

**Supplementary Table 1. *O*- and *N*-methyltransferases show to accept BIA substrates in vitro, which have been characterized at the molecular level.** Specificity refers to regiospecificity (2,6,7,9,3',4'), stereospecificity [(*R*) or (*S*)] and to the number of pre-existing bonds on the nitrogen atom (2'*N*, 3'*N*). † represents features not conclusively demonstrated, ‡ represents activities that have been demonstrated but are quite minor or weak, "*nd*" indicates that no activities have been detected and "not tested" indicates that no *in vitro* assays were performed. Positions denoted by numbering scheme are presented visually in Supplementary Figures 4 and 5. For enzymes with many reported substrates, minor activities are not listed.

Plant species	Short name	Enzyme full name	Genbank ID	<i>In vitro</i> substrate(s)	Specificity	Functional evidence <i>in planta</i>	Reference(s)
<i>Glaucium flavum</i>	GfIOMT1	<i>O</i> -methyltransferase 1	KP176693	( <i>R,S</i> )-Norlaundanosoline ( <i>R,S</i> )-6- <i>O</i> -Methylnorlaundanosoline ( <i>R,S</i> )-6- <i>O</i> -Methylaudanosoline ( <i>R,S</i> )-Tetrahydrocolumbamine ( <i>S</i> )-Reticuline ( <i>R,S</i> )-Scoulerine	6,7,3',4'	High expression in stem & flower bud correlating with accumulation of glaucine	Chang <i>et al.</i> , 2015
	GfIOMT2	<i>O</i> -methyltransferase 2	KP176694	( <i>R,S</i> )-Norlaundanosoline ( <i>R,S</i> )-Scoulerine ( <i>R,S</i> )-Tetrahydrocolumbamine ( <i>S</i> )-Reticuline	6,3',4'	High expression in root tissue	Chang <i>et al.</i> , 2015
	GfIOMT3	<i>O</i> -methyltransferase 3	KP176695	<i>nd</i>		Very low expression in all tissues	Chang <i>et al.</i> , 2015
	GfIOMT4	<i>O</i> -methyltransferase 4	KP176696	<i>nd</i>		Moderate expression level in all tissues examined	Chang <i>et al.</i> , 2015
	GfIOMT5	<i>O</i> -methyltransferase 5	KP176697	<i>nd</i>		Moderate expression level in stem tissue, all others low	Chang <i>et al.</i> , 2015
	GfIOMT6	<i>O</i> -methyltransferase 6	KP176698	( <i>R,S</i> )-Scoulerine ( <i>S</i> )-Reticuline ( <i>R,S</i> )-Tetrahydrocolumbamine ( <i>R,S</i> )-Norreticuline	2,9 7,3'	High expression in root, leaf and flower bud tissues	Chang <i>et al.</i> , 2015
	GfIOMT7	<i>O</i> -methyltransferase 7	KP176699	( <i>R,S</i> )-Scoulerine ( <i>R,S</i> )-Tetrahydrocolumbamine	2,9	Moderate expression level in root, stem and flower bud tissues	Chang <i>et al.</i> , 2015
	GfINMT1	Coclaurine <i>N</i> -methyltransferase		( <i>S</i> )-Coclaurine Heliamine	2' <i>N</i>		Hagel <i>et al.</i> , 2015b
	GfINMT2 (GfTNMT)	Tetrahydroprotoberberine <i>N</i> -methyltransferase		( <i>R,S</i> )-Stylopine ( <i>S</i> )-Canadine			Hagel <i>et al.</i> , 2015b
	GfINMT3	<i>N</i> -methyltransferase 3		( <i>S</i> )-Reticuline ( <i>R,S</i> )-Stylopine	3' <i>N</i>		Hagel <i>et al.</i> , 2015b
	GfINMT4	<i>N</i> -methyltransferase 4		( <i>S</i> )-Coclaurine ( <i>S</i> )-Reticuline	2' <i>N</i> , 3' <i>N</i>		Hagel <i>et al.</i> , 2015b
	GfINMT5	<i>N</i> -methyltransferase 5		( <i>S</i> )-Reticuline ( <i>S</i> )-Coclaurine	3' <i>N</i> , 2' <i>N</i> <sup>†</sup>		Hagel <i>et al.</i> , 2015b
	GfINMT6	<i>N</i> -methyltransferase 6		( <i>S</i> )-Coclaurine	2' <i>N</i>		Hagel <i>et al.</i> , 2015b
<i>Papaver somniferum</i>	Ps6OMT (PsOMT2)	Norcoclaurine 6- <i>O</i> -methyltransferase	AY217335, AY268894	( <i>R,S</i> )-Norcoclaurine ( <i>R,S</i> )-Norprotosinomenine Isorientaline	6,7	Majority of BIAs content reduced by VIGS knockdown	Facchini & Park, 2003; Ounarooun <i>et al.</i> , 2003; Desgagné-Penix & Facchini, 2012
	Ps7OMT (PsOMT1)	Reticuline 7- <i>O</i> -methyltransferase	AY268893	( <i>R,S</i> )-Reticuline ( <i>R,S</i> )-Orientaline ( <i>R,S</i> )-Protosinomenine ( <i>R,S</i> )-Isoorientaline	6,7,4' <i>S</i> > <i>R</i>	<i>N</i> -methylated BIAs (eg. laudanine) content reduced by VIGS knockdown; Morphinans and noscapine content increased by VIGS knockdown	Ounarooun <i>et al.</i> , 2003; Desgagné-Penix & Facchini, 2012
	PsSOMT1	Scoulerine-9- <i>O</i> -methyltransferase	JN185323	( <i>S</i> )-Scoulerine ( <i>S</i> )-Norreticuline ( <i>S</i> )-Reticuline	9,2 3',7	Noscapine, papaverine and pathway intermediates decreased by VIGS knockdown; Transcript abundance correlates with noscapine accumulation in cultivars	Dang & Facchini, 2012
	PsSOMT2	<i>O</i> -methyltransferase 2	JN185324	( <i>S</i> )-Scoulerine	9	Noscapine decreased by VIGS knockdown; Transcript abundance correlates with noscapine accumulation in cultivars	Dang & Facchini, 2012
	PsSOMT3	<i>O</i> -methyltransferase 3	JN185325	( <i>S</i> )-Scoulerine	9	Noscapine decreased by VIGS knockdown; Transcript abundance correlates with noscapine accumulation in cultivars	Dang & Facchini, 2012
	PsN7OMT	Norreticuline 7- <i>O</i> -methyltransferase	FJ156103, XM_026575469	( <i>S</i> )-Norreticuline	7	Transcript abundance correlates with papaverine accumulation in cultivars; Papaverine content reduced by VIGS knockdown	Pienkny <i>et al.</i> , 2009; Desgagné-Penix & Facchini, 2012
	Ps4'OMT1	3'-hydroxy- <i>N</i> -methylcoclaurine 4'- <i>O</i> -methyltransferase 1	AY217333	<i>Not tested</i>			Facchini & Park, 2003

**Supplementary Table 1 (continued)**

Plant species	Short name	Enzyme full name	Genbank ID	<i>In vitro</i> Substrate(s)	Specificity	Functional evidence <i>in planta</i>	Reference(s)
<i>Papaver somniferum</i> (continued)	Ps4'OMT2	3'-hydroxy- <i>N</i> -methylcoclaurine 4'- <i>O</i> -methyltransferase 2	AY217334	( <i>R,S</i> )-3'-hydroxy- <i>N</i> -methylcanadine ( <i>R,S</i> )-laudanosoline ( <i>R,S</i> )-6- <i>O</i> -methylnorlaudanosoline ( <i>R,S</i> )-norlaudanosoline	4 <sup>+</sup>	Expression level correlates with known BIA biosynthesis genes 4'- <i>O</i> -methylcoclaurine and majority of BIAs content reduced by VIGS knockdown	Facchini & Park, 2003; Ziegler <i>et al.</i> , 2005; Desgagné-Penix & Facchini, 2012
	PsOMT2: PsOMT3 dimer	4'- <i>O</i> -desmethyl-3- <i>O</i> -acetyl-papaveroxine 4'- <i>O</i> -methyltransferase	JN185324, JN185325	4'- <i>O</i> -desmethyl-3- <i>O</i> -acetyl-papaveroxine Narcotoline	4'	Narcotoline content increased by VIGS knockdown of OMT2; High narcotoline content in cultivar expressing inactive mutants OMT2b and OMT3b; Co-expression in yeast enables noscapine biosynthesis	Park <i>et al.</i> , 2018; Li & Smolke, 2016
	PsOMT2: Ps6OMT dimer	4'- <i>O</i> -desmethyl-3- <i>O</i> -acetyl-papaveroxine 4'- <i>O</i> -methyltransferase	JN185324, AY217335	4'- <i>O</i> -desmethyl-3- <i>O</i> -acetyl-papaveroxine Narcotoline	4'	Narcotoline content increased by VIGS knockdown of OMT2; High narcotoline content in cultivar expressing inactive mutants OMT2b and OMT3b; Co-expression in yeast enables noscapine biosynthesis	Park <i>et al.</i> , 2018; Li & Smolke, 2016
	PsOMT2b: PsOMT3b dimer		MH029293, MH029295	<i>nd</i>			Park <i>et al.</i> , 2018
	PsCNMT	Coclaurine <i>N</i> -methyltransferase	AY217336	<i>Not tested</i>		N-desmethylated BIA content increased by VIGS knockdown; Induction following elicitor treatment correlates with known BIA biosynthesis enzymes; Expression in yeast enables BIA biosynthesis (various)	Facchini & Park, 2003; Desgagné-Penix & Facchini, 2012; Fossati <i>et al.</i> , 2014; Trenchard & Smolke, 2015; Li & Smolke, 2016
	PsTNMT	Tetrahydroprotoberberine <i>N</i> -methyltransferase	Q108P1	( <i>R,S</i> )-Canadine ( <i>R,S</i> )-Stylophine ( <i>R,S</i> )-Tetrahydropalmatine		Induction following elicitor treatment correlates with known BIA biosynthesis enzymes; Protein level correlates with protoberberine accumulation in <i>P. somniferum</i> tissues	Liscombe & Facchini, 2007
	PsRNMT	Reticuline <i>N</i> -methyltransferase	KX369612	( <i>R</i> )- and ( <i>S</i> )-Reticuline ( <i>S</i> )-Bulbocapnine ( <i>S</i> )-Corytuberine ( <i>S</i> )-Glaucine Tetrahydropapaverine Noscapine	<i>R</i> > <i>S</i>	Magnoflorine content reduced and corytuberine content increased by VIGS knockdown	Morris & Facchini, 2016
<i>Coptis japonica</i>	PsNMT4		KX369613	<i>nd</i>			Morris & Facchini, 2016
	Cj6OMT	Norcoclaurine 6- <i>O</i> -methyltransferase	D29811	( <i>S</i> )- and ( <i>R</i> )-Norcoclaurine ( <i>R,S</i> )-Norlaudanosoline ( <i>R,S</i> )-Laudanosoline ( <i>S</i> )-Scoulerine ( <i>S</i> )- and ( <i>R</i> )-Coclaurine ( <i>R,S</i> )-Laudanosoline	6 <sup>+</sup>	Overexpression in <i>E. californica</i> cells substantially increases BIA content	Morishige <i>et al.</i> , 2000; Tamura <i>et al.</i> , 2006
	Cj4'OMT	3'-hydroxy- <i>N</i> -methyl-coclaurine 4'- <i>O</i> -methyltransferase	D29812	( <i>R,S</i> )-Norlaudanosoline ( <i>R,S</i> )-6- <i>O</i> -Methylnorlaudanosoline ( <i>S</i> )- and ( <i>R,S</i> )-Scoulerine ( <i>R,S</i> )-Reticuline	4 <sup>+</sup>		Morishige <i>et al.</i> , 2000
	CjSOMT (CjSMT)	Scoulerine 9- <i>O</i> -methyltransferase	D29809	( <i>S</i> )- and ( <i>R,S</i> )-Scoulerine ( <i>R,S</i> )-Tetrahydroxyprotoberberine ( <i>R,S</i> )-Norreticuline ( <i>R,S</i> )-norlaudanosoline	9 <sup>+</sup> 7 or 3 <sup>+</sup>	Overexpression in <i>E. californica</i> cells enhanced production of <i>O</i> -methylated protoberberines	Takeshita <i>et al.</i> , 1995; Morishige <i>et al.</i> , 2000; Takemura <i>et al.</i> , 2010
	CjCoOMT	Columbamine <i>O</i> -methyltransferase	AB073908	Columbamine ( <i>S</i> )- and ( <i>R,S</i> )-Scoulerine ( <i>R,S</i> )-Tetrahydroxyprotoberberine	2, other		Morishige <i>et al.</i> , 2002
	CjCNMT	Coclaurine <i>N</i> -methyltransferase	AB061863	( <i>R</i> )- and ( <i>S</i> )-Coclaurine 6,7-Dimethoxyl-1,2,3,4-tetrahydroisoquinoline ( <i>R,S</i> )-Noreticuline ( <i>R,S</i> )-6- <i>O</i> -Methylnorlaudanosoline ( <i>R,S</i> )-Norlaudanosoline	2' <i>N</i> , 3' <i>N</i> <sup>+</sup>	Expression in <i>E. coli</i> enables reticuline biosynthesis; Expression in yeast enables magnoflorine biosynthesis	Choi <i>et al.</i> , 2002; Minami <i>et al.</i> , 2008
<i>Coptis chinensis</i>	Cc6OMT1	6- <i>O</i> -methyltransferase 1	MH165875	( <i>S</i> )-Norcoclaurine	6, 7	Transcript abundance correlates with alkaloid accumulation in tissues	He <i>et al.</i> , 2018
	Cc6OMT2	6- <i>O</i> -methyltransferase 2	MH165876	( <i>S</i> )-Norcoclaurine	6, 7	Transcript abundance correlates with alkaloid accumulation in tissues	He <i>et al.</i> , 2018

**Supplementary Table 1 (continued)**

Plant species	Short name	Enzyme full name	Genbank ID	<i>In vitro</i> Substrate(s)	Specificity	Functional evidence <i>in planta</i>	Reference(s)
<i>Coptis teeta</i>	Ct6/7OMT	Norcoclaurine 7- <i>O</i> -methyltransferase	MH165877	( <i>S</i> )-Norcoclaurine	6,7		He <i>et al.</i> , 2018
	CtSOMT	Scoulerine 9- <i>O</i> -methyltransferase	MH165874	( <i>S</i> )-Scoulerine	9	Transcript abundance correlates with alkaloid accumulation in tissues	He <i>et al.</i> , 2018
<i>Eschscholzia californica</i>	Ec7OMT	Reticuline 7- <i>O</i> -methyltransferase	AB232153	( <i>S</i> )-Reticuline ( <i>S</i> )-Norreticuline ( <i>S</i> )-Scoulerine ( <i>S</i> )-6- <i>O</i> -Methylnorlaudanoline ( <i>S</i> )-Laudanosoline	7 7,3' 2		Fujii <i>et al.</i> , 2007; Purwanto <i>et al.</i> , 2017
	EcS/ROMT (G3OMT)	Scoulerine/reticuline <i>O</i> -methyltransferase	LC171865	( <i>S</i> )-Reticuline ( <i>S</i> )-Norreticuline ( <i>S</i> )-Scoulerine	7,3' 9,2	Expression in yeast enables biosynthesis of chelerythrine; Highly expressed in high macarpine <i>E. californica</i> cell line A5-1	Purwanto <i>et al.</i> , 2017
	EcG2OMT		LC171866	<i>not tested</i>			Purwanto <i>et al.</i> , 2017
	EcG11OMT	10-hydroxy-dihydrosanguinarine 10- <i>O</i> -methyltransferase	LC171864	10-Hydroxydihydrosanguinarine <sup>†</sup>	10 <sup>†</sup>		Purwanto <i>et al.</i> , 2017; Purwanto, 2017
	EcTNMT	Tetrahydroprotoberberine <i>N</i> -methyltransferase	EU882977	( <i>R,S</i> )-Stylopine ( <i>R,S</i> )-Tetrahydropalmatine ( <i>R,S</i> )-Canadine ( <i>S</i> )-Scoulerine		Transcript expression induced by elicitor treatment of cell culture	Liscombe <i>et al.</i> , 2009
<i>Thalictrum flavum</i>	Tf6OMT	Norcoclaurine 6- <i>O</i> -methyltransferase	AY610507	Norlaudanoline		Cellular localization of transcripts correlates with known BIA genes in berberine accumulating organs	Samanani <i>et al.</i> , 2005; Robin <i>et al.</i> , 2016
	Tf4'OMT	3'-hydroxy- <i>N</i> -methylcoclaurine 4'- <i>O</i> -methyltransferase	AY610510	<i>Not tested</i>		Cellular localization of transcripts correlates with known BIA genes in berberine accumulating organs	Samanani <i>et al.</i> , 2005
	TfSOMT	Scoulerine 9- <i>O</i> -methyltransferase	AY610512	<i>Not tested</i>		Cellular localization of transcripts correlates with known BIA genes in berberine accumulating organs	Samanani <i>et al.</i> , 2005
	TfCNMT	Coclaurine <i>N</i> -methyltransferase	AY610508	( <i>R,S</i> )-Norreticuline Dimethoxytetrahydroisoquinoline (±)-Pavine ( <i>S</i> )-Scoulerine	2' <i>N</i>	Cellular localization of transcripts correlates with known BIA genes in berberine accumulating organs	Samanani <i>et al.</i> , 2005; Liscombe <i>et al.</i> , 2009
	TfPavNMT	Pavine <i>N</i> -methyltransferase	EU883010	( <i>S</i> )-Reticuline (±)-Pavine ( <i>R,S</i> )-Stylopine ( <i>S</i> )-Scoulerine	2' <i>N</i> , 3' <i>N</i>		Liscombe <i>et al.</i> , 2009; Torres <i>et al.</i> , 2016
<i>Papaver bracteatum</i>	PbTNMT	Tetrahydroprotoberberine <i>N</i> -methyltransferase	EU882994	( <i>R,S</i> )-Tetrahydropalmatine ( <i>R,S</i> )-Stylopine		Transcript expression induced by elicitor treatment of cell culture	Liscombe <i>et al.</i> , 2009
<i>Psychotria ipecacuanha</i>	IpeOMT1		AB527082	( <i>R,S</i> )-Isococlaurine ( <i>R,S</i> )-Norcoclaurine ( <i>R,S</i> )-Norprotosinomenine ( <i>R,S</i> )-Nororientaline	6, 4'	Not involved in BIA biosynthesis	Nomura & Kutchan, 2010
	IpeOMT2		AB527083	Oripavine	3	Not involved in BIA biosynthesis	Nomura & Kutchan, 2010
	IpeOMT3		AB527084	( <i>S</i> )-Norreticuline ( <i>R,S</i> )-Nororientaline ( <i>S</i> )-Coclaurine	7 5 only	Not involved in BIA biosynthesis	Nomura & Kutchan, 2010
<i>Thalictrum tuberosom</i>	TtOMTII;1		AF064693	( <i>R,S</i> )-Laudanosoline ( <i>R,S</i> )-3'- <i>O</i> -Methylnorlaudanoline ( <i>R,S</i> )-4'- <i>O</i> -Methylnorlaudanoline ( <i>R,S</i> )-3- <i>O</i> -Desmethylcheilanthifoline		Not involved in BIA biosynthesis	Frick & Kutchan, 1999
	TtOMTII;2		AF064694	( <i>S</i> )-Norcoclaurine ( <i>R,S</i> )-4'- <i>O</i> -Methylnorlaudanoline		Not involved in BIA biosynthesis	Frick & Kutchan, 1999
	TtOMTII;3		AF064695	( <i>R,S</i> )-Norisoorientaline		Not involved in BIA biosynthesis	Frick & Kutchan, 1999
	TtOMTII;4		AF064696	( <i>R,S</i> )-Norisoorientaline		Not involved in BIA biosynthesis	Frick & Kutchan, 1999
	Heterologous dimers (various)			Many			Frick & Kutchan, 1999
<i>Ephedra sinica</i>	EsPaNMT	Phenylalkylamine <i>N</i> -methyltransferase	MH029305	( <i>S</i> )-Coclaurine ( <i>S</i> )-Reticuline ( <i>R,S</i> )-Stylopine		Not involved in BIA biosynthesis	Morris <i>et al.</i> , 2018
<i>Dactylicapnos scandens</i>	DsC7OMT	Corytuberine 7- <i>O</i> -methyltransferase		( <i>S</i> )-Corytuberine	7		He <i>et al.</i> , 2017

**Supplementary Table 1 (continued)**

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- Hagel, J. M., Mandal, R., Han, B., Han, J., Dinsmore, D. R., Borchers, C. H., et al. (2015). Metabolome analysis of 20 taxonomically related benzyloquinoline alkaloid-producing plants. *BMC Plant Biol.* 15, 220. doi:10.1186/s12870-015-0594-2.