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 Brundrett 2008

SYMBIOTIC PLANT-MICROBE INTERACTIONS

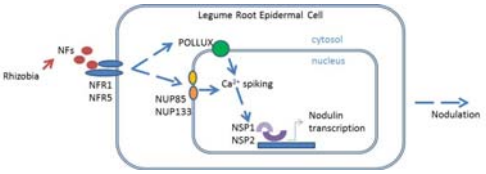
- Nodulation
  - Legume family and specific N_2-fixing soil bacteria (rhizobia)
  - Supression of plant immune responses and formation of specialised N_2–fixing structures = nodules
  - Bacteria receive photosynthate in exchange for Nitrogen source


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

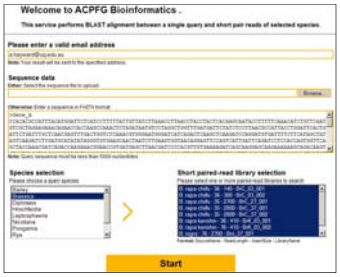
SYMBIOSIS IN THE BRASSICACEAE

- Do not form *SYM* pathway associations

- 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

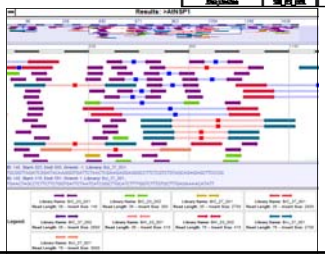
TAGdb: http://flora.acpfg.com.au/tagdb/cgi-bin/index
•Discover putative gene homologues in SGS data for a species of interest symbioses



Useful for:
Any species with available next-generation sequence data
- •Orphan species.
- •Uncharacterised genomes

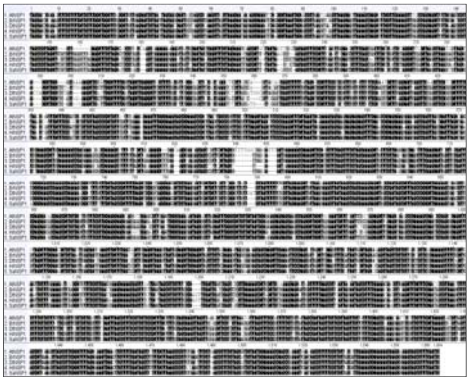
NSP GENES IN THE BRASSICACEAE

TAGdb Brassicaceae libraries:



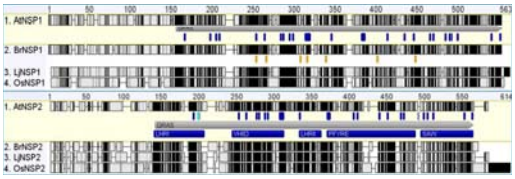
NSP1 and *NSP2* complete coding sequences in *B. rapa*, *B. oleracea* and three wild Brassicaceae species – *Diplotaxis tenuifolia, Hirschfeldia incana and Sinapis alba*

NSP GENES IN THE BRASSICACEAE



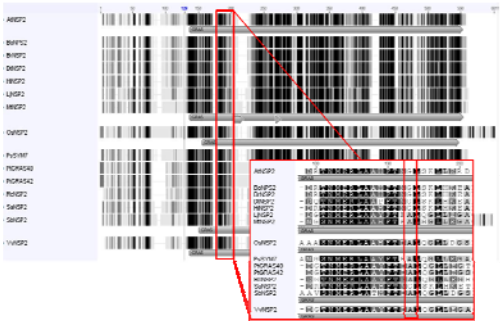
NSP GENES IN THE BRASSICACEAE

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



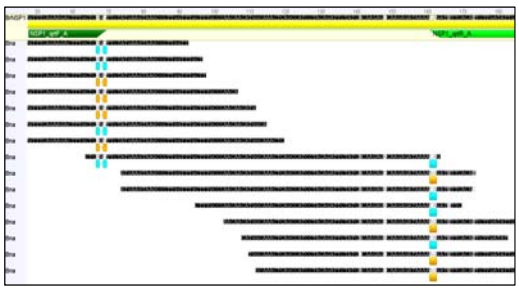
•Alanine residue important for NSP1-NSP2 interaction in *L. japonicus* not conserved in the *Brassicaceae*, but conserved and functional in rice (Yokota et al., 2010)

NSP GENES IN THE BRASSICACEAE

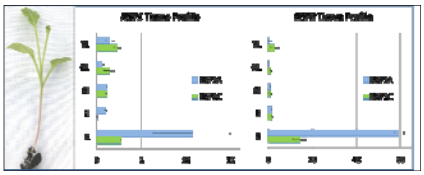
•Degenerate primers to amplify in additional Brassicaceae species

NSP GENES IN THE BRASSICACEAE

•Primers to amplify specific genome copies



NSP GENES IN THE BRASSICACEAE

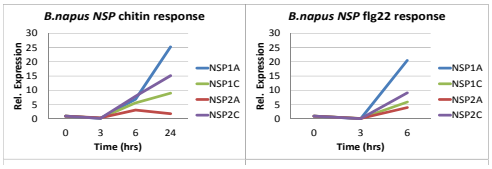


Co-expression of *NSP1* and *NSP2* in *Brassica napus*. Co-expression of *BnNSP1* and *BnNSP2* suggests possible functional interaction in the root. Gene expression in the youngest leaf (YL), oldest leaf (OL), basal internode (BI), hypocotyl (H) and roots (R) of 3-week old *B. napus* cv. Ag Spectrum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

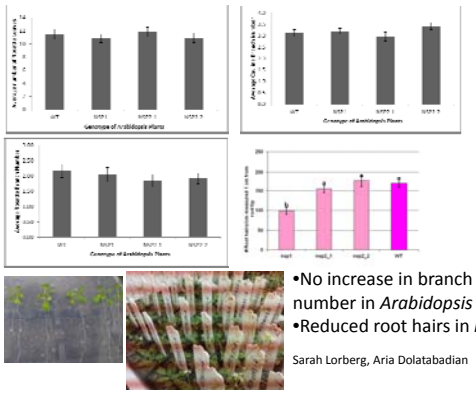
Hayward et al., Plant Biotechnol J. 2012 Aug;10(6):750-9.

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:



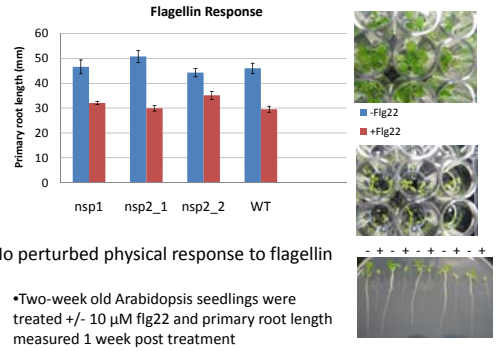
nsp MUTANTS IN THE BRASSICACEAE



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

Sarah Lorberg, Aria Dolatabadian

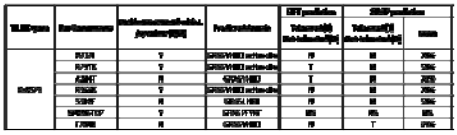
nsp MUTANTS IN THE BRASSICACEAE



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

CURRENT AND FUTURE WORK

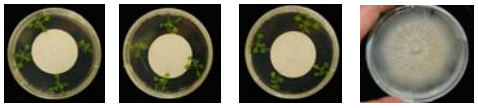
- *B. rapa* TILLING mutants – RevGen UK (JIC)



- 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

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