


Brassica SYM genes

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SYMBIOTIC PLANT-MICROBE INTERACTIONS


- Symbioses between land plants and microbes
 - plants and rhizosphere fungi (mycorrhization)
 - Legume family and rhizobia (nodulation)
- Important impacts on plant productivity and resource use efficiency in agriculture
- Mycorrhizal fungi
 - Phosphorus uptake
 - ~ 460 million years ago
 - Arbuscular mycorrhizae (AM) in ~ 80% of terrestrial plants
 - Generally **non-specific** host selection



Mark Brandreth 2008

SYMBIOTIC PLANT-MICROBE INTERACTIONS

- Nodulation
 - Legume family and specific N₂-fixing soil bacteria (rhizobia)
 - Suppression of plant immune responses and formation of specialised N₂-fixing structures = nodules
 - Bacteria receive photosynthate in exchange for Nitrogen source

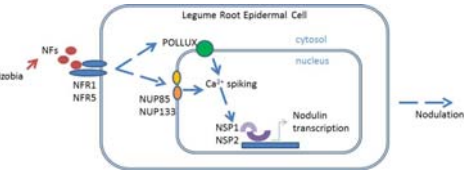


Jiang & Gresshoff 1999

- reduce artificial nitrogen fertilisers
- ~ 60 million years old
- Species-specific** recognition and signaling processes

SYM GENES

- Nodulation pathway evolutionarily recruited from AM symbiosis → common genes between mycorrhization and nodulation
- Major genes required for nodulation and/or mycorrhization include:
 - Nodulation Signalling Pathway 1 (NSP1)*
 - Nodulation Signalling Pathway 2 (NSP2)*



GRAS domain transcription factors

SYMBIOSIS IN THE BRASSICACEAE

- Do not form SYM pathway associations

Mycorrhizae Fungi

N₂ Fixing Bacteria

- BUT have genes conserved with those required for either, or both, of these symbiotic pathways
- homologues also described and functionally conserved in rice
 - Can rescue *L. Japonicus nsp* mutants (Yokota et al., Plant Cell Physiol (2010) 51 (9):1436-1442)
 - Required for strogolactone synthesis in rice and medicago (Liu et al., Plant Cell. 2011 Oct;23(10):3853-65)
- Why are NSP1 and NSP2 so conserved in non-nodulating species?
- Functionally diversified from a minimal set required for plant-microbe interactions?

NSP GENES IN THE BRASSICACEAE

TAGdb: <http://flora.acpfg.com.au/tagdb/cgi-bin/index>

- Discover putative gene homologues in SGS data for a species of interest symbioses

Welcome to ACPFG Bioinformatics .

This service performs BLAST alignment between a single query and short pair reads of selected species.

Please enter a valid email address

Web form input will be sent to the sponsor's address

Sequence data

Please specify the reference file to compare

Reference file name (a sequence in FASTA format)

Species selection

Please choose a query species

Short paired-read library selection

Please select the short paired-read library to search

Start

Useful for:

- Any species with available next-generation sequence data
- Orphan species.
- Uncharacterised genomes

NSP GENES IN THE BRASSICACEAE

TAGdb Brassicaceae libraries:

Species	Accession	Length (bp)	GC (%)	Exons	Intron length (bp)
<i>Brassica napus</i>	BN_01_001	200	35	2	100
<i>Brassica oleracea</i>	OL_01_001	200	35	2	100
<i>Brassica rapa</i>	RA_01_001	200	35	2	100
<i>Brassica campestris</i>	CA_01_001	200	35	2	100
<i>Brassica chinensis</i>	CH_01_001	200	35	2	100
<i>Brassica parachinensis</i>	PC_01_001	200	35	2	100
<i>Brassica pekinensis</i>	PK_01_001	200	35	2	100
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NSP1 and NSP2 complete coding sequences in *B. rapa*, *B. oleracea* and three wild Brassicaceae species – *Diplotaxis tenuifolia*, *Hirschfeldia incana* and *Sinapis alba*

NSP GENES IN THE BRASSICACEAE

NSP GENES IN THE BRASSICACEAE

Accession	Class	Function	Protein Size (aa)	PI	Protein ID
NSP1	GNP-domain TP	Activation of nucleic acid synthesis	476	5.2	Q9L1P1
NSP2	GNP-domain TP	Activation of nucleic acid synthesis	476	5.2	Q9L1P2

Sequence similarity between lotus, rice, Arabidopsis and Brassica NSP-like proteins

Dark blue and grey= active domains; Orange = *B. rapa* TILLING mutations; Light blue = Alanine residue important for NSP1:NSP2 interaction in *L. japonicus*

NSP GENES IN THE BRASSICACEAE

•Alanine residue important for NSP1-NSP2 interaction in *L. japonicus* not conserved in the *Brassicaceae*, but conserved and functional in rice (Yokota et al., 2010)

NSP GENES IN THE BRASSICACEAE

•Degenerate primers to amplify in additional Brassicaceae species

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<i>Brassica napus</i>	BN_01_001	476
<i>Brassica oleracea</i>	OL_01_001	476
<i>Brassica rapa</i>	RA_01_001	476
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<i>Brassica parachinensis</i>	PC_01_001	476
<i>Brassica pekinensis</i>	PK_01_001	476

NSP GENES IN THE BRASSICACEAE

•Primers to amplify specific genome copies

NSP GENES IN THE BRASSICACEAE

Co-expression of *NSP1* and *NSP2* in *Brassica napus*. Co-expression of *BnNSP1* and *BnNSP2* suggests possible functional interaction in the root. Gene expression in the youngest leaf (YL), oldest leaf (OL), basal internode (BI), hypocotyl (H) and roots (R) of 3-week old *B. napus* cv. Ag Spectrum seedlings. Expression of both A-genome (A) and C-genome (C) homeologs was quantified by qRT-PCR normalised to the *BnPP2A* control gene

Hayward et al., Plant Biotechnol J. 2012 Aug;10(6):750-9.

NSP GENES IN THE BRASSICACEAE

- Microbe elicitor response of *NSP1* and *NSP2* expression
- NSP* expression activated by rhizobia/nod factor in legumes
- Brassicaceae *NSP* genes activated by microbial elicitors:

nsp MUTANTS IN THE BRASSICACEAE

- No increase in branch number in *Arabidopsis*
- Reduced root hairs in *nsp1*

Sarah Lorberg, Aria Dolatabadian

nsp MUTANTS IN THE BRASSICACEAE

Flagellin Response

- No perturbed physical response to flagellin
- Two-week old *Arabidopsis* seedlings were treated +/- 10 μ M flg22 and primary root length measured 1 week post treatment

CURRENT AND FUTURE WORK

- B. rapa* TILLING mutants – RevGen UK (JIC)

TILLING region	RevGenAccession	Accession	ProteinAccession	NSP1 position	NSP2 position	NSP3 position
NSP1	R238N	Y	SN5P1V083	107	107	206G
	R238E	Y	SN5P1V083	107	107	206G
	R238T	N	SN5P1V083	107	107	206G
	R238G	Y	SN5P1V083	107	107	206G
	R238P	N	SN5P1V083	107	107	206G
NSP2	R238E	Y	SN5P1V083	107	107	206G
	R238P	N	SN5P1V083	107	107	206G

- TILLING lines for *B. rapa* POLLUX, *NFR5-LIKE* and *NFR1-LIKE* genes

CURRENT AND FUTURE WORK

- Scoring mutant interaction capability with *Brassica* mycorrhizal-like symbiont *Piriformospora indica*.

- Scoring interactions with pathogenic microbes – *L. maculans*, *P. syringae*
- Complementation of *L. japonicus* sym mutants with *Brassica* sequences

THE UNIVERSITY OF QUEENSLAND AUSTRALIA

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RevGenK

