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REVIEW

Current status and future possibilities of molecular genetics techniques in *Brassica napus*

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Abstract As PCR methods have improved over the last 15 years, there has been an upsurge in the number of new DNA marker tools, which has allowed the generation of high-density molecular maps for all the key Brassica crop types. Biotechnology and molecular plant breeding have emerged as a significant tool for molecular understanding that led to a significant crop improvement in the *Brassica napus* species. *Brassica napus* possess a very complicated polyploidy-based genomics. The quantitative trait locus (QTL) is not sufficient to develop effective markers for trait introgression. In the coming years, the molecular marker techniques will be more effective to determine the whole genome impairing desired traits. Available genetic markers using the single-nucleotide sequence (SNP) technique and high-throughput sequencing are effective in determining the maps and genome polymorphisms amongst candidate genes and allele interactions. High-throughput sequencing and gene mapping techniques are involved in discovering new

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alleles and gene pairs, serving as a bridge between the gene map and genome evaluation. The decreasing cost for DNA sequencing will help in discovering full genome sequences with less resources and time. This review describes (1) the current use of integrated approaches, such as molecular marker technologies, to determine genome arrangements and interspecific outcomes combined with cost-effective genomes to increase the efficiency in prognostic breeding efforts. (2) It also focused on functional genomics, proteomics and field-based breeding practices to achieve insight into the genetics underlying both simple and complex traits in canola.

Keywords Breeding · Canola · Quantitative trait locus · Molecular markers · Sequencing · Gene mapping techniques

Introduction

Canola is the second most important crop and source of vegetable oil in the world (Starner et al. 1999). Therefore, it plays a considerable role in global food security from the prospective of growing populations (Shahin and Valiollah 2009). Brassica has approximately 100 species, including *B. napus*, spp. *oleifera*, commonly known as rapeseed, canola and oilseed rape (Rieger et al. 2001). Canola refers to those varieties of *B. napus* that meet a specific limit of erucic acid (< 2% in oil) and glucosinolates (30 μmol/g in toasted oil-free meal).

The canola crop gives more output and returns in the farming community compared to pulses (Norton et al. 1999). With the introduction of hybrid varieties in the farming community, canola production has been enhanced under normal and poor soil conditions, and net returns and share in the GDP have increased (Brandt et al. 2007). In the hybrid development of Brassica, the primary obstacle is to determine the best combination from different cultivars for heterosis, good seed yield and genetic variation. Thus, genetic variation does not always linearly correlate with heterosis. There was a considerable improvement in total additive and non-additive genetic value when seed yield and oil contents were considered in pedigree selection with GE (Genetics × Environments) (Beeck et al. 2010).

In a *B. napus* breeding programme, numerous molecular markers are directly linked with agro-morphological traits, and some of them have been successfully assimilated for marker-assisted selection (MAS), but for quantitative traits, the programme has not yielded satisfactory results. QTL population mapping is not necessarily transferable by molecular markers because flanking distance between the markers is very large. Therefore, integrated approaches are inevitable for canola improvement to collect field trial data along with QTL mapping and constructing a linkage map to find good allele and gene pairs involved for high yield within existing genetic resources. Application of molecular markers for Brassica development has an enormous challenge to figure out the gene of interest and allied lines used for sustainable agricultural programmes. To implement all the breeding and molecular tools for genomic selection in Brassica oilseed crops, a possible approach is using genome by single-nucleotide polymorphism (SNP) markers under a next-generation sequencing platform for effective screening. The present review discusses integrated approaches, such as molecular markers technologies, used to determine genome arrangements and interspecific outcomes crossed with cost-effective genomes in order to increase the efficiency in prognostic breeding efforts. We also discuss modern breeding with an updated synthetic view of how functional genomics, proteomics and field-based breeding practices can effectively improve canola productivity for global needs as well as for sustainable agriculture.

Yield restrictions

In the early 1990s, different legislation was enacted by authorities to regulate Brassica production in the world. The purpose of this legislation was to minimize the cross-pollination conflicts amongst vegetable seed growers, while seed companies producing these important crops (Gulden et al. 2009) acquired increased fuel prices and excellent adaptations of canola amongst growers. The seed yield of any crop is an important but complex characteristic of studies controlled by different alleles, making it possible to identify the right allelic interaction between crop plants (Rehman 2013). Over the last few decades, there has been a dramatic increase in the biology and

chemistry of nucleic acids. It is now possible to isolate the genes of interest and determine their structures by breeding, DNA extraction techniques, polymerase chain reaction (PCR), molecular markers and genetic analysers to determine the desired gene available for canola production (Warwick 2000).

Breeding

The development of genetic diversity-focused crop breeding is a good technique for improving yield and developing resistant varieties against biotic (pest and disease) and abiotic stresses. Their success will depend upon the combined used of genetic engineering and traditional breeding goals (Fahad et al. 2014; Noman et al. 2017). Previously, several millennium crops have been improved regarding quality, resistance against pests and diseases, and higher yield traits. Breeding involved hybridization by selection of the segregated progeny for particular traits of interest. Several modifications have been made in agricultural practices, for instance, increased fertilizer dose for better growth as well as development and introduction of selective herbicides, fungicides and pesticides. Crop composition can be modified by crops grown with high output value (Brandt et al. 2007).

Traditional breeding practices

Ever since the beginnings of the domestication of plants some 10,000 years ago, plant breeding has been particularly effective in developing crops and varieties that have contributed to the development of modern societies. Plant breeding technology has developed from genetics, but crop improvement depends on identification of particular traits required for achieving high-profile features, especially on oil quality, yield and resistance against pests and diseases. Moreover, success in breeding also depends on an understanding of plant physiology, pathology and biochemistry of the specific crop. A number of genes or alleles, present at a specific locus, have different interactions with their function in plants. Traditional plant breeding is concerned mainly with the directed re-assortment of the allelic variants to produce allele combinations suitable for genotypes assumed to be the best in specific environmental conditions. Embryo rupture and ovule culture methods have been employed to enhance the species value by transferring the disease resistance that could be sexually hybridized with oilseed rape. The black leg (*Phoma lingam*) disease resistance was introduced to *B. juncea and B. napus* using embryo culture to increase the survival of the hybrid with backcross, along with reducing the generation time. The hybrids developed, however, gave important information about DNA behaviour and pairing between weak homologous genomes and loci with various allelic interactions that are relatively easy to manipulate by crossing and selecting for a specific condition (Nelson et al. 2004). Molecular marker technology is used to compute the traits and allelic interactions for genetic diversity (Gupta 2006). It could also be helpful in determining the loci number involved in yield, seed weight, seed colour and their shape that could differ from other species. Likewise, the recent integration of advances in biotechnology, such as genomic study, and molecular marker applications with conventional plant breeding practices has opened the basis for molecular plant breeding, a multidisciplinary science that is reforming twenty-first century crop improvement.

The methods of molecular plant breeding are of great interest amongst plant breeders and crop scientists (Varshney et al. 2007). Figure 1 depicts a framework for crossing with different allelic interactions. Brassica lines were produced from substitutes of three different genomes, A, B and C (A = natural Brassica; B = *Brassica napa;* C = *Brassica carinata*). Approximately 16% of self-pollinated offsprings carry 38 chromosomes, whilst the rest of progenies lack the B genome sequence. It has also been reported that B genome chromosomes were easily eliminated during self-pollination (Xiao et al. 2010).

Many elite varieties contain different detrimental alleles. A rigorous and frequent breeding cycle is required to remove the hazardous effects and ultimately discover a new variety adapted to a new environment. To achieve finding elite lines with good characteristics, many constraints associated with interspecific crosses must be overcome. Several comparative studies, to test combinations from different lines, have demonstrated that crosses of canola lines with interspecific hybrids of canola and *B. rapa* species increased the seed yield of canola by almost 90% (Xiao et al. 2010).

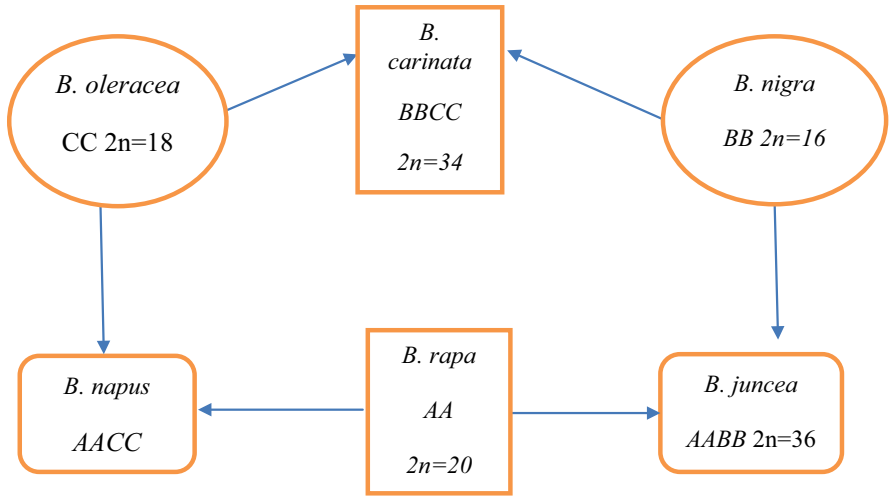

Fig. 1 Crossing with different allelic interactions (Source: Collier and Mullins, 2012)

Breeding improvements and genetic potential

Several breeding modifications for enhancing the yield and oil qualities of the canola crop have been documented by numerous researchers and scientists. Amongst the Brassica relatives, *Sinapis arvensis* (wild mustard) is the most common weedy plant in the world. The interspecific crossing between *B. juncea* × *S. arvensis* backcrossed with *B. Juncea* × *S. arvensis* (Bing et al. 1991) resulted in weak and sterile plants that had no seed production. This cross would not be the result of a natural transfer where the inserted traits into the species are stable. Weed species from the Brassicaceae family, such as *Raphanus raphanistrum* (wild radish) and *Erucastrum gallicum* (dog mustard), are locally quite abundant and were hybridized with canola as a female parent (Gupta 2006). *B. napus* × *R. raphanistrum* cross results showed that F1 hybrids have fertile pollen (0–65.4%) with a low number of F2 progeny (Rieger et al. 2001), while hybrids resulting from the *D. muralis* × *B. napus* and *D. erucoides* × *B. napus* crosses have sterile males (Ringdahl et al. 1987).

Improvements in breeding technology can be considered under several categories such as (1) alien genotypes, (2) high precision and speed of selection, (3) breeding system modification, (4) reduced generation time, and (5) an improved and more precise definition of breeding objectives.

Alien genotypes

Alien variation is a sexual hybridization method introduced in crop plants. Different procedures are being used to overcome the natural hybridization, as some species of *Vicia faba* cannot be related to the hybrid species production. The desired traits could be transferred by selection and a repeated backcross. The repeated backcross method is successful only when detectable gene(s) or allele(s) are easy to transfer and there is a presence of a limited number of genes in the species that can be irradiated or transferred by pollen grain in random order (Warwick 2005). The quantitative characteristics cannot be easily recognized in genomic segments. Two-parent families derived from cross-genetic mapping of candidate genes combine with the family and population genetic analysis for disequilibrium, which strengthen the power of the top QTL to discover more gene(s) in the future for canola improvement (Yu et al. 2008) (Fig. 2). QTL mapping simplifies the analysis value involved for the growth of population exchange with genetic causality established in a single gene (Harjes et al. 2008). In plant breeding technologies, double haploid or F_2 populations were derived from a cross of two inbred lines. An F1 single-genotype individual contains segregating materials; so that association between loci can be predicted based on the mapping distance. In this way, we can generally get a low resolution of QTL localization. The accuracy of the QTL localization can be improved when larger numbers of meiotic events are measured. The value of the recombination

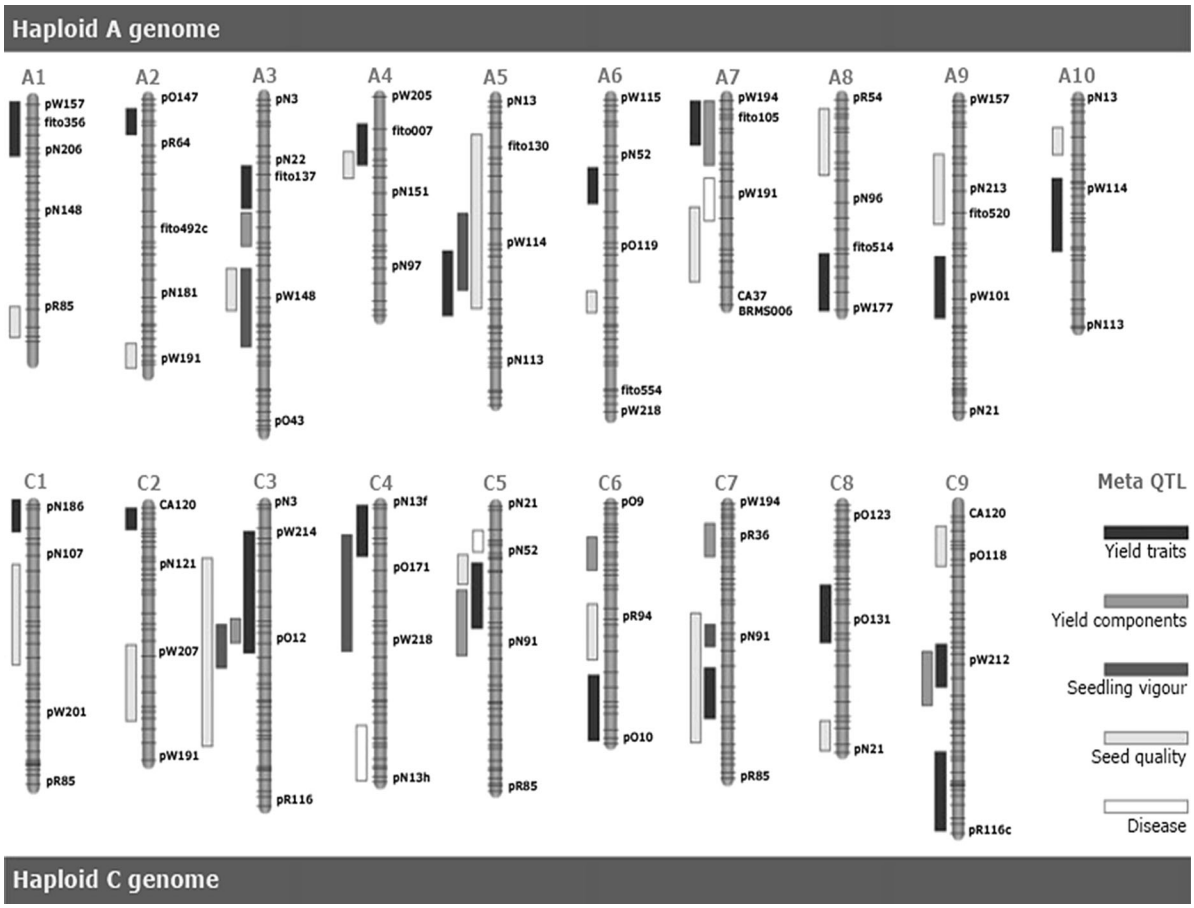

Fig. 2 Meta quantitative trait loci genetic map for yield traits and nutritional characteristics in *B. napus*. Reported while QTL location was confirmed, Radoev et al. (2008) for allelic variation and target enrichment depleted in *B. napus* gene pool (Source Snowdown et al., 2012)

frequency will be greatly undervalued between any QTL and its surrounding regions, as the inbred lines are more active for fine mapping of the QTL(s) (Snowdon and Friedt 2004).

Molecular markers are used to scan the genomes for the selection of individuals containing favourable alleles and transgenes by recurrent selection. Genomics, plant biotechnology and molecular marker application established new ideas, analyses and genetic variations for the development of improved varieties (Collard and Mackill, 2008). QTL history mapping related to agronomy and nutritional characteristics are reported in oilseed crops to evaluate various SNPs sequences, particularly QTL target genomes (Basunanda et al. 2010). Meta-QTL trait genomes are highly interesting for determining the specific target regions important for diversity. Hybridization-based sequence techniques were derived to determine the target genomes by resequencing (Albert et al. 2007) and discover SNPs in canola (Pichon and colleagues conference presentation: http://www.intl-pag.org/18/abstracts/W92_PAG XVIII_643.html). Sequence-captured technologies for polyploidy crops such as canola, in which homologous target loci exhibit imprisonment, can be fixed by interlocus polymorphism later on (Albert et al. 2007).

High precision and selection speed

Plant phenotypic growth characteristics depend on the choice of traditional historical background. In some cases, visual interest is removed to reveal the nature of the organ. Plant phenotypic characteristics are affected by (G × E) interactions by measuring the error

difference and allowing the breeder to find characteristics for crop improvement (Warwick 2005). In the future, it may be possible to identify individual alleles by a DNA product and antibodies probe. Integrated plant breeding approaches decrease the costs of selection and time. The process involved physiological and biochemical characteristics to determine the phenotypic efficiency through strong genetic correlation and high genetic diversity amongst the cultivars. Sequence DNA tags and molecular marker genomic approaches are considered for the selection of phenotypic characteristics, especially for environment indices (Jansen and siesta 2001). Marker-assisted selection strategies are used to accelerate the spread of transgenes in commercial cultivars, which was obtained by backcrossing (Tuberosa et al. 2007). However, in the future, drought tolerance or nutrients might be the main limiting factors for the canola crop. Biotechnological tools provide genetic background information for gene expression to optimize genetic potential for crop improvement. Transgenic modification may alter the phenotype of the individual selected through environment selection.

Breeding systems modification

Breeders adopt various strategies from plant reproductive systems to develop new varieties (Collard and Mackill 2008). These strategies include self-compatibility (movement of pollen amongst flowers of the same plant), where insects facilitate pollination within the flower, and in herkogamous species, where insects move pollen between distanced flower parts, making it more vulnerable to differentiate from autonomous self-pollination (automatically a flower without a pollinator). The selection of a particular strategy largely depends upon good genetic architecture, a self-compatible habitat and homozygous compatibility achieved by the self-pollination process (Howlett 2004).

Gene transfer by self-pollination means more of the genes associated with the desired traits will be transferred. Some traits are not identified by molecular biology, and these are directly involved with self- or cross-pollination. Incompatibility at the molecular basis is not well understood, and more research must be done on the system to determine the desired traits. Literature has reported that self-incompatibility blocks self-pollination at specific stages such as pollination, fertilization and the seed maturation stage. Therefore, self-incompatibility not only aids in finding the genetic information for specific traits, but also enables the gene(s) to transfer at a specific level in the crop (Sivasithamparam et al. 2005).

Reduced generation time

The maximum potential for genetic gain is proportional to the phenotypic variation present in the original source, and subsequent cycles of selection remain proportional to the environmental factors. Phenotypic variation is positively associated with genetic diversity, but at the same time, environmental factors and interactions between genotype and environment also mainly depend on the phenotyping. Alien substances that do not adapt to the environment between the natural crosses or induced mutation lead to the introduction of transgenic events, or combinations of these sources can be derived from the crosses of segregated progeny. In many crops, such as wheat or canola, farmers might be able to reduce the life cycle of the plants.

Figure 3 reveals the generation cycle related to genetic gain, phenotypic variability and length of the generation for a variety. Large genetic correlation and genotypic selection, instead of phenotypic selection during the life cycle, make it possible to reduce the cycle times, while the bad use of phenotyping selection can achieve additional savings in nurseries by combining the scores of phenotypic data and molecular markers. GE interaction has positive effects on oil contents in diploid species. Oil contents of the rape species are controlled by the embryo and cytoplasm, while GE controls the maternal genetic effect, whereas the embryo and cytoplasm play the main role behind the scenes (Wu et al. 2006). Plant yield is dependent on yield components.

Improved and precise breeding goals

Improved and precise breeding goals are more important for canola crop improvement because otherwise it is more difficult to obtain the desired goals. Molecular biology is involved in controlling the growth and development of any studied crop. Some yield traits are directly involved with molecular biology and can be explored by some application processes. Qualitative and quantitative effects are important when numbers

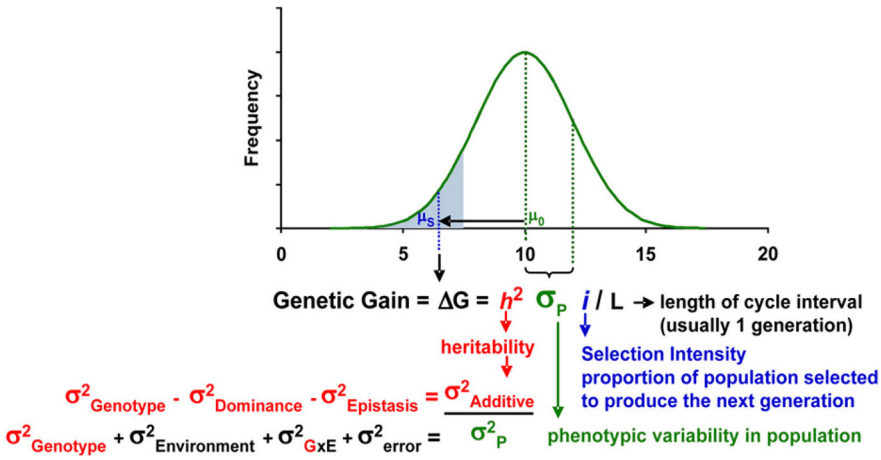

Fig. 3 Generation cycle related to genetic gain, phenotypic variability and length of the generation (Source: Moose and Mumm 2008)

of genes are known, but financial issues hinder the process to adopt a programme in order to recognize those gene pairs controlling the traits. In general, the commercial adoption of new techniques depends on economic conditions. For conventional plant breeding, selection based on phenotype alone has been effective historically.

Molecular biology and canola improvement

Yield and crop quality

Brassica is a member of the intermediate C3–C4 photosynthetic carbon metabolism, and the presence of chloroplasts and mitochondria in its leaf sheath cell tissue indicates the presence of phosphoenol pyruvate (PEP) carboxylase activity. C3 and C4 species have an intermediate carbon dioxide compensation point (Yu et al. 2008). This feature can be useful for rape improvement because of photorespiration, which causes a reduction in net photosynthesis, and thus the crop can withstand low light availability and harsh environmental conditions. In rape, the recipient's homologous genes are turned off or eliminated compared to wheat crops, where they are more affected.

It is more important for genes to disappear in the host cell at the right time, and particularly in the exact cell in the correct amount, they play a significant role if proven effective but are expensive (Nelson et al. 2007). A gene's resistance against herbicides would also helpful for a breeding programme. Herbicide weed control was successful in cereals, so it is possible to identify their desirable genes and transfer them into canola crop for resistance against herbicides. Microorganisms are an alternative source of resistance, which might offer possible utilization for transfer to the genes. Many desirable genes and alleles are available for scientists and researchers with an opportunity to exploit the hybrid. If we find the specific gene or gene sequence, then there is a possibility that a desired gene could be transformed for improving yield and oil quality in canola.

Marker-assisted selection (molecular markers) techniques for *B. napus*

It is a general consensus amongst breeders and researchers that genetic diversity exists in spring canola for a breeding programme. Amongst the canola gene pool, genetic diversity determined by molecular marker techniques (used for clearly distinct canola species) has also been preserved for future use, and these gene pools could be used for canola genome improvement. Unadopted canola germplasm in spring canola breeding could be challenged by the introduction of negative traits/alleles from exotic germplasms in spring canola and would require repeated breeding cycles for crop improvement. The elite species needed for a breeding programme have more problems associated with interspecific hybrid production and introduction of unwanted allele's linkage drag and hybrid sterility. Allelic interactions amongst improved canola with exotic varieties for the improvement of seed yield and other traits are being investigated in

open-pollinated and hybrid cultivars with encouraging results (Rehman 2013).

Molecular markers, used for genome sequencing and characterizing the genetic material from the gene pool, are present in all crops. Some efforts have been made to increase the knowledge about genetic diversity and their future uses for a crop improvement programme. The alleles and nucleotide sequence confirmed by molecular markers provide basic information about genetic diversity from wild ancestors and elite varieties geographically distributed over a wide range that have had their germplasm materials conserved in the gene bank for future use (Buckler et al. 2006). Parental mating detection is used to identify the traits that are involved in QTL, while genomic selection has been proposed to identify these deficiencies so breeding lines are used to determine the phenotypes by molecular marker scores in the population. Some selected important traits are described in Table 1, where genetic markers are used for mapping loci in canola. Restriction fragment length polymorphism (RFLP), amplified fragment length polymorphism (AFLP), simple sequence repeat (SSR), and RAPD primers are used to determine the genetic diversity present in the canola crop (Jesske et al. 2011). High allelic diversity is present amongst winter fodder and vegetable types of the Brassica species (Hasan et al. 2006). Chinese spring canola has showed greater genetic diversity from Swedish spring canola when SSR markers were applied. Thus, improvements can be made with the use of non-oil seed with oil seed types of canola (Zhou et al. 2006). More than 80 spring and winter canola varieties from 18 breeders showed grouping towards cultivars from the same breeder using the AFLP marker system. Hence, genetic variation determined by molecular markers will deliver pedigree information towards a gene pool in the breeding system (Hu et al. 2007). Genetic diversity for *B. napus* grown in specific environmental conditions cannot be passable for permanent improvement for the long term (Hasan et al. 2006).

Molecular biology and disease resistance

Understanding the molecular mechanism against gene resistance is associated with (a) the need for research and (b) research to improve disease resistance in crop plants. There are some necessary things that could be identified; (i) disease-resistant sources (ii) the transfer of resistant genes and (iii) monitoring and evaluation of their activity in the presence of resistant genes. In general, current breeding methods and their selection can be achieved with regular screening for these traits but with varying degrees of success and often only after prolonged effort (Fahad et al. 2014). The chemical fungicides used to control diseases have negative effects on human health and the environment. Therefore, to manage this problem, new strategies were adopted. The constant use of fungicides has

Table 1 Summary of the selected important traits where genetic markers used for mapping

Specific character	**Trait**	**References**
Morphological trait	Plant height	(Ferreira et al. 1995)
	Flowering time	(Somers et al. 2001)
	Seed colour	(Fray et al. 1997)
	Petal flower	
Male sterility	"Ogura" Fertility rest over	(Brown et al. 2003)
	Polymer fertility rest over	(Jean et al. 1998)
Oil quantity	Erotic acid content 1	(Das et al. 2002)
	Glucosinolate contents	(Uzunova et al. 1995)
	Linoleic acid contents	(Hu et al. 1995), (1999)
	Oleic acid content	
Abiotic stress	Cold tolerance, winter hardiness	Kole et al. (2002a)
Disease resistance	White rust, Black leg, turnip mosaic virus, Turnip yellow virus	Kole et al. (2002b)

Loci in *B. napus*

developed resistance to pathogens strains. Bio-control is an alternate viable way to suppress diseases using microorganisms in order to improve plant health and growth and thus increase the canola yield and its oil quality.

Sequence-based techniques for Brassica improvement

Brassica species have greater competition in commercial breeding and consumer demands due to NGS (Next-generation sequencing)-based technology. There is a constantly increasing demand for new varieties from breeders to seek low input for target regions that are directly linked with agronomical and nutritional traits (Collard and Mackill 2008). In 2003, Brassica genomes were sequenced for the first time at a smaller scale with less intricate genomes with two diploid progenitor genomes of canola (Snowdown et al. 2012). Applications of peak next-generation DNA sequencing methodologies have a greater impact on a massive platform in genetics. New DNA-based technologies mainly focus on genome analysis at a greater scale with fine-tuned resolution to obtain single-base accuracy. In modern times, RNA sequences with full-length cDNA analysis, as well as non-coding RNA technologies, are now being developed.

Next-generation sequencing would also help with basic fundamental biological work for a full canola genome sequencing as well as with future concerns of the breeder (Margulies et al. 2005). It will also help in identifying novel genes derived from pre-historic DNA sequences as well as significantly increase the scope of Meta-genomic analysis derived from the environment. Integrated approaches of these technologies have a dramatic change in genetic and biological research. High-throughput screening needs discoveries in SNP (single-nucleotide polymorphism) for all major and minor crops. This technique has been tested in many crops with more or less complex genomes, i.e. canola. Data regarding whole genomes have been financially feasible for reading sequencing of ESTs (expressed sequenced tags) (Parkin et al. 2010), 454 amplicon sequences (Gholami et al. 2012), transcriptome profiling (Bancroft et al. 2011), or array-based techniques, which have been established for mid- to large-scale SNP discoveries for canola lines. Polymorphism and chromosome re-arrangement will help offer a comprehensive description of early and ancient species of *B. napus.*

Sequential differentiation and transcriptome abundance allow the scientist to construct a genetic map of twin single-nucleotide polymorphisms against canola and *B. napus* genomes with its ancestors. There is a dire need to develop a mechanism for tracking the rearrangements and inheritance for completely genomic segments. This technique is cost-effective for a canola breeding crop improvement programme to determine the crop genomes by a transcriptome sequence, consequently increasing the predictive efficiency of the breeding programmes (Bancroft et al. 2011). Expression analysis techniques are being used to determine the redundant transcriptome in allotetrapoloid genomes of canola, preserving multiple copies of the genes. Canola genomes are closely related to the main diploid Brassica species evolved from a hexaploid. Expressed sequenced tags were used to determine the differentiated sets of the transcriptome and the efficacy of identifying the distinguished transcriptome in canola using microarray hybridization. Microarrays did not identify the distinguished transcript that was closely homologous related while expressed differentially towards unique transcripts. Gene expression in polyploidy canola species should be transformed to next-generation sequencing, which is cost-effective in obtaining millions of sequenced tags for whole canola genomes. Triplicate genome segments of *B. oleracea* spp. are paralogous with each other and segmentally duplicated with *Arabidopsis thaliana* genomes. Synonymous analysis-based substitution predicted that the triplicate Brassica segments diverged from a common ancestor after divergence with Arabidopsis and Brassica lineage.

Genome triplication inferred 35% of the gene occurred in Brassica lineage by loss or deletion of the mechanism. Gene encoding in a single transduction or transcriptase was not found to significantly retain those encoding proteins classified with other functions (Town et al. 2006). Sanger sequenced-based technologies with NGS data were assembled, and a press release occurred in August 2011. The data regarding complete, ongoing planned projects for sequencing and resequencing in *B. napus* are presented in Table 2. The countries involved during this consortium for assembling sequenced data genomes for *B. Oleracea* and *B. napus* are China, North America, Australia and Europe. The whole genome of canola was already

Table 2 Some examples of ongoing, planned for sequencing and resequencing projects for Brassica improvements

Accession	Type	Sequencing aim	Platform	Country
DH12075	Spring canola	*B. napus* reference assembly	Illumine, 454 Sanger	CA NSEQ
Damor DH	Winter oilseed rape	*B. napus* reference assembly	Illumine, 454	France
Zhongshuang11	Spring oilseed rape	*B. napus* reference assembly	Illumine, 454	China
Tapidor DH	Winter oilseed rape	*B. napus* reference assembly	Illumine, 454	China, UK
Nagyos 7	Chinese oilseed rape	*B. napus* reference assembly	Illumine, 454	China, UK
51 lines	Diverse *B. napus* lines	Whole genome sequence	Illumine	Germany
123 lines	Diverse *B. napus*	Leaf transcriptase's	Illumine	UK
517	Species wide *B. napus*	Restriction associated DNA	Illumine	Assyst/Germany
Express 617	Winter oilseed rape mapping	RAD	Illumine	Germany, China
10 inbred lines	6 winter and 4 spring	Sequencing capture	454, Illumine	Chile, Canada

publicly released in 2009 by Bayer Crop Science (http://tinyurl.com/28cbe6v) (Snowdown et al. 2012). Research reported on the cost per basis of genomes revealed that cost per base genomes was reduced by one-ninth compared to conventional sequence methods.

A linear decrease in the cost of conventional Sanger sequenced analysis techniques compared with commercialization of NGS for per base sequencing prices for the last few years is shown in Fig. 4. It is evident from Fig. 4 that the cost for sequencing continuously decreases every 2 years, indicating that a clear picture would predict canola chromosome structure variation and DNA sequence variation emerging for a single run of only a few thousand dollars per genomes. By this, we can imagine that we can also judge the polyploidy nature of the canola genome origin and novel features of the crop plant and make it beneficial for breeding purposes (Snowdown et al. 2012).

Proteomics analysis

Ultrastructural protein analysis (proteomic and genomic) revealed that the matured seed of canola had wide ranges of oil contents (36.49–55.19%). The results further predicted that oil bodies are closer in high range rather than in the low oil content line, and these differences depend upon the thickness of the cell wall and cell size. In all, 119 and 32 differentially expressed proteins from total and oil body protein were determined, respectively. QTL mapping analysis for oil contents confirmed that some genes were involved in

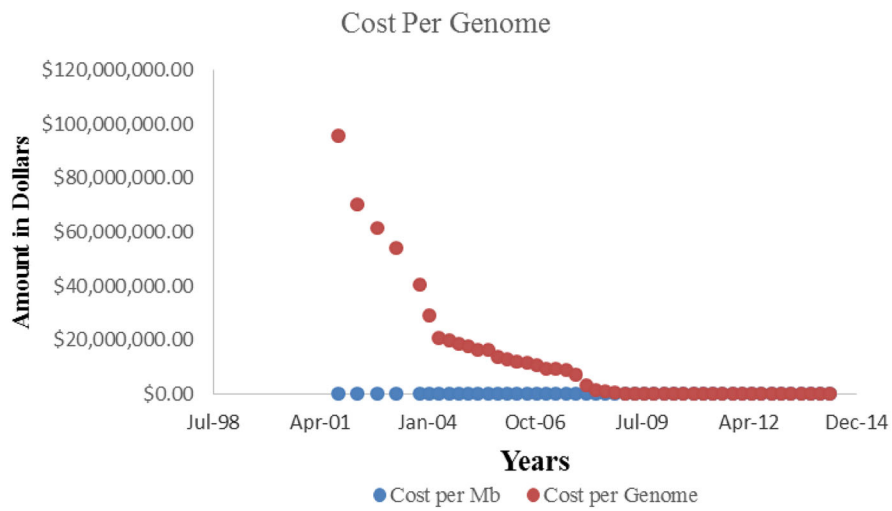

Fig. 4 Cost per Mb and Cost per genomes http://www.genome.gov/sequencingcosts/2014

differentially expressed protein profiles. The function of genes that were involved in coded storage expressed proteins that were examined by analysing full-length cDNA from canola seeds (Gan et al. 2013).

Two-dimensional gel electrophoresis was also used to determine the seed protein structure in Brassica genomes. Developmental expression protein spots (794) were found, and hierarchal clustering analysis was used to find 12 expression trends that were based on Arabidopsis functional schemes. Energy- and metabolism-related proteins were more represented in developing seed, accounting for 24.3 and 16.8%, respectively, of the total proteins (Hajduch et al. 2006).

A biosynthetic pathway was responsible for the accumulation and storage of protein in canola seed (Goffman et al. 2005). Canola embryos revealed that rubisco acts without a Calvin cycle to increase the carbon efficiency during triacylglycerol production (Schwender et al. 2004). Until now, limited information has been available for protein regulation during seed development, i.e. translation and posttranslational pathways for protein regulation. Many gene-encoding enzymes are involved in controlling the seed-filling process for respective pathways (Goffman et al. 2005). cDNA sequencing techniques provide *relevant* information *about the* canola genomes for high-throughput genomic technologies, i.e. proteomics expression gene profiling at the mRNA and gene level. Arabidopsis presents an opportunity for this type of genome profiling for seed development programmes (Aebersold and Mann 2003).

Functional genomics

Functional genomics is an important part of the plant genome that plays a great role in the research leading to crop improvement. Mutant function is a good source that provides valuable information for gene regulation metabolic activity development and chemical that can be used in reference plants, i.e. *Arabidopsis thaliana* (Long et al. 1993). Targeting induced local lesions in genomes (TILLING) is a more efficient genetic approach in the modern era and is being applied in a reference plant (Arabidopsis). TILLING is a screening type of analysis technique, in which the plant population is treated with mutated reference plants, causing point mutation followed by desired gene discovery from the population. This screening collects a series of allelic mutants with varying function. DNA samples that are used during mutational screening from self-fertilization are further used for downstream screening (Henikoff et al. 2004) based on an enzyme with a singleton DNA. Chemical mutants are used to induce the genome cut randomly easy to make larger mutant populations at all gene loci. This technique is also helpful for inducing mutation in all desired genes for all crop plants. It was also reported that duplicate genomes are easier to induce mutation checked by paralogue sets of genes. The canola genome is more complex, with the ability to induce mutants easier than others (Town et al. 2006).

Plant mapping is a powerful tool to determine the processes of genome evolution and genetic resource materials transfer between the species. In the last few decades, comparative mapping has been used against some grasses, i.e. monocot rice (Lukens et al. 2004). The canola genome was mapped with 1000 genetically linked loci at homologous positions with similar sequences. Chromosome conservation at the centromere position occurs within two species, representing that the Brassica diploid species was eluted from hexaploid species. Endosperm is the main part of the seed that transfers nutrients from parental plants to the embryo. Endosperm has a greater impression of the seed phenotype and genetic variation, while maternal testa affects the zygotic endosperm (Penfield et al. 2004). Gene mapping and microarray datasets determine the basic genomes by dissecting the endosperm and collecting EST for canola improvement. Endosperm analysis with gene expression and metabolic processes provide new gates of research for further improvements in the canola genome.

Summary and future prospects

Recent advances and development in molecular plant breeding provide pivotal assistance in current efforts to find gene pairs that lead to significant crop improvements in canola. Genetic variations in canola species for winter and spring must be extended to enhance the productivity as well as biodiversity of crop plants. The Brassica gene pool might be extendable for unadopted as well as allied species for a long-term perspective, where multiple gene pair allelic interactions with right combinations are involved to

obtain maximum productivity. From this prospective, molecular marker techniques are powerful tools in molecular biology to determine variations at the polyploidy level. Moreover, we can obtain the sequence of the whole Brassica genome and help to identify the novel genes that are involved for contributing or controlling the seed yield through agronomic and seed quality traits in advanced breeding technologies. Brassica EST development, along with sequencing techniques for Brassica genomes, is helpful for constructing physical maps to assist in determining the SNPs for a large number of agronomical QTL(s) and candidate genes in the future. High-throughput sequence technology with cost-effective molecular marker techniques is expected to create a new impetus for new canola variety development. The advancement of new technologies and discoveries in molecular biology techniques will benefit all communities. Integrated approaches, such as DNA chip and high-throughput sequencing genotyping studies, provide enough information for the molecular genetics of the Brassica crop. Arabidopsis is a closely related relative and model plant, which can be used to obtain new information and breeding technologies for plant improvement in the Brassica genome. Integrated advanced breeding approaches provide much information; however, a huge gap still exists regarding drought and gene action for canola improvement.

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