Exploring and generating epigenetic variation in *Brassica*

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ROTHAMSTED RESEARCH

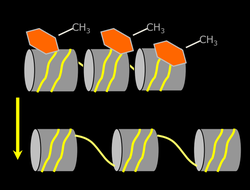
Introduction

It is now apparent that epigenetic regulation, mediated through marks that affect chromatin structure, may play a major role in the control of development and response of plants to environment. An increasing range of agronomic traits are being shown to be affected to some extent by stably inherited epigenetic modifications. Compared with animal genomes, there are important differences in the prevalence and pattern of DNA methylation marks in plant genomes, where epiallelic variation in methylation is also often stably inherited through meiosis. We are generating approaches and resources to explore and exploit variation in DNA methylation within *Brassica* genomes, with a focus on the consequences for seed development and composition.

We have taken two approaches to inducing epi-allelic variation, both targeting ^{5m}CpG sites, which have been shown to be associated in plant genomes with exonic DNA.

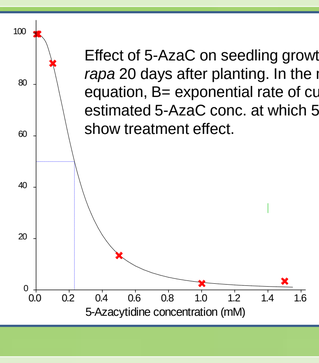
Hypomethylation: ^{5m}CG → CG

- 5-azaCytidine (inhibits methyltransferases)
- EMS TILLING: DNA methyltransferase 1 → *braA.met1.a*, *braA.met1.b*

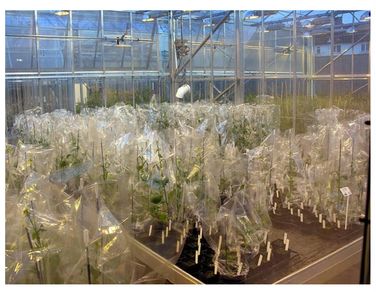


Establishment of a hypomethylated 'epi-mutant' population

We imbibed seeds of *B. rapa* R-o-18 with 5-AzaC at 0.01mM, 0.1mM, 0.5mM, 1.0mM and 1.5mM for 3 days at 20°C (King, 1995). We then established dosage response curves for germination, seedling growth, plant survival, flowering time, seed set, seed size and seed viability, and selected 0.25-0.30 mM as the optimal treatment for generation of an 'epi-mutant' E1 population of 500 lines. A subset of these have been taken through to E3 seed.



Effect of 5-AzaC on seedling growth of *B. rapa* 20 days after planting. In the modelling equation, B= exponential rate of curve, M= estimated 5-AzaC conc. at which 50% will show treatment effect.



Heritable modulation of *Brassica* seed traits

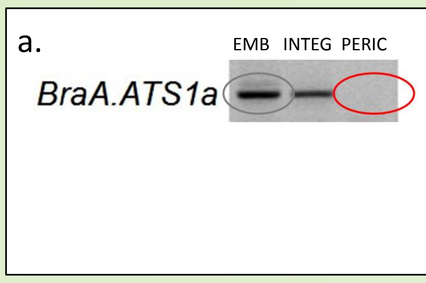
Forward phenotypic screening of E3 seed has identified significant heritable variation for a range of seed traits. These include compositional variation in total oil and protein content, and specific fatty acids. Such variation is associated with a subset of E1 plants (BraROAz_E1) and appears to segregate within the associated E2 families. In addition, we have observed differences in seed size, as well as the variance of seed size.

Mutants and alleles of *B. rapa* DNA methyltransferase I

We sequenced two paralogues of *BraA.MET1* with high similarity to those published by Fujimoto *et. al.* (2006) and used the R-o-18 EMS TILLING platform (Stephenson et al., 2010) to identify two *braA.met1.a* and one *braA.met1.b* non-sense mutations, as well as five additional mis-sense mutations in predicted functional domains. These lines are currently being introgressed into wild-type R-o-18 whilst maintaining heterozygosity to reduce initial collateral effects of down-regulation of ^{5m}C.

Tissue and locus-specific variation in DNA methylation

The triplicated *Brassica* A genome structure contains a series of paralogous genes which may differ in their level of DNA methylation, and so result in locus-specific variation in local chromatin structure and transcriptional competence. We are developing a set of locus-specific assays to investigate how such variation may be modulated in different tissues and at key stages of development. The example below shows tissue-specific variation in DNA methylation within the *BraA.ATS1.a* gene that is consistent with transcriptional modulation (i.e. sites are hypermethylated in pericarp, leading to reduced transcripts).



a) RT-PCR of locus-specific copy of *BraA.ATS1.a* in R-o-18; 30 days after pollination. EMB: embryo, INTEG: integument; PERIC: pericarp tissue.
b) High resolution melt (HRM) of bisulphite-treated DNA isolated from embryo and pericarp tissue at the same stage. A shift of curve to the right indicates higher melting and more ^{5m}C.

Whole epi-genome scanning

In collaboration with Pat Heslop-Harrison and Trudy Schwarzacher (U. Leicester) we have carried out a preliminary analysis of the spatial distribution of ^{5m}C in chromosomes from meiotic pachytene spreads of wild-type *B. rapa* R-o-18 and a small sample of BraROAz_E2 plants. Immuno-labelling with anti-^{5m}C antibody showed clusters of ^{5m}C signal co-localised with heterochromatic regions in both samples, but a qualitative reduction of ^{5m}C signals was observed within the euchromatin of BraROAz_E2 plants. This is consistent with a hypomethylation of CG sites compared with CHG and CHH sites.

More recently we have started to generate whole-genome ^{5m}C data at base-pair resolution, optimising bisulphite sequencing and matching sequences to reference genomes of *B. rapa* Chiifu-401 and R-o-18. Several statistical and informatics challenges that we are addressing, associated with this approach, should be applicable to other complex crop genomes.

Targeted selection of epi-alleles for crop improvement

Epiallelic variation is associated with a range of genes and traits of considerable agronomic importance. Such variation appears stable over several generations and can be induced using chemical intervention or *met1* mutants. There is scope in *Brassica* to select locus-specific epi-alleles that may modulate gene regulation at key developmental stages, in a tissue-specific manner and/or respond to particular environmental cues.

References

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