Mini-Paper 6

The potential for identifying and utilising sources of host plant resistance to the pests and pathogens of *Brassica* crops as a key component of future IPM strategies

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In wild species, host plant resistance is often a key factor limiting pest populations. However, in the last century the 'green revolution' enabled the breeding of crops with high yield under agronomic conditions in which plants have access to sufficient nutrients and water and where pests, diseases and weeds were controlled effectively by agrochemicals or other means. Other factors such as crop quality, including good physical appearance, were also critically important. However, despite the widespread use of pesticides, pests and diseases and competition from weeds are still responsible for billions of pounds worth of crop losses each year. Chemical control is confounded by a number of factors, including loss of efficacy due to the evolution of resistance in the target species (e.g. the moth *Plutella xylostella* and the aphid *Myzus persicae* are reported to have gained resistance to the majority of insecticides in use, and likewise blackgrass (*Alopecurus myosuroides*) has developed resistance to a wide range of herbicides), legislative removal on the basis of environmental concerns, the slow development of the next generation of pesticides, incomplete protection when pesticides are used due to variation in coverage of a crop, and limited availability to farmers in many parts of the world. In addition, pesticide application represents an additional cost to farmers. The number of pests that threaten crops is also likely to be altered by increased pest ranges predicted as a result of climate change (e.g. *P. xylostella* currently does not overwinter successfully in the UK and most damage is due to migration of moths from continental Europe and North Africa (Chapman *et al.* 2002), but warmer winters may enable it to do so and hence increase the number of generations produced in a season).

Many of these issues could be overcome by the incorporation of genetic resistance into cultivars and so this has become a major activity in most crop breeding programmes. Some resistant cultivars have been around for a long while (and indeed some have fallen into disuse – such as the lettuce cultivar Avon Defiance developed in the UK with resistance to lettuce root aphid (*Pemphigus bursarius*) and downy mildew (*Bremia lactucae*)), but there is still considerable interest in identifying further sources of resistance.

Until recently, the majority of breeding for resistance involved the use of single genes as these are easier to identify and to screen for. Dominant R genes are often favoured as they can be phenotypically selected when heterozygous and also because they only need to be bred into one parent of F1 hybrid cultivars. However, as with chemical control, the wide deployment of single gene resistance places a high selection pressure for the evolution of the pests or pathogens to overcome the resistance gene, with the timescale for this often being as short as five years. This has led to an 'arms race' to identify and deploy the next resistance gene (exemplified by the gene-for-gene resistance to downy mildew *Bremia lactucae* of lettuce (Simko *et al.*, 2013)). Another problem with

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single disease resistance genes is that they are often race-specific and so have limitations in the scope of their effectiveness.

One way to overcome the problems with single gene resistance is to use quantitative resistance, which may sometimes be referred to as field resistance, general resistance or partial resistance. This mode of resistance is often incomplete and is based on the presence of multiple quantitative genes in different locations in the plant genome, conferring an additive contribution to the resistance. This character is usually either recessive or co-dominant, which can make it very difficult to introduce in new cultivars. But this type of resistance has the benefit that it is much more unlikely to be overcome by the evolution of resistance-breaking pest populations, leading to greater durability. In addition, cultivars with quantitative resistance are also more likely to be resistant to a broad range of races. Because of the mode of action of this type of resistance, there may also be efficacy against a broad range of biotic, and even abiotic, stresses. Breeding for quantitative genes effective against several economically important pests can therefore be a new way to create durable and widely resistant cultivars. However, the task of finding new sources of resistance is becoming increasingly challenging.

Sources of resistance

If the development of resistant cultivars is to depend on conventional breeding programmes rather than genetic modification (as with cultivars of *Brassica oleracea* that have been engineered with the *Bt* gene, e.g. Zhao *et al.* (2000)) then potential sources of resistance alleles include:

- Cultivars – either in current use or historic and from different parts of the world
- Landraces – the first domesticated cultivars
- Wild species

Cultivars

Over the centuries to millennia of crop domestication, beneficial characteristics have been selected for, but in this process the genetic base of the crop has become restricted (this is referred to as the Founder effect) (Figure 1). For example in Figure 1, alleles G and E were not captured during the domestication process. This reduction in allelic diversity in the domesticated gene pool makes it harder for breeders to respond to the challenges of local and, to a greater extent, global food security where phenotypes that are capable of resisting biotic and abiotic stress may be required (Reeves *et al.* 2012). Current cultivars are generally available from breeding companies and seed merchants, but many historic/heritage cultivars are conserved in genebanks, like the Biological Resource Centre "INRA Bracysol" (BRC). Over a long period, this gene bank has collected a great number of old cultivars. In addition, the BRC has created a network, with the breeding companies and all French partners who work around *Brassica napus,* to collect cultivars as they cease to be cultivated. However, there is no formalised system for this conservation activity to take place and hence many older cultivars may no longer be available. There should be a mechanism put in place for this to happen (which would need the consent of the breeding companies where Plant Breeders Rights are still active).

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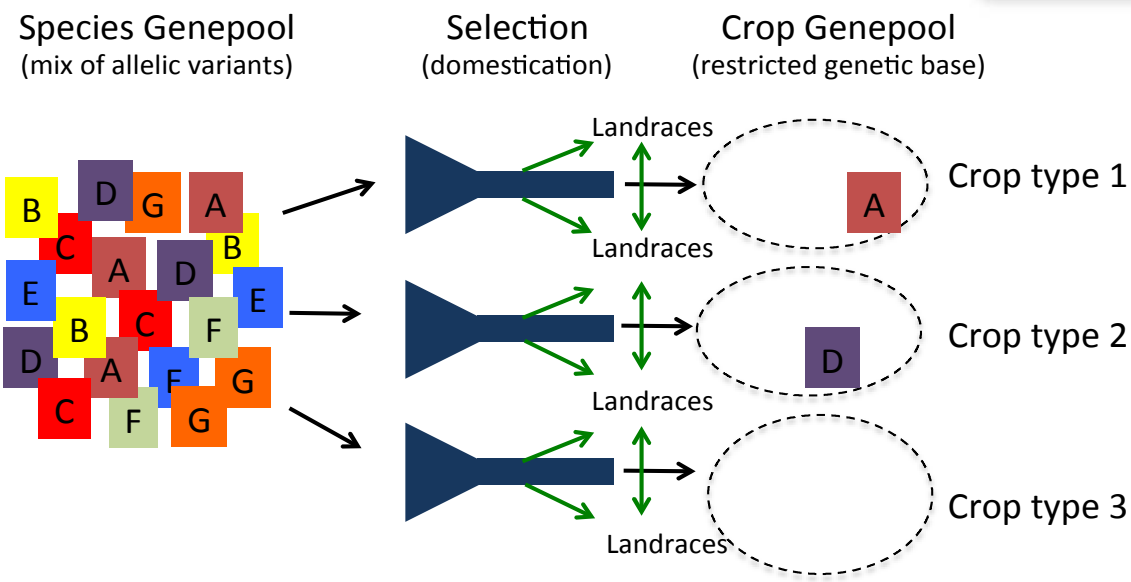

Figure 1 Reduction in allelic variation due to domestication process. In this illustration, alleles G and E have not been captured in the crop types during selection. Landraces that were either used or discarded prior to crop type formation may have valuable alleles that are not captured in the crop types. Adapted from Walley *et al.* (2012).

Landraces

Landraces are variable and identifiable populations, which usually have a local name and lack "formal" crop improvement. They are characterised by a specific adaptation to the environmental conditions of the area of cultivation (tolerant to the biotic and abiotic stresses of that area) and are closely associated with the people who developed and continue to grow them. Their variability means that, although the yields are not high, a reliable yield will be obtained regardless of environmental stresses experienced in any particular season. For historical and geographical reasons the Iberian Peninsula is a centre of genetic diversity for *Brassica oleracea*. It is a region with different linked centres of origin of biodiversity, allowing landraces to evolve adapted to the farming system. Landrace seed is reproduced by the farmers themselves and involves harvesting open pollinated seed (so there is a high degree of heterozygosity and plant-to-plant heterogeneity), but the practice is progressively being abandoned as they are superseded by more profitable modern cultivars that have improved yield, quality and uniformity. For example, broccoli landraces in southern Italy have largely been replaced by F1 hybrids and, hence, are in threat of extinction. In Europe, in 1983-84 there was an EU programme to collect landrace populations of *B. oleracea* before they were completely superseded by hybrid cultivars (van der Meer *et al.*, 1984). This programme involved France, Britain, Germany, Denmark, Belgium, Italy and the Netherlands. These accessions are presently maintained and stored using approaches which preserve the diversity of each population and they can be accessed from the relevant organisations in these countries e.g. the Warwick Genetic Resources Unit, CGN, IPK, Nordgen Bank and INRA Bracysol. There is an urgent need to preserve landraces both on-farm (*in situ*) and *ex situ* and it is suggested that the long-term, dynamic on-farm conservation of landraces should be based on their enhanced use. Landrace protection schemes are presently being developed in Italy through Regional Laws and the National Plan for Agrobiodiversity Conservation (see Ciancaleoni et al.,

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2014). While it is clear that landraces are highly variable, for many crops the degree of variability within landraces has not been characterised with genetic markers. There should also be an analysis of whether there is genetic drift in landraces over time. This source of resistance presents an interesting potential since selection has been done under combined stresses. This may have led to selection of quantitative resistance genes, actively researched in IPM strategies.

Wild species

The genus *Brassica* contains 37 different species (Gomez-Campo, 1980). The wild species of the *B. oleracea* group are found in small isolated areas and form very distinct phenotypes. *B. oleracea* L. is found on the coasts of northern Spain, western France and southern and south-western Britain (Rakow, 2004). All *Brassica* species within this group can be crossed, producing hybrids that are generally fertile. It is thought that *B. napus* was formed on the coast of northern Europe where both *B. oleracea* and *B. rapa* grow wild; others believe that *B. napus* originated in the Mediterranean region or in western or in northern Europe. It is possible that *B. napus* could have formed at different places from crosses between different forms of *B. oleracea* and *B. rapa* (Rakow, 2004).

Wild species have been shown to possess more extensive genetic variation than the crop relatives, even for the very polymorphic group of *B. oleracea* vegetables. The principle is illustrated in Figure 1, and Table 1 shows that *B. oleracea* (both crop and wild accessions) have very limited genetic variability compared to the other wild species relatives. Additional data collected using nuclear SSR markers also show that wild *Brassica* species have much higher allelic variation, including many alleles that are not present in the crops (G. Teakle, unpublished data). This variation is also expected to extend to variation in genes (example of introgression of regulatory alleles from *B. villosa* into broccoli to breed the high glucoraphanin Beneforte broccoli (Traka *et al.*, 2013)) and to the wild species containing additional unique genes. While some collections of wild species are held in gene banks and research collections, the highly variable nature of wild species (Table 1) means that to maximise the potential resource available, wild species should be strategically sampled across their eco-geographic ranges to ensure that the full range of environmentally adapted alleles are captured. One limitation to the use of wild species, and at some point old cultivars, is the presence in their genome of "unwanted genes" sometimes closely linked to "targeted genes". For *Brassica napus*, an amphi-diploid species, no wild genotype has been found. To bring new genetic diversity, one of the strategies is to cross *B.napus* with one of its parent species. A relatively new and attractive strategy is to re-synthetise *B. napus* from its parent species, *B. oleracea* and *B. rapa*.

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Table 1. Survey of chloroplast SSR alleles across a set of crop and wild relative C genome Brassicas (Allender *et al.*, 2007).

Species	Number of Different Chloroplast Types	Number of Accessions Tested
Brassica cretica	6	9
Brassica hilarionis	2	94
Brassica incana	2	11
Brassica insularis	3	4
Brassica macrocarpa	4	16
Brassica rupestris	3	4
Brassica villosa	6	13
Brassica oleracea	**2**	**80**

In combination, this large resource of available germplasm represents too many lines to screen. To address this, the first step is to create a smaller representative core collection (Brown 1989, 1995; Reeves *et al.* 2012; van Hintum *et al.* 2000). The aim of a core collection is to have a sampling of the diversity that encompasses as much of the eco-geographic and morphological variation as possible. The use of DNA genotype data for these collections would enable the percentage of available variation captured in the core collection to be estimated.

However, genetic assessment of much of this variation is complicated by the segregating nature of accessions of outbreeding crops, such as *Brassica*, especially if they are sourced from gene banks where the genetic variation in an accession is preserved, as no individual is the same as its siblings and many alleles may be heterozygous. In the UK, the Vegetable Genetic Improvement Network (VeGIN) http://www2.warwick.ac.uk/fac/sci/lifesci/research/vegin/brassica/ have taken the concept of a core collection one step further for *Brassica* crops, and fixed individuals to create permanent resources called Diversity Fixed Foundation Sets (DFFS) (Pink *et al.* 2008, Walley *et al.* 2012). The lines are homozygous and can be used in replicated experiments, allowing a more accurate estimate of line means and the heritable component of a trait from the environmental variance. The *Brassica* wild species, however, are a difficult resource to manage due to a prolonged vegetative phase and low fertility of self-pollinated plants, so they were first crossed to a rapid cycling line in order to capture segments of the wild species genome in a user-friendly genetic background. Similar activities are ongoing for *Brassica napus* in a related, publicly-funded project, the Oilseed Rape Genetic Improvement Network, OREGIN (www.oregin.info). In a new phase of the project, the assessment of a large number of lines for nitrogen use efficiency and various disease resistances is scheduled for 2015/16, which should provide breeders with essential data and tools.

Thus, altogether, with cultivars, landraces and wild species there is a huge amount of genetic variation that could be screened and tapped. This biodiversity comprises all the different alleles available, but also combinations of alleles, some of which are required together in the same organism for expression of a multi-genic trait. The challenge now is to screen this germplasm for new traits. There are two primary approaches for doing this: forward genetics and reverse genetics. Forward

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genetics refers to finding a plant that possesses the desired trait and then determining the genes that control the trait, while reverse genetics refers to determining how the sequence of a known gene may vary and whether this has an impact on the phenotype of the plant. A classic example of forward genetics is work undertaken at the University of Warwick to identify the genes controlling a seedling vigour QTL for which the genes have now been identified (Finch-Savage *et al.*, 2013). For the latter approach, there is now a wide range of opportunities enabled by next generation sequencing technology. Screening for novel traits will be considered in the next section.

Trait identification and data collection

Screening germplasm for new target traits requires necessary expertise in the trait, together with suitable facilities and resources (e.g. pest or pathogen strains). For many traits of agronomic importance, agreed phenotypes have been established, enabling comparisons between germplasm and across laboratories. However, often the necessary expertise does not reside in breeding companies, which is where academic research can play a pivotal role. Some of this research is the result of spin-off applications from fundamental research programmes, while other work may be in collaboration with breeders who may fund it directly, or it may be through government strategic funding to address a recognised deficiency in industry capability.

One of the difficulties associated with pests and pathogens is the degree of variability within their populations. While screening is often performed under field conditions for some traits, for others, and to make screening more efficient, it is usually desirable to screen initially using standard strains, such as single spore isolates of fungal pathogens or various pest insect collections. Pathogens are often classified into different pathotypes which are defined by their infection phenotype on standard sets of differential plant lines, such as the Turnip Mosaic Virus differential set developed by John Walsh at the University of Warwick, the European clubroot differential set, and *Leptosphaeria maculans* differential lines. Screening needs to take into account a range of factors, including the quantity of inoculum, the developmental stage of the plant and the environment of the screen. It is essential to include suitable experimental replication to ensure sufficient statistical power to measure the traits robustly.

Technology is also having an ever-more significant impact on our ability to increase the throughput and automation of trait screening or phenomics as it is also known. Automated phenomics platforms, such as that produced by Lemnatec, are able to integrate the analysis of a host of measurements. Automated image analysis is also gaining momentum as an important phenotyping tool, such as image analysis of parsnip root cankers being developed in a collaboration between the University of Warwick and Elsoms Seeds. Other technologies include X-ray tomography to non-destructively visualise root architecture in a soil matrix, spectral reflectance analysis as a surrogate to measure crop canopy behaviour, and the development of robots that can automate phenotyping of plants in the field. Other strategies to increase the throughput of traits can be to use seedling screens where large numbers can be readily undertaken. This will also require subsequent evaluation of the small number of candidate genotypes identified to be re-screened at other developmental stages. In addition, if a single pest or pathogen strain has been used in a screen to identify a source of resistance, it is also necessary check the general value of the resistance by testing it against a range of other strains.
It would be interesting to work at the European level on this screening. We could imagine creating a European bank of *Brassica* genotypes, and organizing together their multiplication, the procedures of screening and the data storage and analysis.

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How to speed up the breeding process

Once a plant accession has been identified that contains a trait of interest, the desire is to incorporate it into elite breeding germplasm. However, relying on a phenotypic selection to do this is often not feasible or cost-effective. If the source of the trait is a wild species, then there will also be other non-beneficial alleles carried along with the genes of interest in a process termed linkage drag. This fact is often a deterrent to performing wide crosses as it can take many generations of selection to break the linkage to the deleterious alleles.

To address these issues, breeders are now largely reliant on the use of genetic markers. There are markers which will be specific to the parent of interest and will help select plants genetically closer to this parent and markers linked to the gene(s) controlling the trait of interest coming from the other parent. Selection can be performed at the seedling stage to save plant-raising costs. But before this, markers linked to the genes of interest need to be identified.

The two primary methods for identifying linked markers are by mapping the trait to major genes, or quantitative trait loci (QTL) for traits under multi-genic control, in segregating bi-parental mapping populations, or by using association mapping in diversity collections. While mapping can be performed in F2 or backcross populations, the use of immortal populations, such as doubled haploid or recombinant inbred populations, provides greater power for trait screening and enables the same populations to be screened for many different traits. Historically, research communities have relied heavily on a relatively small number of these types of populations and there is now a growing need to increase the range of populations available.

To perform the mapping, it is also necessary to genotype the populations and the latest advances in DNA sequencing technology are having a big impact in this area. While it is still not cheap to sequence the genomes of all lines in a population, other approaches such as sequencing the transcriptome, strategies that involve a genome complexity reduction process (e.g. genotype-by-sequencing; Elshire et al, 2011), genome capture techniques (e.g. resistance gene enrichment sequencing, RenSeq; Jupe *et al.*, 2013), and a range of high density single nucleotide polymorphism (SNP) detection arrays have enabled the locations in genomes of genes controlling traits to be mapped with high accuracy and, with the assistance of increasing numbers of genome sequences, improved our ability to suggest candidate genes for the traits. These techniques, however, are not suitable for use in a breeding programme. Thus once linked SNP markers have been identified they need to be converted to a low cost assay suitable for screening small numbers of markers across large numbers of plants. One marker type popular with the breeding industry is the KASP assay (LGC Genomics) and standard sets of KASP markers for different species are beginning to be made available to researchers and breeders.

What are the targets for trait identification?

There are a wide range of targets for which the identification of sources of resistance genes would be useful in both *Brassica* vegetables and oilseed rape. Research at the University of Warwick in the UK has focused on sources of resistance to Turnip Mosaic and Turnip Yellows Viruses, *Plutella xylostella* and *Delia radicum*. In addition, resistance traits against *D. radicum* have also been studied at The James Hutton Institute (formerly SCRI) together with Agroscope, Wadenswil (Baur *et al.* 1996), and via public-private partnerships in The Netherlands, involving Wageningen University and Dutch seed companies. Whilst the identification of complete resistance is always exciting, complete resistance, generally dependent on a single gene, is sometimes not sustainable once incorporated into commercial cultivars (Simko *et al.*, 2013). The potential for the breakdown of complete resistance has been demonstrated for both resistance to *Bremia lactucae* (Simko *et al.*, 2013) and resistance to

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Nasonovia ribisnigri in lettuce (G. Hough, PhD thesis, University of Warwick). There is also evidence that in Germany some strains of clubroot are overcoming host plant resistance (Martin Hommes, personal communication). Thus, partial resistance, which is often polygenic, may be a more sustainable solution that can be used as one component of a resistance management strategy.

The way forward

The new technology and improvements in techniques described in this paper mean that it should be 'easier' and quicker to incorporate new traits for pest and disease resistance into commercial crop cultivars. Possibly one of the greatest bottlenecks in the process at the moment is the lack of resources for 'phenotyping' – screening plant material for useful traits. This is often seen as 'routine' and 'non-challenging' science by research funders and so it is difficult to obtain substantial funding for this activity, although in the UK, the Vegetable Genetic Improvement Network (VeGIN) (http://www2.warwick.ac.uk/fac/sci/lifesci/research/vegin/brassica/**)** and the Oilseed Rape Genetic Improvement Network (OREGIN) (www.oregin.info**)** have supported phenotyping on a small group of traits in *Brassica* . This does not apply to *Brassica* crops alone, since at the recent International Symposium on Carrot and other Apiaceae held in France (September 2014), the conclusion of the international group discussing crop improvement in carrot was that 'phenotyping' is now the bottleneck (Rosemary Collier, personal communication).

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