

Utilisation and future needs for molecular markers in *Brassica* oilseed breeding

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ABSTRACT

The majority of the work utilising molecular markers in *Brassica* oilseed breeding has to date been based on genetic mapping using various DNA markers systems, in segregating populations generated by individual users for investigation of particular traits of interest. As PCR techniques have developed over the last 15 years, a wealth of new marker technologies have arisen which have enabled the generation of high-density molecular maps for all the major *Brassica* crop species. Molecular markers linked to a wealth of agronomically important traits have been reported, and a number are now successfully integrated in oilseed breeding programs. This review describes the current use of available genetic marker technologies in practical rapeseed breeding and provides an outlook for potential future uses of new technologies including single nucleotide polymorphisms, haplotyping and allele-trait association studies.

Key words: Molecular markers – genetic maps – marker assisted selection – breeding

INTRODUCTION

Molecular markers have been widely used to map agronomically important genes in *Brassica* genomes and to assist rapeseed breeding and selection procedures. Comparative mapping using molecular marker techniques has also enabled detailed investigations into the complex duplicated structure of *Brassica* genomes and given important insight into their evolution and composition (e.g. Lagerkrantz and Lydiate 1996). The majority of the work utilising molecular markers in *Brassica* oilseed breeding has to date been based on genetic mapping using various DNA marker systems, in segregating populations generated by individual users for investigation of particular traits of interest. As PCR techniques have developed over the last 15 years, a wealth of new marker technologies have arisen which have enabled the generation of high-density molecular maps for all the major *Brassica* crop species. Molecular markers linked to a wealth of agronomically important traits have been reported, and a number are now successfully integrated in oilseed breeding programs. In many cases, however, the implementation of mapping information for marker-assisted selection has not achieved the early expectations, particularly in the case of quantitative trait loci (QTL). QTL observed in a given mapping population are not necessarily transferable to other material, and in many cases the genetic distances between markers flanking QTL are too large to enable either marker-assisted breeding or map-based cloning approaches. Two recent developments may assist in overcoming these shortcomings: Firstly, the close relationship between *Brassica* and the now completely sequenced model plant *Arabidopsis thaliana* (Fig. 1) makes it possible in many cases to transfer *Arabidopsis* genome information to *Brassica* genomes, based on microsynteny between genome regions involved in traits of interest – particularly now that physical genome libraries are becoming more widely available. Secondly, the implementation of haplotype information for allele-trait association studies has the potential to play a major role in *Brassica* breeding, because the genomic information that can potentially be gained will no longer be applicable to only isolated crosses segregating for a few traits of interest. The major challenges now facing *Brassica* geneticists and breeders are (i) to align existing genetic and physical maps in order to agree on a consensus map with a standardised nomenclature, and (ii) to compile and integrate relevant phenological, morphological and agronomic information with allelic information for *Brassica* oilseed germplasm, in order to enable the development of association mapping techniques for exploitation of available genetic resources outside of the narrow rapeseed gene pool. This review summarises important milestones in *Brassica* marker applications and discusses the importance and potential of recent developments with respect to *Brassica* breeding and genetics.



Fig 1. Together with new, highly informative and high-throughput marker technologies, the complete sequence information and gene expression data now available from *Arabidopsis* has enormous potential for application in genetic analysis and breeding of *Brassica* crops, the closest agronomically relevant relatives of *A. thaliana*.

AVAILABLE MARKER SYSTEMS

The field of molecular genetics has developed beyond all expectation since the publication of the structure of DNA by Watson and Crick 50 years ago. Protein and isoenzyme markers still play a role for specific questions in commercial plant breeding, but genome research and marker-assisted applications in *Brassica* first began to flourish in the late 1980s with the development of the first restriction fragment length polymorphism (RFLP) linkage maps for *B. oleracea* (Slocum et al. 1990), *B. rapa* (Song et al. 1991) and *B. napus* (Landry et al. 1991). Shown as an example in Figure 2 is the RFLP map constructed by Uzunova et al. (1995) and extended with additional markers (W. Ecke, University of Göttingen, unpublished results).

With the discovery of the polymerase chain reaction (PCR) the potential arose to greatly increase the marker density in the existing maps, first with randomly amplified polymorphic DNA (RAPD) markers and more recently with amplified fragment length polymorphisms (AFLP) and inter-simple sequence repeats (ISSR). The ability to convert anonymous PCR markers that are closely linked to traits of interest into sequence characterised amplified region (SCAR) or sequence tagged site (STS) markers has provided a platform for the development of simple PCR-based markers that meet the requirements and capacity of commercial rapeseed breeders. Further progress has been achieved with the development of simple sequence repeats (SSR), also known as microsatellites, which – due to their highly polymorphic and robust nature and simple, relatively inexpensive analysis – are a particularly valuable resource for map alignment among different materials. Unfortunately the number of *Brassica* microsatellite primers publicly available (see www.brassica.info/ssr/SSRinfo.htm) is still relatively low in comparison to other important crop species, meaning that a true consensus map and nomenclature has still not been agreed. However, the release into the public domain by a commercial *Brassica* microsatellite consortium (Lydiate and Sharpe, 2003) of a set of robust, highly polymorphic, mapped SSR markers spanning the entire *B. napus* genome will greatly assist the *Brassica* genetics community in mapping and genome integration. Great potential for extremely fine genetic mapping is offered by the highly abundant single nucleotide polymorphism (SNP) markers, which offer the opportunity to uncover allelic variation directly within expressed sequences of candidate genes and to develop haplotypes based on linkage disequilibrium for analyses of quantitative traits. Although SNP detection currently still relies on techniques and equipment that are beyond the scope of the majority of plant breeders, it can be expected that SNP markers will play a major role in *Brassica* genetics and breeding in

coming years, as high-throughput SNP discovery and genotyping methodologies developed for human genetics are made more accessible and cost-effective.

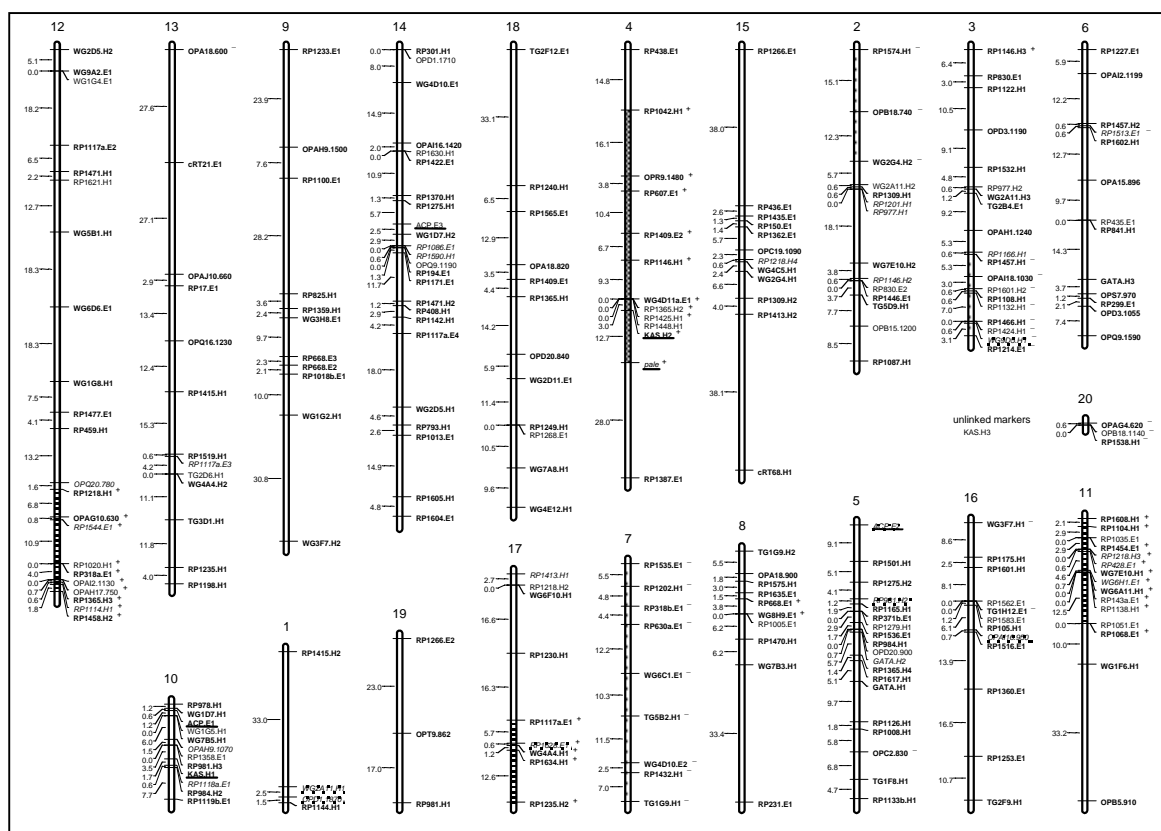


Fig 2. Genetic map of *Brassica napus* constructed from the cross ‘Mansholt Hamburger Raps’ x ‘Samourai’ (see Uzunova et al. 1995). The map consists of 214 RFLP markers (prefix RP, WG or TG), 35 RAPD markers (prefix OP) and one phenotypic marker, *pale* – corresponding to the gene for pale yellow petal colour arising from the ‘Mansholt’ parent. In total 20 linkage groups are formed, whereby one RFLP marker could not be grouped. Alignment with other maps has shown that linkage group 20 belongs to the same chromosome as linkage group 1.

APPLICATIONS OF GENETIC MAPS IN BREEDING

In recent years a number of practical examples have demonstrated the power of high-density genetic maps and candidate gene studies for identification of genetic markers closely linked to important traits in *Brassica* crops. Table 1 shows examples of some of the traits for which genetic mapping has been employed to discover gene loci and identify linked markers. For example, Ecke et al. (1995) localised the genes controlling erucic acid content in *B. napus* and identified loci contributing to seed oil content. Somers et al. (1999) identified RAPD markers associated with linoleic acid desaturation in *B. napus* and found that the gene *fad3* localised near one of the identified QTL for this trait. Hu et al. (1999) also found RAPD markers linked to linoleic/oleic acid content in rapeseed oil, and furthermore developed sequence tagged markers associated with low linolenic acid content that appeared to be linked to an omega-3 desaturase gene. Pilet et al. (2001) identified QTL for field resistance to blackleg in crosses of different genetic background, whereas Plieske and Struss (2001) converted RFLP and RAPD markers linked with B-genome blackleg resistance into STS markers for use in marker assisted backcrossing of the introgressed trait in *B. napus*, and Walsh et al. (1999) mapped genes responsible for turnip mosaic virus resistance in oilseed rape. Availability of genetic markers for resistance genes will enable combination of such genes in order to establish more durable resistance against major oilseed rape pathogens. Numerous studies have concentrated on mapping of genome regions controlling flowering time and vernalisation requirement (e.g. Ferreira et al. 1995b), and molecular markers are widely used by commercial

plant breeding companies to test for fertility restorer genes for male sterility systems. The development of genetic markers is of particular interest in selection for (recessive) complex traits that are strongly influenced by environment, for example yellow seed colour in *B. napus*. Chen et al. (1997) identified RAPD markers linked to a C-genome gene involved in seed colour expression, and Somers et al. (2001) also used RAPD markers to identify a major locus associated with yellow seed colour in *B. napus*. The work described by Badani et al. (2003) and Lotz et al. (2003) on genetic characterisation of seed colour is a good example of an integrated genetic mapping and candidate gene approach using *Arabidopsis* resources and genome tools to identify gene loci involved in complex traits. Homoeologous *B. napus* loci involved in flavonoid biosynthesis, identified by screening a *B. napus* BAC library with specific *A. thaliana* *tt*-gene primers, are to be localised in genetic maps in order to identify associations between individual *tt*-loci and the major QTL associated with seed colour. In future it is such integrated methods that are likely to have the best success in the study and isolation of gene loci involved in important quantitative traits in rapeseed.

Table 1: Selected examples of some of the important traits for which genetic maps have been used to discover gene loci or QTL and/or identify closely linked markers in *B. napus*.

Character	Specific trait	Selected references
Disease resistance	Blackleg (<i>Leptosphaeria maculans</i>)	Pilet et al. (2001)
	White rust (<i>Albugo candida</i>)	Ferreira et al. (1995a)
	Turnip mosaic virus (TuMV)	Walsh et al. (1999)
	Turnip yellows virus (TuYV)	Dreyer et al. (1996)
Oil content/quality	Erucic acid content	Ecke et al. (1995)
	Glucosinolate content	Uzunova et al. (1995)
	Oleic acid content	Hu et al. (1999)
	Linoleic acid content	Hu et al. (1999)
	Linolenic acid content	Tanhuanpaa et al. (1995)
Abiotic stress	Cold tolerance, winter hardiness	Kole et al. (2002)
Cytoplasmic male sterility	'Ogura' CMS fertility restorer	Delourme et al. (1998)
	'Polima' CMS fertility restorer	Jean et al. (1998)
Morphological traits	Plant height (dwarf locus)	Foisset et al. (1995)
	Flowering time	Ferreira et al (1995b)
	Petalless flower	Frey et al. (1997)
	Seed colour	Somers et al. (2002)

OUTLOOK

The growth of *Brassica* EST collections and initiatives to sequence *Brassica* genomes and develop integrated physical maps will doubtless assist in the identification of single nucleotide polymorphisms for a large number of agronomically relevant candidate genes in coming years. By combining SNP haplotype data with pedigree and trait information from large sets of rapeseed genotypes it will be possible, hopefully in the not to distant future, to elucidate the genetic control of a large number of important traits via allele-trait association studies. Together with developing DNA chip technologies and using the enormous progress already made in *Arabidopsis thaliana* as a foundation, the next ten years should see enormous gains in knowledge of the molecular genetics of *Brassica* crops. As the most important close relative of *Arabidopsis*, oilseed rape is likely to be one of the major crops to benefit most from the new information and technologies becoming available in the field of plant molecular genetics. Thus it can be expected that practical rapeseed breeding will in the next decade reap considerable benefits from molecular genetic research, technology and marker development.

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