10.1071/FP15045_AC

Supplementary Material: *Functional Plant Biology*, 2015, 42(8), 792–801.

Supplementary Material

Alkaloid production and capacity for methyljasmonate induction by hairy roots of two species in Tribe Anthocercideae, family Solanaceae

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Supplementary File 1. Partial molecular analysis of *QPT* gene activity in Anthocercideae hairy roots of *Anthocercis ilicifolia* subsp, *ilicifolia* and *Cyphanthera tasmanica*

Partial *QPT* cDNA sequences were recovered from *QPT* specific transcript pools of *A. ilicifolia* (*Ai*) and *C. tasmanica* (*Ct*) hairy root cultures. Briefly, total RNA was isolated from hairy roots primed for first strand cDNA synthesis using AMV-reverse transcriptase (Promega) in accordance with manufacturer's instructions and using a reverse oligonucleotide (5'-CCGTTAGATAATGGAACAACCA-3') representing a conserved region in exon 8 of *Nicotiana QPT*1 and *QPT*2 genes (Ryan *et al*. 2012). One μL of total cDNA reaction was then used in a standard PCR reaction (94^{o}C 30 sec; 51^{o}C 1 min; 72^{o}C 1 min; 30 cycles) using oligonucleotides representing conserved regions of *Nicotiana QPT* genes in exon1 and exon 6 (Forward = 5'-CCAAGGTTGGTGGTGAAAATG-3' and Reverse = 5'-GGAGCAGTTTTCCTAGTCTCC-3'). Two closely related forms of QPT cDNA sequences were recovered from hairy root cDNA of both Anthocercideae species which were designated *AiQPT*i and *AiQPT*ii for sequences from *A.ilicifolia* and *CtQPT*a and *CtQPT*b for sequences from *C.tasmanica*. Supplementary Figure 1 shows a

comparison of these gene sequences (minus sequences of the oligonucleotides used to recover them by PCR) with each other and also homologous regions of corresponding regions of *NtQPT*1 and *NtQPT*2 genes from *N. tabacum*, previously identified as being derived from the *N. sylvestris* ancestral parental genome (Ryan *et al*., 2012).

A cDNA capture and sequencing approach was then used to estimate relative expression of each *QPT* gene type in hairy roots of both Anthocercideae species, as described by Ryan *et al*. (2012) to elucidate *QPT* version and sub-genome representation in transcripts from leaf and root tissues of *N. tabacum* varieties. Briefly, PCR products were purified (PCR purification kit, QIAgen) and ligated into pGEM-T easy Vector System (Promega). Ligation reactions were transformed into $CaCl_2$-heat shock competent *E. coli* XL-1 cells and approximately 20 individual colonies, growing on LB Amp medium, were randomly selected for plasmid isolation and DNA sequencing of inserts as described previously (Ryan *et al*. 2012). Results indicated that approximately 75% of *QPT* transcripts recovered from hairy roots of *A.ilicifolia* were of the *AiQPT*i type form, both in control hairy roots and those harvested 16 h after MeJa treatment (Supplementary Table 1). In contrast, approximately 90% of recovered transcripts from hairy roots of control hairy roots of *C.tasmanica* belonged to the *CtQPT*b class. Additionally, all sequences that were recovered from MeJa-treated hairy roots of *C.tasmanica* were observed to of *CtQPT*b type. Previous studies have reported the existence of duplicate functional paralogues of the *QPT* gene in *Nicotiana* species, designated *QPT*1 and *QPT*2, with the latter being preferentially upregulated following wounding or MeJa treatment of alkaloid-producing tissues (Shoji *et al*. 2011; Ryan *et al*. 2012). It may be noteworthy however that the characteristic indel in exon 2, which distinguishes *QPT*1 from *QPT*2 in *Nicotiana,* is not a feature of cDNA sequences representing *AiQPT*i and *AiQPT*ii or *CtQPT*a and *CtQPT*b (Supplementary Figure 1). Other indel differences do exist in exon 2 between the *Nicotiana* and Anthocercideae cDNA sequences noted here, but in each case are characteristic of both *C. tasmanica* sequences and both *A. ilicifolia* sequences (Supplementary Figure 1). Further work is required to determine whether there is any functional significance of these observations

relating to the capacity of hairy roots of Anthocercideae species to produce pyridine alkaloids in response to wound-associated stress.

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10          20          30          40          50          60          70
NtxQPT1   GTTGTGAAAA TGTCAGCAAT AGCCACCAAA AATGCA---G TGGAGTCATT AGTAGTGAAG ---CCACCAG
NtxQPT2   GTGGTGAAAA TGTCAGCAAT AGCCACCAAG AATACAAGAG TGGAGTCATT AGAGGTGAAA ---CCACCAG
AiQPTi    GTGGTGAAAA TGTCAGCAAT AGCGGCAAAG AATGCAACAA TGGAGTCATT AGCAATGAAG GCTCCTCCAG
AiQPTii   GTGGTGAAAA TGTCAGCAAT AGCGGCAAAG AATGCAACAA TGGAGTCATT AGCAATGAAG GCTCCTCCAG
CtQPTa    GTG---AAAA TGTCAGCAAT AGCCGCAAAG AATGCAACAG TGGAGTCATT AGCAGTGAAG GCTCCTCCAG
CtQPTb    GTG---AAAA TGTCAGCAAT AGCCGCAAAG AATGCAACAG TGGAGTCATT AGCAGTGAAG GCTCCTCCAG
          Ex-2

                 80          90         100         110         120         130         140
NtxQPT1   CACACCCAAC TTATGATTTA AAGGGTGTTA TTCAACTTGC CCTCTCTGAA GATGCTGGGG ATTTAGGAGA
NtxQPT2   CACACCCAAC TTATGATTTA AAGGGAGTTA TGCAACTTGC ACTCTCTGAA GATGCTGGGA ATTTAGGAGA
AiQPTi    CACACCCAAC TTATGATTTA AAGAGTGTTA TTCAACTTGC ACTCTCTGAA GATGCTGGGA ATTTAGGAGA
AiQPTii   CACACCCAAC TTATGATTTA AAGAGTGTTA TTCAACTTGC ACTCTCTGAA GATGCTGGGA ATTTAGGAGA
CtQPTa    CACACCCAAC TTATAATTTA AAGGGTGTTA TTCAACTTGC ACTCTCCGAA GATGCTGGGG ATTTAGGAGA
CtQPTb    CACACCCAAA TTATGATTTA AAGGGTGTCA TTCAACTTGC ACTCTCCGAA GATGCTGGAA ATTTAGGAGA
                                                                            Ex-3

                150         160         170         180         190         200         210
NtxQPT1   TGTGACTTGT AAGGCAACAA TTCCTATTGA CATGGAATCC GAAGCTCATT TTCTAGCAAA GGAAGACGGG
NtxQPT2   TGTGACTTGT AAGGCGACAA TTCCTCTTGA TATGGAATCC GATGCTCATT TTCTAGCAAA GGAAGACGGG
AiQPTi    TGTGACTTGT AAGGCGACAA TTCCTGTTGA TATGGAATCC GAAGCTCATT TTCTAGCAAA AGAAGACGGG
AiQPTii   TGTGACTTGT AAGGCGACAA TTCCTGTTGA TATGGAATCC GAAGCTCATT TTCTAGCAAA AGAAGACGGG
CtQPTa    TGTGACTTGT AAGGCGACAA TTCCTGTTGA TATGGAATCC GAAGCTCATT TTCTAGCAAA AGAAGATGGG
CtQPTb    TGTGACTTGT AAGGCGACAA TTCCTGTTGA CATGGAATCC GAAGCTTATT TTCTAGCAAA AGAAGACGGG

                220         230         240         250         260         270         280
NtxQPT1   ATTGTAGCAG GAATTGCACT TGCTGAGATG ATATTCGCAG AGGTTGATCC TTCACTAAAG ATGGAGTGGT
NtxQPT2   ATCATAGCAG GAATTGCACT TGCTGAGATG ATATTCGCGG AAGTTGATCC TTCATTAAAG GTGGAGTGGT
AiQPTi    ATTGTAGCAG GAATTGCACT TGCTGAGATG ATATTCGCAG AAGTTGATCC TTCACTAAAG GTGGAGTGGT
AiQPTii   ATTGTAGCAG GAATTGCACT TGCTGAGATG ATATTCGCAG AAGTTGATCC TTCACTAAAG GTGGAGTGGT
CtQPTa    ATTGTAGCAG GAATTGCTCT TGCTGAGATG ATATTCGCAG AAGTTGATCC TTCACTAAAG GTGGAGTGGT
CtQPTb    ATTGTAGCAG GAATTGCACT TGCTGAGATG ATATTTGCAG AAGTTGATCC TTCACTAAAG GTGGAGTGGT
                                                                        Ex-4
                290         300         310         320         330         340         350
NtxQPT1   CTATAAATGA TGGTGATAAA GTTCATAAAG GCTTGAAATT CGGCAAAGTA CAAGGAAAGG CTCACAGCAT
NtxQPT2   ATGTAAATGA TGGCGATAAA GTTCATAAAG GCTTGAAATT TGGCAAAGTA CAAGGAAACG CTTACAACAT
AiQPTi    CTATAAAGGA TGGTGATGTA GTTCATAAAG GCTTGAAGTA TGGTAAAGTA CAAGGAAATG CTTACAACAT
AiQPTii   CTATAAAGGA TGGTGATGTA GTTCATAAAG GCTTGAAATA TGGTAAAGTA CAAGGAAATG CTTACAACAT
CtQPTa    CTATAGAGGA TGGTGATATA GTTCATAAAG GCTTGCAATA TGGTAAAGTA CAAGGAAATG CTCACAGCAT
CtQPTb    CTATAAAGGA TGGTGATATA GTTCATAAAG GCTTGAAATT TGGTAAAGTA CAAGGAAATG CTCACAGCAT
                                                                Ex-5

                360         370         380         390         400         410         420
NtxQPT1   TGTTATAGCT GAGAGAGTTG TTCTCAATTT CATGCAAAGA ATGAGCGGAA TAGCTACACT AACTAAGGCG
NtxQPT2   TGTTATAGCT GAGAGGGTTG TTCTCAATTT TATGCAAAGA ATGAGTGGAA TAGCTACACT AACTAAGGAA
AiQPTi    TGTTATAGCT GAGAGGGTTG TTCTCAATTT TATGCAAAGA ATGAGTGGAA TAGCTACACT AACTAAGGCA
AiQPTii   TGTTATAGCT GAGAGGGTTG TTCTCAATTT TATGCAAAGA ATGAGTGGAA TAGCTACACT AACTAAGGCA
CtQPTa    TGTTATAGCT GAGAGGGTTG TTCTCAATTT TATGCAAAGA ATGAGTGGAA TAGCTACACT AACTAAGGCA
CtQPTb    TGTTATAGCT GAGAGGGTTG TTCTCAATTT TATGCAAAGA ATGAGTGGAA TAGCTACACT AACTAAGGCA
                                                                            Ex-6

                    430         440         450
NtxQPT1   ATGGCAGATG CTGCACACCC TGCTACCATC TT
NtxQPT2   ATGGCAGATG CTGCACACCC TGCTTACATC TT
AiQPTi    ATGGCAGATG CTGCACACCC TGCTTTTATC TT
AiQPTii   ATGGCAGATG CTGCACACCC TGCTTTTATC TT
CtQPTa    ATGGCAGATG CTGCACACCC TGCTTATATC TT
CtQPTb    ATGGCAGATG CTGCATACCC TTCTTATATC TT
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Fig. S1. Partial cDNA sequence information of *QPT* cDNA sequences recovered from control hairy roots of *A.ilicifolia* (*AiQPT*) and *C.tasmanica* (*CtQPT*) compared to homologous regions *QPT*1 and *QPT*2 genes from *N.tabacum* (Ryan *et al*. 2012). The first base of relevant exons is shown in blue bold type, with **Ex-2-Ex-6** (below) representing the beginning of Exon 2-Exon 6. *Nicotiana QPT* genes consist of 10 exons (Ryan *et al*. 2012).

Table S1. Percentage of each form of *QPT* recovered from *QPT* cDNA pools derived from RNA of ethanol control or 2.5µM MeJA-treated tissues of *A. ilicifolia* and *C. tasmanica* hairy root cultures

n = number of individual bacterial colonies randomly picked from selection medium and inserts sequenced to identify *QPT* cDNA type which had been sub-cloned

	Treatment	***QPTa***	***QPTb***	***QPTi***	***QPTii***
A. ilicifolia	EtOH control (n=20)	–	–	75	25
	MeJA treated (n=20)	–	–	75	25
C. tasmanica	EtOH control (n=21)	9	91	–	–
	MeJA treated (n=20)	0	100	–	–