



QTL mapping for seed weight and seed yield-related traits in oil-seed crop rapeseed (*Brassica sp.*)

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ABSTRACT

Vegetable *Brassica* is one of the world's most vital oil-seed crops and provides the basis of world supplies, so to satisfy the popularity of live stocks for vegetable oil and good fats, it is essential to cultivate and improve the production of rapeseeds. Quantitative trait loci (QTL) is a viable tool for characterizing large loci and also important in molecular marker assisted breeding programs. Seed yield (SY) is a complex trait controlled by different loci and directly influenced by the seed weight, the number of seed per silique, and silique per plant. SY is also affected by some other yield related traits like (plant height, silique length, biomass weight, flowering time and seed size). Among all the SY traits thousand seed weight (TSW) is one of the most leading factors in rapeseed crop and is significantly correlated with yield per plant. Thus to improve the seed weight (SW), different SW and seed size genes have been successfully cloned. This study records the known QTLs of seed-yield that are frequently associated with rapeseed features. As a total of 972 QTLs for seed-yields and yield related are identified on 16 chromosomes and aligned in to the physical map of *Brassica napus* darmor-bzh. Moreover in 65 regions 147 potential candidate genes are identified, where 131 QTLs are renowned overlapped and is supposed to effect nine different traits. (flowering time, number of primary branches, plant height, pod numbers, seed number per pod, silique per unit area, seed weight, seed yield and test weight). Current such findings is studied and revised in this paper form pervious published research papers and explain the mechanisms of the genes for SW of oilseed rape that has been cloned as well as QTLs with the help of molecular markers assisted breeding for yield-related traits and this work also provide the basic knowledge for the cloning of genes responsible for seed weight (SW) and yield related traits that could be used for improvement in *Brassica* genus as well as *B. napus*.

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INTRODUCTION

Rapeseed (*Brassica napus* L.) is an agricultural crop, source of proteins, oil for human and animal fodder and utilized worldwide to make vegetable oil and biofuels

(Solangi et al., 2018). The polyploid *B. napus* (AACC, $2n = 38$) derived by hybridizing turnip rape and cabbage. Supporting the development of high yielding varieties is the primary task of plant breeders. To enhance the seed yield per plant, three fundamental traits are seed weight (SW), silique per plant (SPP), and seed per silique (SS) that influence the yield. Seed weight is the most Seed-yield

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(SY) is also affected by some other yield related traits like (plant height, silique length, biomass weight, flowering time and seed size) (Long et al., 2007) important trait in crops and is one of the three major components for oilseed rape. Therefore increasing seed weight is primary approach to improve oilseed rape production (Geng et al., 2016). *B. napus* records the least period and antiquity of plant generation development restrictions in *Brassica* (Jesske et al., 2013; Chalhoub et al., 2014).

Winter varieties of *B. napus* are identified as high yielding varieties than of the spring cultivars of *B. napus* (Ali et al., 2003) and sown in late summer, while spring varieties *B. napus* are sown at the end of winter in the northern latitudes (Asia, Eastern Europe, Australia and Canada) (Nesi et al., 2008).

So breeding of high yielding cultivars is key goal for breeders. Rapeseed yield is limited by silique number/plant, seeds/silique/plant and seed weight. The quantity of seeds is indisputable, that depends on the number of seeds per plant (Ding et al., 2010, Chen et al., 2007).

Importance of rapeseed

Most vegetable oils can be utilized as cooking oils, fuel oils, and diesel oils. The most generally known oils are canola oil, palm oil, soya oil, and sunflower seed oil. However the most commonly used cooking oil is canola oil, also known as rapeseed oil. It is extracted from rapeseed on heating and crushing and have higher content of omega-3 (linolenic acid) and omega-6 (linoleic) and low saturated fatty acids (Shahbandeh, 2020). Sometimes peoples became confused in usage of the term rapeseed, as rapeseed is common/ traditional name for the brassicaceae oilseed crops. There are two types of rapeseed oil (1) industrial oil and (2) canola oil. The seeds of both types are visually alike, but the thing that determine the difference between them is their individual chemical profile regarding fatty acid and glucosinolates. The term Canola refers to the edible oil crop and was registered in 1979 in Canada, while industrial oil refers to the chemical industries and machine usage (for example, engine lubricant and biodiesel) (Friedt et al., 2018). Similarly, in order to provide biodiesel oil, rapeseed can be used, while rape cake, as a source of bio-methane, significantly improves the energy balance but is of little benefit to radiations (Thamsiriroj and Murphy, 2010). The side effect is that the oil extracted from rapeseed gives up the nutritious biological feed. For cows, this protein-loaded animal feed is incredible (Patterson et al., 1999a). Likewise, rapeseed oil was used as compelling oil simply if oil lights were to occur. It was also used for the collection of detergents and plastics (Johnson, 1999) and medicinal food plant in Middle Asia, North Africa and West Europe (Saeidnia and Gohari, 2012). The increase of erucic acid corrosive substances in characteristic rapeseed oil is

harmful to individuals. Therefore plant breeder and breeding programs are engaged to improve the rapeseed oil quality and yielding traits (Hatzig et al., 2018).

Six interrelated species (the triangle of the *Brassica* family)

The mustard family involves roughly 300 ages (*Brassicaceae*, *Cruciferae*) and about 4000 species. A scope of farming plants incorporates either crop (vegetables, flavors, and oil) or weeds such as *Sinapis arvensis*. During the 1930s, cytological examination mainly the chromosome number and genetics relationship within the *Brassica* variety was established (Nagai and Tsunetaro., 1930). There are two degrees of ploidy; (i) according to three main species: *B. Brassica oleracea* (CC, n = 9) as well as *B. nigra* (BB, n = 8), (ii) three species of secondary origin: *B. napus* (AC, n = 19), *B. juncea* (AB, n= 18) and *B. carinata* (BC, n = 17), respectively. Re-synthesis confirmed the latter is amphidiploids (allotetraploids) derived from the primary species. *B. napus* and other allotetraploid ('U triangle in Figure 1), (Nagaharu, 1935) progressing inherited and molecular assessments give more through comprehension into the arranged associations between the species and associated genera (Figure 2) (Mei et al., 2010; Liu et al., 2016). As according to the previously studies, it is proved that approximately 7500 years ago, diploid species (*Brassica rapa* and *B. oleracea*) were hybridized to produce *allopolyploid B. napus* (Chalhoub et al., 2014). While the identification of comparative genome biology and phylogenetic restorations from whole genome sequences of brassica diploids are consistent with their common origin from an ancient paleohexaploid followed by two whole genome duplications (WGD) and whole genome triplication (WGT) (Zhang et al., 2018). Therefore at present the natural allopolyploid species offers the distinctive opportunity to study the genome evolution due to its vigorous nature of progenitor genomes accompanies the speciation by interspecific hybridization.

Along with early polyploidizations, a total of 72x genome multiplication is discussed since the origin of angiosperms and high gene content. In the previous studies and researches, it is valued that within the Mediterranean basin wheat-growing area characterizes 27% of the arable land (Royo et al., 2017), while in case of rapeseed it is important crop of all over the Europe in accord of vegetable-oil production as well as animal protein. Along with Europe, France, Germany, Poland and United Kingdom are also main producers of rapeseed vegetable-oil (Canola) (Baker and Charlton, 2020).

Attribution of candidate gene

As previously described, 425 genes have been found in

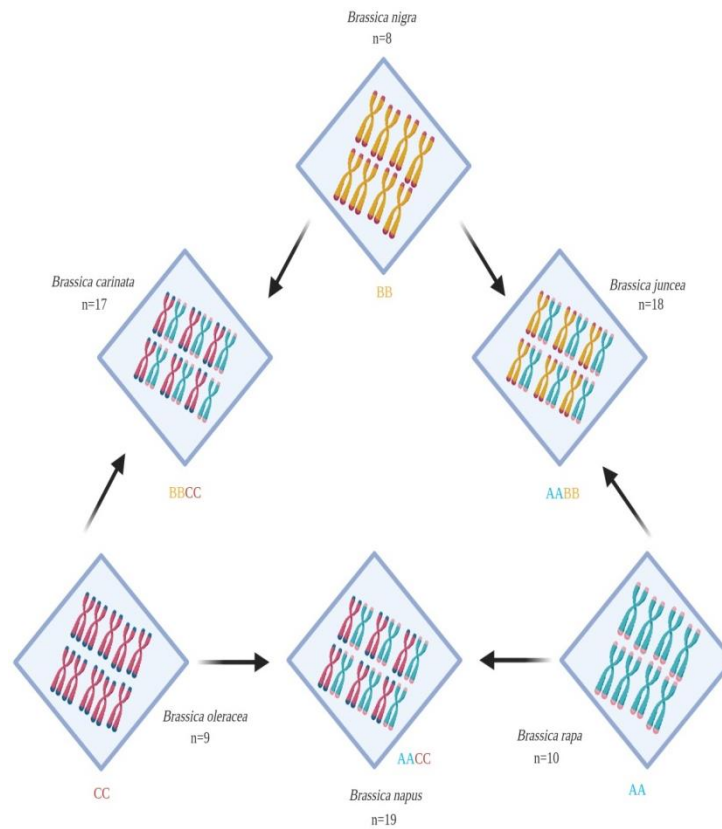


Figure 1. The U-triangle diagram shows the genetic relationships between six *Brassica* genus species. Different colors represent chromosomes from each genome (Nagaharu, 1935).

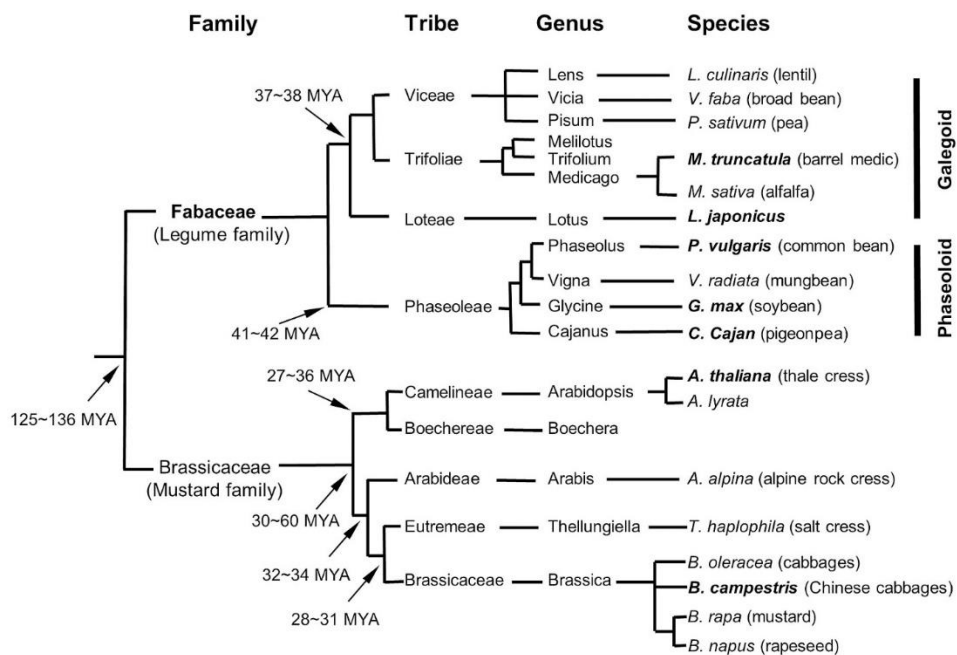


Figure 2. The taxonomic relationship between the two families, the *Fabaceae* and *Brassicaceae* of the main crop and model organisms (Hyung et al., 2014).

the model plant *A. thaliana* related to seed number and seed weight, and seed-yield (SY) (Shi et al., 2009), yielding characteristics were distinguished. In previous studies, identifying yield and yield-related traits in *B. napus* of these 425 homologous genes have not discovered before.

It is essential to reproduce high yielding characteristics in the selection of this strategy. Cultivars from *B. napus*. This is because sufficient information has been made better to understand the SY hereditary cycles and yield-identified characteristics. Presently, due to *B. napus* genome sequences, it is feasible to find the candidate gene using *B. napus* physical map. Position of QTL flanking markers were examined on *B. napus* physical map by Blastn and E-PCR (Schuler, 1997). The genes those were found in the QTL region are being considered as potential candidate genes. In addition, the same method was used before by Chao et al. (2017) to detect candidate genes for seed oil content in *B. napus*. This type of technique is also helpful to find out the candidate genes of QTLs from several linkage maps that could be combined in one map.

The identified 82 regions of disguising QTLs and 218 and-candidate genes in 162 QTLs regins, 76/218 candidate genes were found inside 57region of overlapping QTLs, which were for oil content trait (Rabonatahiry et al., 2017). Up-and-comer characteristics of covering QTLs were certified to be steadier than self-sufficient QTL, and if candidate characteristics of free QTLs were considered, different results could be obtained. Contender features are subject to QTL and are responsible for the quality assortment (Tabor et al., 2002). Similarly, in my assessment, these candidate features that may affect one or different aspects may be used as a tool to request high seed yield to aggregate any selected credits. Besides, the chief beneficial quality blend can be used to support the most substantial advantage, especially those characteristics found in the region covering QTLs, including multiple attributes.

Molecular markers

In terms of plant breeding, genetic markers are the dynamic developments (Kebriyae et al., 2012). They are closely linked with the target gene and act as a sign. A genetic marker is a gene or DNA sequence with a known chromosome location controlling a specific gene or trait (Collard et al., 2005). Biochemical markers and DNA based markers are available for genetic studies (Boopathi, 2020). Innate qualities are the characteristic features that are constrained by allelic characteristics or inherited loci and can be transmitted from one age to a substitute and will be used as experimental tests or names as needed to remain a private track, a tissue, a cell, a center, a

chromosome or a quality. Since the dissemination of the DNA structure 50 years ago (Watson and Crick, 1953), genetic characteristics have shaped one of the world's most fundamental developments. DNA marker technology has changed the research of plant genome and has been widely used in recent years. In the brassica genome, nuclear markers are widely used to design large agronomic traits and assist in the replication and assurance of rapeseed. Marker's progress and the organization have revealed critical information on the structure of those genomes. They are baffling genomes, uncommonly duplicated with intra-and inter-genomic linkage block preservation that licenses homologous recombination. Besides, chromosome maps are being used in vegetable and oilseed crops for regions of significant characteristics.

The transcendent perceptible ones are among the traits arranged by different examination locations, sickness barrier, cytoplasmic male sterility restorers, and oilseed quality. Report markers identified with an omega-6 obsession with unsaturated fats and desaturases as a logical examination. *B. napus* and *B. oleracea* markers associated with those characteristics will eventually be used for marker assurance, quality separation, sub-nuclear representation, and control purposes. The *B. oleracea*, that is chromosomes were investigated; $2n=18$ from the resynthesized amphidiploid *B. napus* Hakuran by repeated crossings (McGrath et al., 1990). A movement of monosomic outcast chromosome extension line plants ($2n=21$) was created by campestris ($2n=20$). Eighty-one putative loci have been identified by morphological, isozyme, and (RFLPs Restriction fragment length polymorphism). Seven of the nine potential synteny social affairs were addressed within the 25 attempted monosomic development plants. Among the 61 DNA clones collected by the same linear method, the homology was 26% (about 80% of them were cDNA clones), indicating that the replication value was improved. In four $2n = 21$ plants, odd sytonic associations were recognized. Two markers from one B were shown by one of these plants. The chromosome of *B. oleracea* was related to a second all-out group of *B. oleracea* sytonic, suggesting development or recombination in Hakuran or the backcross auxiliaries between non-homologous chromosomes.

Genetics of yield-related traits and seed weight in *Brassica* spp.

Materials and Methods

Recent studies have identified several genes and pathways that underlie seed weight control (Li and Li, 2015; Zuo and Li, 2014). To sort out the genetics for seed weight and seed-yield related traits through molecular

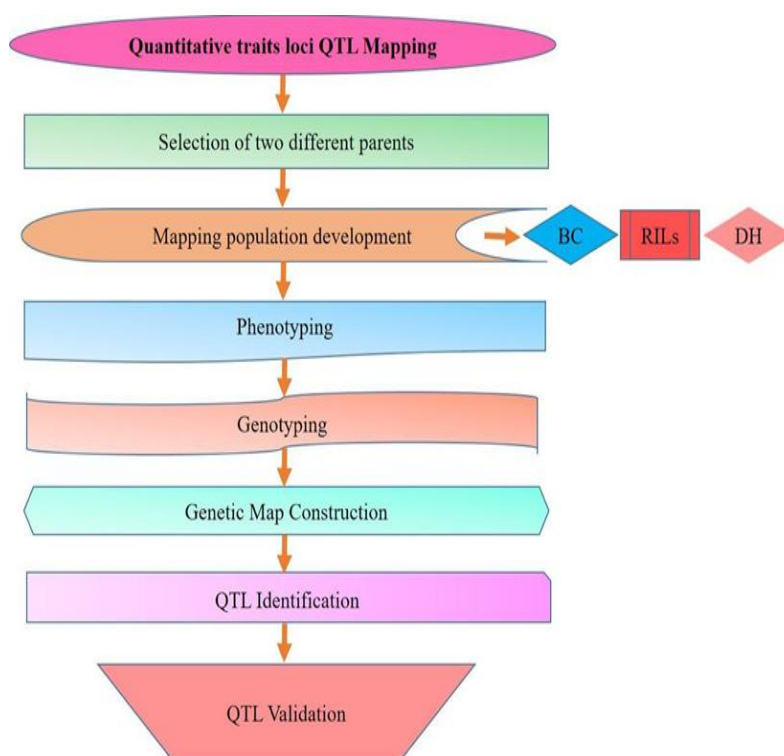


Figure 3. Plan of QTL Mapping (Jiang, 2013).

genetics (QTL mapping and Fine QTL mapping) many researchers have done studies using various methods and materials. For example, QTL for seed yield and yield-related traits obtained from 22 populations aligned with physical map *B. napus* winter type *Darmor-bzh*, the alignment was done through the QTL flanking markers to positioned the markers on map through E-PCR (Raboanatahiry et al., 2018). Because of the background, one marker on the genome could be recognized due to corresponding primers. In this way, primer sequences are identified on *Darmor-bzh* to spotting the markers. Wang et al. (2020) conducted quantitative trait loci (QTL) mapping method to uncover the genetic mechanism of thousand seed weight (TSW) using a double haploid population as a material derived from a cross between an elite line ZY50 and a pol cytoplasmic male sterility restorer line. In this way different study by various aspects to disclose the mechanism of seed weight and seed-yield related traits through different morpho-physiological and molecular method is done. Figure 3 shows the QTL mapping plan (Jiang, 2013).

QTLs identified for seed weight in *Brassica* spp.

Seed weight (silique per plant, seed per silique and seed weight) is one of three yielding parts of rapeseed plant. It

is additionally associated with the sorghum sum (Clarkei and Simpson, 1978). More efforts are being undertaken within *B. napus* genetic back ground than in normal oilseed rape (Morgan et al., 1998) to design seed size/weight QTLs for *Brassica* spp and the sequences and observation of the rice genome is now greatly enhanced by the method and technique improvements done in model plant *Arabidopsis* (Rensink et al., 2004, Marriage et al., 2009, Schmidt et al., 2001)

Materials for plants and phenotypic assessment

A 190-line Double Haploid (DH) individual was brought from microspore culture to be named SJ-DH population with an F₁ cross in SW Hickory (a spring type *B. napus* variety) and JA177 (a winter-type *B. napus* pure line). This population has been used for genetic and QTL mapping by (Cai et al., 2012). Each plant's seed weight from the population was measured based on 500 seeds with three replication. The standard seed weight for each plant has been converted to thousand seed weight (TSW). The mean of 10-15 plants thousand seed weight were used for the quality assessment for the parents, F₁, and SJ DH lines from each plot. The detailed information for the production of population, field trial and sampling procedures for seed weight is described before.

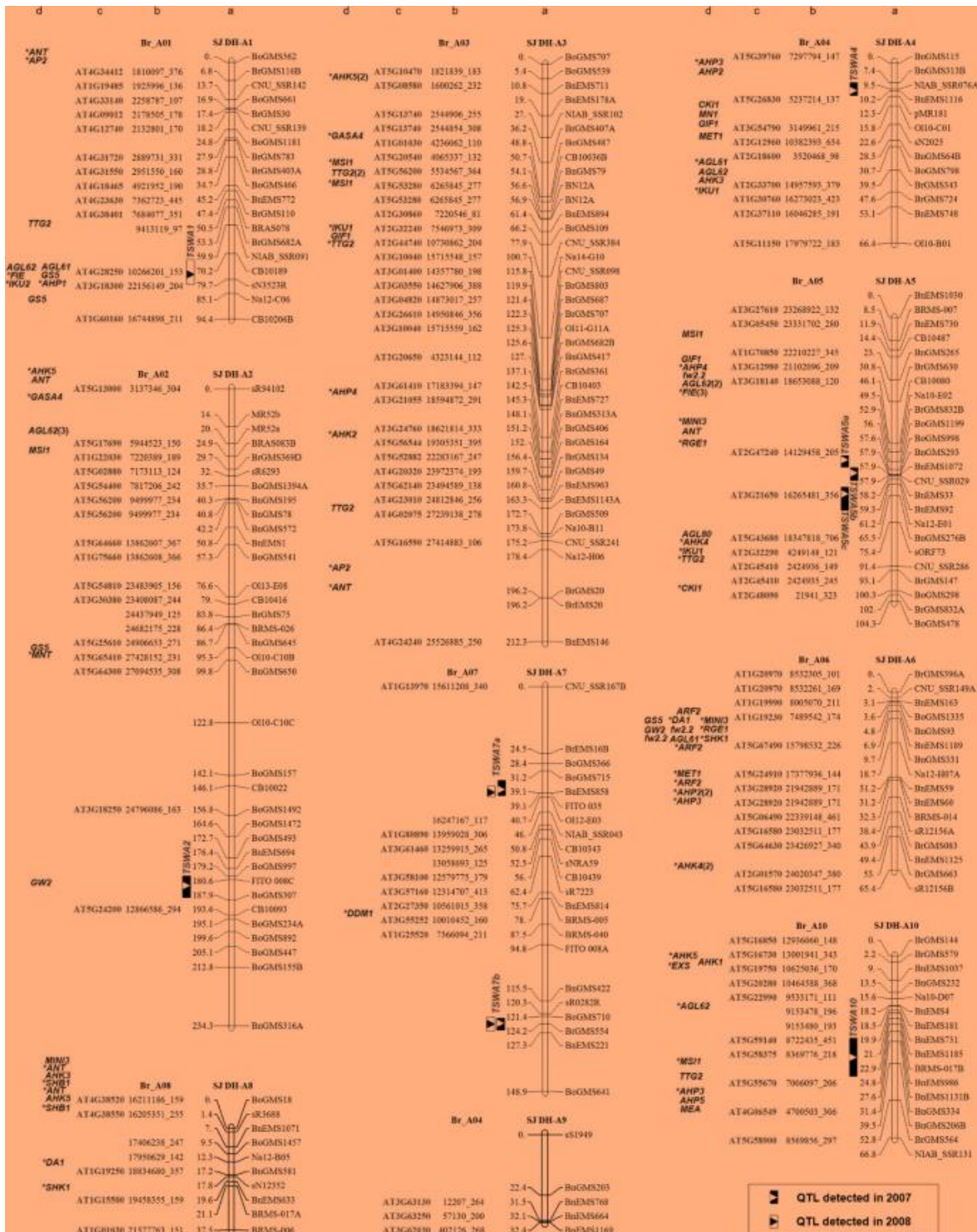


Figure 4. Seed weight/size gene distribution map of *B. napus* (A genome).

The seed size/weight distribution map has been used to identify candidate genes for TSW QTLs. The Blueprint of *B. napus* explained in Figures 4 and 5. The closest genes on sides

of *TSWA1* were *LG A1*, *TTG2*, and *GS5*. While *GW2* was set at the same location of *TSWA2* (Figure 1), *CK1* and *MN1* were close to the top of *TSWA4*. *MINI3*

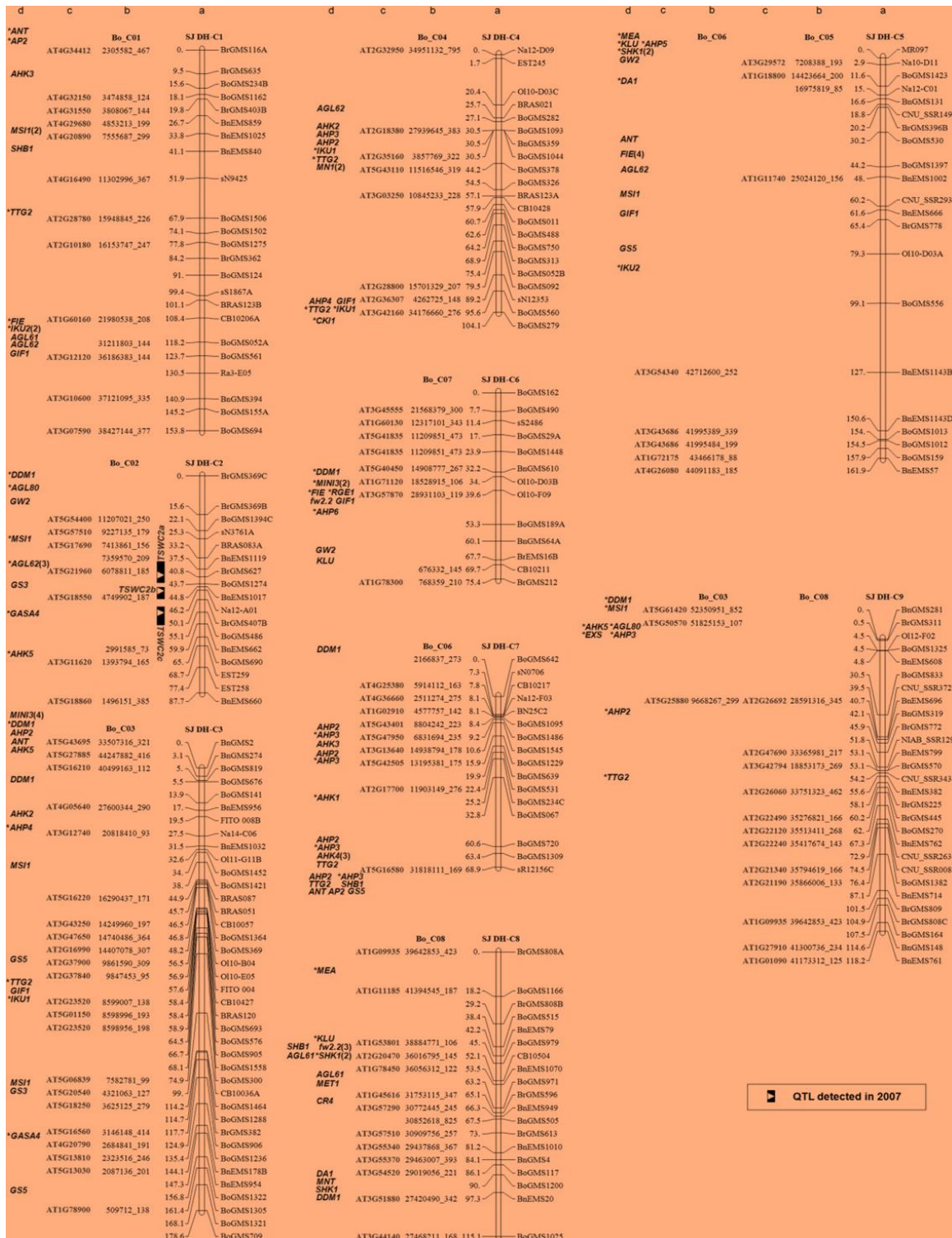


Figure 5. Seed weight/size quality circulation guide of *B. napus* (C genome).

and *FIE* were noted nearby *TSWA5a*, and *TSWA5b*. The *MINI3* genes were positioned within interval of *TSWA5b*.

However, the *FIE* gene came into confidence interval of *TSWA5*(Figure 4). Within a similar interval of *TSWA10*,

three genes, AHP3, AHP5, and MEA, were identified (Figure 4).

Furthermore, the analysis identified the potential target genes mapped QTLs, giving valued clues for more assessment of sequences differences of two parental lines of SJ DH population. The expressing of LGs is reliable with the standards proposed by the overall *Brassica* genome undertaking. All 19 linkage groups (LGs) are being allocated 397 SSR loci and 6 gene specific markers, the markers covered a genetic distance of 2,216.4 cM with a distance average of 5.36 cM and all SSR markers were constantly distributed throughout the whole genome of *B. napus* along with 199 and 198 SSR loci on genome A and C respectively. Besides to identify the putative seed size/seed weight genes in *B. napus*, the homologous sequences were first investigated by (Altschul et al., 1997) through the BLASTn program. The identified sequences from results were first mapped on the chromosomes of *B. rapa* or *B. oleracea* and then on the behalf of homologous collinear associations between *B. napus* and *B. rapa* or *B. oleracea*, these sequences were mapped on *B. napus* linkages groups (LGs). Moreover, *AGL62*, *GS3*, and *GASA4* were identified in interval of three new distinguished/identified QTLs (TSWC2a, TSWC2b and TSWC2c, Figure 5), (Cai et al., 2012).

Segment a presents the hereditary linkage gathering of the SJ DH population. The classification of LGs adheres to the principles proposed by the Multinational Brassica Genome Project (Parkin et al., 1995) Genome 38:1122-1133ch) of the LGs is spoken to with a vertical bar with locus position (in cm) on the left and SSR loci names on the right. The QTLs data (pinnacle, span, and name) of TSW were on the left-hand of the LGs. Section b records the homologous collinear loci in *B. rapa* or *B. oleracea*. The numbers assign the actual situation in *B. rapa* or *B. oleracea* chromosome with the length of intensification piece. Segment c is the Arabidopsis quality codes comparing to the homologous loci. The section records the homologous qualities of seed size or weight distinguished in *B. rapa* and *B. oleracea*. Qualities that are in the solid match into Arabidopsis and *B. rapa* /*B. oleracea* actual positions are apart with bullets. Numbers in sections are the couple rehash seasons of the pair rehashed qualities (Marriage et al., 2009).

In conclusion, the above review clearly identified the potential objective characteristics of the planned QTLs, and provided important insights for the additional correlation of sequence comparison between the two parental lines.

However, no known data on applicant characteristics can be interpreted from the mape for both significant QTLs that were *TSWA7a* and *TSWA7b*, recommending those QTLs could also be extraordinary in *B. napus* or either *B. rapa* Genome. The seed size/weight quality and *DDM1* on LGA7 was distant from these two QTLs, thus unlikely the candidate gene for the 2 QTLs as explained in Figure 4.

Fine mapping of significant QTLs

Due to their high heritage, both the SW and the SL of the plants are critical consideration of the yield probability in rapeseeds, and they are of worthy concentrations for raising assurance (Zhou et al., 2006). Generally, an increase in the silique length can increase source causing higher seeds (Chay and Thurling., 1989). Both seed weight and silique length are quantitatively inherited and are controlled by different QTLs, mainly additive effects (Meijuan and Jusheng, 1992) and in rapeseed only linkage analysis is used for mapping QTLs of seed weight and silique length (Fan et al., 2010). Fine mapping is based on recombinant individual screened from a large scale near isogenic lines (NIL) following the initial linkage mapping and needs various rounds of successive backcrossing and self-crossing as well as the genotyping of thousand individuals (Salvi and Tuberosa, 2005). Therefore this traditional NIL based fine mapping is time consuming and a labor costing. However, for high-throughput genome-wide linkage detection for a specific fine mapping of one QTL is wasteful and not important. Though, fine mapping QTLs using a linkage analysis requires a population with thousands of individuals, and the limited polymorphic loci between the two parents will influence the mapping accuracy (Holland, 2007). Fine mapping and map-based cloning have been proved to be one of the most efficient ways to disclose the genetic bases of quantitative traits and identification of genes underlying these traits (Takeda and Matsuoka 2008; Miura et al., 2011).

Several major QTLs for pod number and seed number per pod had been identified in previous QTL mapping studies (Zhang et al., 2011; Qi et al., 2014; Zhang et al., 2012; Wang and Guan, 2010) but none have been fine-mapped and further cloned. Up to now, only one gene (*ARF18*) associated with thousand seed weight has been cloned in oilseed rape group *B. napus*, on A09 chromosome, associated with seed weight and silique length (Liu et al., 2015).

Conclusions and their future perspectives

This detailed study is about QTLs identified in *Brassica* for the yield related attributes. Although rapeseed oil content breeding has made good progress worldwide in the past years still potential for further improvement is needed. Synchronous improvement of multiple traits is the current trend for high oil breeding and could be modified and consolidated. In these identified stable QTLs, new applicant qualities could also be recognized. Moreover, the availability of Next-generation sequencing (NGS) and Single Nucleotide polymorphism (SNP) arrays will accelerate research on molecular regulatory mechanisms that may help to improve rapeseed agronomic traits.

Genetic engineering, molecular marker technology combined with a conventional directional selection strategy will be the trend in rapeseed breeding.

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