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REVIEW

Molecular regulation and genetic improvement of seed oil content in *Brassica napus* L.

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Abstract As an important oil crop and a potential bioenergy crop, *Brassica napus* L. is becoming a model plant for basic research on seed lipid biosynthesis as well as seed oil content, which has always been the key breeding objective. In this review, we present current progress in understanding of the regulation of oil content in *B. napus*, including genetics, biosynthesis pathway, transcriptional regulation, maternal effects and QTL analysis. Furthermore, the history of breeding for high oil content in *B. napus* is summarized and the progress in breeding ultra-high oil content lines is described. Finally, prospects for breeding high oil content *B. napus* cultivars are outlined.

Keywords breeding, maternal effects, oilseed rape, QTL

1 Introduction

Oil is one of the three major nutrients required for human survival. As the third major source of edible oil after soybean and palm, *Brassica napus* L. (rapeseed) accounts for about 15% of the world total production[1]. High-quality *B. napus* oil, with low saturated fatty acid content, is considered as one of the healthiest edible vegetable oils. *B. napus* is mainly produced in Asia, North America, and Europe, where its production accounts for 31%, 24% and 32%, respectively, of world total production in 2015, according to data from the USDA Foreign Agricultural Service (World Agricultural Production: http://www.fas.usda.gov/data/world-agricultural-production). The two main rapeseed producing countries in Asia are China and India. In China, the rapeseed cultivation area of about 7 million hectares yearly accounts for about 21% of the total world production. Rapeseed oil is also the largest domestic source of edible vegetable oil in China (http://www.fas.usda.gov/data/world-agricultural-production).

Oil production is the primary target trait of rapeseed breeding. Rapeseed oil production efficiency is determined by seed oil content and seed yield[2]. These two factors are therefore important breeding objectives for improving oil production. Traditional breeding for over 60 years in China has increased the seed yield 2–3-fold compared to initially introduced rapeseed varieties that yielded about 750 kg·hm^{-2}[3]. Recent studies suggest that increased oil content may be a more efficient approach to increase oil production per unit area; an increase in oil content by 1% was estimated to be equivalent to increasing the seed yield by 2%–3%[4]. Therefore, efforts to understand the regulatory basis of oil content and to breed high oil content varieties are objectives of current rapeseed research. With the development of biotechnology, the wide application of second-generation technology and SNP markers has greatly promoted the study of rapeseed oil content in the world. Especially in China, rapeseed oil content research and breeding have made good progresses in recent years, and the details are described in the following sections.

2 Regulation of *B. napus* oil content

2.1 Genetic analysis on seed oil content in *B. napus*

Plant seeds are complex structures that consist of three major components: the embryo, the endosperm, and the seed coat. In mature rapeseed seeds, the endosperm degenerates and the seed coat enwraps the embryo tightly. Based on the results from Hu et al.[5], most of the oil body organelle accumulates in the embryo and a small proportion of oil body was also observed in the aleuronic

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cells of the seed coat. Seed oil content is a complex quantitative trait controlled by multiple genes that undergo complex interactions with the environment. Genetic control involves a combination of seed embryo genetic effects, and maternal nuclear and cytoplasmic genetic effects. The genetic effects on seed oil content vary because of different research methods and materials. Gan and Lin[6] showed that seed oil content in *B. napus* was influenced by maternal genotype, whereas Wang[7] found that the oil content in hybrids was affected by embryo genotype as well as the maternal genotype. Another study by Wu et al.[8] indicated that oil content was determined by embryo genotype, cytoplasmic and maternal genotype effects, and interaction of genotype and environment. The above results suggest that studies in different genetic backgrounds might be able to detect one effect but detection of others might be limited. To avoid the influence of genetic background, Wang et al.[9] chose 10 representative lines with different oil contents from 3000 *B. napus* accessions to perform a genetic analysis. They found that the maternal genotype contribution to average oil content was as high as 86%, and that the xenia effect accounted for only 14%. Moreover, by comparison of oil contents between reciprocal F_1 and F_2 populations, cytoplasmic effects were also found to have an important influence on oil content[9].

2.2 The effects of the embryo oil synthesis process on seed oil content

Oil synthesis in plant seeds is a complex physiological and biochemical process involving two steps: fatty acid synthesis (FAS), and triglyceride (TAG) accumulation. The biochemical pathways of the two processes have been well studied[10–12]. The key genes encoding lipid synthesis and regulation have been successfully cloned and characterized in *Arabidopsis* including glycolysis-related genes and fatty acid synthesis-related gene ACCase (Acetyl-CoA carboxylase)[13], G3PDH (sn-Glycerol-3-phosphate dehydrogenase)[14], TAG biosynthesis genes DGAT1 (diacylglycerol acyltransferase 1)[15–17] and DGAT2 (diacylglycerol acyltransferase 1)[18], GPAT (glycerol-3-phosphate acyltransferase)[19], and LPAAT (lysophosphatidic acid acyltransferase)[20,21]. Genetic transformation studies confirmed that these genes can increase seed oil content to various degrees[22]. For example, seed obtained from transgenic *B. napus* expressing the cytosolic ACCase in the plastid resulted in a relative increase in oil content of 5%[13]; overexpression of a mutated yeast sn-2 acyltransferase gene (SLC1-1) in *B. napus* and *Arabidopsis* induced the mature seed from the various transgenic lines to produce relative increases in oil content ranging from 8% to 48%[20]; *Tropaeolum majus* DGAT1 (TmDGAT1) has also been overexpressed in high-erucic acid *B. napus* resulting in relative seed oil content increases of 11%–30%[23].

The flow of carbon into storage lipid is also influenced by other metabolic pathways which draw upon cellular carbon. In plants, glycolysis takes place in the cytosol and plastid, with both compartments linked by transporters in the plastidial envelope[24]. Pyruvate kinase catalyzes the irreversible production of pyruvate and ATP, which are utilized in numerous biochemical pathways. Andre et al.[25] disrupted the gene encoding the β1 subunit of plastidial heteromeric pyruvate kinase complex in *Arabidopsis* which resulted in mature seed displaying a 60% reduction in seed oil content. Mitochondrial pyruvate dehydrogenase kinase (PDHK) downregulates the mitochondrial PDH complex by phosphorylation. Seed-specific antisense repression of the gene encoding mitochondrial PDHK during seed maturation has been shown to result in increased seed oil content and seed weight in *Arabidopsis*[26,27]. Wakao et al.[28] have suggested that cytosolic Glu6PDH plays a role in provision of reducing power (in the form of NADPH) for fatty acid (FA) biosynthesis in maturing seeds where photosynthesis may be restricted.

Certain transcription factors regulate lipid synthesis pathway genes and can greatly influence metabolic processes involved in seed oil accumulation. In *Arabidopsis* and rapeseed, there are five such transcription factors that control downstream target genes, namely *WRI1, LEC1, LEC2, ABI3,* and *FUS3*[29]. *WRI1* encodes an AP2/EREB domain transcription factor; the *wri1* mutant cannot convert sucrose into precursors for fatty acids, leading to increased insoluble sugar contents and reduced seed oil content[30,31]. Overexpression of rapeseed *WRI1* in *Arabidopsis* increased not only seed oil content but also seed weight[32]. *LEC1*, encoding CCAAT-box binding factor *HAP3* subunit homolog plays an important role in the formation and maturation of the seed embryo[33,34]. Overexpression *of LEC1* in rapeseed upregulates many key genes that are involved in glycolysis, lipid synthesis, and oil accumulation, leading to a significant increase in fatty acid levels[35]. *LEC2*, *ABI3,* and *FUS3* belong to plant-specific transcription factor families. The transcription level of the *LEC2* gene was positively correlated with oil accumulation[36–38]. *FUS3* and *ABI3* have important roles in late-stage seed development[39]. TRANSPARENT TESTA2 (TT2), regulating biosynthesis of proanthocyanidins (PAs) in the seed coat of *Arabidopsis* causes inhibition of fatty acid (FA) biosynthesis in the seed embryo. TT2 is expressed in embryos at an early developmental stage; it directly binds to the regulatory region of FUSCA3 (FUS3) and mediates expression of numerous genes in the FA biosynthesis pathway[40]. These genes and their respective functions are summarized in Table 1.

2.3 Maternal effects on seed oil accumulation

Maternal effect refers to the effect that the cultivar genotype and the environmental response of the maternal

Table 1 Identification and function of genes affecting seed oil content

Organ	Function	Gene	Specie	Reference No.
Embryo	Fatty acid synthesis	Acetyl-CoA carboxylase (ACCase)	*Arabidopsis*	[13]
	TAG synthesis	Acyl-CoA:sn-glycerol-3-phosphate acyltransferase (GPAT)	*Arabidopsis*	[19]
		sn-Glycerol-3-phosphate dehydrogenase (G3PDH)	Rapeseed	[14]
		Acyl-CoA:lysophosphatidic acid acyltransferase (LPAAT)	*Arabidopsis*, rapeseed	[20,21]
		Type 1 acyl-CoA: diacylglycerol acyltransferase (DGAT1)	*Arabidopsis*, rapeseed, maize	[15,16]
		Type 2 acyl-CoA: diacylglycerol acyltransferase (DGAT2)	Soybean	[18]
	Glycolysis related	Mitochondrial pyruvate dehydrogenase kinase (PDHK)	*Arabidopsis*, rapeseed	[26,27]
		Cytosolic D-glucose-6-phosphate dehydrogenase (Glu6PDH)	*Arabidopsis*	[28]
		Plastidial heteromeric pyuvate kinase complex	*Arabidopsis*	[25]
	Transcription regulation	Leafy contyledon 1 (LEC1)	*Arabidopsis*, rapeseed, maize	[34,35,37]
		Leafy contyledon 2 (LEC2)	*Arabidopsis*	[36]
		Transparent tetsa 2(TT2)	*Arabidopsis*	[40]
		Wrinkled 1(WRI1)	*Arabidopsis*, rapeseed, maize	[30,32,37]
Seed coat	Seed size regulating genes in embryo	Apetala 2 (AP2)	*Arabidopsis*	[41]
		CYP78A5 (KLU)	*Arabidopsis*	[42]
		Transparent testa 8 (TT8)	*Arabidopsis*	[43]
Mother plant	Photosynthesis	Growth-regulating factor 2 (GRF2)	Rapeseed	[44]
All	Cytoplast effect	ORF188, a mitochondrial gene	Rapeseed	[45]

parent have on traits of the offspring[46]. The seeds of rapeseed develop and mature within maternal tissue surrounded by the maternal silique wall (Fig. 1). The development of the embryo depends on the direct supply of nutrients from the plant, hence the physiological metabolism and gene expression in the silique wall or seed coat (maternal tissue) can influence the accumulation of seed nutrients (Fig. 1). Wang et al.[9] showed that the maternal plant genotype controls the oil content of *B. napus* F_1 seeds. After flowering, the green silique wall becomes a major organ for photosynthesis, and provides more than 70% of the energy for seed development[47,48].

Thanks to high-throughput sequencing and transcriptome gene expression analysis, studies with rapeseed lines zy036 and 51070 have confirmed that the silique wall photosynthesis regulates accumulation of seed oil[49]. Seed coat-specific gene expression can regulate the supply of nutrients and influence the maturation process, affecting the final development of the endosperm and embryo[50–52]. For example, *AP2* and *KLU* regulate glucose metabolism, and affect the seed oil content in the *Arabidopsis* seed coat[41,42,53]. Tissue culture studies using rapeseed siliques, ovules and embryos from rapeseed lines with different oil content have confirmed that seed coat affects oil accumulation by regulating sucrose concentration[44,54]. TRANSPARENT TESTA 8 (TT8) in the seed coat was also reported to act maternally to affect seed FA biosynthesis and inhibited seed FA accumulation by downregulating a group of genes including *LEC1*, *LEC2*, *FUS3*, and *CDS2*, which are transcriptional factors important for seed development or FA biosynthesis[43]. Wang et al.[9] showed that the cytoplasm significantly regulates rapeseed oil content in lines 51218 and 56366. Cytoplasmic genome sequencing of these two lines led to the development of useful molecular markers[55]. Cytoplasmic effect and marker linkage analysis between the two materials and resource populations helped to identify the key gene (*ORF188*) that affect oil content[45]. The locations of the molecular regulation pathways are indicated in Fig. 1.

2.4 QTL mapping on seed oil content

Seed oil content is a complex quantitative trait controlled by multiple genes; therefore, high oil content *B. napus* lines from different sources show significant genetic variations. In the past 20 years, researchers have used DH populations, recombinant inbred lines and F_2 populations from parents with different oil contents to locate genes/QTLs affecting oil content. A large number of QTLs have been identified across almost all chromosomes, with each study identifying 3–27 loci[56–66]. For example, Qiu et al.[58] localized QTLs in a DH population to linkage groups N1, N3, N4, N8, N12, N13, and N17. Delourme et al.[59] located 14 and 10 QTL regions in two DH populations. The genetic basis of rapeseed oil content has also been studied using correlation analysis. Five years ago, most of the maps were constructed on the basis of the SSR markers. With the development of the GWAS, the QTLs maps depend on it more and more. For example, Zou

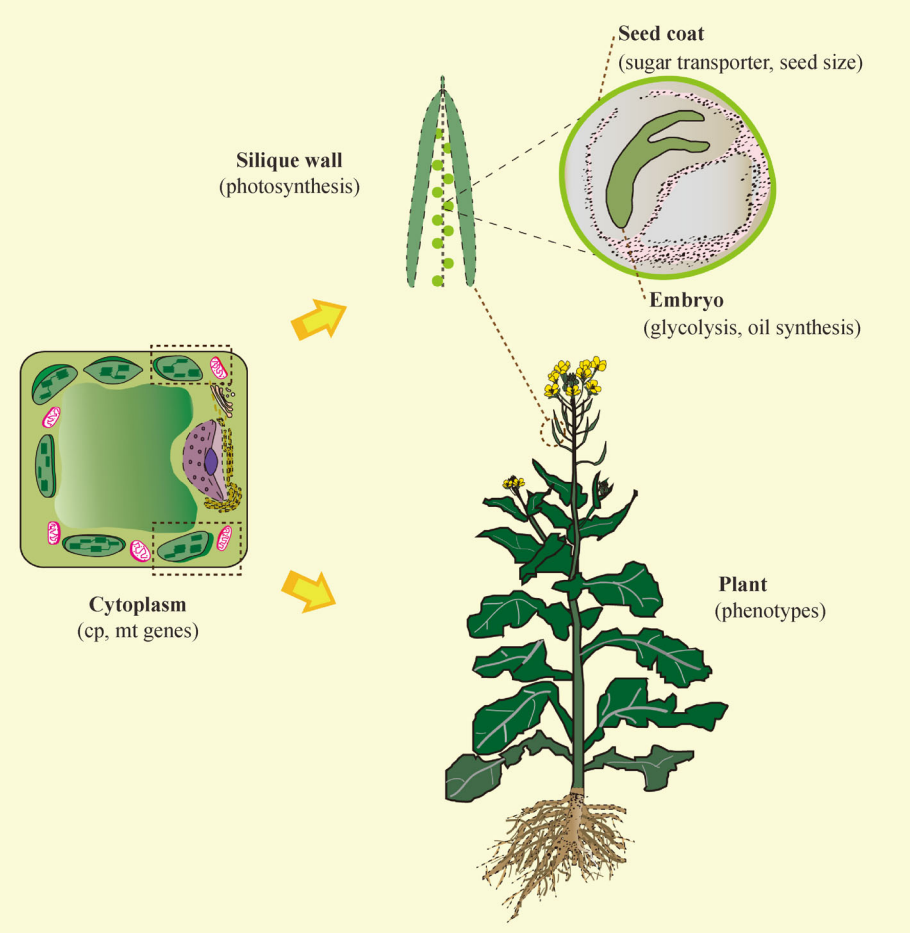

Fig. 1 Regulation model of seed oil content in *Brassica napus*. Boldfaces indicate major organs or factors controlling the seed oil content and their relative regulating pathways are listed in the parenthesis.

et al.[67] used 116 molecular markers linked to oil content QTL to conduct a GWAS analysis on 172 rapeseed lines from around the world. Their study indicated that about 50% of the markers were correlated with oil content. Sun et al.[68] analyzed 14 markers for candidate oil synthesis genes with seven major QTL peak areas or within the confidence intervals in 81 rapeseed core germplasm accessions. They found that six markers in four QTL regions were associated with oil content, and that combinations of four of these markers could increase oil content by 5%. Although previous studies found many loci associated with oil content, the phenotypic variability and additive effect of the detected QTLs for oil content were relatively small. As a consequence, there has been little application of markers for oilseed content in rapeseed breeding. Recently, using a Brassica 60K SNP array, three segregating populations with high oil (zy036, 6F313, and 61616) and one low oil content (51070) strain were used to identify oil content QTLs where differences in oil content reached 15%. They obtained six QTLs from the three different high oil strains that contributed more than 20% of the variation which providing a good resource for marker-assisted breeding of high oil rapeseed[69,70]. These results are summarized in Table 2.

2.5 Relationships between oil content and other traits

As seed oil content and seed yield traits are closely related to each other, clarification of relationships between various traits and oil content is essential for breeding for high oil content. Investigation of the correlation of seed oil content with several major yield traits in 16 varieties suggested positive contributions in the following order: pod number > lodging rate > 1000 grain weight > yield > main inflorescence fruitless rate > silique number[72]. Another study showed that oil content was significantly positively correlated ($r = 0.421$) with seed number per silique while it only had a small positive correlation ($r = 0.172$) with single seed weight[61]. The correlation between oil content and inflorescence yield in segregating DH populations varied with geographic location[73]. In this work a positive correlation was found between oil content and main inflorescence yield in one location whereas oil content in the other three locations was not significantly correlated

Table 2 QTLs affecting seed oil content in *B. napus*

Oil content of parents/%	Localization method	QTL number	Position	Contribution/%	Reference No.
43.60, 42.00	DHP, ML	7	N1,N3,N4,N8,N12,N13,N17	2.40–15.70	[58]
48.00, 46.00	DHP, ML	15	N1, N2, N3, N5, N6, N10, N12, N13, N15, N16, N17	1.20–13.40	[59]
47.50, 41.70	DHP, ML	8	N1, N4, N6, N12, N16, N17, N19	1.77–27.57	[59]
43.28, 37.03	DHP, ML	5	N1, N8, N10, N13	5.21–10.17	[64]
39.70, 34.80	DHP, ML	7	N4, N7, N11, N16, N17	3.73–10.46	[63]
39.70, 34.80	DHP, ML	9	N1, N3, N4, N5, N7, N13, N14	5.19–13.57	[65]
N/A	DHP, ML	19	N1,N2,N3,N4,N5,N6,N7,N8,N10,N12,N13,N14,N16, N18	4.20–30.20	[66]
49.53, 39.42	DHP, ML	12	A2, A3, A5, A6, C2, C5, C8, C9	9.15–24.56	[69]
30.00–52.00	NP, GWAS	12	A1, A3, A9, A10, C2, C3	3.00–15.00	[67]
	NP, GWAS	26	A1, A3, A4, A9, A10, C2, C3	5.00–15.00	[67]
32.66–46.73	NP, GWAS	4	A1, A5, A7, A8	4.42–13.13	[68]
34.20–51.40	NP, GWAS	1	A8	6.22	[71]

Note: DHP, DH segregative population; ML, map-based localization; NP, natural population; GWAS, genome-wide association studies.

with inflorescence yield. The study concluded that the oil content and yield did not have a strong negative correlation, thus setting a theoretical foundation for synchronized oil content and yield breeding.

Physiological and biochemical studies showed that *Sclerotinia* resistance and lodging resistance are important factors affecting oil content in rapeseed[74]. With the aid of high-throughput sequencing and transcriptome gene expression analysis, two functional genes (*HSP17.8* and *DDT*) were identified in *B. napus* that could stabilize oil content under stress by improving the heat and drought resistance of plants (unpublished data). The above results demonstrated that plant resistance and oil content are positively correlated; thus, providing a theoretical and technical basis for synchronized improvement in *B. napus* varieties.

2.6 Relationships between environment and oil content

As a quantitative trait, rapeseed oil content is controlled by multiple genes and influenced by environmental factors. Because of the wide and varied planting area in China, rapeseed oil contents in diverse ecological areas show great differences. Some studies suggest that most of the QTL loci of the same population detected in different environments were environmentally specific, indicating that the adaption to different environmental factors has an important role in fostering high oil content varieties bearing strong alleles[71]. Temperature and light are the two greatest environmental factors influencing oil production of rapeseed[75–77]. High temperature during rapeseed maturation is not conducive to the accumulation and transformation of nutrient material into seed[75]. Especially in China, in the Yangtze River Basin, which contains the main Chinese Rapeseed producing areas, temperatures often rise too fast. High temperature forces seeds to mature earlier. As a result, seed weight and oil content decrease sharply. Some studies also show adequate lighting and long-time illumination can increase the efficiency of lipid accumulation[76,77]. Currently, research on the mechanism whereby environmental factors affect seed oil content is insufficient. Yu et al.[78] analyzed the global transcription profiles of 20 d-old siliques of *B. napus* after heat stress using a *Brassica* 95K EST microarray and found that many HSF/HSP transcripts and other heat-related marker genes were upregulated. Other upregulated genes including some transcription factors and potential developmental regulators were preferentially expressed in the heat-stressed silique wall or seed.

3 Progress and prospects for genetic improvement of oil content in *B. napus*

3.1 Creation of ultra-high oil *B. napus* resources

Breeding high oil rapeseed varieties ten years ago largely depended on conventional plant breeding methods such as continuous individual plant selection in varietal populations, hybrids between varieties, mutagenesis, yellow seed, and heterosis. For example, in 2007, Dr. Fu Tingdong at Huazhong Agricultural University bred high oil (up to 54.72%) and double low rapeseed line by hybridization and a series of selections[79]. Using similar technology, Fu et al.[80] at the Chinese Jiangshu Academy of Agricultural Sciences used a high oil content population as the selection group to positively screen for seed oil content and obtained a high oil line HOC3 with an oil content of 54.52%.

In recent years, conventional breeding methods to improve oil content in rapeseed have encountered increasing bottlenecks. More biotechnology approaches, such as microspore culture and molecular marker-assisted

selection breeding are urgently needed to support high oil content breeding. Zhu et al.[81] reported that a combination of gene pyramiding and microspore culture helped to increase oil content to 52.38% in the restorer material strain T057-7. Li et al.[82], at the Shanxi Provincial Hybrid Rapeseed Research Center, increased the oil content of *B. napus* germplasm materials from about 40%–61.7% using single crosses, backcrosses, ecological breeding, yellow seed breeding and DH line breeding methods. Recently, by combing the microspore culture and marker-assisted selection, Wang's group at the Oil Crops Research Institute of the Chinese Academy of Agricultural Science used four special aforementioned resources (zy036, 6F313, 61616, and 61218) to create five new strains with oil contents of 60%, among which YN171 has an oil content of up to 64.8%[5], a new world record.

Hu et al.[5] conducted anatomical analysis on the seeds with different oil contents, and proposed a forecasting model of rapeseed oil content. Oil content = cotyledon ratio × oil body organelles ratio in cotyledon cell + radicle ratio × oil body organelles ratio in radicle cell + 1/3 × seed coat ratio × oil body organelles ratio in aleurone cells. According to this formula, if the maximum values of the various components of rapeseed currently observed were considered the predicted oil content could be increased to 75%, indicating further potential for improvement in rapeseed oil content.

3.2 Breeding of high oil content *B. napus* cultivars

If high oil varieties are to be practical, they must also have other excellent agronomic traits. Until now, high oil breeding has experienced two phases: The first phase was to identify high oil content cultivars with low erucic acid and low glucosinolate contents, high levels of which are harmful to human health), and the second phase was high oil content taking into account other characteristics such as high yield and other desirable agronomic traits. For phaseI, Canada and Europe achieved the goal much earlier. Well known varieties includs the Canadian double low varieties *Zephyr* and *Midas* both with oil contents exceeding 50%[83], and French double low variety *Major* with an oil content over 50%[76]. Since the early 1980s, high oil rapeseed breeding work in China has included consideration of erucic acid and glucosinolate contents. After 20 years, high oil rapeseed varieties in China have basically achieved the double low character, and average oil content in varieties has been improved by 2%–3% over nearly 10 years, from 40.5% to 43%[23,84].

Current breeding objectives for high oil rapeseed varieties are currently in the second phase, which takes into account high oil content and high yield combined with desirable agronomic traits including *Sclerotinia* resistance, pod shattering resistance, and lodging resistance. Good progress has been made; 24 high oil containing varieties were identified during the national validation of winter rapeseed varieties in China during 2001–2006. Among them, Zhongshuang 9 was obtained from a multiple cross, microspore culture, and different generations screening for target traits. The integrated traits include yield, resistance, and quality to achieve rapid pyramiding of multiple target traits with good qualities such as high oil content, high yield, high disease resistance, high lodging resistance, high protein content, low erucic acid, and low sulfur glucoside content. During 2007–2010, China produced 44 winter rapeseed varieties with high oil content. Among them, Zhongshuang 11 was the first released OP variety with oil content > 49%; it combines outstanding traits such as high oil content, strong shattering resistance, lodging resistance and resistant to *Sclerotinia*. Zhongshuang 11 overcame the contradictions between phenotypes of high oil content and yields, resistance traits through combining approaches such as microspore culture and marker assistant selection.

4 Conclusions and future perspectives

Although rapeseed oil content breeding has made good progress worldwide in the past ten years, there is still potential for further improvement. Synchronous improvement of multiple traits is the current trend for high oil breeding. The completion of rapeseed genome sequencing projects will further help in identifying and isolating the corresponding genes from oil trait QTLs. Moreover, the availability of NGS and SNP arrays will accelerate research on molecular regulatory mechanisms that may help to increase rapeseed oil content. Genetic engineering, molecular marker technology combined with a conventional directional selection strategy will be the trend in breeding high oil content rapeseed.

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