Harnessing and Characterising Genetic Diversity within the *Brassica* A and C genomes

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Brassica biodiversity:

Background

- The modern cultivars of brassicas represent a restricted genetic base in relation to the wider *Brassica* genepool.

- Researchers require access to novel variation, but in a form that can be readily exploited in a reasonable time scale.

- Warwick HRI is pioneering the development of Diversity Fixed Foundation Sets (DFFS).

- We are producing three *Brassica* DFFS in complementary projects funded by the UK Department for Environment Food and Rural Affairs (Defra): a *Brassica napus* DFFS (under the OREGIN project – see **http://www.oregin.info**), a *B. oleracea* DFFS and a further C genome-DFFS across 9 wild *Brassica* species.

Rationalising the assessment and use of Biodiversity

Genebanks
Warwick HRI Genetics Resources Unit > 6000 *Brassica* accessions

Core collections
Reference sample representing diversity in *ex situ* collections
However, genetic heterozygosity/heterogeneity within accessions

Diversity Fixed Foundation Sets (DFFS)
Reference sample representing diversity in *ex situ* collections
Genetically homozygous (fixed) immortal lines
High throughput compatible
Public domain

Brassica DFFS

- **A DFFS is an informative set of genetically fixed lines representing a structured sampling of diversity across the relevant genepool.**

http://www.brassica.info/diversity/diversity_sets.htm

- Fixed lines, either doubled haploid (DH) or inbred, enable trait and genotype data to be accumulated over time for common genetic material, and provide a number of advantages over diversity collections containing heterozygous or mixed lines.

- The DFFS are organised in nested subsets of 94 lines (equivalent to a microtitre plate with control wells) – for compatibility with high throughput screening approaches.

- Cumulative trait and molecular data will be analysed and stored in databases to provide information relevant to future crop improvement.
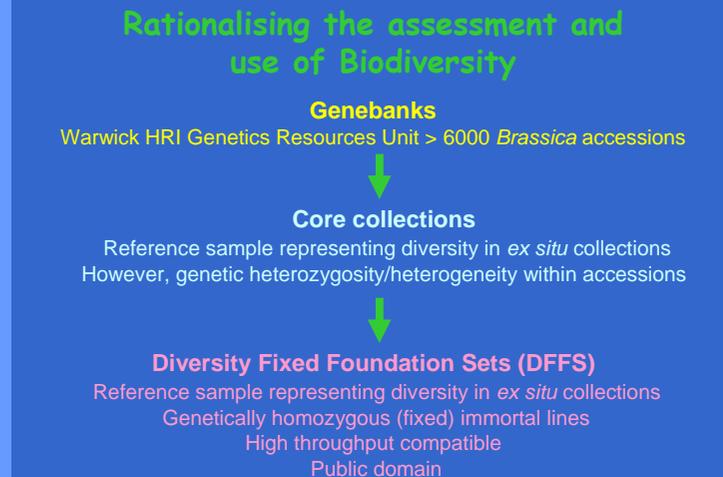
B. oleracea DFFS (BoDFFS)

- 4 nested subsets - 376 lines in total
- The first 2 subsets will be fixed by microspore or inbreeding
- The 3rd and 4th subsets will be fixed by inbreeding only

Breakdown of the composition of the BoDFFS

		FFS-94	FFS-188	FFS-282	FFS-376
acephala	kale	10	19	28	38
alboglabra	Chinese kale	3	7	10	13
botrytis	cauliflower	21	46	73	100
capitata	cabbage	12	28	43	57
gemmifera	Brussels sprout	10	19	29	38
gongyloides	kohlrabi	3	7	10	13
italica	Calabrese	18	40	63	84
tronchuda	Trunchuda kale	4	9	13	18
C spp		8	8	8	10
A spp		3	3	3	3
B spp		2	2	2	2
Total		94	188	282	376

B. napus DFFS (BnDFFS)

- 2 nested subsets – 188 lines in total
- Will be fixed either by microspore or inbreeding

The *B. napus* types are included in the BnDFFS are :

- Winter oilseed rape
- Spring oilseed rape
- Swede
- Fodder/forage rape
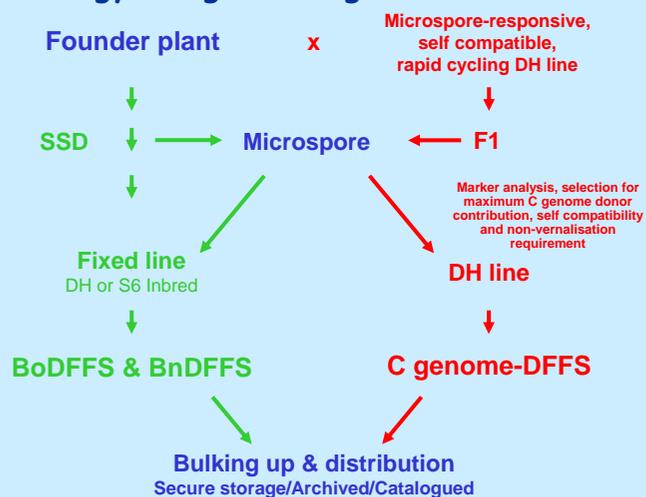- Hannover salad/Siberian kale
- Resynthesised *B. napus*

OREGIN

Brassica C Genome-DFFS

- One set of 94 lines
- Founder plants will first be crossed to a microspore-responsive, rapid cycling, self compatible DH *B. oleracea* line
- Microspore culture will be performed on F1s of these crosses and marker analysis used to select self compatible DH lines that contain the most donor C genome and that do not require vernalisation to create a population of more easily managed lines

The C genome species are included are:

- *B. balearica*
- *B. bourgaei*
- *B. cretica*
- *B. hilarionis*
- *B. incana*
- *B. insularis*
- *B. montana*
- *B. macrocarpa*
- *B. villosa*

Strategy for generating fixed lines

Founder plant   x   **Microspore-responsive, self compatible, rapid cycling DH line**

SSD → Microspore ← F1

Marker analysis, selection for maximum C genome donor contribution, self compatibility and non-vernalisation requirement

Fixed line
DH or S6 Inbred

DH line

BoDFFS & BnDFFS

C genome-DFFS

Bulking up & distribution
Secure storage/Archived/Catalogued

Additional information

- Marker analysis, e.g. with microsatellite (SSR) markers, will be used for quality control and for allelic diversity assessment.

- Allelic diversity data will enable us to establish a framework to generate 'graphical genotypes' for each line - these provide a picture of the mosaic of inherited chromosomal regions and are valuable in determining their genetic descent and comparing the allelic distribution between the different subgroups of *B. napus*.

- Seed of fixed lines, DNA and information will be available in the **public domain** to encourage adoption as a reference resource for crop improvement and for understanding the genomic and genetic basis of agronomic traits.