

Present status of Rapeseed Mustard oil Quality Improvement

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ABSTRACT: In India, traditional indigenous species such as brown sarson, black mustard, yellow sarson, toria, Indian mustard, and taramira are grown, as well as non-traditional species such as white mustard, gobhi sarson, and karan rai, or Ethiopian mustard. Since around 3500 BC, indigenous species have been cultivated. Oilseed crops, behind cereals, are the second most influential determinant of the agricultural economy in the field crop sector. India also imports a lot of vegetable oils. Vegetable oil use has increased in recent years, both for culinary and industrial purposes. The demand-supply mismatch in edible oils has required massive imports, which now account for 60% of the country's needs. Major achievements in genetic resource management, quality improvement, varietal development, hybrid development, improving tolerance to abiotic and biotic stresses, seed production, and the convergence of conventional and biotechnological approaches. In oilseeds brassica improvement, the production of 203 agro climatic specific varieties, including four hybrids, five low erucic, five low erucic, and five low glucosinolate varieties, as well as the registration of 42 genetic stocks, has been outstanding. In addition, the report tries to give a future vision and plan for rapeseed-mustard research in order to improve productivity and quality while maintaining yield improvements. Major challenge in our study is to find the correct data related to the study but it become possible through some international author's publications.

Keywords: Oilseeds, quality control, fatty acid, erucic acid, glucosinolate, PUFAs, MUFA.

INTRODUCTION

India is one of the world's greatest producers of oilseeds, contributing significantly to Indian agriculture and providing a source of income for rural communities (Rai *et al.*, 2016). Brassica oil is a valuable source of energy and nourishment for humans, while degreased cakes are used as feed in animal nutrition (Baltrukoniene *et al.*, 2015). Brassica produces roughly a third of India's total oil, making it one of the country's most important edible oilseed crops, ahead of peanuts. The fat composition of oils derived from Brassica seeds for culinary and industrial purposes determines their worth. out of the major oilseed crops extensively grown in India, rapeseed and mustard inhabit the second most important place after soybean because of its greater sustainability and adaptability to diverse agro-ecological situations (Choudhary *et al.*, 2022). Indian mustard (*Brassica juncea* (L.); *B. campestris* L. ecotypes toria, brown sarson, and yellow sarson; gobhi sarson (*B. napus* L.), Ethiopian mustard (*B. carinata*), and taramira (*Brassica juncea* L.) their Rapeseed

mustard is used to make animal feed, vegetable oil, and biodiesel (Rai *et al.*, 2016). From the above mentioned Brassica species, in the Indian subcontinent, *B. juncea* is one of the dominant species expanding along with *B. rapa* (syn. *B. campestris* L.) and *B. napus* L., and these are the important sources of edible oil in India. *Indian mustard* is the scientific name for *Brassica juncea*. It has 36 chromosomes and derived as an amphidiploid of *B. rapa* ($2n = 20$; genome AA) and *B. nigra* ($2n = 16$; genome BB) (genome AABB) (Rai *et al.* 2017). Indian mustard (*Brassica juncea* L.) is largely self-pollinated with limited outcrossing ranging from 10–18% of the total seed set (Rai *et al.*, 2012).

Organic acids produced from triglycerides and phospholipids are known as fatty acids. Unsaturated fatty acids have one carboxyl (-COOH) group and a lengthy carbon chain connected by double bonds, while saturated fatty acids have single bonds. Fatty acids have been the most common kind of dietary fat. Mustard seeds typically contain 37 to 42 percent oil, as well as the principal kinds of fatty acids derived from Brassica species, including palmitic acid (16:0), linoleic acid

(18:2), stearic acid (18:0), oleic acid (18:1), eicosanoic acid (22:0), linolenic acid (18:3), and erucic (22:1) acid. Brassica species are widely farmed for edible and industrial oils produced from the seeds all over the world, however also contain considerable levels of unpalatable fatty acids, such as erucic acid, which are toxic to humans (Snowdon *et al.*, 2007). Brassica oil has higher genetic variation in its fatty acid content than other vegetable oils. Brassica oil has more long-chain monounsaturated fatty acids than any other commercially produced plant oil, such as erucic acid (22:1). The oil is good for industrial use but not for human consumption due to the high quantity of erucic acid in it.

As a result, producing variants free of erucic acid is required, and lowering high erucic acid levels is a desirable breeding goal for Brassica oilseed crops. For human nutrition, crop species with greater amounts of C18:1 and C18:2 fatty acids have recently attracted more attention. Oils with a high oleic and linoleic acid content are more resistant to oxidation and produce fewer unwanted byproducts when deep fried. As a result, significant breeding objectives in *Brassica juncea* entail increasing oil content and enhancing the fatty acid composition of the seed oil.

The following are the goals of the rapeseed oil and seed meal quality enhancement programme:

— The yield and quality characteristics of *B. juncea* and *B. napus* lines with low erucic acid and low glucosinolate were evaluated.

— Rapeseed and mustard cultivars with decreased erucic and/or glucosinolate contents are being developed.

— Basic research to better understand the genetics and breeding behaviour of erucic acid and glucosinolate content, as well as information on the reaction types "0" and "00" to endemic pests and illnesses.

The study of indigenous and foreign germplasm indicated a wide range of fatty acid variance in *Brassica juncea*.

The FAO/WHO recommends a greater ratio of MUFA/SUFA in human nutrition, as well as a large proportion of PUFAs, such as C18:2 and C18:3 with a needed ratio between 5:1 (x-6) and 10:1 (x-3) in Table 1. The oil content of Indian mustard lacks the optimal composition of fatty acids which is essential for the human energy and nourishment, as can be seen from the preceding guidelines. High erucic acid, in particular, is nutritionally undesirable, whereas high erucate levels in mustard oil are hazardous to various mammalian health (Somerville *et al.*, 2000).

Table 1: The fatty acid profile is tabulated FAO/WHO also recommends Indian mustard oil (*B. juncea*) for human nutrition.

	Indian mustard seed oil has an average amount of	FAO/WHO suggestions
Saturated fatty acids (SFA, C16:0 + C18:0)	Very low level (\10%)	Low level
Mono-unsaturated fatty acid (MUFA, C18:1)	Low level (*15%)	High amount
Polyunsaturated fatty acids (PUFA, C18:2 + C18:3)	Low level (*15–20%) with almost equal proportion	Moderate level with a desirable ratio (5:1 to 10:1)
Very-long-chain-unsaturated fatty acids (VLCUFA, C22:1)	Very high content (*40–50%)	Absence of VLCUFA

a) According to Sinha *et al.*, (2007), fatty acids are present in mol% of cultivated varieties.

b) The UN Food and Agriculture Organization and the World Health Organization collaborated on a report on "Fats and Oils in Human Nutrition," which was presented in Rome on October 19–26, 1993.

Fatty Acid in Brassica Species: According to ideal fatty acid composition for safe human health, the unsaturated fatty acids in Brassica species are linoleic (20%), oleic (65%), linolenic (9%), and erucic acid (2%), while the saturated fatty acids are palmitic (6%) and stearic (2%). The fatty acid content of Brassica seed oil determines its nutritional value, with specific focus dedicated to the quantities of oleic, linoleic, linolenic, and erucic acids, which are all vital in human health and nutrition. In Indian mustard, oleic acid had a positive association with linoleic acid and a negative correlation with erucic acid. Linoleic acid exhibited a

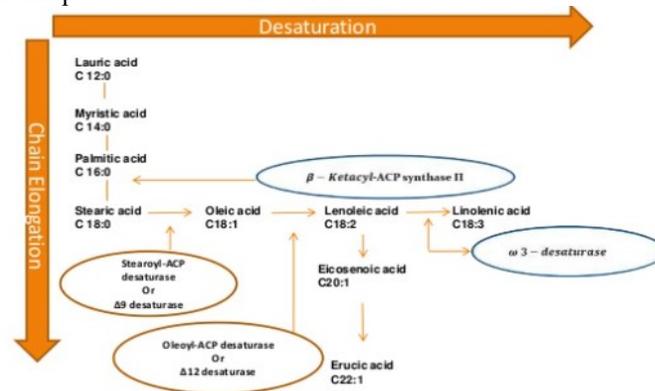
negative and significant association with erucic acid across the crops as well as in Indian mustard. Erucic acid in Indian mustard and glucosinolate content in toria were also negatively and significantly associated with linolenic acid.

Biosynthesis of Fatty Acids and their Analysis:

Dimov and Mollers (2010) investigated the genetic diversity of saturated fatty acid concentration in two sets of oilseed rape (*B. napus*) cultivars. In terms of total saturated fatty acids, the results showed that there are extremely significant genetic variations among the cultivars, ranging from 6.8 to 8.1 percent, with a mean of 7.4 percent. According to the characterization of mutants lacking in long-chain fatty acids in *Arabidopsis thaliana*, the Fatty Acid Elongase 1 (FAE1) gene is responsible for elongation of C18:1 (oleic acid) to C22:1 (erucic acid) (Lassner *et al.*, 1996). The gene encodes β -ketoacyl-CoA synthase (KCS), a seed-

specific condensing enzyme that works as a rate-limiting enzyme in erucic acid biosynthesis and is also implicated in the first stage of the fatty acid elongation pathway (Lassner *et al.*, 1996). The embryonic control of erucic acid led to the development of the half-seed technique (Harvey and Downey, 1964) for the determination of the fatty acid composition of individual seeds. This technique was used worldwide in

the genetic analysis of erucic acid and the development of zero-erucic acid *Brassica* cultivars. This method enables the plant breeder to analyse the fatty acid content of one cotyledon while retaining the other cotyledon with the embryo for planting. This approach was used by Kirk and Hurlstone (1983) to develop low erucic acid *B. juncea* lines.



Different types of fatty acids in *Brassica juncea*:

1. Palmitic acid (C16:0) and stearic acid (C18:0) are two types of fatty acids: Fats that are rich in palmitic or stearic acids find their applicability in various foods for consumption. Stearic acid, in comparison to palmitic acid, has lower low-density lipoprotein (LDL) cholesterol, which is well-known for causing coronary heart disease. Thus, replacing palmitic acid with stearic acid lowers LDL-cholesterol levels, lowering the risk of the disease. Saturated fatty acids palmitic acid (C16:0) and stearic acid (C18:0) are widely consumed in Western nations (Ervin *et al.*, 2004). Some studies indicate that palmitic acid is more cholesterol-raising in comparison to stearic acid (Mensink 2016).

2. Oleic acid: Oleic acid is some kind of unsaturated fatty acid that plays a significant function in human nutrition. Oleic acid-rich fats and oils are very resistant to heat and oxidation, making them ideal for a wide range of applications. Because oleic acid-rich oils have thermal stability comparable to or equal to saturated fats, they are ideal for commercial food-service applications that replace saturated fats, meaning long-term stability. It allows you to heat them to a higher temperature without smoking, which helps to cut down on cooling time and food oiling. Unsaturated fats and oils, such as oleic acid oil, have the ability to reduce cholesterol levels, but saturated (palmitic and stearic) fatty acids have the ability to significantly elevate blood cholesterol levels. Vegetable oils with a high C18:1 concentration is becoming increasingly desirable in both nutritional and industrial uses.

3. Linoleic acid: Increased dietary required linoleic acid (C18:2) and decreased linolenic acid have a significant impact on the nutritional quality of Brassica oil (C18:3). Linoleic acid and its derivatives are recognized as essential fatty acids since they cannot be

generated by the human body and must be gained from diet. Furthermore, the edible oil's high amount of linoleic acid decreases blood cholesterol and prevents atherosclerosis. As a result, edible oils rich in linoleic acid are regarded as premium oils. Although linolenic acid is an important fatty acid, it can produce rancidity and off-flavor in the oil.

4. Linolenic acid: Linolenic acid (C18:3) is a fatty acid found in many food oils. Linolenic acid is quickly oxidised due to its three double bonds, reducing the oil's shelf life. As a result, one of the most important breeding goals is to lower down the quantity of linolenic acid obtained from rapeseed. Because genetic diversity in linolenic acid content is restricted, x-rays and chemical mutagens are used to create low-linolenic acid mutants, which are predominantly seen in oilseed rape (*Brassica napus* ssp. *oleifera*) (Scarath *et al.*, 1988). Linolenic acid is produced either by desaturation of linoleic acid (C18:2) or, possibly, by elongation of C16:3 (Thompson, 1983).

5. Erucic acid: In general, erucic acid concentration varies greatly in the genus Brassica, depending on the genotype's allelic composition, ploidy level, genetic background, and environmental effect. Its ingestion is unsatisfactory, resulting in a variety of human health problems. As a result, genotypes and varieties with low levels of this fatty acid are nutritionally graded as high. Zero-erucic acid genotypes were found to belong to *B. napus*, *B. rapa*, and certain *B. juncea* and *B. carinata*, according to studies. Plasticizers, detergents, surfactants, and polyesters, among other goods, rely heavily on high erucic acid oil. As a result, producing genotypes with high erucic content is a major priority in today's brassica breeding. One of the most advantageous breeding programmes is the resynthesis of the amphidiploid species *B. napus* with high erucic

acid content from genotypes of their diploid progenitors, *B. oleracea* and *B. rapa*.

The high oleic acid concentration in seed oil makes it more heat stable, making it better for cooking. Oleic acid makes seed oil more suitable for industrial usage, in order to boost up the efficiency of cooking oil. Furthermore, higher amount of oleic acid is considered to be nutritive for human consumption since they increase the levels of high-density lipoproteins (HDLs) in the blood while decreasing the levels of low-density lipoproteins (LDLs) (Chang and Huang 1998). When present in higher proportions in edible oil, erucic acid, another important MUFA, is known to be anti-nutritional and inappropriate for human consumption. Erucic acid levels in cooking oil are higher because humans have a larger cardiac conductance, which leads to increase in level of blood cholesterol (Sinha *et al.*, 2007).

Several genotypes with increased erucic acid levels will be essential for a variety of sectors. The plastic, tannery, cosmetic, polyester, and detergent industries all employ erucic acid-rich oil as a raw material (Coonrod *et al.*, 2008). The genotypes Pusa 30, PM-21, and PM-24 with low erucic acid will be extremely important in the future when Brassica breeding programmes focus on the establishment of zero-erucic lines for nutritional objectives.

Polyunsaturated fatty acids (PUFAs) are recognized to be precursors of long-chain fatty acids, which are used to make physiologically important compounds like prostaglandins. Cooking oil should be low in polyunsaturated fatty acids (PUFAs) such linoleic and linolenic acids. Linoleic and linolenic acid levels were 11.00–45.30 percent, 11.10–26.72 percent, 18.57–26.93 percent, 9.99–17.23 percent, 14.08–18.18 percent, 9.82–26.66 percent, and 14.08–18.18 percent respectively, in *Brassica juncea*, *Brassica napus* and *Brassica rapa* genotypes. Linoleic acid is an essential fatty acid that the human body cannot generate on its own and must be obtained from food.

High quantities of linoleic acid in edible oil have been shown to lower blood cholesterol and prevent atherosclerosis. Despite the fact that linolenic acid known to be an essential fatty acid, its existence in the oil can cause rancidity as well as a bad taste (Sharafi *et al.*, 2015). Brassica cultivars with low erucic acid levels and high linoleic acid levels can also be employed in various Brassica breeding programmes aimed at improving the quality and quantity of oil for nutritional and industrial uses.

Canola: Canola is a vital source of oil for both edible and industrial uses and research to increase quality of oil is critical for maintaining its repute as a high-quality vegetable (Brassica) oil. Palmitic acid, linoleic acid, stearic acid, oleic acid, arachidic acid, linolenic acid, and erucic acid are some of the fatty acids contained in canola oil (Wang *et al.*, 2017). Canola oil is used in human diets because of its high nutritional content, and

it's been found to decline the plasma cholesterol levels when compared to nutrient heavy in saturated fatty acids. It has been shown that canola oil consumption affects biological activities that impact a variety of different disease risk indicators (Lin *et al.*, 2013).

The oil's fatty acid content is genetically regulated, and it has been successfully altered to make products that are specially customized for their intended application. Canola oil typically includes <2% erucic acid, 5-8% saturated fats, 60-65% mono-saturated fats, and 30-35% polyunsaturated fats. Double zero oil is commonly used in cooking, salad toppings, and the production of margarine. This appeals to clients that cannot risk their health since canola has the lowest saturated fat ratio among all the prime edible vegetable oils.

Canola cultivars having enhanced oil profiles used in high-temperature or saying continuous frying are sometimes referred to as "specialty canola." Oils produced by specialty canola cultivars typically contain less than 4% of linolenic acid (18:3) and more than 70% of oleic acid (18:1). These cultivars' oils are more temperature stable and have a longer shelf life.

The researchers has developed transgenic (genetically modified) canola seeds with much increased oil content. When the truncated canola storage protein 2S-1 promoter, also known as the napA promoter, is at an appropriate level, seed-specific over expression of BnLEC1 and BnLIL genes (from canola) under the control of the promoter significantly increases the seed oil content of the transgenic oilseed plant without affecting other major agronomic traits.

Qi *et al.* (2012) isolated the motifs for RNA-binding No.2 (RRM2) from the flowering control locus A (FCA) protein (FCA-RRM2) from canola variety No. 1 (i.e., "Nannongyou"), then used *Agrobacterium rhizogenes* to introduce it into cotyledon nodes, where it was fixed under a 35S-35S promoter (a variant of the cauliflower promoter) (for plant transformation selection). Canola FCA-RRM2 enhances plant growth, its organ size, cell structure, plant productivity, and their oil content, according to the researchers. These findings, according to the study's author, present a realistic way for improving the plant's genetics.

Fatty acid content. Now, people are well known about their healthy diet as consumption of any unhealthy food without essential fatty acids causes a number of diseases related to blood, endocrine, immune function and metabolic effects etc. (Bhoge, 2015). The utilization of brassica oil by humans is dependent on brassica oil. The majority of monounsaturated fatty acids (MUFAs) are utilized largely as a source of edible oil due to the larger concentration of 16 and 18 carbon unsaturated fatty acids in vegetable seeds (Simopoulos, 2008; Ramos *et al.*, 2009; Priyamedha and co-workers 2014). Linolenic acid (C18:3), for example, is an undesired fatty acid for this content since it affects the oil's durability, despite the fact that it is an important dietary fatty acid. Furthermore, erucic acid (C22:1)

includes about half of the total fatty acid, which is harmful to humans since it has been linked to myocardial lipidosis. Although erucic acid is abundant in wild forms of rapeseed and mustard (about 40% of total fatty acids), it is rarely found in rapeseed produced for human use.

According to the annual report of ICAR-DRMR, 2020, the fatty acid profile of various genotypes, as determined by gas chromatography, revealed palmitic and stearic acid as major saturated fatty acids, while oleic, linoleic, and linolenic acid constituted prominent unsaturated fatty acids but nutritionally desirable fatty acids, according to the annual report. Erucic acid, on the other hand, was found to be less than 2% in some genotypes.

According to DRMR Barotpur's annual report (ICAR-DRMR, 2020), (Table 2) the fatty acid content of PM 29, PM 30, PDZ 1, and Kranti has changed dramatically. The PDZ 1 variety has lower levels of erucic acid and eicosenoic acid than the other three varieties, and it has been found to be best suited for the timely sown and irrigated conditions of the National Capital Region of Delhi and its surrounding areas in the states of Haryana, Rajasthan, and Uttar Pradesh. It bears yellow seeds with a concentration of 40.56 percent of oil. PM 29 and PM 30 are low erucic acid (single zero) cultivars of Indian mustard, according to the most current annual report (ICAR-DRMR, 2020), with 1.96 and 1.56 percent, respectively.

Table 2: Fatty acid composition of various *Brassica* varieties.

Sr. No.	Varieties	Palmitic acid	Stearic acid	Oleic acid	Linoleic acid	Linolenic acid	Eicosenoic acid	Erucic acid
2017								
1.	RH-749(ZC)	2.69	1.57	11.26	18.15	13.45	6.01	45.62
2.	NRCHB-101	2.56	1.60	12.68	19.21	14.37	5.15	42.89
3.	Kranti(NC)	3.08	1.74	10.53	17.83	15.08	3.52	45.29
4.	PM-21(QC)	3.56	2.13	39.36	36.76	12.27	2.26	0.86
5.	PDZ-1	3.69	2.73	45.03	35.60	9.85	2.67	2.33
6.	PM-29(QC)	3.90	2.57	37.07	38.08	12.33	3.22	1.45
7.	PM-30(LR)	3.26	2.54	41.41	32.32	16.18	1.80	2.51
2018								
8.	RH-749	3.08	1.28	12.27	17.34	17.49	3.95	43.54
9.	PM-30	3.96	1.93	40.24	30.48	18.90	2.61	1.27
10.	Kranti(NC)	3.50	1.29	10.57	19.28	18.25	4.08	41.46
11.	NRCHB-101	2.84	1.29	14.27	17.80	16.16	4.95	39.42
12.	PM-29(QC)	4.62	1.62	41.10	34.33	15.86	2.17	1.01
2019								
13.	RH-749(ZC)	2.89	0.86	9.88	15.33	15.75	8.35	45.28
14.	Kranti(NC)	3.18	0.83	14.59	19.51	15.86	8.09	35.46
15.	PDZ-1(QC)	3.65	1.30	42.25	33.60	10.42	5.37	4.05
2020								
16.	PM 29	3.54	1.24	39.13	35.32	11.87	6.46	1.96
17.	PM 30	3.67	1.18	38.27	30.80	18.46	8.97	1.56
18.	PDZ 1	3.34	2.08	43.33	34.06	12.12	6.10	1.44
19.	Kranti	2.81	1.03	12.97	18.85	15.40	7.51	40.84

These two have a lot of oleic acid in their fatty acid makeup (38.27–39.13), which is helpful since studies suggest that oleic acid consumption can help with cancer, inflammatory, and auto-immune illnesses. In the case of linolenic acid, which is thought to be harmful to human health, PM29 concentration has decreased from 15.86 (2018) to 11.87 (ICAR-DRMR, 2020).

Using genetic and molecular methodologies, nutritional value has indeed been improved

1. Decline in erucic acid content at molecular level:

TAGs carrying erucic acid (22:1) are esterified at the sn-1 and sn-3 positions within the glycerol backbone are naturally accumulated in the seeds of ancient cultivars, accounting for 45-50 percent of the overall fatty acid composition. Erucic acid concentration is genetically controlled in rapeseed by two additive loci (EA & EC) on A- and C-genomes, which combined account for 90% of the total variation in erucic acid, but

evenly not (Jourden *et al.*, 1996). Using a QTL method, the two loci were found in rapeseed (Jourden *et al.*, 1996). When rats were feeded with HEAR (high erucic acid rapeseed) oil, they developed heart lesions and abnormal fat accumulation in their body, as well as a decrease in body weight (Badawy *et al.*, 1994). Despite the fact that this adverse nutritional impact has never been identified in humans, recessive alleles (eA and eC) were inserted at both loci implicated in 22:1 concentration, Low Erucic Acid Rapeseed (LEAR) cultivars were selected.

2. Seed oil content is determined by genetics: The primary goal of oilseed rape breeding is to increase seed oil content. The content of seed oil, on the other hand, is determined by a intricate genetic determinism that is still unknown. In order to improve the genetic development of the crop, breeders will require a good grasp of the genetic determinism of various products especially the oil. Using multiple segregating rapeseed

populations, recent research has discovered many QTL (7 to 14 regions per study) involved in the control of oil content, which is consistent with the trait's polygenic determinism. Each QTL contributed for less than 10% of the overall variance in oil content, according to Delourme *et al.* (2006).

Some of these QTL aligned to erucic acid content loci, suggesting that it is a key predictor of oil content in oilseed rape (Burns *et al.*, 2003). The major factor affecting oil content was found to be additive effects (Delourme *et al.*, 2006), with individual additive effects of the various alleles ranging from 0.2 to 1.2 percent. Variations in oil content are also influenced by significant environmental factors (Delourme *et al.*, 2006).

3. Transgenic approaches to improve content of seed oil: The majority of efforts to improve the seed's eventual lipid content have concentrated on metabolic pathways involved in Fatty Acid synthesis and TAG formation. Overexpression of a lysophosphatidate acyltransferase gene from yeast (*Saccharomyces cerevisiae*) in oilseed rape significantly improves seed oil content in controlled circumstances. These transgenic lines showed an increase in oil content of roughly 10% when tested under field settings. Individual FA biosynthesis genes, on the other hand, did not significantly boost lipid accumulation in seeds (Thelen and Ohlrogge 2002). Glycerol-3-phosphate (Gly3P) supply has recently been identified as a limiting factor for lipid synthesis (Vigeolas and Geigenberger 2004). In rapeseed, overexpression of a yeast Gly3P dehydrogenase gene resulted in a three- to fourfold rise in Gly3P concentration, resulting in a 40% increase in lipid content (Vigeolas *et al.*, 2007).

CONCLUSION

Rapeseed breeding for enhanced seed quality resulted in the emergence of double zero cultivars with low 22:1 as well as low seed GSL concentrations. A comprehensive and powerful investigation on seed quality in *Brassica napus* enabled us in finding of many generic regions implicated in seed quality among diverse mapping populations.

FUTURE SCOPE

Integrating this information to create a uniform QTL map for seed quality will be a future challenge. This will surely draw attention to the genetic factors that influence seed development. Furthermore, the Arabidopsis genome sequence completion might aid in the identification of seed quality QTL. Finally, the whole *B. rapa* (A genome) sequence will be available soon (Hong *et al.*, 2008), opening up new avenues for seed quality QTL identification and characterization.

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