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# Structure and expression of the quinolinate phosphoribosyltransferase (*QPT*) gene family in *Nicotiana*

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ABSTRACT

Synthesis of wound-inducible pyridine alkaloids is characteristic of species in the genus *Nicotiana*. The enzyme quinolinate phosphoribosyltransferase (QPT) plays a key role in facilitating the availability of precursors for alkaloid synthesis, in addition to its ubiquitous role in enabling NAD(P)(H) synthesis. In a previous study, we reported that *Nicotiana tabacum* L. var. NC 95 possesses a *QPT* RFLP pattern similar to its model paternal progenitor species, *Nicotiana tomentosiformis* Goodsp. Here we show that although some varieties of *N. tabacum* (e.g. NC 95 and LAFC 53) possess *QPT* genomic contributions from only its paternal progenitor species, this is not the case for many other *N. tabacum* varieties (e.g. Xanthi, Samsun, Petite Havana SR1 and SC 58) where genomic *QPT* sequences from both diploid progenitor species have been retained. We also report that QPT is encoded by duplicate genes (designated *QPT1* and *QPT2*) not only in *N. tabacum*, but also its model progenitor species *Nicotiana sylvestris* Speg. and Comes and *N. tomentosiformis* as well as in the diploid species *Nicotiana glauca* Graham. Previous studies have demonstrated that the *N. tabacum QPT2* gene encodes a functional enzyme via complementation of a *nadC*⁻ *Escherichia coli* mutant. Using a similar experimental approach here, we demonstrate that the *N. tabacum QPT1* gene also encodes a functional QPT protein. We observe too that *QPT2* is the predominate transcript present in both alkaloid and non-alkaloid synthesising tissues in *N. tabacum* and that promoter regions of both *QPT1* and *QPT2* are able to produce GUS activity in reproductive tissues. In *N. tabacum* and in several other *Nicotiana* species tested, *QPT2* transcript levels increase following wounding or methyl jasmonate treatment whilst *QPT1* transcript levels remain largely unaltered by these treatments. Together with conclusions from recently published studies involving functional interaction of MYC2-bHLH and specific ERF-type and transcription factors with *QPT2*-promoter sequences from *N. tabacum*, our results suggest that whilst both members of the *QPT* gene family can contribute to the transcript pool in both alkaloid producing and non-producing tissues, it is *QPT2* that is regulated in association with inducible defensive pyridine alkaloid synthesis in species across the genus *Nicotiana*.

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## 1. Introduction

Alkaloids are a structurally diverse group of nitrogen-containing secondary metabolites that have a widespread distribution in the plant kingdom [1–3]. Pyridine alkaloids such as anabasine, anatabine, nicotine and its de-methylated derivative nornicotine, are characteristically found in leaves and/or roots of the ~75 *Nicotiana* species that are naturally distributed in North and South America, mainland Australia, a few Pacific Islands and Southern Africa [4–7]. It is generally accepted that pyridine alkaloid synthesis in *Nicotiana* species represents a resource expensive, but evolutionary adaptive response, capable of protecting plants against excessive insect herbivory [8–10]. Reduction in pyridine alkaloid levels, produced via the down-regulation of *PMT* transcript levels, was associated with a diminished capacity of transgenic *Nicotiana attenuata* plants to resist insect damage in their native habitat [11]. In a separate study, low alkaloid mutants of *Nicotiana tabacum* were observed to be more susceptible to insect attack than their near-isogenic high alkaloid counterparts [12]. Acting agonistically at acetylcholine (cholinergic) receptors, pyridine alkaloids are also harmful to vertebrates and consumption of alkaloid-containing leaves of *Nicotiana* has been linked with teratogenic, abortive

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or fatal consequences in both grazing mammals and humans [13–17].

Classical experiments undertaken by Dawson and co-workers more than half a century ago showed that nicotine is produced in roots of *N. tabacum*, from where it is transported to and stored in aerial tissues [18–20]. The capacity for nicotine synthesis has been shown to increase in healthy roots of *N. tabacum* and *Nicotiana sylvestris* following damage to aerial tissues [21,22], or surgical removal of the shoot apex [23]. Experiments involving *Nicotiana* plants [9,24–28], cell cultures [29–33] and axenic root cultures [28,34–36] have also indicated that the wound hormone methyl jasmonate acts to stimulate expression of key genes needed for alkaloid synthesis in these tissues.

Quinolinate phosphoribosyltransferase (EC 2.4.2.19) (QPT) functions as the anapleurotic enzyme of the pyridine nucleotide cycle and is thus a key component of primary metabolism [37–39] and also secondary metabolism in pyridine alkaloid producing species [40–42]. *QPT* transcript is readily detectable in alkaloid-synthesising root tissues of a number of *Nicotiana* species, and levels are enhanced in roots of *N. tabacum* and its maternal progenitor species *N. sylvestris*, following damage to aerial tissues [43,44]. Low levels of *QPT* transcript are also present in leaf tissues of *N. sylvestris* and *N. tabacum* but, in contrast to roots, do not increase in response to wounding [34,35]. Antisense-mediated suppression of *QPT* gene activity has been reported to enable production of *N. tabacum* with extremely low levels of nicotine levels in leaf tissues [45]. Following wound-associated stress, elevated *QPT* transcript levels are correlated with increased expression of other key structural alkaloid biosynthetic genes, such as *ODC, MPO, PMT* and *A622* [29,30,34–36,46–50]. Recent analyses have indicated that MYC2-bHLH and specific AP2/ERF transcription factors play a key role in mediating jasmonate-induced expression of these genes [51–57].

*N. tabacum* is an allotetraploid species, derived from parental species closely related to modern *N. sylvestris* and *Nicotiana tomentosiformis* [58–60]. Consistent with a previous report [44], we observe here that the high alkaloid variety NC 95 and its near isogenic low alkaloid derivative LAFC 53, possess *QPT* genomic contributions from only one progenitor species (*N. tomentosiformis*). In contrast several other commonly studied varieties of *N. tabacum* (e.g. Xanthi, Samsun, Petite Havana SR1 and SC 58) contain *QPT* genomic contributions from both model progenitor species *N. tomentosiformis* and *N. sylvestris*. We identified two distinct classes of *QPT* genes (*QPT1* and *QPT2*) in *N. tabacum*, and also its model progenitor species. As previously demonstrated for *QPT2* [44], we show that *QPT1* from *N. tabacum* encodes functional QPT, via complementation of a *nadC*$^{-}$ *Escherichia coli* mutant. In *N. tabacum*, both *QPT* genes are expressed in alkaloid and non-alkaloid synthesising tissues, however, *QPT2* is the predominant transcript present and is strongly induced in response to wounding or methyl jasmonate treatment in alkaloid synthesising root tissues. Predominant expression and wound-associated induction of *QPT2* was also observed in alkaloid-producing tissues from other members of the genus *Nicotiana*.

## 2. Materials and methods

### 2.1. Plant materials, wounding and jasmonate treatments

Laboratory seed stocks from selfed individuals of *N. tabacum* varieties, *Nicotiana debneyi* Domin., *N. tomentosiformis*, *N. sylvestris* and *Nicotiana glauca* have been described previously [34,44,61]. A single, non-flowering plant of *Nicotiana suaveolens* Lehm. was sourced from a native population on Flinders peak in the You Yangs regional park near Melbourne, Victoria, Australia (37°57′S 144°25′E), and seeds were collected after growth and selfing in an insect-proof greenhouse. Seeds of *N. sylvestris* 56A, *N. otophora* Griseb. 38A, together with seeds of many varieties in the *N. tabacum* var. NC 95 lineage were kindly provided by Dr Verne Sisson, previously of North Carolina State University and curator of the National Plant Germplasm System's *Nicotiana* collection. Seeds of *Solanum lycopersicum* cv. Roma were obtained commercially (Yates, Australia). Growth of plants in soil/hydroponics and wounding treatments were undertaken as described previously [34,35].

Clonal hairy root cultures of *N. tabacum* var. NC 95 and var. SC 58, *N. suaveolens* and *N. debneyi* were generated from *Agrobacterium rhizogenes* LBA 9402 leaf disc infections as described previously [61–64]. To stimulate alkaloid synthesis, healthy root cultures at the early stages of the rapid growth phase of each culture (10–12 days after subculture), were treated in duplicate with 2.5 μM and also 25 μM of methyl jasmonate for 16 h as described previously [35,65].

### 2.2. Southern hybridisation analysis

Restriction digest analysis of genomic DNA by Southern hybridisation was undertaken using $\alpha^{32}$P-dATP labelled DNA probes [44] generated using coding sequences of *QPT, PMT, ADC*, *ODC*, *SamS* and *SamDC* genes as described elsewhere [63]. These probes had previously been shown to detect these genes on Southern blots [65,66].

### 2.3. Isolation of QPT gene sequences from N. tabacum and N. glauca genomic libraries

Genomic phage libraries of *N. tabacum* cv. Xanthi (Clontech, USA) and *N. glauca* [67], each representing 3–4 genomic equivalents, were screened at high stringency using *N. rustica QPT* cDNA [44] as a probe. Two classes of *QPT* genes were identified from each species, and designated as either *QPT1* or *QPT2*. Representative genes were sequenced and likely intron/exon boundaries identified by comparisons to previously determined *Nicotiana QPT* cDNA sequences ([44]; accession numbers AJ243436 and AJ243437). Intron/exon boundaries were confirmed via subsequent sequencing of the cDNA sequences recovered by RT-PCR from each species. *QPT* genomic sequences from *N. tabacum* and also *N. glauca*, each containing 10 exons and ~1–2 kb of 5′ regulatory sequence were deposited in the NCBI database (*N. tabacum* accession numbers; AJ748262 (*NtQPT1a*) and AJ748263 (*NtQPT2a)*; *N. glauca* accession numbers AM922108 (*NgQPT1*) and AM922107 (*NgQPT2*)).

### 2.4. QPT phylogenetic analysis

Genomic DNA was isolated from the leaves of healthy plants using a standard CTAB-based extraction method [68]. PCR amplification of genomic *QPT* sequences was undertaken using a forward oligonucleotide within the first exon (either QPTexon1F1: *5′ GAATTCTATGTTTAAAGTTTTTCCTTTCACTG 3′*; QPTexon1F2: *5′ GAATTCTATGTTTAGGGCTCTTCCTTTCAC 3′*; or QPTexon1F3: *5′ GCATCCATATGCAATTAC 3′*) in conjunction with a reverse oligonucleotide in exon 3 (QPTexon3R1: *5′ CGCGAATATCATCTCAGCAA 3′*). PCR conditions were 94 °C 2 min, 1 cycle; followed by 30 cycles of 94 °C 0.5 min, 52–55 °C 1 min, and 72 °C 1 min: 72 °C 5 min 1 cycle. PCR products were ligated into pGEM-T easy (Promega), and following transformation into chemically competent *E. coli* (DH5α) cells, plasmid DNA was isolated (Promega Wizard® Plus SV Minipreps DNA Purification System). The PCR cDNA inserts were sequenced and trimmed of oligonucleotide binding sites, to produce partial genomic *QPT* sequences each of ~1.1 kb in length, spanning the 3′ end of exon 1 into the 5′ region of exon 3. *QPT* sequences were aligned manually in BioEdit version 7.0.41 [69] and gaps encoded as binary characters using GapCoder [70]. *PAUP** version 4.0 beta

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