

# Genomics-led radiation mutagenesis in rapeseed

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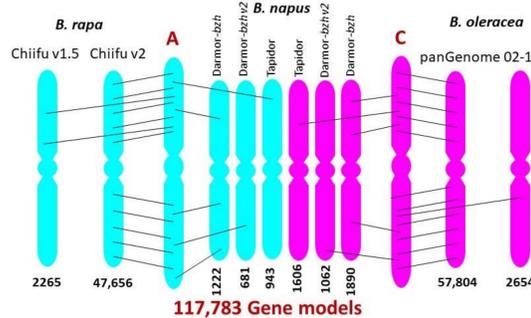
## Introduction

Rapeseed is the most agronomically important crop type of the species *B. napus*, a recently formed allotetraploid ( $2n=38$ , AACCC) with short evolutionary history and limited genetic diversity. Both diploid progenitors, *Brassica rapa* (AA) and *Brassica oleracea* (CC) underwent whole-genome triplication (WGT) event prior to rediploidization caused by extensive genomic rearrangements, gene losses and homoeologous exchanges (HE). Polyploid redundancy supports the viability of genome structural variation frequently occurring in rapeseed as result of natural non-homologous translocations, duplications and deletion events as well as sustains vitality in artificially mutagenized plants with high mutation rates. The advent of Next Generation Sequencing (NGS) technology allowed us to revise our understanding of the impacts of ionizing radiation on plant genomes. We developed a radiation mutagenesis panel of  $M_2$  rapeseed lines derived from a current cultivar and re-sequenced the genomes (to ~12x redundancy) for ~600 lines. The range of mutations induced, including single nucleotide variants, frame shift-inducing InDels and segmental duplications, as well as a broad range of structural deletions is described. The resource represents a powerful genomics-led reverse genetic platform for predictive mutation breeding.

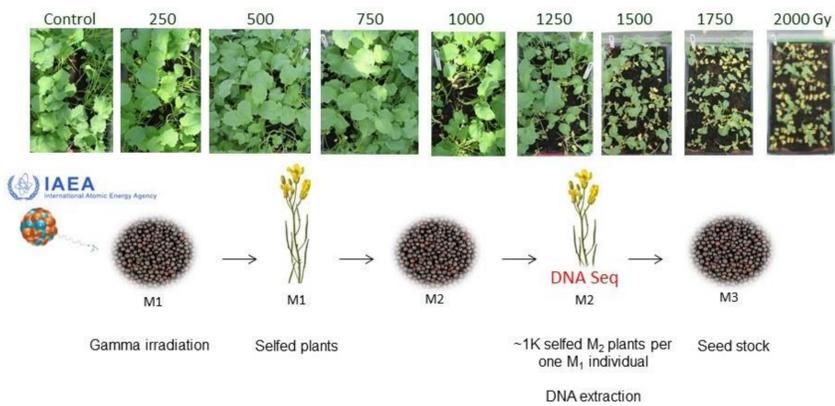
## Material and Methods

- Our current **AC pan-transcriptome**<sup>1</sup> sequence of *B. napus* consists **117,783 Gene models**

York Brassica genomics platform: AC pan-transcriptome reference sequence for *B. napus*



- Rapeseed seeds were irradiated with 8 different doses of **gamma rays** (<sup>60</sup>Co) at the International Atomic Energy Agency (IAEA) Austria.



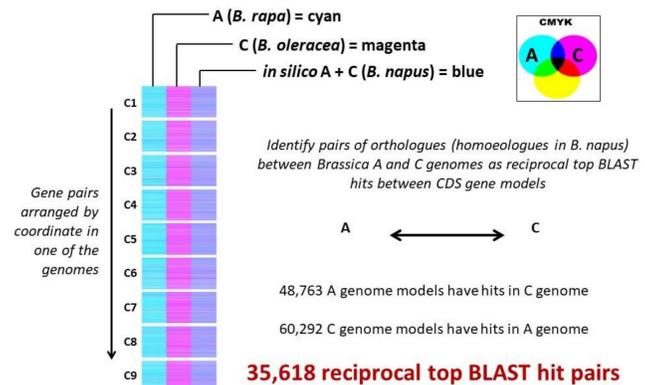
- DNAseq data from genome re-sequenced radiation mutagenesis panel of ~600  $M_2$  rapeseed plants (dosage 1750-2000Gy) mapped to AC pan-transcriptome reference sequence for **SNP and InDel identification**.

A -> C/M	12,574
A -> G/R	36,510
A -> T/W	15,250
C -> A/M	13,530
C -> G/S	11,592
C -> T/Y	46,986
G -> A/R	42,079
G -> C/S	13,836
G -> T/K	15,366
T -> A/W	13,707
T -> C/Y	49,035
T -> G/K	13,680

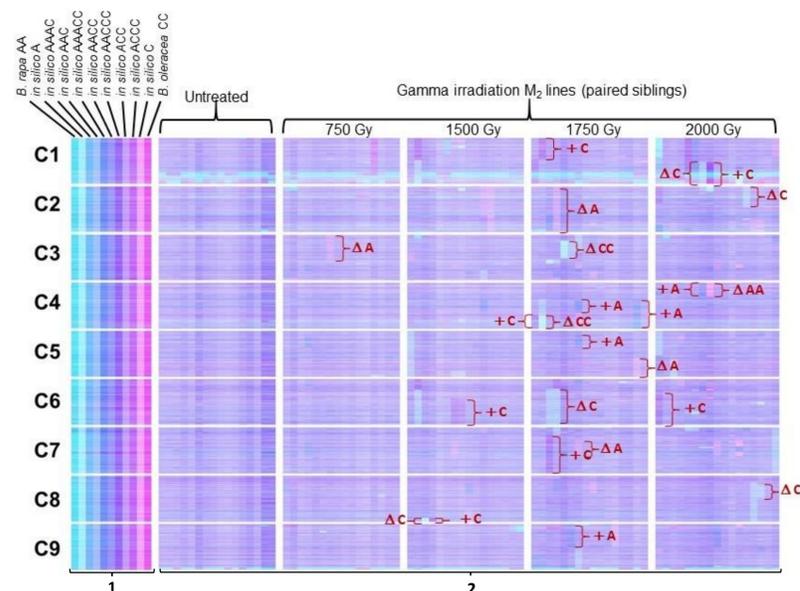
Each line contained ~9,000 single base mutations covering the complete spectrum of changes and ~350 InDels that result in frame-shifts expected to knock out gene function

- Identification of genome structural variation**

Identifying homoeologous gene pairs between *B. napus* A and C genomes as reciprocal top BLAST hits between CDS gene models revealed **35,618 homoeologous gene pairs**. Gene pairs were assigned to colour (A genome cyan and C genome magenta) and arranged by coordinate in one of the genomes.

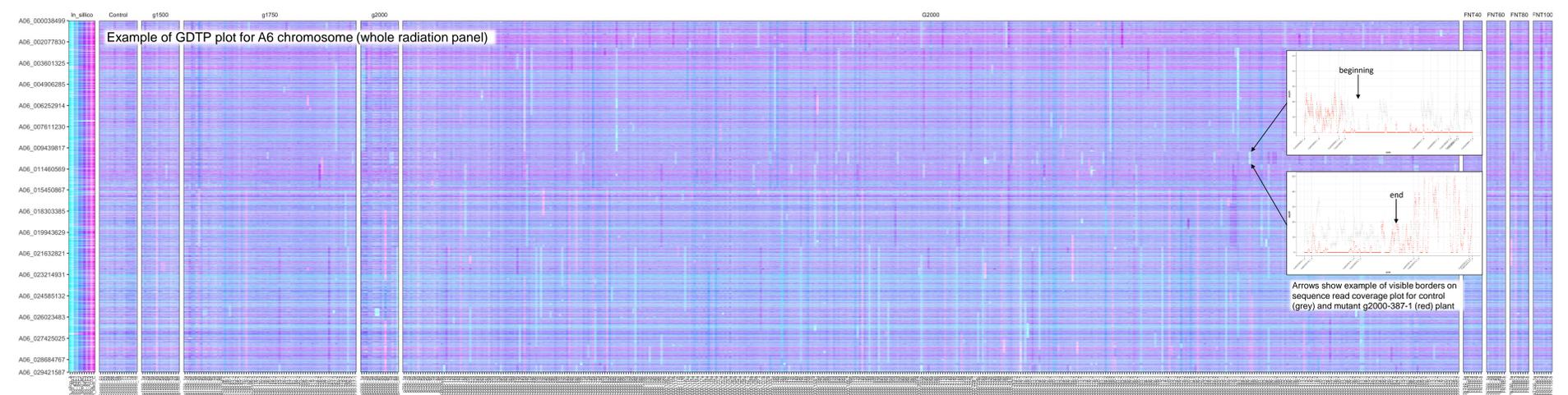


- We predicted situation of prevalent or missing genomes as results of megabase-scale genome duplication or deletion as result of loss or incorrect joining of double-strand breaks
- We visualised RNAseq/DNAseq data by using **Transcriptome/Genome Display Tile Plot**<sup>2,3</sup> (TDTPs/GDTPs)



## Conclusion

Rapeseed is the UK's third most valuable crop. Genome re-sequenced radiation panel provides state-of-the-art resources available to encourage the hypothesis-driven investigation of gene function and to underpin the development of new cultivars with improved characteristics.



## Reference

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- Harper AL, Trick M, He Z, Clissold L, Fellgett A, Griffiths S, Bancroft I. (2016) Genome distribution of differential homoeologue contributions to leaf gene expression in bread wheat. *Plant Biotechnol J* 14:1207-1214.
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