CHARACTERIZATION OF THE A/B REGULON IN TOBACCO
(Nicotiana tabacum)

Deborah G. Reed

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_____
John Jelesko, Chair

_____ _____
Carole Cramer Craig Nessler

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(ABSTRACT)

Plant alkaloids are secondary metabolites that may be synthesized in an inducible defense response to herbivory (Baldwin 1999). Genetic engineering of secondary metabolic pathways in plants to enhance or reduce metabolite production is limited by the current understanding of these pathways and their regulation in response to environmental conditions. This study was intended to provide new insights into the mechanism and regulation of alkaloid biosynthesis in *N. tabacum* by identifying genes that are coordinately regulated during conditions that induce alkaloid biosynthesis and by comparing their expression in regulatory mutant backgrounds that differ at two quantitative alkaloid loci, *A* and *B*. In order to identify novel genes that are differentially expressed during alkaloid biosynthesis, the transcriptional profiling procedure, fluorescent differential display (FDD), was used to screen total RNA isolated from Burley 21 (WT, *AABB*) and LA21 (low alkaloid regulatory mutant, *aabb*) tobacco root cultures that were induced for alkaloid synthesis. Four of thirteen cloned FDD fragments showed sequence homology to genes with defense-related functions. The differential expression of genes represented by selected FDD gene fragments was confirmed by comparing Northern blots of transcripts of those genes to known alkaloid biosynthetic genes, putrescine methyl transferase (*PMT3*), ornithine decarboxylase (*ODC3*), arginine decarboxylase (*ADC1*), and quinolinate phosphoribosyltransferase (*QPRT*). The role of the *A* and *B* loci in differential expression of genes represented by FDD clones and of known nicotine biosynthetic genes was examined using quantitative real time polymerase chain reaction (QRT-PCR) to measure transcript levels of these genes in four tobacco genotypes differing in alkaloid content, Burley 21(*AABB*), HI21 (*AAbb*), LI21(*aaBB*), and LA21 (*aabb*). Results of this study suggest that the *A/B* regulon is not limited to

alkaloid biosynthetic genes, but includes multiple genes with defense-related functions. QRT-PCR analysis of nicotine biosynthetic genes and genes represented by confirmed differentially expressed FDD clones showed increased mRNA accumulation in response to alkaloid induction in all the tested genotypes, which suggests that the *A* and *B* mutations affect overall mRNA accumulation levels, rather than gene inducibility, per se.

Baldwin, I.T. 1999. Inducible nicotine production in native Nicotiana as an example of adaptive phenotypic plasticity. Journal of Chem. Ecol. 25: 3-30.

Dedication

To Mike, Matt and Wyatt,
What better way to say, “I love you,” than with a thesis?

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Abbreviations

ACS = Acridone Synthase

ADC = Arginine Decarboxylase

β-ATPase = β-Adenosine Triphosphatase

AtPUP = *Arabidopsis thaliana* Purine Permease

B21 = Burley 21 Tobacco

BSA = Bovine Serum Albumin

BY-2 = Bright Yellow 2

CDP = (Disodium 2-chloro-5-[4-methoxyspiro(1,2-dioxetane-3,2'-[5chlorotricyclo[3.3.1.1]decan)]-4-yl)-1-phenyl phosphate)

CESA = Cellulose Synthase

CSL = Cellulose Synthase-like

CHS = Chalcone Synthase

cDNA = Complement Deoxyribonucleic Acid

DAO = Diamine Oxidase

DFMA = α-Difluoromethylarginine

DFMO = α-Difluoromethylornithine

DIG = Dioxygenin

DNase = Deoxyribonuclease

dNTP = 2'-Deoxynucleotide 5'-Triphosphate

DTT = Dithiothreitol

dUTP = Deoxyuridine 5'-Triphosphate

EREB = Ethylene Responsive Element Binding Protein

EST = Expressed Sequence Tag

F = M13 Forward Primer

Fd-GOGAT = Ferredoxin Glutamate Synthase

FDD = Fluorescent Differential Display

GBPLN = GenBank Plant Database

HI21 = High Intermediate 21 Tobacco

HPLC = High Performance Liquid Chromatography

HF = Hormone Free

IBA = Indolebutyric Acid

JA = Jasmonic Acid

JERE = Jasmonate- and Elicitor-Responsive Element

LA21 = Low Alkaloid 21 Tobacco

LB = Luria-Bertani Medium

LI21 = Low Intermediate 21 Tobacco

LOX = Lipoxygenase

LRP = Leucine Rich Protein

MAPK = Mitogen-Activated Protein Kinase

MAPKK = Mitogen-Activated Protein Kinase Kinase

MAPKKK = Mitogen-Activated Protein Kinase Kinase Kinase

MeJA = Methyl Jasmonate

MOPS = 3-(*N*-Morpholino)propanesulfonic Acid

MPO = Methyl Putrescine Oxidase

mRNA = Messenger Ribonucleic Acid

ODC = Ornithine Decarboxylase

ORCA = Octadecanoid-Responsive *Catharanthus* AP2-Domain Proteins

ORF = Open Reading Frame

PC = Proprotein Convertases

PCR = Polymerase Chain Reaction

PhyA = Phytochrome A

PMT3 = Putrescine Methyltransferase

QPRT = Quinolinate phoshphoribosyltransferase

QRT-PCR = Quantitative Real Time Polymerase Chain Reaction

R = M13 Reverse Primer

RNA = Ribonucleic Acid

RNasin = Ribonuclease Inhibitor

SA = Salicylic Acid

SAM = S-Adenosyl Methionine SPDS = Spermidine Synthase

SDS = Sodium Dodecyl Sulfate

SIPK = Salicylic Acid-Induced Protein Kinase

STR = Strictosidine Synthase

TAE = Tris Acetate Ethylenediaminetetraacetic Acid Buffer

TAP = Tomato Anionic Peroxidase

TBE = Tris Borate Ethylenediaminetetraacetic Acid Buffer

TIA = Terpenoid Indole Alkaloid

WIPK = Wound-Induced Protein Kinase