ABSTRACT

Erythropoietin (*EPO*) is a glycoprotein hormone which plays a vital role in the regulation of the formation of red blood cells in mammals. Inadequate production of *EPO* was found to be one of the major causes of anaemia. Anaemia is also a frequent complication to many other diseases such as AIDS, chronic renal failure, rheumatoid arthritis, etc. In the mid-1980s, anaemia was treated with blood transfusion which however brought about many other problems. The advent of recombinant DNA technology proved to be a useful method for the mass production of recombinant *EPO*. However, finding a stable biofactory for the production of this protein was essential as the expression system needed to have certain attributes for the large and safe production of *EPO*. In this proof of concept experiment, the use of banana (*Musa acuminata*) has shown to be of great potential as an expression system for the production of recombinant *EPO.* Particle bombardment was also a suitable transformation method for the transient expression of the *EPO* gene. The transformation success of about 17-80% success (based on GFP expression and RT-PCR results) proved that meristems of banana were able to express the human *EPO* gene in its system in the period studied. Nevertheless, the production of the recombinant protein appeared to have stunted the growth of the banana plant and decreased the number of multiple shoots formed from the meristem compared to the controls. The use of the KDEL sequence in the gene construct potentially enables the retention of the *EPO* protein in the endoplasmic reticulum for higher expression levels. This study however did not analyse the expression levels of *EPO* with and without the KDEL in its construct. However, the transcription of *EPO* in both the constructs was observed based on the RT-PCR results further suggesting the successful transformation of the cells. This study shows that the

human *EPO* gene can be transformed in banana meristems and the *EPO* mRNA in the transformants were also shown to be successfully transcribed.

ABSTRAK

Erythropoietin (*EPO*) adalah suatu hormone glikoprotein yang memainkan peranan penting dalam mangawalatur proses pembentukan sel darah merah. Kekurangan penghasilan *EPO* merupakan salah satu sebab anemia. Anemia juga sering dikaitkan dengan pelbagai penyakit lain seperti AIDS, sakit buah pinggang yang kronik, rheumatoid artritis dan sebagainya. Pada pertengahan tahun 1980, pesakit anemia dirawat dengan transfusi darah yang kemudian didapati membawa kepada banyak masalah lain. Penemuan rekombinan DNA merupakan suatu kaedah yang amat berguna dalam penghasilan secara besaran protein *EPO*. Namun mencari suatu sistem pengekspresan yang stabil bagi penghasilan protein ini adalah penting kerana sistem ini haruslah mempunyai beberapa ciri-ciri yang penting bagi menghasilkan *EPO* yang banyak dan stabil. Dalam eksperimen pembuktiaan konsep ini, penggunaan meristem pisang (*Musa acuminata*) sebagai sistem pengekspresan telah dibuktikan sebagai suatu pilihan sistem yang efisen bagi produksi *EPO* rekombinan. 'Particle bombardment' merupakan suatu kaedah transformasi yang efisen bagi pengekspresan transient gen *EPO*. 17-80% kejayaan yang didapati dalam kajian ini membuktikan bahawa pisang adalah suatu sistem pengekspresan yang dapat mengekalkan gen *epo* manusia dalam jangka masa kajian ini dijalankan. Namun, produksi protein rekombinan ini telah membantutkan pertumbuhan pisang dan juga mengurangkan pertumbuhan mercu pucuk daripada meristem jika dibandingkan dengan kawalan. Penggunaan KDEL dalam vektor mempunyai potensi untuk mengekalkan produksi *EPO* di retikulum endoplasma (ER). Namun dalam kajian ini tahap ekspresi *EPO* dengan dan tanpa KDEL tidak dibuktikan. Tetapi, pengekspresan *epo* dalam kedua-dua jenis vektor ini dapat diperhatikan daripada keputusan RT-PCR. Kajian ini membuktikan bahawa kehadiran gen *EPO* manusia

adalah stabil dalam sistem kultur tisu pisang dan kehadiran mRNA *EPO* juga dapat dikenalpasti.

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Appendix

Abbreviations

BAP	6-benzylaminopurine
BLAST	Basic local alignment search tool
cDNA	complementary deoxyribonucleic acid
cm	centimetre
DNA	deoxyribonucleic acid
dNTP	deoxynucleotriphosphate
dH_20	distilled water
EPO	erythropoietin
ER	endoplasmic reticulum
et al	*et alia* (and other people)
GFP	green fluorescent protein
hr	hour
HDEL	His-Asp-Glu-Leu
hEPO	human erythropoietin
kb	kilo base
KDEL	Lys-Asp-Glu-Leu
LB	Luria-Bertani
MS	Murashige and Skoog media
mRNA	messenger ribonucleic acid
mg	milligram
mm	milimeter
mL	mililiter
mM	mili molar
M	molar
min	minute
NaOH	sodium hydroxide
NaCl	sodium chloride

ng	nanogram
PCR	polymerase chain reaction
pCEPO	pCAMBIA1304-epo
pCEPOKDEL	pCAMBIA1304-epo-KDEL
RT-PCR	reverse transcriptase polymerase chain reaction
rpm	revolutions per minute
SDS	sodium dodecyl sulphate
sec	second
TAE	trisacetate
TBE	trisborate
UV	ultra violet
µL	micro liter
°C	degree Celsius
%	percentage