Nutraceutical Potential of Rapeseed: Breeding and Biotechnological Approaches

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Contents

Abstract

Rapeseed (*Brassica napus* L.) is a prime oil crop of the world that also provides proteins for the livestock feeding. This crop has achieved remarkable success over the past few decades by development of canola types with low erucic acid

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and glucosinolate contents. Considerable progress has also been made to enhance the oleic acid content of seed oil. In contrary, little attention has been paid to improve other health-promoting constituents such as phytosterols and tocopherols of the oil. Following oil extraction, rapeseed meal is an excellent source of vitamins, minerals, and high-quality proteins that can help to mitigate human malnutrition. However, poor digestibility, dark color, and bitter taste of rapeseed meal due to anti-nutritional factors like phenolics, phytates, and crude fiber make the meal unacceptable for human consumption. It is crucial to further improve the nutraceutical and commercial value of rapeseed by increasing the content of health-benefitting components while simultaneously minimizing the antinutritive components to the acceptable amounts. To achieve the objective, it is imperative to understand the consolidated genetic architecture of oil and meal quality traits in connection to each other. Recent advances in next-generation sequencing technologies, availability of pan-genome of *B. napus* together with improved bioinformatic and genome editing methodologies would be very useful to reveal the genetic networks and identify high-resolution sequence-based markers for marker-assisted breeding for quality traits in rapeseed.

Keywords

Rapeseed · Nutraceutical · Marker-assisted selection · Erucic acid · Glucosinolates

1 The Crop Rapeseed

Brassica napus L. (AACC, 2n = 38) is one of the six species comprising the well-known U's Triangle of *Brassica*, a genus that belongs to the plant family Brassicaceae. It is an allopolyploid crop species that possesses the full chromosome complements of two diploid species, known to be *B. rapa* and *B. oleracea* (Chalhoub et al. 2014). A few interspecific hybridization events followed by genome duplication are believed to occur in the recent past (<10,000 mya), when two progenitor species (*B. rapa* and *B. oleracea*) were growing in close geographical proximity in Mediterranean area to originate *B. napus* (Friedt and Snowdon 2010). *B. napus* encompasses two subspecies – *B. napus* ssp. *oleifera* (commonly known as rapeseed/oilseed rape) and *B. napus* ssp. *rapifera* (swede and rutabaga) cultivated for oil and fodder purposes, respectively. Of these two subspecies, *B. napus* ssp. *oleifera* carries abundant amount of oil (45–50%) in its mature seeds that has both nutritional and industrial applications depending upon fatty acid composition of oil (Nesi et al. 2008). Also, meal/cake, a by-product left after oil extraction, holds great promise as an excellent protein source (38–40%) for animal nutrition. Presently, rapeseed crop represents the third largest valuable source of vegetable oil (after soybean and oil palm) and the second largest source of protein rich extraction meal (after soybean) in the world (Tang et al. 2021). The crop is cultivated over a large geographical spread of around 33 million ha in Europe, America, China, and India. Different ecotypes of rapeseed, i.e., winter,

semi-winter, and spring types, are raised in diverse ecologies of the world (Song et al. 2020). Winter-type rapeseed with strong vernalization requirement is predominantly grown in Europe, parts of China, and eastern USA, whereas, very early flowering spring forms with no vernalization requirement are cultivated in Canada and Northern Europe. The rapeseed cultivation in Australia and China is largely based on semi-winter types that need mild vernalization (Friedt and Snowdon 2010). Winter cultivars give higher seed yield than spring types owing to advantage of lengthy crop season of over 300 days occupied by winter lines in contrast to only 120–190 days taken by spring cultivars. Historically, rapeseed oil was used widely as lamp oil for European domestic and railway coaches lighting or for some other technical purposes, i.e., to produce soaps, inks, candles, and lubricants. It was of limited value for edible use because of the presence of appreciable amount of two anti-nutritional components – erucic acid (EA) and glucosinolates (GSLs) in the seed, which have detrimental health effects on humans and animals, respectively. The huge success of rapeseed crop for edible oil and animal feed was achieved during just the past four decades after dramatic reduction of these two antinutritive components in the seeds. A trade name "Canola" or double zero/"00" was registered in Canada in 1979 that refers to the edible oil crop carrying low EA (<2%) in the oil and low level of GSLs in the extraction meal (<30 μmol/g of defatted meal). Tower was the first canola rapeseed cultivar, commercially released in Canada in 1974. Thereafter, canola forms largely replaced the traditional rapeseed in all rapeseed-producing areas in Australia, Canada, and Europe and to a very large extent in China (Friedt and Snowdon 2010). The cultivation of canola rapeseed has started in India from 2007 (Chauhan et al. 2010).

2 Nutraceutical Profile of Rapeseed Oil and Meal

Rapeseed oil and meal constitute excellent source of nutraceuticals that are crucial for human nutrition and health as discussed below.

2.1 Rapeseed Oil

In the rapeseed oil, there are triacylglycerols (fatty acids), representing the major component (98%) and non-glyceride fractions (phytosterols, tocopherols, pigments (carotenoids and chlorophylls), and waxes), that constitute the remaining minor fraction (2%) of oil.

2.1.1 Fatty Acids

The fatty acid composition which includes saturated fatty acids (palmitic (PA; C16:0) and stearic acids (SA; C18:0), monounsaturated fatty acid (MUFA) such as oleic acid (OA; C18:1), polyunsaturated fatty acids (PUFA) like linoleic (omega-6; LA; C18:2) and linolenic acids (omega-3; LiA; C18:3), and very long-chain fatty acids (VLCFA) like eicosenoic acid (C20:1) and erucic acid (EA; C22:1) determines the nutritional quality of rapeseed oil as seen in other vegetable oils. The rapeseed oil carries a healthy

lipid profile, fit for human consumption (Russo et al. 2021). The rapeseed oil is low in saturated fatty acids (<7%) and high in MUFA and PUFA, with OA accounting for 61%, LA for 21%, and LiA for 11%, that help in balancing cholesterol level. LA and LiA are two essential fatty acids as they are not synthesized by our body and need to be obtained from the diet. Rapeseed oil contains considerable amount of these two fatty acids with a nearly ideal ratio (5–10:1) to avert cancer, heart, and autoimmune-related diseases. High content of MUFA imparts stability to oil by making it resistant toward oxidation at high temperature and ambient storage. Earlier, rapeseed oil was not acceptable for edible purpose in many countries around the world due to the occurrence of high level of EA (>50%), which is believed harmful to health. EA has slow tendency to oxidize, thus, it accumulates in arterial lining resulting in fibrotic heart lesions. It was found responsible for body weight reduction and abnormal accumulation of fat in laboratory rats (Charlton et al. 1975). Although the negative effects of high EA have never been observed in human beings, it is still suggested to keep the level of EA low (<2%) in rapeseed oil for safe human consumption. Conventionally bred canola rapeseed cultivars lack this nutritionally undesirable long-chain fatty acid (<2% of C22:1), thus making the oil suitable for human consumption.

2.1.2 Phytosterols

Phytosterols in rapeseed occur as free sterols or combined in esters, glucosides, and esterified glucosides. Phytosterols comprise of 45–60% sitosterol, 29–43% campesterol, and 5–13% brassicasterol, with minor fractions of avenasterol (3–7%), stigmasterol (<1%), and cholesterol (<1%). Brassicasterol is a distinct type of phytosterol reported only in Brassicaceae family. Significant amount of phytosterols is present in oil of rapeseed cultivars (0.5–1.1%) (Piironen et al. 2000). Rapeseed oil is reported to contain highest phytosterol content just next to corn oil as revealed by biochemical analysis on ten different oil types (Gordon and Miller 1997). Phytosterols are known to reduce low-density lipoprotein cholesterol and total cholesterol (treatment of hypercholesterolemia) in humans, thus reducing the risk of heart diseases (Cabral and Klein 2017). They are proved to play a vital role as anticarcinogenic, anti-inflammatory, and anti-oxidation agents. They are also known to impart beneficial effects on dementia. Phytosterols are the essential membrane constituents. They protect the skin from UV rays by quenching singlet oxygen. Phytosterols need to be procured from the diet as they cannot be synthesized by our body.

2.1.3 Tocopherols

Rapeseed oil is one of the richest natural sources of tocopherols. The major form of tocopherol in rapeseed oil is γ-tocopherol followed by α- and δ-tocopherols. α-tocopherol is the biologically active form of vitamin E. Vitamin E has potential antioxidant properties and are helpful in avoiding aging-related diseases, Alzheimer's disease, and cardiovascular diseases (Gugliandolo et al. 2017). An adequate intake of vitamin E can also help to reduce chances of neurological disorders, cataracts, and cancer (Schneider 2005). γ-tocopherol is also important as it imparts oxidative stability to rapeseed oil at higher temperatures.

2.2 Rapeseed Meal

Hulls (seed coat) and embryos together constitute the de-oiled rapeseed meal with hull contributing nearly 30% of the total meal weight. Most of the carbohydrates including fiber and phenolic compounds are present in hull portion with tiny fraction of them in embryo part. On the other hand, embryo portion is mainly rich in protein which is less in hull part.

2.2.1 Proteins

The seeds of *B. napus* hold near 45% oil and 25% seed storage proteins. After oil extraction, the meal carries up to 40% of protein. Cruciferins-12S globulins (60%) and napins-2S albumins (20%) are the major proteins account for 80–83% of the total protein content (Swati et al. 2015). Other proteins such as oleosins and lipid transfer proteins constitute the minor proportion of total proteins. Rapeseed protein has a high biological value due to its nearly ideal amino acid composition, which is scarce in cereal proteins, making it an excellent addition to cereal-based diets. Slightly lower levels of essential sulfur containing amino acids (methionine and cystine in napin) have been reported in rapeseed protein as compared to soybean protein. Rapeseed meal protein shows amino acid composition comparable with milk protein casein. The cultivation of modern canola cultivars with low glucosinolate level decreased the amount of the desired napin in seeds (Malabat et al. 2003). The pathways of glucosinolate and amino acid biosynthesis are interconnected and share common enzymes, so manipulation of glucosinolate in canola cultivars could affect the content of napin (Nesi et al. 2008). Peptide mixtures of rapeseed are proved to show antidiabetic, anorexigenic, antiviral, anticancer, antioxidant, and bile acid binding properties (Alashi et al. 2014). Presently, the rapeseed meal is being used solely for livestock feeding. However, it can be served as a potential protein source for poultry and for human nutrition as well. To achieve this, there is need to decrease the content of most limiting anti-nutritional components (GSLs, fiber, sinapates, and phytic acid) responsible for unpleasant bitter taste, dark color, and reduced digestibility of rapeseed meal.

2.2.2 Vitamins and Minerals

Besides proteins, the rapeseed meal is also an abundant source of vitamins (A and C) and minerals. The calcium and phosphorus content of 0.64% and 1.03%, respectively, has been reported. These values are relatively higher than present in soybean meal.

2.2.3 Fiber

Rapeseed meal contains approximately 11–13% dietary fiber. Dietary fibers are of two types, soluble dietary fiber (SDF) and insoluble dietary fiber (IDF). IDF contains large amount of lignin and cellulose whereas SDF is comprised of mainly hemicelluloses. IDF can significantly reduce the digestibility of other essential nutrients, although SDF also have considerable ability to reduce the digestibility. The fiber negatively influences the seed oil and protein content, as the biosynthesis of fiber and oil/protein compete for same photosynthetic assimilates. The yellow-seeded genotypes of rapeseed contain less fiber and phenolics and more oil and protein contents

due to thinner and translucent seed coat resulting in a larger embryo and lower hull fraction. So, seed quality in rapeseed can be improved by breeding for yellow color seed coat.

2.2.4 Glucosinolates (GSLs)

GSLs are diverse glucose and amino acid derived secondary metabolites, specific to order Brassicales only. They are nitrogen and sulfur containing compounds comprising a thioglucose and a sulfonated oxime linked to the chain elongated amino acid. Over 130 diverse GSL types have been discovered from 16 dicot angiosperms, most of which are raised for edible purposes (Blažević et al. 2020). Based upon precursor amino acid, GSLs can be aliphatic (derived mainly from methionine), aromatic (phenylalanine or tyrosine derived), and indolic (tryptophan derived) (Halkier and Gershenzon 2006). However, this classification has little significance. Later, Blažević et al. (2020) gave classification based on three criteria, i.e., amino acid precursor, degradation product, and presence or absence of an aromatic moiety. Using these criteria, 130 GSL types were divided into 9 panels from A to I. Fifteen main GSL types are recorded in rapeseed, achieving content ranging from 60 to 100 μmol/g dry weight in seeds. Aliphatic GSLs are the predominant ones, comprising up to 92% of whole GSL types. The GSL biosynthetic pathway can be divided into three steps: (1) chain elongation, i.e., addition of a methylene group into the chain elongated amino acids, (2) formation of core structure of GSL, i.e., addition of the sulfur group and S-glucosylation, and (3) modification of the core structure (i.e., secondary side chain modifications) – benzoylation, desaturation, hydroxylation, methoxylation, and oxidation depending upon GSL type. Intact GSLs are chemically stable and relatively biologically inactive. However, the enzymatic hydrolysis of GSLs by endogenous myrosinase upon physical injury or tissue damage (cutting, chewing, and mixing) releases biologically active compounds such as isothiocyanates (ITC), oxazolidine-2-thiones, thiocyanates (SCN), nitriles (NI), and epithionitriles (Blažević et al. 2020). Isothiocyanates and its derivatives are known for their therapeutic properties such as biocidal, chemopreventive, antioxidant, and antimutagenic effects (Grundemann and Huber 2018). GSLs and their hydrolysis products also known to protect the plants against bacterial and fungal pathogens and generalist herbivores. Some GSL types such as sulforaphane and indole-3-carbinol possess potential anticarcinogenic properties. However, hydrolysis of other GSLs such as progoitrin and epiprogoitrin gives goitrogenic products. Numerous adverse metabolic effects such as reduced appetite, retarded growth, and liver and kidney damage have been recorded in poultry, fish, and pigs (Kaiser et al. 2021). Studies proving the harmful effects of GSLs in humans are limited. Traditional rapeseed carries high level of GSLs (>50 μmol/g of defatted meal). However, canola forms of rapeseed have a much reduced content of GSLs (<30 micromoles/gm of defatted meal), thus making the meal fit for animal nutrition.

2.2.5 Phenolic Compounds

The main phenolics accumulate in rapeseed are phenolic acids along with soluble and insoluble forms of condensed tannins. The phenolic acids exist in the three forms – free, esterified, and insoluble-bound form. Free phenolic acids comprise about 15% of the total phenolic acid content, whereas nearly 80% of total phenolic acids occur in the

esterified form. Sinapic acid is the prime phenolic acid that constitutes around 90% of the total present free phenolic acids and 70.9–96.7% of esterified phenolic acids (Naczk et al. 1998). Tannins are widely distributed complex polyphenolic compounds showing molecular weights ranging from 500 to 6000 Da. They can be classified into insoluble and soluble forms as per their structure and reactivity response to hydrolytic agents. Majority of the tannins in oilseed rape are proanthocyanidins, also known by the name of condensed tannins. They are formed by polymerization of flavan-3-ols or flavan-3,4-diols. The content of tannins in rapeseed meal varies from 0.2% to 3% of defatted meal (Naczk et al. 1998). Rapeseed meal accumulates more phenolic compounds than any other oilseed (Nowak et al. 1992). Phenolic components contribute to astringency, off-flavor, and dark color of meal. Sinapine, an unpalatable phenolic compound, is reported to impart adverse effects on feed intake and body weight gain with diets containing rapeseed meal (Tangtaweewipat et al. 2004). Egg tainting has been observed when tannin-rich rapeseed meal is fed to laying hen (Shahidi and Naczk 1992). Phenolics are known to affect overall protein digestibility and amino acid availability. However, phenolic compounds are also known to possess antioxidant properties and work as radical scavengers and quenchers. They exhibit protection against heart diseases. Rapeseed extracts with sinapine showed excellent antioxidant activities towards liposomes oxidation and low-density particles. Nowak et al. (1992) have reported phenolic compounds to carry antimicrobial properties, effective against gram-negative bacteria (*E. coli* and *Enterobacter aerogenes*) and gram-positive bacteria (*Bacillus subtilis* and *Streptococcus lactis*).

2.2.6 Phytates

In rapeseed, majority of organic phosphorus (up to 90%) is stored in embryo contained phytic acid. The content of phytic acid ranged from 2% to 4% in seeds, 2–5% in the oil-extracted meal, and 5–7% for the protein concentrates of rapeseed (Lickfett et al. 1999). It is biosynthesized through two distinct metabolic pathways that involve seven enzymes – *MIPS*, *MIK*, *IMP*, *ITPK*, *IPK2*, *2-PGK*, *IPK1*, and *MRP5*, a multidrug resistance-associated protein. Phytic acid is believed to act as an anti-nutritional component but also has anticancer and antioxidant activities. Phytates have a high tendency to bind to metal ions, such as calcium, phosphorus, magnesium, iron, and zinc, owing to their strong chelating properties, which ultimately reduce the bioavailability of essential minerals. Phytates are poorly metabolized by humans and non-ruminant chicken and pigs. Thus, phytases are added externally to rapeseed meal to improve the digestibility of phytates and availability of free phosphorus. Genetically eliminating or reducing the phytic acid content in the rapeseed would be an effective approach to make rapeseed meal nutritious for human consumption.

3 Growing Importance of Rapeseed Nutraceuticals in Face of Chronic Diseases and Malnutrition

Inadequate intake of vitamins and minerals, often known as micronutrient malnutrition or hidden hunger, has a negative impact on human health. Even mild level of micronutrient deficiency may promote disease development and affect cognitive

ability. Hidden hunger affects around two billion people worldwide, with the African continent and South Asia having the highest frequency. Micronutrient malnutrition often leads to stunted growth in children (starting from fetal development to 4 years of age), as already observed in developing countries (Branca and Ferrari 2002). Chronic conditions/diseases arising as a result of malnutrition can be effectively treated with the healthy plant-based diets and via biofortification of the important staple crops. Biofortification is the method of breeding food crops that are naturally enriched with micronutrients/macronutrients in the adequate quantity in their edible parts (in a bioavailable form) to mitigate the hidden hunger. Now, the focus of agricultural sector has been inclined towards developing nutrient supplement food crops rather than just increasing the supply of food crops. Biofortification approach targets on enhancing the nutritional value of the food crops that people already consume rather than attempting to add some health-promoting components to the diet. This approach is certainly the most sustainable and cost-effective approach as it is a one-time investment and gives long-term results to alleviate nutrient deficiency. The rapeseed crop has gained substantial importance over the years owing to the health-promoting effects of various bioactive compounds present in it as already mentioned above. Great efforts have been done in the past to improve the composition of rapeseed oil and meal to meet the dietary requirements of the consumers. Levels of anti-nutritional compounds such as EA and GSLs have been reduced to acceptable amounts. However, further interventions are required to convert this crop species into a high-quality food-grade crop in which desirable components should be high enough to support human and animal health but at the same time should not be too high that they become toxic. The prime objectives to enhance the nutraceutical value of rapeseed are to improve the content and composition of the seed oil and meal for food and feed purposes.

4 Attempts to Enhance Seed Oil Content in Rapeseed

Increase in oil content has been one of the most important goals of rapeseed breeding. Variation for seed oil content has been reported in *B. napus* germplasm. It varies from 26% to 50% (Xiao et al. 2019). The trait is under the control of complex regulatory mechanisms that is still not completely understood. Genetic studies have revealed the polygenic inheritance of the trait, which is frequently influenced by the environment.

4.1 Mapping for Oil Content

Numerous QTLs (quantitative trait loci) ranging from 3 to 27, each with small effect of $<10\%$, have been detected to control the variation for oil content in *B. napus* in many biparental QTL mapping studies (Zhao et al. 2012; Jiang et al. 2014). These have been visualized on all 19 chromosomes of *B. napus*. However, many QTLs,

i.e., 67, including a few QTLs with more than 20% phenotypic variation explained, were identified in a doubled haploid (DH) population generated from the cross between KenC-8 and N53-2 varieties of *B. napus* (Chao et al. 2017). The study also found some common QTLs that affected both oil and protein contents but in an opposite manner. Four QTLs on chromosomes A08, A09, C03, and C06, explaining >10% effects for oil content consistently over more than four trails, were further screened to detect candidate genes controlling oil content by integrating genome resequencing and transcriptomic analysis of parental genotypes (Yan et al. 2022). More recently, genome-wide association studies (GWAS), which exploit historical recombination events and provide high mapping resolution, have been attempted to reveal the genetic basis and identify candidate genes for seed lipid content in *B. napus.* A patatin-like lipase (*PTL*) gene on C07 was found to be associated with oil content using GWAS in a collection of 290 rapeseed germplasm accessions (Wang et al. 2021). Another major QTL (*qA07.SOC*) on A07 has been detected for seed oil content based on the GWAS and RIL population (derived from *B. napus* and *Sinapis alba*) based mapping (Zhao et al. 2022). Tang et al. (2021) also provided a deeper understanding of oil accumulation in rapeseed by associating genome variants and transcriptomes (of developing seeds) of a large natural *B. napus* population (505 inbred lines) with multiyear, multilocation seed oil content data. Previously reported 27 QTLs and some novel QTLs for seed oil content were detected in the study. QTL regions under breeding selection were also underpinned based on selection intensity of identified QTLs. A pair of homologous genes, *BnPMT6s* encoding a S-adenosyl-L-methionine-dependent methyltransferase, was demonstrated to negatively affect the oil content.

4.2 Characterization of Genes Involved in Oil Biosynthesis

A few genes regulating oil content have been cloned in *B. napus* (Liu et al. 2019). *ORF188*, a novel chimeric mitochondrial gene cloned using comparative genomics and transcriptome analysis, increased oil content by 8% in *B. napus* (Liu et al. 2019). Ding et al. (2019) used RNA interference (RNAi) technology to elucidate *BnLACS2* to be the key element for seed oil production. *BnLACS2* is a long-chain acyl-CoA synthetase, whose overexpression increased the oil content, whereas its silencing lowered oil content in rapeseed plants. Transgenic oilseed rape lines showed a near 10% increase in oil content by overexpression of a lysophosphatidate acyltransferase (*LPAT*) gene from yeast (*Saccharomyces cerevisiae*) (Zou et al. 1997; Taylor et al. 2002). About 40% surge in oil content has been recorded by an increase in Gly3P content upon overexpression of a yeast *Gly3P* dehydrogenase gene in rapeseed (Vigeolas et al. 2007). Seed-specific overexpression of the *BnLEC1* and *BnL1L* genes at an appropriate level under the control of the two truncated canola storage protein 2S-1 promoters, also known as the *napA* promoters, significantly promoted the seed oil content in rapeseed with no detectable negative effects on other major agronomic traits, including protein content (Tan et al. 2011). The CRISPR-CAS9 has emerged as a powerful technology to study gene function and produce desired traits

by efficient mutagenesis of targeted genes (Zhang et al. 2021). The technology has been used recently for functional characterization of genes involved in oil biosynthesis. Knocking out many *BnSFAR4* and *BnSFAR5* genes at the same time improved seed oil content of *B. napus* without affecting seed germination or vigor (Karunarathna et al. 2020). Silencing of the *BnLPAT2* and *BnLPAT5* genes caused decreased oil content and enlarged oil bodies (Zhang et al. 2019).

5 Improvement in Fatty Acid Composition of Rapeseed Oil

5.1 Reduction in Erucic Acid (EA)

EA is synthesized from OA by elongation process catalyzed by a rate-limiting enzyme *β*-ketoacyl-CoA synthase (KCS) encoded by the gene *FAE1*. Mutants for FAE1 led to decreased content of longer chain fatty acids (Lassner et al.1996). A mutant identified from German spring rapeseed cultivar 'Liho' is regarded as the only donor source for low EA to all canola quality rapeseed cultivated today. 'Oro' and 'Zephyr' were the first low EA rapeseed cultivars, followed by many, that were developed through conventional breeding efforts and released first in Canada. Content was found to regulate by two loci with additive effect as F_1 seeds from cross between low and high EA genotypes depicted intermediate EA content between corresponding parents and segregation ratios in F_2 and F_3 seeds were in accordance to expected ratios under control of two genes (Fourmann et al. 1998).

5.1.1 Mapping for EA Content

Various molecular markers have been used for mapping the EA content. Two additive loci (E^A and E^C on A and C genomes of *B. napus,* respectively) were identified that on collective basis explained 90% of whole EA variation in *B. napus*, while contributing unequally to the final EA content (Jourdren et al.1996). The two genes *BnFAE1.1* on A08 and *BnFAE1.2* on C03*,* encoding *β*-ketoacyl-CoA synthases, were cloned and revealed to co-segregate with E^A and E^C, respectively (Fourmann et al. 1998). GWAS studies have also been carried out to identify loci for EA content of rapeseed oil. Candidate genes *BnaA08g11130D* and *BnaC03g65980D*, orthologous to Arabidopsis *FAE1*, and *BnaA08g11140D* and B*naC03g66040D*, orthologous to *KCS17*, were identified through GWAS (Zhu et al. 2019).

5.1.2 Characterization of *FAE* Genes

There are six gene copies encoding *FAE1* proteins in *B. napus* (Wu et al. 2008; Cao et al. 2010). Of these, *BnaA8.FAE1* and *BnaC3.FAE1* are the major ones that control erucic acid synthesis in seeds. Comparison of *FAE1* sequences between HEA (high erucic acid) and LEA (low erucic acid) genotypes of *B. napus* indicated nucleotide variations. Functional activity of *FAE1* was lost *via* deletion of two to four bases in C genome homolog (Fourmann et al. 1998; Wu et al. 2008) or substituting C/T in A genome homolog (Han et al. 2001). Single SNP substitution causing amino acid change from serine to phenylalanine led to the

development of LEA *B. napus* cv. Oro (Katavic et al. 2002). Nineteen mutants for the *BnFAE1* fragment were detected through the targeting induced local lesions in genomes (TILLING) approach during the screening of 1344 M_2 plants. Of these, three M_3 plants showed reduced EA (Wang et al. 2010). The low level of EA content in *B. napus* was achieved by inhibiting the gene expression of *FAE1* genes through RNAi (Peng et al. 2010; Shi et al. 2017) and CRISPR/Cas9 technologies (Liu et al. 2022a).

5.2 Progress to Increase Oleic acid (OA) or/and Reduce Linolenic Acid (LiA) in Rapeseed: HOLL (High Oleic and Low Linolenic) Varieties

Increased levels of MUFA (OA) and reduced content of PUFA (LiA) in rapeseed oil are vital to impart thermal stability to the oil so that it can be utilized for food applications that need high cooking and frying temperatures and for salad dressings. Presently, canola quality rapeseed cultivars contain approximately 55–65% OA. There is a necessity to further increase the OA content in rapeseed oil. Thus, breeding rapeseed cultivars with high oleic acid (HOA) and low linolenic acid (LLiA) content is an important objective. The objective can be achieved by developing high OA germplasm combined with improved understanding of genetic control of trait. In the fatty acid biosynthesis pathway, activity of fatty acid desaturases (*FADs*) regulates the amount of OA, LA, and LiA. The OA is desaturated to LA by inserting double bond at delta 12 position mediated by an enzyme oleoyl-PC D12-desaturase encoded by gene *FAD2* (Hu et al. 2006; Peng et al. 2010; Yang et al. 2012), whereas LA is further desaturated to LiA by inserting double bond at the delta 15 position mediated by another enzyme, delta-15 desaturase encoded by gene *FAD3* (Yang et al. 2012; Lee et al. 2016). HOA and LLiA rapeseed mutants were identified in the physically and chemically mutagenized populations of rapeseed (Bai et al. 2019; Fu et al. 2021). Mutants harboring >80% OA were demonstrated to display reduced agronomic potential (lower seedling vigor, delayed flowering, and reduced plant height) and oil content (Bai et al. 2019). Depending on mutants, one or two major loci for *FAD2* controlling OA (Falentin et al. 2007), while two loci for *FAD3* genes for LiA were revealed (Barret et al. 1999). The first LLiA *B. napus* cultivar, Stellar, was identified in 1987 (Scarth et al. 1988) followed by Scarth with very LLiA (Scarth et al. 1995).

5.2.1 Mapping for OA and LiA Content

Both linkage and association studies have been used to map QTLs for OA in *B. napus* (Hu et al. 2006; Yang et al. 2012; Wen et al. 2015; Qu et al. 2017; Bao et al. 2018; Fu et al. 2021). The QTLs detected through linkage analysis were mapped on chromosomes A01, A05, C01, and C05 of *B. napus*. GWAS for fatty acid composition in a set of 520 genetically diverse rapeseed accessions identified a total of 62 MTAs distributed over 18 chromosomes which explained 2.31–14.48% of the phenotypic variance for seven fatty acids: PA, SA, OA, LA, LiA, EiA, and EA (Qu et al. 2017). However, only five genomic regions present on chromosomes, A02, A08, A09, C01, and C03,

were common in the two years. Six haplotype regions on chromosomes A02, A07, A08, C01, C02, and C03 were identified as significantly associated with OA through GWAS of haplotype blocks (Yao et al. 2019). Further, whole-genome sequencing of 50 rapeseed accessions revealed three genes: *BnmtACP2, BnABCI13*, and *BnECI1* in the haplotype region of A02 and two genes, *BnFAD8* and *BnSDP1* in the C02 responsible for variation in OA content. Association studies in a diversity set comprising 435 rapeseed accessions identified 149 quantitative trait nucleotides (QTNs) for fatty acid content and composition, of which 34 QTNs were overlapped with previously reported loci (Guan et al. 2019). Candidate genes *BnaA08g08280D* and *BnaC03g60080D* for OA indicated genome sequences differences between HOA and LOA genotypes. A novel QTL for *FAD2* explaining 6.2–11.7% of phenotypic variation over three crop seasons was revealed on A09 through GWAS analysis using 375 low EA *B. napus* genotypes. Transfer of this region together with previously mapped QTLs could facilitate increase in OA to 80%. Additionally, fine mapping of identified QTL unraveled three promising candidate genes that could also be validated via gene expression studies. Closely linked marker, *BnA129,* was also designed to enable marker-aided selection for OA (Zhao et al. 2019). Candidate gene-based association study in 324 rapeseed accessions identified three candidate genes present on chromosomes A07, A08, and C03 to be associated with oleic acid (Zafar et al. 2020). In the abovementioned studies, two major QTLs located on chromosomes A05 (Hu et al. 2006; Yang et al. 2012) and C01 (Hu et al. 2006) were found to harbor homologs to *AtFAD2* gene of Arabidopsis, that catalyze OA into LA acid and hence plays crucial role in controlling OA content in seed oil.

5.2.2 Characterization of *FAD* Genes

Four *AtFAD2* orthologs (the major effect *BnFAD2.C01* and *BnFAD2.A05,* plus their homeologous copies *BnFAD2.A01* and *BnFAD2.C05*) have been identified in allotetraploid *B. napus* (Yang et al. 2012; Gacek et al. 2017). Three copies of *BnFAD2* (*BnFAD2.C01*, *BnFAD2.A05*, and *BnFAD2.C05*) are functional while one copy *BnFAD2.A01* seems to be a pseudogene. Mutations in the *BnFAD2* copies have been revealed to influence OA quantity. A single nucleotide polymorphism (SNP) in the coding region of copy *BnFAD2.C01* caused surge in OA content up to 77%, whereas for *BnFAD2.A05* copy, a single-nucleotide substitution or a 4-bp insertion (Hu et al. 2006; Yang et al. 2012) in the coding region caused increase in OA quantity up to 75%. Fu et al. 2021 also confirmed the role of *BnFAD2* copies to control OA by revealing SNPs in *BnFAD2.A05* and *BnFAD2.C05* copies. Loss of function of *BnFAD2* copies in *B. napus* via RNAi (Peng et al. 2010), gene knockouts (Wells et al. 2014), and CRISPR/Cas9-mediated genome editing (Okuzaki et al. 2018; Huang et al. 2020; Liu et al. 2022b) again confirmed the significance of *FAD2* genes in increasing OA amount up to 84–85%. CAPS markers (Falentin et al. 2007), Illumina qPCR-based assay (Hu et al. 2006), SNP markers (Yang et al. 2012), and KASP (Fu et al. 2021) markers have been developed for marker-assisted selection of desirable *FAD2* alleles for high OA. Gene sequence comparison of *FAD3*copies also indicated single nucleotide mutations. For direct selection of *FAD3* allele, restriction site generating–polymerase chain reaction (RG-PCR) (Barret et al. 1999) and SNaPshot (Spasibionek et al. 2020)

have been developed. These markers have been successfully employed for selection of high OA and LLiA content in winter rapeseed.

5.3 Increase in Eicosapentaenoic Acid (EPA) and Docosahexaenoic Fatty Acid Levels

Long-chain unsaturated fatty acids (i.e., EPA and DHA) rich oil is in high demand because of its numerous health benefits. These fatty acids were once acquired exclusively from fish oil. Attempts were made to obtain EPA and DHA from rapeseed oil through transgenic approach. Using six different gene constructs from *Thraustochytrium* sp., *Pythium irregular*, and *Calendula officinalis*, levels of arachidonic acid (AA) and EPA were increased up to 25% and 15% of total seed fatty acids in rapeseed, respectively (Wu et al. 2005). Similarly, transgenic *B. napus* expressing microalgal PUFA synthases (*OrfA*, *OrfB*, and hybrid *OrfC*) from *Schizochytrium* sp. ATCC 20888 showed 2.87–3.43% average DHA content in T2 seeds of inbred lines of selected transformation events (Walsh et al. 2016). The total DHA and EPA content was around 4.4% in the field-produced canola oil (Walsh et al. 2016). In the latest transgenic DHA canola variety, seven fatty acid biosynthesis genes from yeast and microalgae were used as single fragment of around 19 Kb to transform *B. napus*, which increased the DHA levels from 9% to 11% like those obtained from fish (Petrie et al. 2020).

6 Minor Oil Components

In addition to total oil content and fatty acids composition, some minor oil components, such as phytosterols (Amar et al. 2008) and tocopherols (Fritsche et al. 2012; Gugliandolo et al. 2017), have also recently drawn the interest among rapeseed researchers to study and improve their content and composition owing to numerous health-benefitting potentials associated with them. The studies executed on phytosterols and tocopherols are discussed below.

6.1 Phytosterols

Abidi et al. (1999) recorded phytosterol content varying from 7659 to 14,023 mg/kg oil in 12 different spring canola varieties. In another study, a twofold variation was observed in the phytosterol content, varying from 4475 to 9380 mg/kg oil among the lines of three winter-type rapeseed DH populations (Amar 2007). Canola cultivars have been observed to contain higher (3565–4800 mg/kg) phytosterol content than traditional non-canola (2079–4329 mg/kg) cultivars (Amar et al. 2009). In a winter rapeseed DH population segregating for EA, QTL mapping results depicted colocalization of two of the three QTL identified for total phytosterol content and two of the four QTL detected for sinapate ester content with two EA genes on A08

and C03 (Amar et al. 2008). A close negative association of phytosterol and sinapate ester content with EA content was found. The study suggested the pleiotropic effect of the two EA genes on phytosterol and total sinapate ester content in seeds of rapeseed. This was supported by the fact that common cytoplasmic acetyl-CoA precursor is required for the synthesis of both EA and phytosterols. To further understand the genetics of phytosterols and their association with other important seed quality traits (oil content, fatty acids content, protein content of the defatted meal, and seed weight), a DH population constructed from the two-canola quality winter rapeseed cultivars, Sansibar and Oase, was used for QTL mapping (Teh and Mollers 2016). The parental genotypes were differed with respect to both oil and phytosterol content. Variable number of QTLs ranging from one to six were identified for the evaluated traits. QTLs governing large effects ($R^2 \geq 25\%$) were all identified on the A genome. QTLs identified for one trait often found to colocalize with QTLs for other traits.

6.2 Tocopherols

Rapeseed germplasm has been investigated for variation in tocopherol content and composition in some instances. Significant variation in alpha, gamma, and total tocopherol content that varied from 63 to 157 ppm, 114–211 ppm, and 182–367 ppm, respectively, has been revealed in a diverse panel comprising 87 winter rapeseed genotypes in a study by Goffman and Becker (2002). Alpha−/gamma tocopherol content ratio found to be varied from 0.36 to 1.23. However, groups with different seed quality types (double zero, single zero, and conventional) showed no significant differences for tocopherol content and composition. The study showed lack of correlation between alpha and gamma tocopherol contents. However, a positive correlation of 0.34 was observed between gamma-tocopherol content and oil content. In another study, a broader range of variation (197.5–460.1 ppm) was reported for total tocopherol in a worldwide germplasm collection of 299 rapeseed accessions (Fritsche et al. 2012). A candidate gene-based association mapping study performed on 96 accessions of this wide set identified between 26 and 12 SNPs within tocopherol biosynthesis genes (*BnaX.VTE3.a* and *BnaA.PDS1.c*) that explained 16.93% of total genetic variance for tocopherol composition and up to 10.48% for total tocopherol content. Gene-based markers were also designed and validated on the remaining accessions of the set to facilitate the selection of rapeseed genotypes with enhanced tocopherol qualities. Endrigkeit et al. (2009) cloned the first gene *BnaA.VTE4,* involved in tocopherol biosynthesis pathway in rapeseed using sequence information of Arabidopsis *VTE4* orthologs. The function of gene was also verified by an *A. thaliana* transgenic approach leading to 50-fold increase of α-tocopherol in seeds of *BnaA.VTE4.a1* overexpressing plants. A marker assay was developed for the gene and mapped to the position of two QTLs on chromosome A02 of Tapidor X Ningyou7 genetic map to facilitate marker-assisted selection for increased tocopherol content. Wang et al. in 2012 used Tapidor X Ningyou7 derived DHs and its reconstructed F_2 population and a panel of 142 rapeseed accessions to

decipher the genetic control of seed tocopherol amount and composition. They found 33 unique QTLs contributing towards phenotypic variation in tocopherol content and composition in biparental populations. Furthermore, they shortlisted 7 QTLs that colocalized with potential candidate sequences in association with tocopherol biosynthesis based on in silico and linkage mapping studies. GWAS performed on diverse panel of 142 rapeseed accessions revealed 61 loci linked with tocopherol content and composition out of which 11 were located within the regions of QTLs detected in biparental populations. The relative abundance of individual tocochromanol species in the seed oil has been modified by overexpression of chimeric TC gene constructs in developing seeds of transgenic rapeseed plants (Kumar et al. 2005). Co-overexpression of *PDS1*and *VTE2* genes have also been attempted in *B. napus* to augment total tocopherol content (Raclaru et al. 2006).

7 Progress to Improve Rapeseed Meal Value

At present, the use of rapeseed meal is restricted to feed of ruminants only, but it can be used as a high-quality protein source for poultry and hog nutrition and even for human consumption by reducing the content of most limiting anti-nutritional factors such as GSLs, fiber, phytic acid, sinapates, and non-starch polysaccharides (NSP) (Wanasundara et al. 2016). Therefore, the present objective in rapeseed meal improvement is to decrease these undesirable components in the seed. Concurrently, there is a great interest to elevate the total seed protein content and manipulating the protein composition by altering napins and cruciferins ratio.

7.1 Reduction of GSLs

Rapeseed meals with high GSLs are not considered good for animal health. So, rapeseed breeding programs are always aimed at reducing GSL content in the seeds. Breeding for low GSL content was initiated after the discovery of Bronowski, a Polish cultivar of *B. napus* (Finlayson et al. 1973). The low GSL content of Bronowski was successfully incorporated into elite zero EA germplasm of summer and winter rapeseed through conventional cross-breeding and pedigree selection (Kondra and Stefansson 1970; Wittkop et al. 2009). The reduction in GSL content occurred due to decrease in aliphatic type of GSLs (Kondra and Stefansson 1970). Today all cultivated rapeseed depicts low EA (<2%) and GSL (<15 μmoles/g of defatted meal) levels in the oil and meal, respectively. The lower level of GSLs in seeds has also raised the concern regarding increased susceptibility of cultivars to pests, pathogens, and birds owing to simultaneous decrease of GSL content in leaves. Thus, it would be desirable to improve the protective effects of *B. napus* by genetic manipulation of the leaf GSL profile without affecting seed quality. A few investigations have suggested the lack of correlation between GSLs of seeds and leaves and proposed the possibility of tissue specific manipulation of GSL profiles (Fieldsen and Milford 1994). Although GSLs can be traced in all parts of the plant,

their concentration varies throughout the plant body, i.e., unequal amount is present in different parts of plant that may also differ with respect to plant development stages and in response to various biotic and abiotic factors (Wittstock and Burow 2010). For instance, the GSL content was found higher in seeds as compared to leaves in *B. napus*. GSL content of leaves varied between 0.6 and 6.9, whereas in seed, it varied from 10.8 to 57.9 μmol/g of dry material (Baaij et al. 2018). Moreover, profile of GSL also varies in different plant tissues. Aliphatic GSLs predominates both in leaves as well as in seeds, whereas indole GSLs are found more abundantly in leaves than in seeds (Velasco et al. 2008). The differences of GSL content and profile may be attributed to diverse functions of different parts of plants. Total GSL content show quantitative inheritance. Profiling of GSLs in *B. napus* indicated three main types: gluconapin, glucobrassicanapin, and progoitrin. The content of these GSLs was found to be determined by the genotype of the mother parent. Three, four, and five loci were reported to control gluconapin, glucobrassicanapin, and progoitrin, respectively. Higher content of gluconapin and glucobrassicanapin showed partial and overdominance over low content of GSL, whereas partial dominance was indicated for high content of progoitrin over absence of progoitrin (Kondra and Stefansson 1970).

7.1.1 Mapping for Glucosinolate Content and Composition

To reveal the genetic control of GSL synthesis and accumulation in vegetative tissues or/and seeds of rapeseed, several studies have been carried out using biparental linkage mapping and GWAS approaches. Available reports indicate that four major genomic regions present on chromosomes A02, C02, A09, and C09 to be the most likely regions to control seed GSL content variation in *B. napus*. Two loci have been revealed to control side chain elongation and hydroxylation in leaves or seeds of populations derived from natural and resynthesized *B. napus* genotypes (Magrath et al. 1994; Parkin et al. 1994). Uzunova et al. (1995) identified four QTLs on rapeseed linkage groups 2, 9, 16, and 18, which together accounted for 61.7% of observed phenotypic variation for seed GSLs in a DH population. Similarly, four QTLs were revealed by Howell et al. (2003) in linkage groups 2, 7, 9, and 19 that together explained 76% variation for seed GSL content in two backcross populations. A total of 39 QTLs governing variation for seed GSL content were detected, 28 of those were mainly found on 4 chromosomes (A02, A09, C02, and C09), that explained 10.2–35.3% of phenotypic variation on individual basis in the TNDH population (Feng et al. 2012). A major effect QTL, *qGSL-C2*, explaining 30.88–72.87% of the phenotypic variation was identified on chromosome C02 in a DH mapping population developed from cross of two *B. napus* accessions that differed in their seed GSL contents (Liu et al. 2020a). The gene, *BnaC2.MYB28/HAG1*, orthologous to the *AtMYB28,* responsible for aliphatic GSL synthesis, was also suggested as promising candidate gene for the detected QTL.

Three loci on chromosomes A09, C02, and C09 were found to be in association with seed GSL content by Harper et al. (2012) in a small set of 53 *B. napus* lines using associative transcriptomics. They also revealed that deletions of orthologs of the gene *AtMYB28* on chromosomes A09 and C02 led to low seed GSL content

(Harper et al. 2012). Two *MYB28* copies on chromosomes A09 and C02 were identified as key regulators for aliphatic GSL variation in rapeseed leaves though associative transcriptomic analysis on 288 accessions of *B. napus* (Kittipol et al. 2019). Additionally, *Bna.HAG3/MYB29.A3*, was revealed to control root aromatic GSL variation. A candidate gene *Bna.MAM3.A3* was also proposed to have a role in phenylalanine chain elongation for aromatic GSL biosynthesis based on root expression data. Lu et al. (2014) inferred 26 candidate genes including orthologs of gene *AtMYB28*, i.e., *BnaA.GTR2a* and *BnaC.HAG3b*, for low seed GSL content in a larger set of 101 *B. napus* lines. Further, Li et al. (2014) also suggested that different copies of *AtMYB28* on chromosomes A09, C02, C07, and C09 responsible for the seed GSL content using a Brassica 60 K SNP array on a set of 472 rapeseed genotypes. Wang et al. (2018) identified 49 loci and 27 candidate genes for association with seed GSL content using GWAS in a diversity set. Further, 5 common and 11 tissue-specific loci were associated to total leaf and seed GSL content using GWAS analysis in 366 accessions of *B. napus* (Liu et al. 2020b). A candidate gene *BnaA03g40190D (BnaA3.MYB28)* responsible for high leaf and low seed GSL content was also validated by gene sequence polymorphism and expression assays.

7.1.2 Characterization of GSL genes

Specific gene regulatory mechanisms combined with environmental influences control GSL biosynthesis, transport, and accumulation, resulting in varying content and distinct GSL profile in different tissues of Brassica species (Mitreiter and Gigolashvili 2021). The glucosinolate biosynthetic pathway has been well characterized in the model species *A. thaliana.* Almost all genes involved in three biosynthesis stages, i.e., side chain elongation of amino acids, core structure formation, and modification of secondary side chain, have been revealed (Sonderby et al. 2010). An intricate network of TFs together with biotic and abiotic stimuli and hormonal and epigenetic factors governs the spatiotemporal synthesis of GSLs (Hirai et al. 2007). The three types of GSLs (aliphatic, indolic, and aromatic) are independently regulated by different group of genes (Sonderby et al. 2010). Three TFs, *MYB28, MYB76*, and *MYB29*, belonging to the *R2R3-MYB* family, regulate biosynthesis of aliphatic GSLs (Gigolashvili et al. 2007, 2008a, b). They are also denoted as *HIGH ALIPHATIC GLUCOSINOLATE (HAG) 1, 2,* and *3,* respectively. Of these, TF*MYB28 (HAG1)* is the prime transcriptional regulator that upregulate most of the genes involved in aliphatic GSLs biosynthesis, including side chain elongation (*MAM1* and *MAM3*) and core structure development (*CYP79F1*, *CYP79F2*, *CYP83A1, ST5b*, and *ST5c*) (Gigolashvili et al. 2008a, b; Baskar and Park 2015). Owing to closest evolutionary relationship of *A. thaliana* with Brassica crops, many *MYB28*orthologues have been identified in Brassica species, including four in *B. rapa* (Kim et al. 2013), three in *B. oleracea* (Augustine et al. 2013), five in *B. juncea* (Yin et al. 2017), and six in *B. napus* (Long et al. 2016). The DNA sequences of *MYB28* members in Brassica crops are found to be highly conserved. The coding region varies from 1350 to 1630 bp in length. The inferred *BnMYB28* protein sequences of *B. napus* share percent identity ranging from 59% to 78% with *AtMYB28* and from 59% to 98% with each other (Long et al. 2016). Many genes explaining variation for GSL content in *B. napus* have been identified via

QTL mapping, genome-wide association, and associative transcriptomic studies as already described above. These studies indicated the significant role of *MYB28* homologs in aliphatic glycoside biosynthesis. Aliphatic as well as total GSL content were also reduced by silencing of *MAM* gene family through RNAi (Liu et al. 2011). Independent knockout mutants of genes *MYB28* and *CYP79F1* have been verified to carry decreased aliphatic GSL content by 55.3% and 32.4%, respectively, compared to control plants in *B. napus* (Jhingan et al. 2023). Genes encoding TFs *MYB34/ATR1 (altered tryptophan regulation 1)*, *MYB51 (HIG1)*, *MYB122 (HIG2)*, and cytochrome P450 enzymes, *CYP79B2, CYP79B3*, and *CYP83B1*, are associated with biosynthesis of indolic GSLs (Celenza et al. 2005; Gigolashvili et al. 2007; Frerigmann and Gigolashvili 2014). Overexpression of allele, *atr1D,* altered the expression of *CYP79B2, CYP79B3*, and *CYP83B1*, which led to increased levels of indolic GSLs, whereas, loss of function mutants resulted in decreased levels of indolic GSLs (Celenza et al. 2005). Redirection of tryptophan into tyramine transformed rapeseed with *TDC* (*tryptophan decarboxylase*) gene resulted in lower levels of indolic GSLs (Chavadej et al. 1994). Limited information is available for synthesis of aromatic type of GSLs. It is obvious that GSLs biosynthesis pathway in Brassica crops is extremely complicated than *A. thaliana* due to presence of polyploidization and multiple genomic rearrangements events. Spatial-temporal transcriptional regulation of different *MYB28* homologues has been commonly observed in Brassica, suggesting the occurrence of functional divergence of *MYB28* copies after genome polyploidization (Kim et al. 2013). Genetically, removal of GSLs in seeds while maintaining them in leaves could be achieved in *A. thaliana* through manipulation of two transporters, *GTR1* and *GTR2*, that control GSL accumulation in seeds (Nour-Eldin et al. 2012). GSLs are primarily synthesized in leaves and silique walls (source tissues) and then translocated to embryos via phloem by two transporters, *GTR1* and *GTR2* (Nour-Eldin and Halkier 2009). The *gtr1 gtr2* double mutant of *A. thaliana* failed to accrue GSLs in its seeds; however, it over-accumulated GSLs higher than tenfold in the source tissues, leaves, and silique walls (Nour-Eldin et al. 2012). In the similar way, low level of GSLs has been achieved through gene editing of transporter genes, *GTR 2* gene in *B. napus* (He et al. 2022). Overexpression of STM gene caused reduction in seed GSL levels (Elhiti et al. 2012). Modified GSL content was also achieved by engineering leaf cotyledon 1 gene (Elahi et al. 2016).

7.2 Reduction of Other Antinutritive Compounds

Breeding of rapeseed cultivars with reduced phenolic compounds (sinapates, tannins, and proanthocyanidins), fiber, and phytates in seeds is important as these components limit the usage of rapeseed meal as a quality grade protein source for human and animal consumption. Interestingly, seed coat color is found to be associated with some of these antinutritive seed constituents in Brassica species. Proanthocyanidins and tannins are the prime components responsible for seed coat pigmentation. Black/brown-seeded genotypes have more proanthocyanidins, tannins, and fiber deposited in their seed coats. In contrast,

yellow-seeded lines with thinner seed coats display improved quality of the seed (higher proportions of oil (also more transparent) and protein) and derived meal (lower quantities of tannins, proanthocyanidins, and fiber). Much attempts have been carried out for the development of yellow-seeded genotypes in oilseed rape. There is no naturally occurring germplasm with yellow seed color in *B. napus.* Most of the yellow-seeded *B. napus* has been derived from interspecific/intergeneric crosses of *B. napus* with related species *B. rapa, B. juncea, B. carinata, B. oleracea* spp. *Alboglabra*, and wild crucifers (*Sinapis alba* and *Descurainia sophia*) (Wen et al. 2012). Although seed coat color/seed pigmentation is a morphological marker, it is difficult to select owing to its low heritability, polygenic inheritance, and maternal and environmental effects (temperature and light) controlling the trait (Stein et al. 2013). So, molecular markers linked to seed coat color were also established in rapeseed (Rahman et al. 2010). Transparent testa mutants (TT), including early (EBGs) and late (LBGs) biosynthesis genes, are known to mainly regulate variation in seed coat color (Appelhagen et al. 2014). The EBGs include *TT4, 5, 6*, and *7,* and LBGs include *TT3, 12, 18, 19, BAN*, and *AHA10.* A few seed coat color genes *TT1TT2, TT10, F3'H, PAL, BAN*, and *TTG1* have also been cloned and revealed to be involved in flavonoid biosynthesis pathways in *B. napus* (Zhang et al. 2013; Lian et al. 2016). Concurrently, genetic mechanisms of seed fiber (Behnke et al. 2018; Miao et al. 2019; Gacek et al. 2021) and condensed tannins (Lipsa et al. 2012) have also been studied together with seed color, oil, protein, and glucosinolate content in order to improve oil content and meal value of rapeseed. QTLs of one trait were repeatedly found to be colocalized with other traits because of interlinked biochemical pathways involved in the synthesis of these components from common substrates/precursors. A lignin biosynthesis gene, *BnCCR1*, on A09 was reported to affect both seed color and lignin content (Liu et al. 2012). More recently, a QTL cqSC-A09 has been detected which simultaneously explained variation for seed color, fiber, and oil content with a large effect (Chao et al. 2022). Most of the studies revealed one major locus on chromosome A09 that explained most of the variation for seed color and meal quality traits (Rahman and McVetty 2011; Liu et al. 2012; Kebede et al. 2012).

Significant genetic variation for phytate content has been reported across 505 genotypes of rapeseed. It ranged from 0.41 to 0.97 mg/5 seeds (Liu et al. 2021). A multidrug resistance-associated protein 5 (*BnaA9.MRP5*) gene was also identified as a candidate gene, with eight distinct haplotypes in association with both seed phytate content and concentration using GWAS in 505 accessions. RNAi-mediated downregulation of *MIPS* genes has been known to decrease phytate amount in a patent (Georges et al. 2006). Phytic acid mutants with knockout mutations in the six genes *BnMIPS, BnMIK, Bn2-PGK*, *BnIPK1, BnIPK2*, and *BnMRP5* were identified in an EMS (ethyl methane sulfonate) mutagenized population of rapeseed after implementation of high-throughput NGS screening protocol (Sashidhar et al. 2020). The study also identified double mutants of *Bn.2-PGK2*, which revealed substantial decrease in phytic acid content. In order to decrease the phytic acid content in rapeseed, three functional paralogs of an important enzyme

BnITPK (inositol tetrakisphosphate kinase) were knocked out using CRISPR-Cas9 mutagenesis in spring rapeseed cultivar Haydn. The mutants with low phytic acid and presence of free phosphorous were obtained.

7.3 Seed Storage Protein (SSP) Content and Composition

Substantial genetic variation for protein content and quality has been observed in rapeseed germplasm (Schatzki et al. 2014; Stolte et al. 2022). The content of cruciferin and napin ranged from 25.5 to 34.7 μg/sample and from 20.0 to 26.4 μg/sample, respectively, across 30 winter rapeseed hybrids of canola quality (Stolte et al. 2022). However, a few genomic studies have been carried out to reveal the molecular regulation of seed storage protein content and its composition. The outcomes of those studies revealed common or closely linked QTLs for seed oil and protein content but controlling the traits in an opposite manner. This is usually expected as both oil and protein compete for common substrates in the metabolic pathway, thus must be partly regulated by the same genes. Also, seed oil is significantly negatively correlated to protein quantity (Gül et al. 2003). *BnFus3* mutant seeds of *B. napus* depicted declined level of oil proportion with increased protein content. Decreased expression of sucrose photo-assimilation and glycolysis pathway related genes was observed in the seeds. This might have affected the synthesis of both oil and protein to alter their content in the seeds (Elahi et al. 2015). To identify the unique/separate QTLs for oil and protein content, Zhao et al. (2006) used a conditional mapping approach. The study detected five QTLs localized on chromosome A07, A09, C01, C08, and C09 for protein synthesis uncommon to QTLs for oil content (Zhao et al. 2006). Three QTLs for napin were envisioned on chromosomes A02, C06, and C09, and two QTLs for cruciferin content were observed on A02 and C19 (Schatzki et al. 2014). Four QTLs for protein content of defatted meal were found on chromosomes A01, A07, and C03. Of these, QTL located on chromosome A01 was associated to five traits, i.e., PA, OA, LA, oil content, and protein content. The variation in the region resulted in decreased PA, LA, and protein content in the defatted meal and increased oil content in the seed (Teh and Mollers 2016). Potential candidate genes encoding seed storage 2S, caleosin, oleosin, and cruciferin were identified for 38 QTLs regulating seed protein content in *B. napus* by Chao et al. (2017). Some promising attempts have also been undertaken to engineer rapeseed with more desirable levels of napin through introducing a Brazil nut (*Bertholletia excelsa*) 2S gene (Guerche et al. 1990, Altenbach et al. 1992) or expression of an antisense gene for cruciferin (Kohno-Murase et al. 1994; Hannoufa et al. 2014). In these cases, transgenic plants showed increased levels of lysine, cysteine, and methionine essential amino acids which led to more napins in their seeds. Also, the increase in napin content was counterbalanced by a decrease in cruciferins, thus suggesting that the 12S/2S ratio is tightly regulated. The lysine content of canola meal protein has also been improved by disrupting the feedback regulation of lysine during biosynthesis (Falco et al. 1995).

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