# Construction of a Quantitative Genomic Map Gives Insight on Rapeseed Genetic Characteristics and Direction for Future Breeding

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**Research**

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## Abstract

# Background

Rapeseed is the second most important oil crop in the world. Improving seed yield and seed oil content are the two main highlights for researches. Unfortunately, rapeseed development is frequently affected by different diseases. Extensive researches have been made through many years to develop elite cultivars which could produce high oil, high yield or be resistant to disease. QTL analysis has been one of the most important strategy in genetic deciphering of agronomic characteristics. In order to comprehend the distribution of these QTLs and to uncover the key regions that could simultaneously control multiple traits, 4555 QTLs that have been identified during the last 25 years were aligned in one unique map, and a quantitative genomic map which involved 128 traits from 79 populations developed in 12 countries was constructed.

# Results

The present study revealed 517 regions of overlapping QTLs which harbored 2744 candidate genes and might affect multiple traits, simultaneously. They could be selected to customize super-rapeseed cultivars, including the cultivars which produce high oil content for biofuels production. The gene ontology and the interaction network of those candidates revealed genes which highly interacted with the other genes and might have strong influence on them. The expression and structure of these candidate genes were compared in eight rapeseed accessions and revealed genes of similar structure which were expressed differently.

# Conclusion

The present study enriches our knowledge on rapeseed genome characteristic and diversity, and it also provided indications for rapeseed molecular breeding improvement in the future.

## Background

Rapeseed (*Brassica napus*, AACC = 38) is a tetraploid species derived from natural hybridization between turnip rape (*B. rapa*, AA = 20) and cabbage (*B. oleracea*, CC = 18) [1, 2, 3]. Both of *Brassica* species and the model plant *Arabidopsis thaliana* belong to the *Brassicaceae* family, their separation took place about 14 to 20 million years ago [4, 5]. Rapeseed is the second most important oil crop in the world, which could supply 13% of the world vegetable oil [6, 7]. Rapeseed utilization is not limited to oil source, it also can be used for food and energy production, remediation and as sightseeing attraction [8]. To fulfil the global high demand of oil, the main objectives of researchers are to discover ways to increase the oil content and to develop high yielding varieties, to succeed in sustainable manufacture in the future. Unfortunately, abiotic and biotic factors frequently weaken the rapeseed development, such as the invasion of

*Sclerotinia sclerotiorum* (steam rot) and *Plasmodiophora brassicae* (blackleg disease), which resulted in yield losses of 10–80% [9] and 20–30% [10], respectively, in China. It would be of great interest to find the genetic loci that could control the traits associated with seed yield and quality, and disease traits, simultaneously, for artificial selection breeding. Rapeseed has experienced selection which contributed to the diversification of winter and spring types. Selection also caused region restructuring where genes controlling agronomical important traits are located. Thus, intensive breeding allowed to optimize those important traits such as oil content, flowering time and pathogen resistance [3]. Actually, diversity in the same species is due to the fact that every individual has their uniqueness starting from their genome and it is reflected into their traits characteristic. Diversity among different species could be understood by analyzing their genomes. Variations within genome are a reflection of breeding events. Genome diversity might be exploited to detect beneficial phenotype associated to specific loci on the genome and linked to environmental conditions.

Quantitative trait loci (QTLs) are correlated with variation of phenotype, and is extensively used for agronomic traits analysis and in plant breeding. Lots of phenotypic traits are usually responsible for improvement of most crops, they are quantitative in nature, and are influenced both by many genes and environmental conditions [11]. QTL mapping could be used to decipher regulatory loci and genetic mechanism of traits [12], to identify genomic regions which are responsible for trait variation, and to establish link between phenotype and polymorphic markers in random biparental populations [13, 14]. Several researches have revealed that the phenotypic effect of QTLs for one characteristic in one genetic background might produce different phenotypic effect in another genetic background, for example, *KN* (*KenC-8 × N53-2*) and *TN* (*Tapidor × Ningyou7*) are two populations which were both cultivated in China, the oil content (OC) and detected QTLs were not similar: 41 QTLs for OC were found with a maximum OC of 50.9% in the 202 *TN-DH* lines [14], whereas in 348 *KN-DH* lines, 63 OC-QTLs were found with maximum OC of 54.8% [15], and in 300 *KN-DH* lines, 67 QTLs were detected with a maximum OC of 57.17% [16]. To uncover similarity and difference between the discovered QTLs, a consensus map which displays multiple QTLs from different genetic and environmental background is indispensable.

Earlier, building a consensus map was possible, but it was limited by the difference of markers which were used in different studies [17]. Now, it can be overcome with QTLs alignment map which has been used for seed oil content and seed yield QTLs by using *B. napus Darmor-bzh* as reference genome [16, 18, 19, 20]. The advantage of QTL alignment is to allow an easy comparison between QTLs and the regions of overlapping QTLs, and can be used to uncover the “stable” or “specified” regions for a trait or for an environment, but also to detect the pleiotropic loci, i.e regions that control multiple traits, simultaneously. In our previous studies, regions of overlapping QTLs were displayed: in one hand, the regions involved QTLs of the same traits but originated from different populations (e.g. OC-QTL from KN and TN overlapped in the same region), these regions can be qualified as “stable” for Chinese environment despite the change in populations, or if the QTLs were from two populations which were developed in two different environments (e.g. KN in China and PT in Canada), these regions were “stable” for the studied trait (e.g. oil content). In other hand, QTLs of different traits which overlapped in the same region were also found, and they might have a pleiotropic effect for those multiple traits [19, 20].

QTLs investigation and discovery of related candidate genes can be done together, this strategy helps to comprehend the authority of these genes over traits [11, 21]. Identification of candidate genes implies the detection of important genes for agricultural and economic quantitative traits. Candidate genes are present within QTL region and they are responsible for phenotype variation [22]. The effect of these genes on variation of phenotype could be elucidated through investigation on the gene arrangement and interaction of loci affecting this variation [11]. This technique has already been used to identify potential candidate genes in *B. napus Darmor-bzh*, for instance, Chao et al. used this technique to identify potential candidate genes for seed oil content trait, and found 448 genes underlying 41 oil content QTLs [16]. More, 76 candidate genes were found for 57 QTLs for oil content and fatty acids [19], and 147 candidate genes were discovered in a region of 131 yield QTLs which were overlapping [20]. Candidate genes can be manipulated to get the most beneficial gene combination to get maximum profit, especially those genes which were found in region of overlapping QTLs involving many traits. Additionally, the release of various *B. napus* genome sequences: *Darmor-bzh* (3), *Tapidor* [23], *Zhongshuang11 – ZS11* [24], *Gangan, Shengli, Zheyou7, Quinta, No2127, Westar* and 1689 other accessions [25] represent a precious resource which will have a tremendous impact on understanding rapeseed accessions diversity, notably the structural variation of regions which are associated with agronomic traits.

In our previous study, overlapping QTLs for single trait were detected (e.g. oil content or seed yield). In the current study, the purpose was to construct a quantitative genomic map and to detect genomic regions that might control multiple traits associated with seed, yield, hormones level and diseases, simultaneously, and the related candidate genes were also identified. Moreover, structural variation and gene expression level of those candidate genes were studied in eight different accessions. Consequently, the ultimate objectives were: (1) to build a quantitative genomic map of QTLs associated to agronomic and disease related traits in order to display overlapping regions with multiple traits; (2) to reveal the candidate genes within those regions of overlapping QTLs for the purpose of finding genes that might have pleiotropic effects on seed composition, seed yield, hormones and disease; (3) to identify the homologous of these candidate genes in eight rapeseed accessions and to analyze the gene expression and the structure variation. The present study would enhance the knowledge of rapeseed genome characteristic and diversity, the findings can be used to develop molecular markers associated with the studied traits, and also can provide some guidance for molecular design for breeding. Identified candidate genes might be used to target for genomics-based improvement and better seed yield, seed composition and disease resistance in the future.

# Results

### A quantitative genomic map of QTLs controlling seed yield, seed components, hormone level and disease related traits

4555 QTLs of 128 agronomic and disease related traits, developed from 79 different populations of three different ecotypes and grown in 12 different countries (Additional file 3: Table S1) were gathered and combined together in one unique map (Fig. 1). A total distance of 978.4Mb on the physical map of

*Darmor-bzh* was covered (Fig. 2A, Additional file 1: Figure S1, Additional file 4: Table S2). Further observation revealed that 2695 and 1860 QTLs were located on A and C genome, respectively. A3 and C3 chromosomes contained the highest number of QTLs, with 430 and 399 QTLs, respectively, whereas A10 and C4 chromosomes had the longest coverage distance (159.33Mb and 122.42Mb, respectively). A9, A3 and C3 chromosomes contained the highest number of traits (80, 71 and 69 traits, respectively) (Fig. 2A). Obviously, most QTLs for seed components, seed yield, hormones level and disease related traits were found on A genome rather than in C genome.

It is crucial to locate regions of the genome where multiple traits overlapped the most. Thereby, the above-mentioned 128 traits were subdivided into five categories: abiotic factor (A), biotic factor (B), hormones level (H), seed components (S) and yield related traits (Y). The total number of QTLs in each category were 349 (A), 334 (B), 42 (H), 1392 (S) and 2438 (Y). Each region on *Darmor-bzh* genome was carefully observed in order to detect regions where QTLs of more than one category of trait could overlap, i.e. regions with two, three, four or five categories of traits, which were present in one region, simultaneously. A total of 517 regions which hosted overlapping QTLs were observed (Fig. 2B, Additional file 1: Figure S1). The region of overlapping QTLs on each chromosome, the number of QTLs and the categories of traits are summarized on Additional file 5: Table S3. First, eight regions were found to harbor all the five categories of studied traits (A, B, H, S and Y) (Additional file 6: Table S4). Those eight regions were located on six chromosomes: one region was found on each of A1 (1.71–1.71 Mb, with 40 QTLs), A2 (2.31–2.31 Mb, with 20 QTLs), A10 (11.78–11.87 Mb, with 14 QTLs) and C3 (5.09–5.33 Mb, with 11 QTLs), and two regions were found on A6 (21.68–21.95 Mb with 15 QTLs and 22-22.30 Mb with 13 QTLs), and on A9 (8.12–9.87 Mb and 20.76–22.51 Mb, with 34 QTLs on each of them). Second, 107 regions which contained four categories of traits were found in all 19 chromosomes. Number of region in each chromosome were respectively of 11 on C3, nine on each of A6 and A9, eight on A7, seven on each of A2 and A8, six on each of A6, A10 and C4, five on C1 and C2, four on each of A1, A3, A4 and C9, three on each of C5 and C8, and one on C6. For example, 28 QTLs of four categories of traits (1A, 12B, 5S, 10Y) overlapped on A2 (1.49–2.31 Mb). Note that the region on A2 (1.71–22.04 Mb) included 288 overlapping QTLs (12A, 22B, 63S, 191Y), which was the richest region of overlapping QTLs in *B. napus* genome. Third, 225 regions on all 19 chromosomes were found to have overlapping QTLs involving three categories of traits: 22 on C3, 20 on C4, 18 on A7, 16 on C5, 15 on C9, 14 on each of A6, C2 and C8, 13 on each of C1 and C7, 11 on A8, ten on A3, nine on A9, eight on A5, seven on each of A2 and C6, six on each of A4 and A10, and two on A1. For instance, on a region of A5 (3.49–5.29 Mb), 40 QTLs of three categories of traits (5B, 12S, 23Y) overlapped. Fourth, 177 regions were found to contain overlapping QTLs which involved two categories of traits: 20 on C4, 17 on C5, 16 on C9, 15 on C8, 14 on each of C6 and C7, 13 on A7, 11 on C1, ten on each of C2 and C3, seven on each of A6 and A8, five on each of A4 and A5, four on A3, three on A1 and A9, two on A10, and one on A2. As example, 13 QTLs of two categories of traits (10B, 3Y) overlapped on C4 (20.66–20.70 Mb).

Note that some QTLs might overlap multiple times with other QTLs in different regions because of their extended length, for example, a QTL for C16:0 was located on A1 (2.25–19.86 Mb) and it could overlap two times with QTLs in region which involved five and four categories (1.71–1.71 Mb and 1.71–22.04

Mb, respectively). Then, the most abundant and the most overlapping categories of traits were S and Y categories, they were found in 403 among the 517 regions of overlapping QTLs detected in this study. The H category of trait was found rarely in overlapping region since the identified QTLs in early published papers were few (42 QTLs), so far, this H category were found in 39 among the 517 regions of overlapping QTLs of this study. Otherwise, regions of overlapping QTLs which involved one environment or one population were observed. No specified region was found exclusively for one population. Also, only specified regions for China were found in 11 areas of the genome: four areas on C3 (36.94–37.27 Mb, 37.27–38.94 Mb, 39.94–40.21 Mb and 41.40-46.52 Mb), two areas on A8 (16.87-17.37Mb and 17.37-18.00Mb), and one area on each of A7 (17.48–18.48 Mb), A10 (0.14–1.64 Mb), C4 (42.73–44.22 Mb), C6 (8.43–9.43 Mb) and C7 (24.87–25.45 Mb) (Additional file 5: Table S3). For instance, the region on A8 (16.87-17.37Mb) had four QTLs (3S, 1Y), which were all found with Chinese experimental field. Ultimately, the rapeseed genome had been finely dissected to unveil regions that harbored multiple traits, simultaneously. It would be crucial to couple those findings with the identification of genes that were located within those regions to understand the influence of those genes over those traits.

### Candidate genes identified within regions of overlapping QTLs, and their interaction network

Totally, 3181 genes which are associated to oil biosynthesis, yield and disease related traits were aligned to the physical map of *Darmor-bzh*, and a total of 2744 candidate genes were found within overlapping QTLs of two to five categories of traits (Fig. 3A, Additional file 7: Table S5). A total number of genes of 26 (1%), 729 (47%), 1291 (27%) and 700 (25%) were found for five, four, three and two categories of traits, respectively (Fig. 3B).

Eight regions of overlapping QTLs of five categories of traits (A, B, H, S, Y) were found in six chromosomes (A1, A2, A6, A9, A10 and C3). A total of 26 candidate genes were found on three among those six chromosomes: seven genes on A6 (four on 21.68–21.95 Mb and three on 22-22.30 Mb), 18 genes on A9 (six on 8.12–9.87 Mb and 12 on 20.76–22.51 Mb) and one gene on C3 (5.09–5.33 Mb). For example, three candidates were found on A6 (22-22.30 Mb) which were DHLAT-BnaA06g33300D, RLK-BnaA06g33320D and AAPPT-BnaA06g33540D. Meanwhile, 729 candidate genes were found within overlapping QTLs of four categories of traits in all 19 chromosomes, and they were respectively of 129 (A1), 120 (A9), 71 (A8), 58 (A10), 46 (C3), 44 (A5), 43 (A3), 38 (A6), 37 (A2), 28 (A7 and C1, each), 22 (A4), 15 (C5), 12 (C9), 11 (C4 and C7, each), 10 (C2), four (C6) and two (C8). As example, three candidate genes (CCT-BnaA03g14860D, RLK-BnaA03g15210D and KAT2-BnaA03g15290D) on A3 (6.84–7.12 Mb) were found within 19 overlapping QTLs (2B, 1H, 5S, 11Y). Moreover, 1289 candidate genes were located within overlapping QTLs of three categories of traits, and they were found on all 19 chromosomes: 169 (C3), 129 (A3), 121 (A2), 104 (C2), 77 (A5), 74 (C4), 70 (C8), 66 (A6 and C6), 61 (A7), 56 (A4), 53 (C1), 48 (C9), 47 (A9), 44 (C7), 40 (A10), 37 (C5), 24 (A8) and three (A1). For instance, two candidate genes (ADC2-BnaC01g03710D and FAE-BnaC01g04130D) were found on C1 (1.93–2.16 Mb) involving 13 overlapping QTLs (4B, 1S, 8Y). At last, overlapping QTLs of 2 categories of traits contained 700 candidate genes in 18 chromosomes (excluding A2): 110 (C9), 75 (C5), 61 (C4), 59 (C7), 51 (A7), 48 (C2), 42 (C3), 41 (C1), 40 (C6), 31 (A3), 29 (A6), 28 (C8), 21 (A5), 19 (A8), 16 (A4), 15 (A10), 9 (A1) and 5 (A9). For example, two

candidate genes (RLK-BnaC07g13860D and RN-BnaC07g14020D) were found on C7 (19.60-19.79 Mb) involving two overlapping QTLs (1 A and 1 B). In assumption from those findings, important genes which were located within regions of overlapping QTL with multiple traits were identified. They might have influence on more than one category of traits, and they could be selected according to the desired improvement of two or multiple traits.

Interaction network analysis of the 2744 candidate genes were made with their 1555 orthologous genes in *A. thaliana* because *B. napus* is not available on String database. Gene ontology (GO) analysis indicated that the 1555 genes could be classified into 16 categories, according to Panther GO-slim biological process's classification (Additional file 8: Table S6), it included the cellular process (GO:0009987), biological phase (GO:0044848), reproductive process (GO:0022414), multi-organism process (GO:0051704), localization (GO:0051179), interspecies interaction between organisms (GO:0044419), reproduction (GO:0000003), biological regulation (GO:0065007), response to stimulus (GO:0050896), signaling (GO:0023052), developmental process (GO:0032502), rhythmic process (GO:0048511), multicellular organismal process (GO:0032501), metabolic process (GO:0008152), growth (GO:0040007), immune system process (GO:0002376). Other genes which could not fit into those categories were classified "Others".

The interaction network was visualized with Cytoscape, 1271 nodes and 10101 edges were displayed (Fig. 4). In this network, 11 genes might be more influential over other genes (degree layout, DL ≧ 70): AP2 (DL = 103), FT (DL = 100), AUX1 (DL = 90), KASIII (DL = 89), CO (DL = 80), MCAT (DL = 79), KASI (DL = 78), AGL20 (DL = 74), PHYA (DL = 72), COP1 (DL = 71) and ACP (DL = 70). Those genes belonged to the GO categories of metabolic process (KASI and AGL20), multicellular organismal process (CO) and other category (AP2, FT, AUX1, KASIII, MCAT, PHYA, COP1 and ACP4). Those most influential genes had function related to oil biosynthesis and yield related traits: ACP, MCAT, KASI and KASIII are both plastidial genes which are involved in fatty acid biosynthesis. ACP act as carrier of acyl intermediates, then MCAT catalyzes the synthesis of malonyl-ACP and CoA from malonyl-CoA and ACP. KASI and KASIII both act on fatty acid elongation. Then, the other seven influential genes were related to yield traits: AGL20 promote flowering and inflorescence meristem in *Arabidopsis*, AP2 are involved in floral organ specification, in floral meristem establishment and in ovule and seed coat development. AUX1 are auxin transporters and have influence on lateral root initiation and positioning, CO regulate flowering during long days, COP1 act as suppressors of photomorphogenesis and stimulate skotomorphogenesis in the dark, FT also promote flowering as AGL20, and PHYA regulate photomorphogenesis. Those most influential genes had different functions and were involved in different metabolism pathways, yet they might have higher effect over other genes, this might indicate that the simultaneous control of multiple categories of traits might be affected at different path of metabolisms.

### Gene expression and structural variation of candidate genes in eight rapeseed accessions.

One copy of each homologous of the 11 most influential genes of the interaction network in *B. napus* was selected for the current gene expression and structural variation analyses. Additionally, the 26

candidate genes which were found within QTLs of five categories (A, B, H, S and Y) were also added to the analyses. Thus, a total of 37 genes of *Darmor-bzh* genome were searched in eight rapeseed accessions by using of BnPIR database [25], including two winter-types (*Quinta* and *Tapidor*), two spring-types (*Westar* and *No2127*), and four semi-winter types (*ZS11, Zheyou7, Shengli* and *Gangan*) (Additional file 9: Table S7). The tissues used in the determination of gene expression on BnPIR database were collected throughout the flowering process of the plant from the eight accessions of this study. They were collected at five different post sowing days: T0: 24 days post sowing; T1: 54 days post sowing; T2: 82 days post sowing; T3: 115 days post sowing; T4: 147 days post sowing. Gene expressions are displayed on Additional file 2: Figure S2.

Particularly, six genes displayed the most significant difference in expression among them, while considering the difference in FPKM value and the expression profile during different post sowing days: ACP4, AGL20, FRB12, LTA2, PP2A and RLP46. The six homologous genes were located on four different chromosomes in *Darmor-bzh* (A1, A5, A6 and A9), but in five chromosomes (A1, A6, A9, C3 and C4) in the other eight rapeseed varieties (Fig. 5, Additional file 10: Table S8). The expression of those six genes is illustrated on Fig. 6 and nucleotide sequence identity is shown on Additional file 11: Table S9. ACP and AGL20 were among the above mentioned most influential genes in the interaction network and their roles were cited earlier. Besides, the four remaining genes were located in regions of overlapping QTLs of five categories of traits: FRB12 encodes a translation initiation factor which is involved in apoptosis in response to infection from *Pseudomonas syringae*, LTA2 is involved in embryo formation and development, PP2A modulates protein phosphoregulation in several cellular processes such as growth and developmental processes, hormone and environmental responses, and RLP46 are trans-membrane receptors which are involved in cellular signaling.

ACP4 gene expression showed dissimilarity at T1, with the highest expression in *Gangan*, followed by *No2127, Westar, Zheyou7* and *ZS11*, while expression of ACP4 in *Quinta, Shengli* and *Tapidor* were lower, the other stages displayed comparable profile. Comparison of nucleotide sequences showed that in one hand ACP4 in *Gangan, Shengli, Westar, Zheyou7* and *ZS11*, and in other hand ACP4 in *Quinta* and *Tapidor*, shared 100% identity, respectively. Similarly, expression levels of FBR12 in *Shengli, Tapidor, Quinta* and *ZS11*, which shared 100% of identity, showed a high peak at T1, whereas at T3, *Westar* FBR12 expression level was slightly elevated compared to the other FBR12. *Westar* FBR12 shared 100% identity with the FBR12 of other genomes except with *Zheyou7* FBR12 which shared 92% identity with *Westar* FRB12. Else, AGL20 of *No2127* particularly displayed significant high expression levels at T2, T3 and T4, while others showed relatively similar expression. *No2127* AGL20 shared 100% of identity with AGL20 of *Shengli* and *Zheyou7*, 97% of identity with *Gangan, Westar, Tapidor* and *ZS11*, and 95% of identity with *Quinta.* Besides, *Shengli* LTA2 expression level went from the highest at T0 to the lowest at T1 in comparison to LTA2 of other genomes. *Zheyou7* and *ZS11* also displayed a succession of increase and decrease of expression level during the five post sowing stages, they though shared 96% of identity, whereas *Shengli* LTA2 shared 100% of identity with *Quinta, Tapidor, Westar* and *ZS11* LTA2, 98% identity with No2127 LTA2 and 96% identity with *Gangan* and *Zheyou7* LTA2. Then, PP2A in *Shengli* and *No2127* displayed alternate increase and decrease, but reverse expression level (i.e. when one increased, the other

decreased). However, they shared 100% of identity between them, and with PP2A of other genome, except with *Westar* PP2A which shared 99% identity with them. *Westar* PP2A expression level remained low from stage T1 to T4. At last, a peak of high expression level was noticeable in RLP46 of *Tapidor* and *Quinta* at T3, while those of *Westar, Zheyou7* and *ZS11* peaked at T2. *Tapidor* and *Quinta* RLP46 shared 100% identity between them, and with *Gangan, Westar* and *ZS11*, and 99% with *No2127, Shengli* and *Zheyou7.* It was noticeable than in genes *Zheyou7* and *ZS11* always had the same expression level, even when the nucleotide sequence identity were less than 100%. Those findings suggested that similar gene sequence might display different expression profile, and reversely, different gene sequence might show similar expression profile.

# Discussion

The current study aimed to combine QTLs for seed component, seed yield, hormones and disease related traits, which were detected in previous studies, in one physical map in rapeseed, to identify the related candidate genes and to analyze their expression and structural variation in different rapeseed accessions. The same strategy was used to find region that might control multiple traits of one category, i.e seed oil [19] and seed yield [20]. In those two studies, some regions were suggested to possibly contribute to the improvement of one trait or multiple traits of one category, and some regions were supposed to be stable for one given environment. For example, a region on A1 (2.50-2.99Mb) had overlapping QTLs for plant height, which were from two populations developed in China (*Tapidor×Ningyou7* and *Express617×V8*), so this region might affect the plant height and it is a stable region for Chinese environment [20]. More, QTLs for C16:0, C18:0, C18:1, C18:2, C20:0, and C22:1 were overlapping on C3 (53.75 Mb to 58.29 Mb), thus, this region might control those six traits, simultaneously [19]. The current study was made in higher level because it was not limited to one category of trait as the previous studies, but five categories which involved all studied traits in rapeseed.

Actually, increasing seed oil and seed yield are among the main focus of researcher on rapeseed, in order to cater the increasing demand of oil. However, usage of rapeseed is not limited as a biomass for oil, but also for multiple purposes, such as protein, carbohydrate and vitamins sources, and many more [8]. Despite the effort in improving seed components and seed yield, rapeseed crops are under attack from various diseases that resulted in huge crop loss. For example, *Leptosphaeria maculans* causes blackleg disease [26], which has created an economic loss of $900 million per growing season in the world [27]. Despite the fact that resistant cultivars have been developed and cultivated since year 1990s in Canada [28], which has decreased the yield loss of 1% [29]. Abiotic stresses has also caused about 50% yield reduction in major crops [30]. Actually, extensive researches are still undertaken to take total control of those biotic and abiotic disease, via selective breeding. Otherwise, phytohormones play important roles in plant growth and development, such as IAA [31], but also on plant adaptation to assure survival face to the environment fluctuation. ABA respond to both of biotic and abiotic stresses [32], which have influence on one another [33]. Those phytohormones support agronomic trait improvement and response to disease. Therefore, all the five categories of traits analyzed in the current study are correlated and are pivotal for rapeseed crop improvement.

# Dissection of rapeseed genome revealed regions controlling multiple traits

The current study is the first study to gather all the QTLs of important agronomic and disease related traits discovered in *B. napus* over 25 years, in order to construct a quantitative genomic map, which is crucial to uncover similarity and difference in QTLs detected from different populations and environments, but also to reveal the regions that might control multiple beneficial traits, simultaneously.

It was obvious that most of the QTLs were found on A rather than C genome (2695 vs 1860 QTLs, respectively). Selection has played important role in improving *B. napus*. It was reported that C genome rather than A genome, contained extended breeding regions (51.15 Mb on C vs 16.80 Mb on A) which might contribute more to alleles producing elite traits [34]. However, a recent investigation on the origin of *B. napus* and the genetic loci that contributed to its improvement had revealed that parallel selection of A and C genome had let to seed quality improvement in *B. napus* [35]. In fact, A genome specific-selection greatly enhanced disease resistance, oil accumulation and environment adaptation of *B. napus* during its first stage of improvement, while C genome had improved developmental traits. This might explain the fact that most of the QTLs of studied traits in the current study could be found on A genome. Particularly, for Asian *B. napus* varieties, it was reported that they have experienced strong artificial selection from A genome which contributed to their adaptation following their introduction from Europe [36].

Apart from that, 517 regions were found with overlapping QTLs involving at least two categories of traits. Those regions might be suitable for selection to improve two or more desired traits, simultaneously, for example, to improve both of abiotic stress response, seed component and seed yield (A4:3.07–4.11 Mb). Several studies have already investigated on co-location of QTLs from different category of traits [37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48]. Those studies demonstrated the importance of analyzing multiple traits, at the same time, to target the loci for breeding cultivars with the most advantageous profile. For example, a study which focused on flowering time (FT) and *Sclerotinia* stem rot resistance (SSR) reported that early FT might increased susceptibility to *S. sclerotiorum*, and regions of co-location of FT and SSR resistance traits were found which were crucial for breeding early maturing and SSR resistance cultivars. More, four co-localized QTL hotspots of SSR resistance and FT on A2 (0–7.7 Mb), A3 (0.8–7.5 Mb), C2 (0–15.2 Mb), C6 (20.2–36.6 Mb), which were consensual with previous studies [47]. In the current study, QTL of SSR and DIF (FT) were also co-localized in those regions.

Particularly, seed components and seed yield traits often overlapped in this study. In earlier studies, yield traits such as flowering time, morphology of root and plant growth environment could affect seed quality traits such as erucic acid, oil, protein and glucosinolate contents [13, 49, 50, 51, 52]. In oil crops, QTLs that could have influence on both seed quality and yield traits had already been discovered in several studies, in a positive or negative way. For instance, oil and protein contents were positively correlated with seed weight in eleven *Brassica carinata* lines developed in Canada [53]. Zhao et al. found evidence of positive correlation between oil content and seeds per silique while evaluating 282 DH lines from cross between *Sollux* and *Gaoyou* (*B. napus*), and developed in Germany and China [54]. In a study performed by Chen

et al. in a DH population derived from cross between high and low oil content *B. napus* and developed in Canada, oil content and flowering time were negatively correlated [55]. In that study, QTLs for oil content, flowering time and seed yield were co-localized on a small region of LG7 where alleles of low oil content, early flowering time and higher seed yield were found together. However, QTLs for high oil content and early flowering time were found in co-location on LG2.

Overlapping QTLs of multiple traits might happen when gene alteration frequencies at nearly linked loci occurs, but also, it might be caused by pleiotropic effect when an appropriate substitution of genes occurs [56]. Also, pleiotropy or/and linked genes might have caused this phenomenon.

Region on the chromosome which was exclusively for QTLs from one population was not found, and those exclusively for one environment was for China only (fixed environment for China). This indicated that those QTLs remained unchanged, despite the variation of population and environment.

# Identified candidates genes might be pleiotropic or linked genes?

In previous studies, 110 genes in *Arabidopsis* were identified to be involved in oil formation [57] and 439 homologous genes were found in *B. napus* [19]. More, 425 yield genes which were related to branch number, flowering time, maturity time, plant height, pod number, seed number, seed weight, and seed yield in *Arabidopsis* were identified [58] and 1398 homologous genes were detected in *B. napus* [20]. Dolatabadian et al. found 1344 resistance genes in *B. napus* [59]. Those genes had relationship with the current studied traits, thus, they were selected to uncover the candidate genes .

A total of 2744 genes related to oil, yield and resistance were found within overlapping QTLs involving two to five categories of traits. As mentioned above, overlapping QTLs might be caused by pleotropy or linked genes. Pleiotropy is when one gene can control multiple unrelated phenotypic traits [60, 61, 62, 63]. Pleiotropy is largely distributed due to biochemical and developmental systems and it affects development and evolution, and creates correlations between genes and phenotype, and it affects selection and impose the accessibility of the evolution extent [64, 65]. The pleiotropic organization of traits (dominant or epistatic) can be modified by selection and inbreeding [66, 67, 68]. Linked genes are genes located closely to each other on the same chromosome and are inherited together during meiosis. Genes might separately control different phenotype but are found closely located on the same region of a chromosome.

Candidates found within region of overlapping QTLs with five categories of traits attracted more our attention, since they might be more influential than the others over multiple traits. A total of 26 candidates were found on five regions distributed on A6, A9 and C3 chromosomes, and they would be the most recommended in this study, for selection to target multiple traits simultaneously (Fig. 7). They belonged to different families and might have different distinct roles, but the way they act to influence each other or to affect the traits still need a deep investigation. Functional investigation of each gene

would be indispensable to comprehend their influence over the studied traits, and would reveal whether they were pleiotropic genes or linked genes.

Gene interaction network revealed that 11 genes might have more influence over the other genes. Genes are responsible for genetic variation of traits [22], and structure and dynamism of genetic regulatory network have impact on quantitative traits [69]. In this study, KAS, ACP, AUX1, CO, FT, PHYA, AGL20 were also identified as most influential genes in our previous studies [19, 20]. Despite the number of genes identified in this study was far larger than those of the previous study, and genes function were also broader, those eight genes of different functions still had higher influence over the other genes, indicating that simultaneous control of multiple traits might be affected at different metabolism pathway.

Expression and structural variation analysis of genes in eight rapeseed varieties showed that some genes which had 100% sequence similarity displayed different expression profile, and some other genes with different sequence showed similar expression profile. Epigenetic factors might be responsible for this phenomenon, i.e. alteration in gene expression while preserving the primary DNA sequence or genotype [70, 71]. Epigenetic mechanisms include DNA methylation which commonly induces gene silencing by blocking transcription binding site, histone modification which alters chromatin structure and accessibility of genes for transcription, and non-coding RNA-associated gene silencing which targets mRNA transcripts for destruction induce and preserve epigenetic change [72, 73]. Even if epigenetic change is natural and regular, it can also be influenced by environmental factors [74, 75]. In the case of our study, the eight accessions were produced with different genetic and environmental backgrounds, thus difference in gene expression even with similar sequence was expected.

# Breeding a super-rapeseed cultivar that meets expectations

The current study uncovered regions, with two, three, four or five categories of traits which can be chosen and used for marker assisted selection, to produce a customized rapeseed cultivar with desired traits. For instance, stresses imposed by heat is detrimental for seed yield and quality (reviewed by Sehgal et al. [76]). In order to control these traits at once, the region on A3 (11.40-12.47 Mb) could be selected for fine-mapping, since it contained overlapping QTLs for heat, seed yield and seed composition. Candidate genes included in this region could be cloned and validated through functional analysis, in order to understand the related molecular mechanism.

Another innovation of the current study is the usage of the rapeseed pan-genome of BnPIR to compare gene expression and gene structure of candidate genes. This strategy aimed to comprehend how same genes of different accessions would be expressed, and how their structures are different. This might serve later to explain their functions. Since numerous rapeseed accessions have been sequenced, performing the same study as our current study is now feasible in those other accessions. It would enhance our understanding of rapeseed genome variation.

Besides, compared to rice, rapeseed breeding program needs more effort and innovation. Until now, rapeseed research focuses on QTLs and the studied traits were repetitive. However, rice breeding program

already focuses on QTG (quantitative trait gene) and QTN (quantitative trait nucleotide) for improvement of this crop [77]. This effort was made in order to further close the gap between genomic studies and practical breeding, and to facilitate the localization of causative variants of all known traits. A collection of rice varieties with those variation was made and a genome navigation system was established for breeding. Thus, research on rapeseed should switch progressively into those QTG and QTN analyses. Actually, multiple rapeseed accessions are also available and a collection of variation should be implanted for breeding.

Finally, the current study has enhanced our knowledge on rapeseed genome characteristics and diversity. Co-localized QTLs might have ally or antagonistic effect. For the usage in practical breeding, identification of the most favorable alleles combinations which will produce maximum profits is still crucial.

# Methods

### Alignment of QTLs on the physical map of Darmor-bzh

Extensive literature inquiry allowed to identify more than 350 papers which reported on GWAS and QTLs analyses in *B. napus* over the last 25 years (1995–2020). They were manually sorted according to data availability. Research articles with missing information were removed (absence of flanking markers, marker sequence or physical position of QTLs on *Darmor-bzh*). QTLs/GWAS with just one flanking marker were kept and given an area of 1cM from the unique marker as loci. 4555 QTLs for seed, yield, hormones and disease related traits were collected from 145 research articles, involving 79 different populations of three different ecotypes and grown in 12 different countries (Table S1). They were aligned in the physical map of *Darmor-bzh*. Location of QTLs flanking markers on the physical map was detected via e-PCR [78, 79], and method of alignment was as similar as our previous studies [19, 20]. The map was build using Circos software [80].

# Identification of candidate genes

Genes in *B. napus* which were reported in three different literatures were selected as basis for the identification of candidate genes in the current study: 439 genes were related to oil formation [19], 1398 genes were related to yield traits [20], and 1344 genes were resistance genes [59]. They were aligned into the physical map of *Darmor-bzh*, and the genes located within overlapping QTLs were identified as candidates for the traits.

# Construction of gene interaction network

The gene interaction network was predicted using STRING (http://string-db.org/) [80]. Orthologous genes in *A. thaliana* were used to perform the analysis, because *B. napus* is still not available on STRING database, and *A. thaliana* genes have more accurate known functions rather than those of *B. rapa* and *B.*

*oleracea*. The genes were clustered using Panther GO-slim biological process (http://pantherdb.org/) [82], and the interaction was visualized with Cytoscape_V3.6.0 [83].

# Gene expression and structural variation analyses of candidate genes

Identification of homologous candidate genes in *Gangan, No2127, Quinta, Shengli, Tapidor, Westar, Zheyou7* and *ZS11* was made with blast tool in BnPIR database (http://cbi.hzau.edu.cn/bnapus/) [25], using *Darmor-bzh* gene sequences as probe. *In sillico* gene expression analysis was made using “gene expression” tool of BnPIR. The identity percentage between gene sequences was calculated using Vector NTI Advance 11.5.1. The heatmap was build using Heatmapper ( http://www2.heatmapper.ca/) [84].

# Abbreviation List

ACP - acyl carrier protein

AGL20 - AGAMOUS-LIKE 20

AP2 - APETALA 2

ASI - ketoacyl-ACP synthase I

AUX1 - AUXIN RESISTANT1

CO - CONSTANS

COP1 - CONSTITUTIVE PHOTOMORPHOGENIC 1

FRB12 - FUMONISIN B1-RESISTANT12

FT - FLOWERING LOCUS T

KASIII - ketoacyl-ACP synthase III

LTA2 - PLASTID SUBUNIT OF PYRUVATE DECARBOXYLASE

MCAT - malonyl coa-ACP malonyltransferase

PHYA - PHYTOCHROME A

PP2A - PROTEIN PHOSPHATASE 2A

RLP46 - RECEPTOR LIKE PROTEIN 46

# Declarations

## Authors' contributions

NR collected and analyzed the data, and wrote the manuscript, HC, JH and HL performed E-PCR and draw the figures, ML supervised the work and revised the manuscript.


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Not applicable.


## Competing interests

The authors declare that they have no competing interests.

## Availability of data and materials

Not applicable.

## Consent for publication

All authors consent for publication.

## Ethics approval and consent to participate

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# Figures

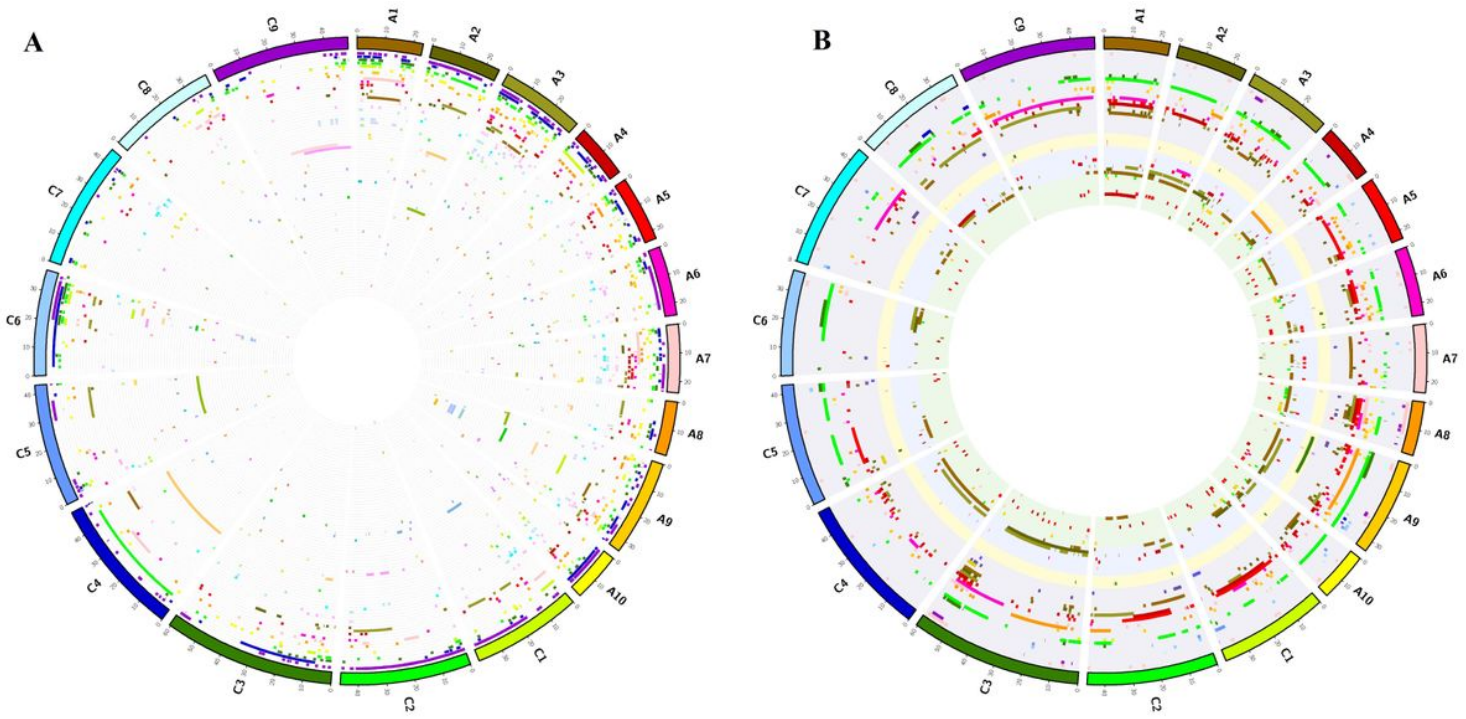
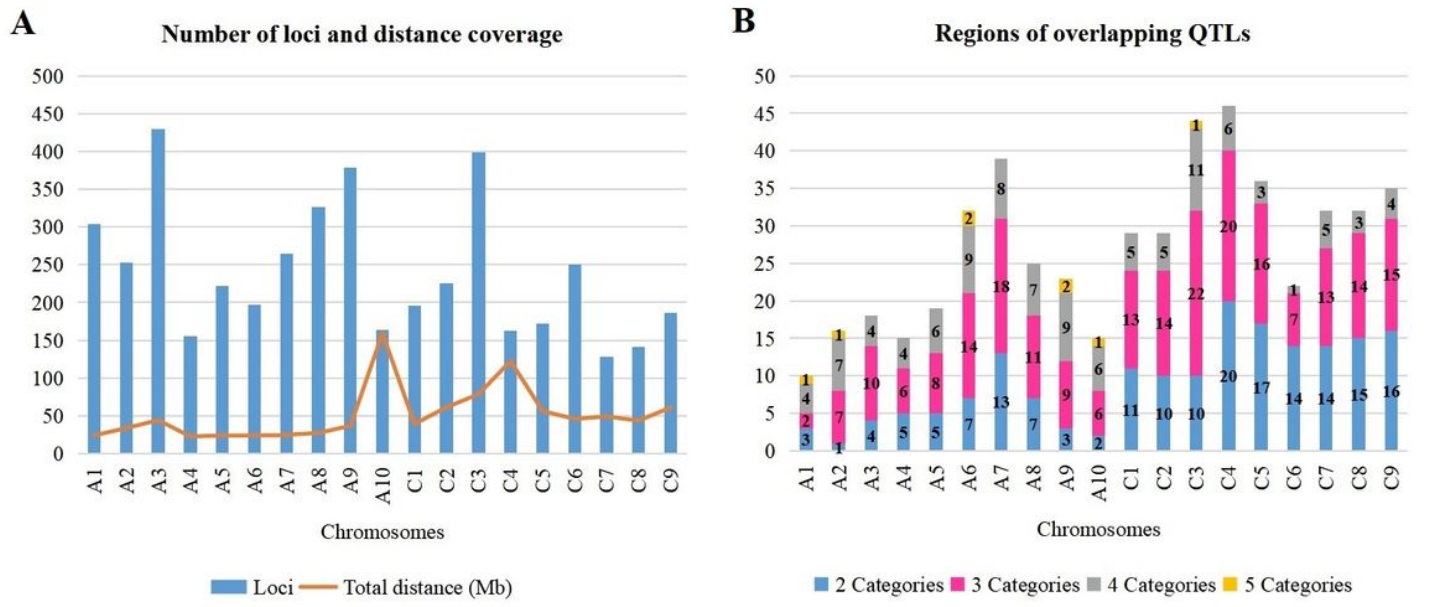



**Figure 1**

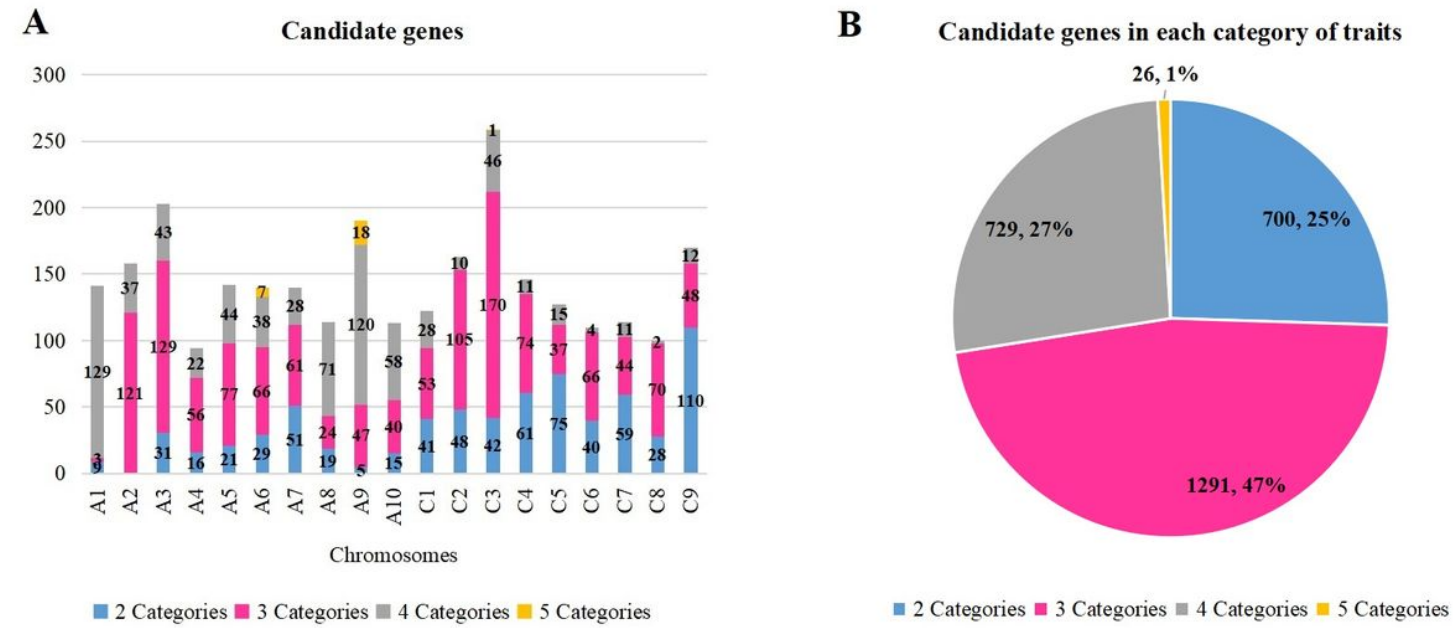
QTLs alignment of agronomic and disease related traits on the physical map of Darmor-bzh A- QTLs alignment for yield related traits. B- QTLs alignment for disease, hormones and seed related traits.

Different colors represent different trait. QTLs were arranged from inner to outer circle according to their apparition on the physical map of Darmor-bzh. The map was built using Circos software.



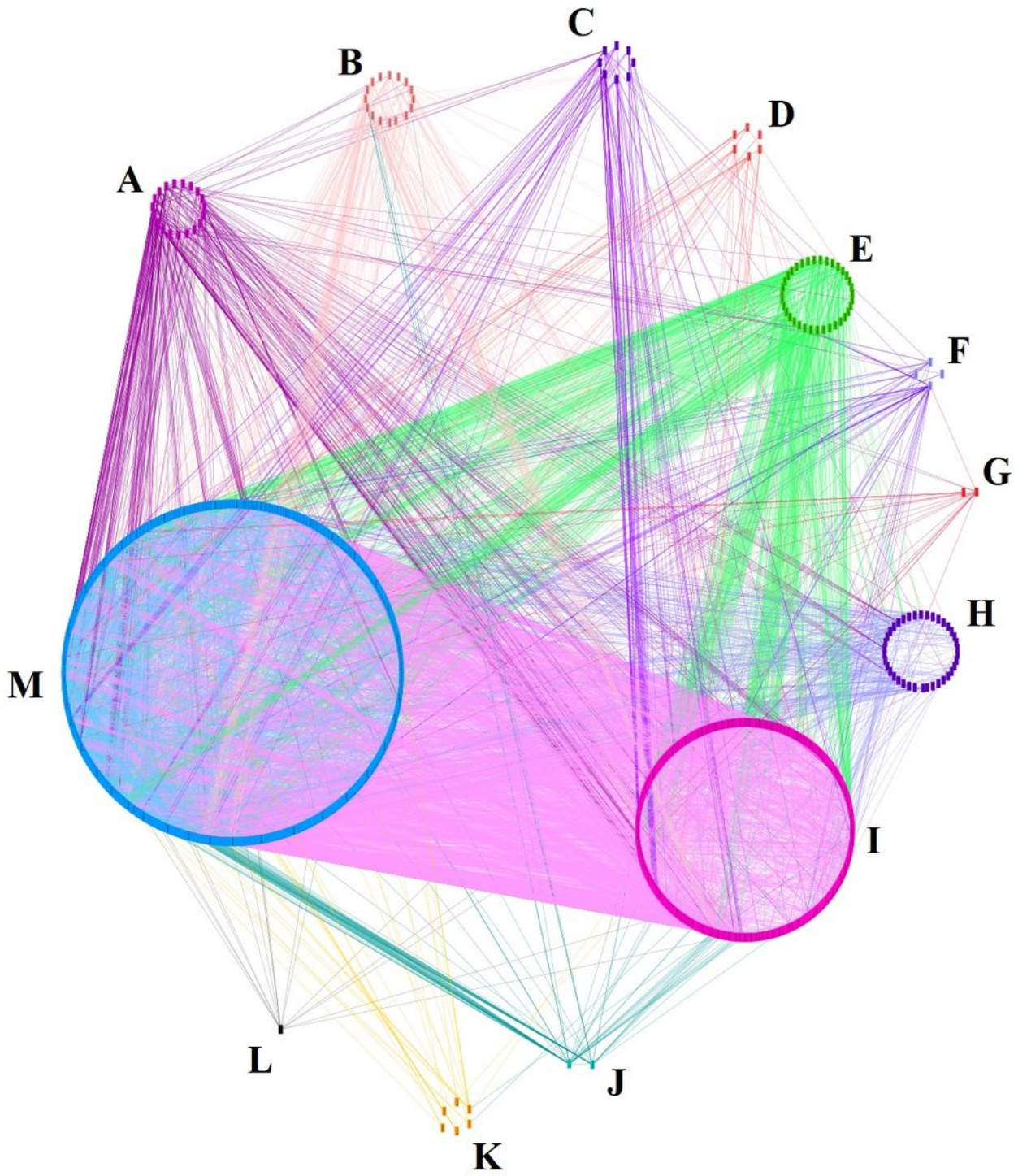

## Figure 2

Dissection of rapeseed genome. A- Number of loci and total distance. B- Regions on chromosomes with overlapping QTLs.




## Figure 3

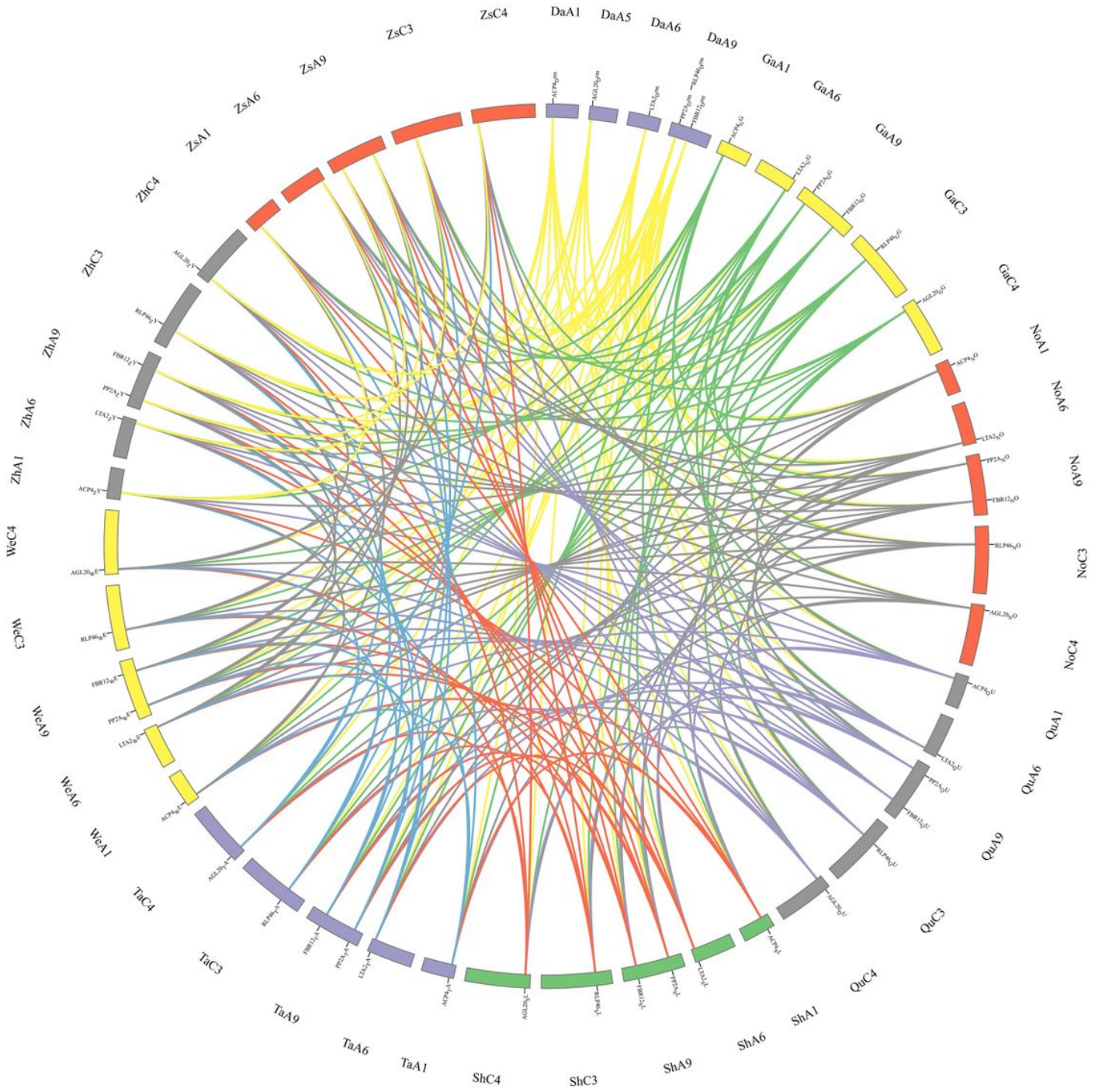
Candidate genes in each categories of traits.




## Figure 4

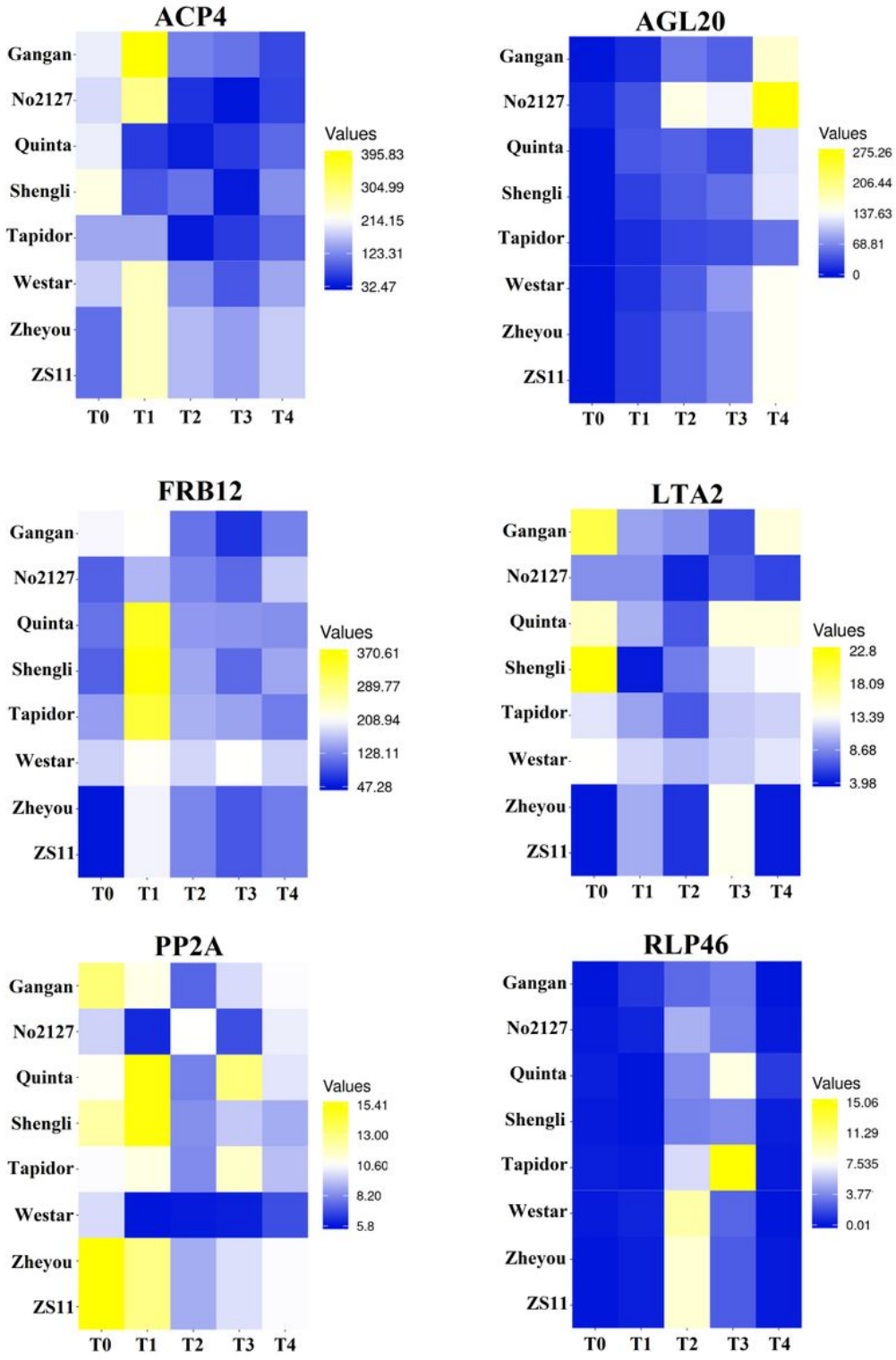
Candidate genes interaction network. The interaction analysis was made with orthologous A. thaliana genes by using STRING (http://string-db.org/) and visualized with Cytoscape_V3.8.2. 1271 nodes and 10101 edges are shown. Eleven categories of genes are displayed according to their GO term enrichment. A-Signalling, B-Multicellular organism, C-Growth, D-Biological regulation, E-Cellular process, F-

Developmental process, G-Immune system process, H-Localization, I-Metabolic process, J-Rythmic process, K-Response to stimulus, L-Reproduction, M-Others.




## Figure 5

Synteny of the six genes with significant expression difference in nine rapeseed varieties. The map was built with TBtools software, with Darmor-bzh (Drm), Gangan (GG), No2127 (NO), Quinta (QU), Shengli (SL), Tapidor (TA), Westar (WE), Zheyou (ZY), Zhongshuang 11 (ZS). Chromosome location is displayed near the genome name.




## Figure 6

Expression of six genes with significant expression difference. The heatmap was build using Heatmapper (http://www2.heatmapper.ca/).

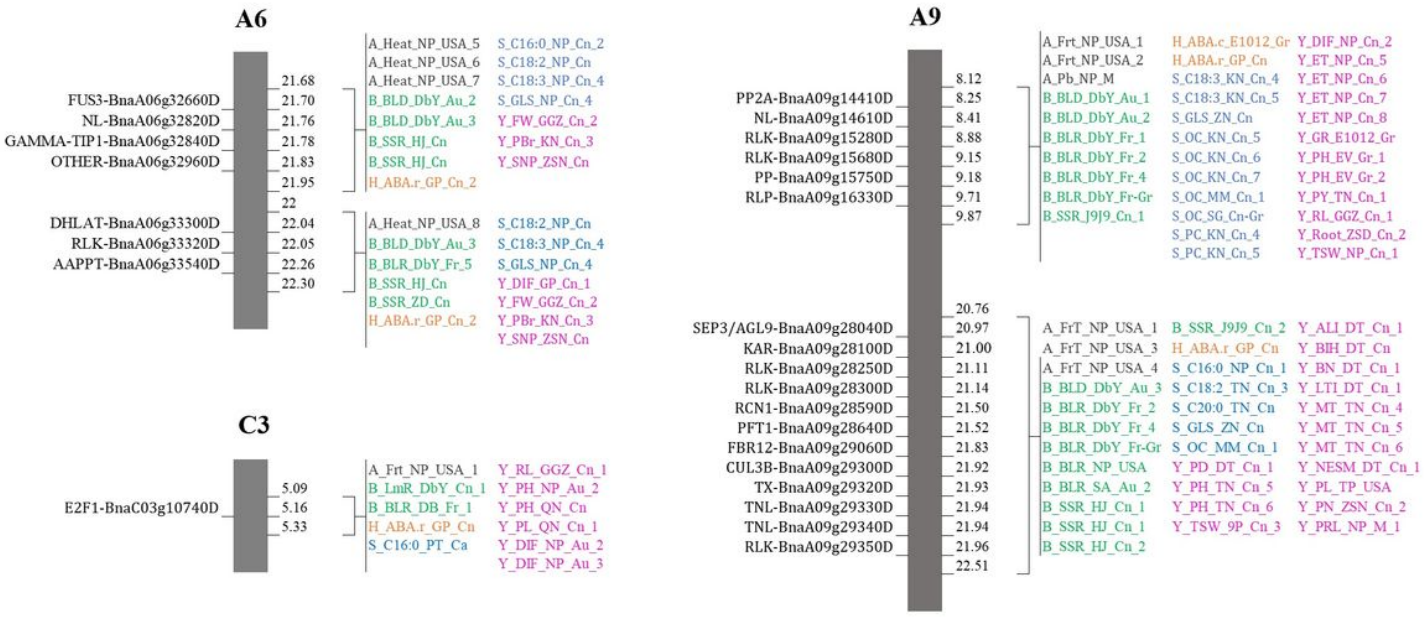



**Figure 7**

Regions on A6, A9 and C3 chromosomes where QTLs of five categories overlapped.

# Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Supplementaryfigure1.pdf
- Supplementaryfigure2.pdf
- Supplementarytable1.xlsx
- Supplementarytable2.xlsx
- Supplementarytable3.xlsx
- Supplementarytable4.xlsx
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- Supplementarytable6.xlsx
- Supplementarytable7.xlsx
- Supplementarytable8.xlsx
- Supplementarytable9.xlsx