Phytochemistry 94 (2013) 10–27

Contents lists available at ScienceDirect

Phytochemistry

journal homepage: www.elsevier.com/locate/phytochem

Review

Molecular genetics of alkaloid biosynthesis in *Nicotiana tabacum*

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ARTICLE INFO

Article history:
Received 22 March 2013
Received in revised form 28 May 2013
Available online 15 August 2013

Keywords:
Nicotiana tabacum
Solanaceae
Nicotine
Nornicotine
Tobacco-specific nitrosamines
Alkaloid biosynthetic genes

ABSTRACT

Alkaloids represent an extensive group of nitrogen-containing secondary metabolites that are widely distributed throughout the plant kingdom. The pyridine alkaloids of tobacco (*Nicotiana tabacum* L.) have been the subject of particularly intensive investigation, driven largely due to the widespread use of tobacco products by society and the role that nicotine (16) (see Fig. 1) plays as the primary compound responsible for making the consumption of these products both pleasurable and addictive. In a typical commercial tobacco plant, nicotine (16) comprises about 90% of the total alkaloid pool, with the alkaloids nornicotine (17) (a demethylated derivative of nicotine), anatabine (15) and anabasine (5) making up most of the remainder. Advances in molecular biology have led to the characterization of the majority of the genes encoding the enzymes directly responsible the biosynthesis of nicotine (16) and nornicotine (17), while notable gaps remain within the anatabine (15) and anabasine (5) biosynthetic pathways. Several of the genes involved in the transcriptional regulation and transport of nicotine (16) have also been elucidated. Investigations of the molecular genetics of tobacco alkaloids have not only provided plant biologists with insights into the mechanisms underlying the synthesis and accumulation of this important class of plant alkaloids, they have also yielded tools and strategies for modifying the tobacco alkaloid composition in a manner that can result in changing the levels of nicotine (16) within the leaf, or reducing the levels of a potent carcinogenic tobacco-specific nitrosamine (TSNA). This review summarizes recent advances in our understanding of the molecular genetics of alkaloid biosynthesis in tobacco, and discusses the potential for applying information accrued from these studies toward efforts designed to help mitigate some of the negative health consequences associated with the use of tobacco products.

Contents

Abbreviations: ADC, arginine decarboxylase; BBL, berberine bridge enzyme-like; bHLH, basic helix–loop–helix; DMN, dihydrometanicotine; ERF, ethylene response factor; JA, jasmonic acid; JAZ, jasmonate ZIM-domain; MAPKK, mitogen-activated protein kinase kinase; MATE, multidrug and toxic compound extrusion; MeJA, methyl jasmonate; MPO, *N*-methylputrescine oxidase; MTHFR, methylenetetrahydrofolate reductase; NAB, *N*-nitrosoanabasine; NAMN, nicotinic acid mononucleotide; NaNG, nicotinic acid ß-N-glucoside; NAT, *N*-nitrosoanatabine; NND, nicotine *N*-demethylase; NNK, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone; NNN, *N*-nitrosonornicotine; NS, nicotine synthase; NUP, nicotine uptake permease; ODC, ornithine decarboxylase; PMT, putrescine methyltransferase; PON, pseudooxynicotine; QPT, quinolinate phosphoribosyltransferase; RNAi, RNA interference; TSNA, tobacco-specific nitrosamine.

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http://dx.doi.org/10.1016/j.phytochem.2013.06.002

1. Introduction

Tobacco products have been smoked or chewed by Native Americans for thousands of years for medicinal, spiritual, and recreational purposes (Winter, 2000). After discovery of the new world, tobacco was introduced to Europe and rapidly spread throughout the world. Currently, there are about 1.3 billion people, roughly one fifth of the world population, who use tobacco products (Rabinoff, 2007), despite the fact that the majority of users are aware of the negative health and economic consequences associated with smoking. The popularity and widespread use of tobacco products can be attributed to the stimulatory and addictive effects of the pyridine alkaloid nicotine (16) via the action of this compound on neuronal nicotinic acetylcholine receptors in the brain (Stein et al., 1998).

Commercial tobacco cultivars typically produce alkaloids at levels between 2% and 4% of total dry weight, with nicotine (16) accounting for ~90% of the total alkaloid content (Saitoh et al., 1985). The structurally related compounds nornicotine (17), anatabine (15) and anabasine (5) constitute nearly all of the remaining 10% of the alkaloid pool. The ratios of the various pyridine alkaloids vary greatly among the different species of *Nicotiana*. In surveys of 60+ members of the genus *Nicotiana*, nicotine (16) was shown to be the predominant alkaloid in over half of the species, while nornicotine (17) was the most abundant alkaloid in about 40% of the species tested (Saitoh et al., 1985; Sisson and Severson, 1990). In a small number of species (e.g. *N. glauca* and *N. debneyi*), anabasine (5) is the major alkaloid produced.

Pyridine alkaloids serve as defensive compounds against herbivores, both within the context of native plant habitats (Baldwin et al., 2001; Voelckel et al., 2001; Steppuhn et al., 2004) and when used as insecticides in agricultural applications (Schmeltz, 1971). When plants of some *Nicotiana* species are attacked by insects or other animals, alkaloid levels become quickly elevated. The insecticidal properties of nicotine (16) are believed to be mediated through its stimulation of acetylcholine receptors, resulting in a continual excitation of neurons that leads to insect paralysis and ultimately death (Baldwin et al., 2001).

Historically, research on nicotine (16) and other tobacco alkaloids has focused mainly on either the pharmacological or insecticidal properties of these compounds. However, as progress has been made toward elucidating the specific components of tobacco products (and the derivative smoke) that are associated with disease, considerable attention has also been directed toward the role of tobacco alkaloids as precursors in the formation of a class of compounds termed tobacco-specific nitrosamines (TSNAs). Green tobacco plants are virtually devoid of TSNAs, but during post-harvest curing and/or fermentation of the leaf, reactions between the pyridine alkaloids and nitrosating species such as NO, NO_2, N_2O_3 and N_2O_4 lead to their formation (Bush et al., 2001). The observations that certain of these TSNAs are able to induce a variety of tobacco-associated cancers across multiple animal systems, and form mutagenic DNA adducts in smokers, have sparked much interest in efforts to reduce their levels in tobacco products (Hecht, 1998, 2003, 2008).

In this review, recent advances in the molecular genetics of tobacco alkaloids will be summarized, with particular emphasis given to the genes responsible for the production and regulation of nicotine (16) and its demethylated derivative nornicotine (17). Insights relevant to the molecular evolution of tobacco that have been revealed as a result of the investigation of alkaloid biosynthetic genes will also be highlighted. Finally, the potential for using the existing gene information toward lessening the harmful effects of tobacco use will be discussed.

2. Classical genetic studies

2.1. A and B loci

During the 1930s, certain Cuban cigar tobaccos being grown in Germany were found to exhibit very low alkaloid contents. The low alkaloid trait was introduced into several commercial U.S. varieties in anticipation that products from these lines might be desired by consumers who were particularly sensitive to throat irritation associated with nicotine (16) (Valleau, 1949). Through the crossing and selection of the low alkaloid trait from Cuban cigar varieties into cultivar Burley 21, it was firmly established that the alkaloid content of tobacco is genetically controlled to a large extent by two non-linked loci, designated *A* and *B* (Legg et al., 1969). Since 1994, the *A* and *B* genetic loci have been frequently cited in the literature using the alternative nomenclature *Nic1* and *Nic2*, respectively, to reflect their close relationship with plant nicotine (16) content (Hibi et al., 1994). Given that subsequent studies have shown that these regulatory loci also control the expression of numerous genes unrelated to nicotine (16) biosynthesis (Kidd et al., 2006; Shoji et al., 2010), the original *A* and *B* terminology is arguably more appropriate than the *Nic1*/*Nic2* designation for these loci, as the latter implies function restricted to the control