% setahun sehingga 2030. Oleh itu, penghasilan padi perlu ditingkatkan antara 25 sehingga 45% untuk memenuhi keperluan dunia. Endosperm merupakan platform terbaik dalam penghasilan protein rekombinan kerana menjimatkan kos, meningkatkan hasil pertanian, mempunyai kadar penyimpanan berskala besar dan selamat daripada pengaruh biotik dan abiotik, contohnya suhu dan patogen. Namun begitu, hanya sesetengah promoter spesifik endosperma dapat dikenalpasti. Penemuan terbaru dalam kajian ini menunjukkan kejayaan di dalam penghasilan plasmid rekombinan, pCAMGpro daripada vektor pengklonan pCAMBIA1305.2 yang merangkumi promoter spesifik endosperma α -globulin (AsGpro). AsGpro ini telah berjaya diamplifikasi daripada pmCACA:GFP dengan menggunakan pencetus kehadapan pendahulu, AsGproF_*HindIII* (5' CACAAACGTGCAAA**AAGCTT**AATTCG 3') dan primer pembalik, AsGproR_*BamHI* (5' GAC**GGATCC**GAGATTGTAGAAGG 3') pada suhu 55°C. Saiz promoter dianggarkan bersaiz 848 bp. Analisis bioinformatik menunjukkan homologi nukleotid *A. sativa* (Glo1) adalah sebanyak 98%. Fragmen ini seterusnya diklonkan ke dalam pMR104a untuk menghasilkan plasmid rekombinan pMRGpro. Kemudiannya, plasmid rekombinan ini diekspreskan ke dalam vektor pengklonan, pCAMBIA1305.2 untuk menghasilkan plasmid baru pCAMGpro. Penemuan di dalam kajian ini juga membuktikan pengubahsuaian genetik beras menggunakan promoter spesifik endosperma mampu meningkatkan dan seterusnya membantu menyelesaikan masalah krisis makanan pada masa akan datang.

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LIST OF SYMBOLS/ABBREVIATIONS

AsGpro	-	<i>Avena sativa</i> globulin promoter fragment
NOS	-	Nopaline synthase terminator
AsGpro:NOS	-	<i>Avena sativa</i> globulin promoter+Nopaline synthase terminator cassette
pMRGpro	-	Recombinant plasmid from pMR104a containing AsGpro
pMRGpro	-	Recombinant plasmid from pCAMBIA1305.2 containing AsGpro:NOS cassette
<i>E. coli</i> DH5 α	-	<i>Escherichia coli</i> strain DH5 α
<i>A. tumefaciens</i>	-	<i>Agrobacterium tumefaciens</i>
<i>A. sativa</i>	-	<i>Avena sativa</i>
$^{\circ}\text{C}$	-	Degree Celcius
hrs	-	Hours
min	-	Minutes
sec	-	Seconds
dNTPs	-	Deoxyribo Nucleotide TriPhosphates
PCR	-	Polymerase Chain Reaction
DNA	-	Deoxyribonucleic Acid
g	-	Gram
mg	-	Milligram
μg	-	Microgram
ng	-	Nanogram
gmol^{-1}	-	Grams per mole
gcm^{-1}	-	Grams per cubic centimeter
bp	-	Base pair
Kb	-	Kilo base
β	-	Beta
α	-	Alfa
L	-	Liter

ml	-	Mililiter
μl	-	Microliter
μm	-	Micrometer
M	-	Molar
mM	-	Milimolar
μM	-	Micromolar
OD	-	Optical density
dH ₂ O	-	Deionized water
CaMV35S pro	-	Cauli Flower Mosaic Virus 35S promoter
MgCl ₂	-	Magnesium chloride
CaCl ₂	-	Calcium chloride
LB	-	Luria Bertani medium
%	-	Percent
IPTG	-	Isopropyl Thiogalactosidase
X-Gal	-	5-Bromo-4-Chloro-3-Indolyl-Beta-D-Galactopyranoside
EDTA	-	Ethylene Diamine Tetra-Acetic acid
NaCl	-	Sodium chloride
w/v	-	Weight per volume
MCS	-	Multiple Cloning Site
AMP	-	Ampicillin
~	-	Approximately
5'	-	5 prime-end
3'	-	3 prime-end
g	-	Gravity
rpm	-	Rotations per minute
V	-	Volts
TAE buffer	-	Tris-acetate -EDTA buffer
TE buffer	-	Tris-EDTA buffer
A	-	Adenine
C	-	Cytosine
G	-	Guanine
T	-	Thymine
BLAST	-	Basic Local Alignment Search Tool
MEGA	-	Molecular Evolutionary
ES	-	Endosperm
ESP	-	Endosperm Specificity Palindrome
EtBr	-	Ethidium bromide

NCBI	-	National Centre for Biotechnology Information
M. W.	-	Molecular weight
GOI	-	Gene of Interest
GMP	-	Genetically Modified Products

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CHAPTER 1

INTRODUCTION

1.1 Background of study

The seeds of cereals such as rice, wheat, barley and maize are the main source of food, animal feeds and the raw materials of food and fiber-based industries worldwide. The most important part of the seed acting as a food reservoir for both mankind and during plant growth and development is the endosperm (Berger, 2003).

According to reports from the Food and Agricultural Organization (2011), the world population is expected to rise by an additional 2 billion by 2030. The statistical reports also show that by October 2008, thirty-six countries from Africa, Asia and Latin America were in food crisis. The current world food production cannot support our escalating population.

Rice is a staple food to more than half of the world's population and therefore stands out as the best candidate for biofortification and biotechnological improvements. This will play a pivotal role in alleviating the world's nutritional deficiency challenges. The demand for rice by rice consumers is projected to increase by 1.8% annually.

The control of powerful endosperm-specific promoters may provide answers to how seed-specific proteins can be increased. A sound understanding of the seed initiation and the control of early endosperm development will be important for increasing cereal crop yield and improving grain quality (Li, 2011). Tissue-specific promoters have the potential to increase recombinant protein stability and deliver targeted gene expression to only specific parts of the plant (Facy, 2009). Different research outcomes have illuminated the importance and role of several endosperm-specific promoter regions to engineer transgenic crops with improved endosperm-specific biosynthesis (Furtado *et al.*, 2009, Hwang *et al.*, 2002, Kawakatsu and Takaiwa, 2010, Furtado *et al.*, 2008, Sunilkumar *et al.*, 2002, Vickers *et al.*, 2006).

Construction of the α -globulin promoter (AsGpro) into the expression vector pCAMBIA1305.2 will allow other researchers to further use this plasmid construct to understand the promoter activity more deeply. In their research, Vickers *et al.* (2006) showed that α -globulin promoter maintained its endosperm-specific activity in both wheat and barley and its endosperm-specific activity is attributed to the Endosperm Specificity Palindrome (ESP) element (**ACATGTCATCATGT**) at position -91 while the expression strength was attributed to the GCN4 element. Mutation of GCN4 decreased expression to ~60% while ESP mutation led to loss of endosperm-specific expression. Mutation of both ESP and GCN4 led to total loss of expression. The activity of the α -globulin promoter has not been tested in rice. This new recombinant vector, pCAMGpro bearing the α -globulin promoter and a gene of interest, can be transformed into rice and the expression quantified in relation to other endosperm-

specific promoters and the constitutive promoters. If α -globulin endosperm-specific promoter shows significantly higher endosperm-specific expression activity, it can be employed in industrial biosynthesis of food and pharmaceutical proteins. This will alleviate the food crisis facing the globe.

1.2 Problem statement

Rice is a staple food to more than half of the world's population and over 600 million human beings suffer from hunger on a daily basis. The world's rice production cannot support both the world's growing population which is expected to rise by an additional 2 billion by 2030 and the rice demand by rice consumers which is projected to increase by 1.8% annually.

1.3 Objectives of study

- i. To amplify the endosperm-specific α -globulin promoter (AsGpro) from pmCACA:GFP plasmid.
- ii. To clone the PCR product (AsGpro fragment) into pMR104a and clone the AsGpro:NOS cassette into the expression vector, pCAMBIA1305.2.
- iii. To analyze the α -globulin promoter sequencing results through bioinformatics and construction of Phylogenetic tree.

1.4 Scope of study

The scope of this research encompassed amplification and subsequent cloning of the endosperm-specific α -globulin promoter fragment (~823 bp) into the intermediate vector, pMR104a. The AsGpro:NOS cassette (~1,111 bp) will be ligated into the pCAMBIA1305.2 expression vector to produce the recombinant plasmid, pCAMGpro (~12,981 bp) followed by Bioinformatics analysis of the AsGpro fragment.

1.5 Significance of study

The endosperm represents approximately 60% of the world's food supply (Xu *et al.*, 2010) yet only a few strong endosperm-specific promoters have been identified. This research is significant in availing a strong endosperm-specific promoter (*Avena sativa* α -globulin promoter) that will increase the production of endosperm-specific proteins in transgenic rice that would consequently contribute to improving the nutritional value and quantity of rice which must be increased between 25 - 45% to match-up the growing population of rice consumers (1.8% annually). This will also alleviate the food crisis facing the entire world.

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