Transformation of *Nicotiana tabacum* cv. Samsun with melanin and indigo genes

by

Anton Jordaan

A thesis submitted in partial fulfilment of the requirements for the degree of

Magister Scientiae

in the Faculty of Natural and Agricultural Sciences,

Department of Genetics,

University of Pretoria,

Pretoria

March 2003

Supervisor: Prof. A-M. Oberholster

UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

Declaration

I, the undersigned, hereby declare that the thesis submitted herewith for the degree Magister Scientiae to the University of Pretoria contains my independent work and has not been submitted for any degree at any other university.

Anton Jordaan

March 2003

nce upon a time there lived a young scientist

who dreamed of going on great adventures. Sometimes he would spend the whole day just reading stories of knights, dragons and wizards. And then he would wonder what he himself should do for his first great adventure. Are there still any beautiful princesses to rescue? Evil dragons to slay? Forgotten treasures to discover?

Then, one day, he thought again of all the stories that he has read about mysterious, magical flowers. He remembered that the stories tell of beautiful flowers with such strange and wonderful powers that many brave knights and mighty wizards went on dangerous quests, hoping to find even just one of these magical flowers. And the stories also tell of two flowers that were the most mysterious and magical of all: the blue rose and the black rose. But it is only in fairytales and legends that brave knights could ever hope of finding any of these mythical flowers. In this world no one has ever seen a real blue rose or black rose.

So the young scientist decided that he, too, will go on a quest to find the fabled blue and black roses. He knew that all the adventurers before him have failed, but that did not bother him too much. He was not going to search through faraway kingdoms like they did, or try to use some strange magic spell. Instead, his clever plan was to use the exciting new science of genetic engineering to find ways of turning flowers blue or black that no one else has ever thought of before…

Contents

List of abbreviations

3GT	UDPG-flavonoid-3-*O*-glucosyltransferase
A. tumefaciens	*Agrobacterium tumefaciens*
ANS	anthocyanidin synthase
bp	base pairs
BX1	indole synthase
CaMV35S	Cauliflower mosaic virus 35S
CHI	chalcone-flavanone isomerase
CHS	chalcone synthase
CTAB	cetyltriethylammonium bromide
cyt-P450	cytochrome P450
DFR	dihydroflavonol 4-reductase
DHI	5,6-dihydroxyindole
DHICA	5,6-dihydroxyindole-2-carboxylic acid
DHN	1,8-dihydroxynaphthalene
DIMBOA	2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one
DMF	dimethyl formamide
DMSO	dimethyl sulfoxide
DNA	deoxyribonucleic acid
dNTP	deoxyribonucleoside triphosphate
DOPA	dihydroxyphenylalanine
DTT	dithiothreitol
E. coli	*Escherichia coli*
EDTA	ethylenediamine tetra acetic acid
F3H	flavanone 3-hydroxylase
F3'H	flavonoid 3'-hydroxylase
F3'5'H	flavonoid 3',5'-hydroxylase
FAD	flavin adenine dinucleotide
FMN	flavin mononucleotide
GHB	glutaminyl-4-hydroxybenzene
HMW-PEG	high molecular weight polyethylene glycol
ido	*Rhodococcus* indole dioxygenase gene
IGL	indole-3-glycerol phosphate lyase
IPTG	isopropyl-β-D-thiogalactopyranoside
kDa	kilo Dalton

L-DOPA	L-dihydroxyphenylalanine
LB	Luria Bertani
melC1	*Streptomyces castaneoglobisporus* copper-transfer chaperone gene
mM	millimolar
MS	Murashige and Skoog
NADP	nicotinamide adenine dinucleotide phosphate
NOS	nopaline synthase
PCR	polymerase chain reaction
pg	picogram
PLP	pyridoxal phosphate
RNA	ribonucleic acid
RT-PCR	reverse transcription polymerase chain reaction
tnaK	tryptophanase gene
TSA	tryptophan synthase α subunit
TSB	tryptophan synthase β subunit
tyrC	*Streptomyces castaneoglobisporus* tyrosinase gene
U	units
w/v	weight/volume
µg	microgram
µl	microlitre
µM	micromolar

List of figures

List of tables