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Breeding for quality in rapeseed-mustard: A review

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Abstract
Rapeseed-mustard is the world's third most important source of vegetable oil in Brassicaceae family and is cultivated in more than 50 countries. As erucic acid and glucosinolate are two main anti-nutrional factor in the oil and seed meal. Canola (00) varieties have oil with less than 2% erucic acid and glucosinolates of less than 30 µ moles/g defatted seed meal and command a premium in the international market. But Indian cultivars possess significantly higher levels of erucic acid (about 50%) and glucosinolates (100-280 u moles/g defatted seed meal).Plant breeders are currently facing the most daunting challenge: improving the long-term output of crop types. Groundwork and researches are in progress to strengthen the agronomic base of low yielding zero erucic lines, as well as to recombine low erucic acid and low glucosinolate to develop '00' varieties. This paper overviews the base work, on-going research, limitations and future prospects for yielding better oil and seed meal quality to increase the international market value and demand of various cultivars.

Keywords: Brassicaceae, erucic acid, anti-nutritional factors, Canola, glucosinolates, defatted seed meal

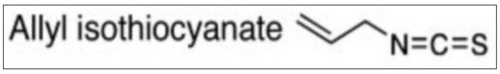
Introduction

Brassicas are considered as one of the earliest crop plants that humans have domesticated. Brassicaceae is one of the ten most commercially important plant families, with approximately 3500 species and 350 genera. The existence of co-duplicate cotyledons (i.e., cotyledons that are longitudinally folded around the radical) and/or two segmented fruits (siliquae) with seeds in one or both segments, as well as just simple hair, if present, characterizes it. Oilseed Brassicas, frequently referred to as rapeseed mustard, are a major category of oilseed crops in the globe. They are made up of eight cultivated crops belonging to the Brassiceae tribe of the Cruciferae family (Brassicaceae) (Willis, 1973) [1]. Indian mustard, toria, yellow sarson, brown sarson, gobhi sarson, karan rai, black mustard, and taramira are eight different kinds of rapeseed mustard that are grown in 53 countries around the world. The oilseed crop rapeseed and mustard are recognized for the quantity and quality of oil and protein in the seed. Since a long time, the primary objectives of the rapeseed-mustard breeding programmes in India and around the world have been to increase oil content and improve the quality of oil and seed meal. The nature and amount of fatty acids determine the quality of oil. Erucic acid levels in Indian rapeseed mustard oil are relatively high (Chauhan *et al*., 2011) [2]. Rapeseed-mustard ranks second among the seven edible oilseeds produced in India, accounting for 28.6% of total oilseed production, following groundnut, which accounts for 27.8% of the Indian oilseed economy. Oilseeds represent around 14.1 percent of India's total cultivated land, with rapeseed-mustard accounting for 3% of that. During 2018-19, the world's estimated rapeseed-mustard acreage, output, and yield were 36.59 million hectares (mha), 72.37 million tonnes (mt), and 1980 kilograms per hectare, respectively. India is responsible for 19.8% of global acreage and 9.8% of global production, respectively (USDA). Productivity has improved significantly over the last eight years, from 1840 kg/ha in 2010-11 to 1980 kg/ha in 2018-19, and production has improved from 61.64 m t in 2010-11 to 72.42 m t in 2018-19. In India, rapeseed–mustard crops are grown in a variety of agro-climatic conditions, from the north-eastern/northwestern hills to the south, in irrigated/rainfed, timely/late sown, saline soils, and mixed cropping systems. During the 2018-19 crop season, Indian mustard accounts for roughly 75-80 percent of the 6.23 million acres under these crops in the country (www.drmr.res.in). Due to high levels of erucic acid (40–50 percent erucic acid in the seed oil) and glucosinolates (up to 300 mg/g glucosinolates in the deoiled powder), Indian cultivars receive little attention in the international market. Canola grade unusual rapeseed cultivars, often known as double low or '00,' are used in a variety of cooking applications and contain 2% erucic acid and 30 moles/gm glucosinolates.

Because *B. juncea* is the most widely cultivated of the oilseed Brassicas in our country, improving the nutritional content of *B. juncea* is critical to meeting our needs.

Chemical composition

Mustard seeds have a chemical composition that benefits them, such as high protein content and a fairly well-balanced amino acid composition, as well as being high in dietary fibre and natural antioxidants. Mustard seed flour has several interesting functional features in addition to its nutritional benefits, thus it might be used in a variety of foods (Ildiko *et al*., 2006) [3]. In general, rapeseed and mustard seeds have 35-45 percent oil, 17-25 percent protein, 8-10 percent fibres, 6-10 percent moisture, and 10-12 percent extractable chemicals. The chemical allyl isothiocyanate is the major component responsible for pungency of mustard seed. The organosulfur chemical allyl isothiocyanate has the formula $CH_2=CHCH_2N=C=S$ and is one of the most frequent isothiocyanates found in nature (ITCs). It is recognised to have anticarcinogenic properties because it reduces carcinogen activation and increases detoxification.



Structure of Allyl isothiocynate (Diana *et al*., 2005)

Triacylglycerol fatty acids (Ct6-C22) make up the majority of oil (92-98%). The remaining lipid molecules in the oil include unsaponifiable hydrocarbons, tarpins, sterols, tocopherols, glycolipids, and phospholipids, among others.

Oil quality

Oil quality is defined not only by its fatty acid composition, but also by other factors, the most important of which are the triacylglycerol profile and the amount and composition of tocopherols. Mustard oil has a special fatty acid composition, it contains about 20–28% oleic acid, 10–12% linoleic, 9.0–9.5% linolenic acid, and 30–40% erucic acid, which is indigestible for human beings and animals. The fatty acid composition and distribution pattern of fatty acids within the triacylglycerol molecule impact the nutritional and functional qualities of oils (Pham and Pham, 2012) [5]. In rapeseed-mustard oil, a high percentage of important fatty acids (linoleic acid; C18:2 and linolenic acid; C18:3) makes it nutritionally beneficial, but a high level of erucic acid; C22:1 (40-57 percent) reduces its utility as an edible oil (Agnihotri *et al*., 2007 and Singh *et al*., 2014) [6, 7]. The two fatty acids present in mustard oil in high amount are oleic acid and erucic acid in which oleic acid is the most broadly distributed of all the fatty acids, occurring in all oils and fats to some level. It, $CH_3(CH_2)_7CH=CH(CH_2)_7CO_2H$, is found as an ester of glycerol—that is, as a glyceride or as an ester of a long-chain alcohol—rather than in its free state, as do other fatty acids. Oleic acid is a low-melting-point solid having two crystalline forms (-form, melting point 13.4 °C [56 °F] and -form, melting point 16.3 °C [61 °F]). It is a long-chain carboxylic acid with one cis-configured double bond between C9 and C10.

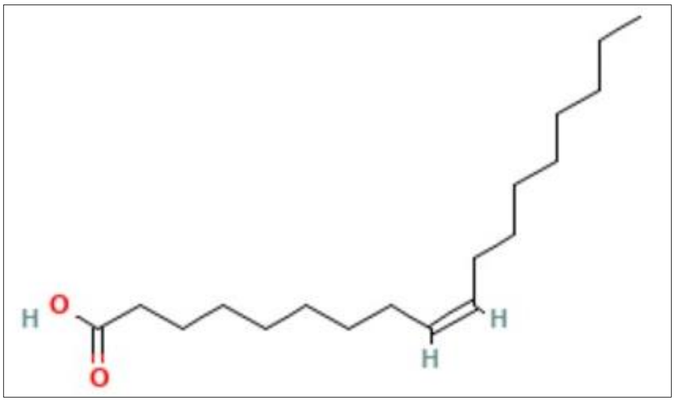
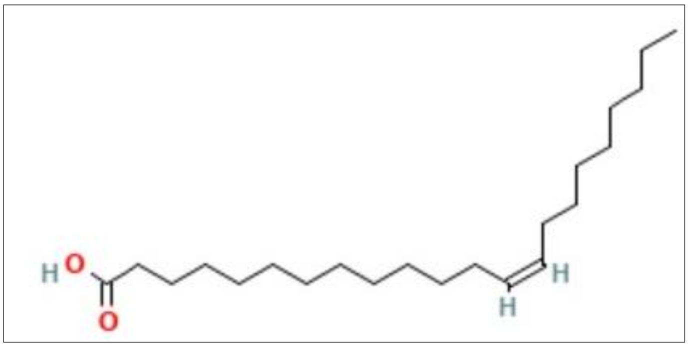
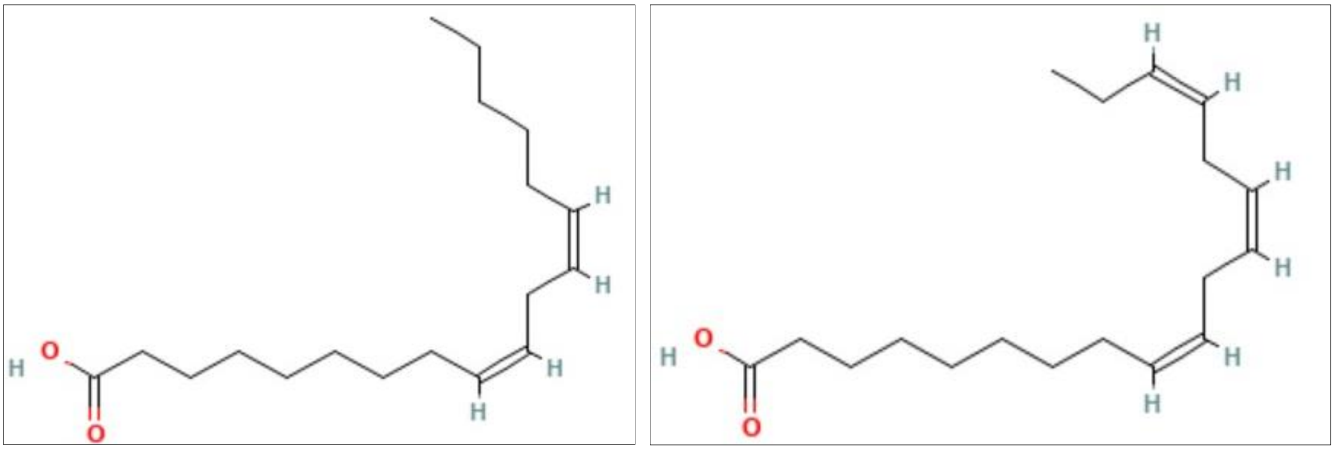


Fig 1: Structure of oleic acid

Erucic acid, often known as 22:19, is a monounsaturated omega-9 fatty acid of chemical formula $CH_3(CH_2)_7CH=CH(CH_2)_{11}COOH$. It is found in wallflower seed and other Brassicaceae plants, with a level of 20 to 54 percent in high erucic acid rapeseed oil and 42 percent in mustard oil (Sahasrabudhe *et al*., 1977) [8]. Brassidic acid is the trans isomer of erucic acid, which is also known as cis-13-docosenoic acid. It has been related to myocardial lipidosis and cardiac diseases in experimental rats, making it unfit for human intake. As a result, its presence in oils and fats has been prohibited in numerous countries. The high erucic acid content of mustard seed can be lowered through breeding, and several genotypes with low erucic acid content are being grown in several countries. Mustard oil is also high in tocopherols, which act as a preservative against rancidity due to their antioxidant properties (Moser *et al*., 2009) [9].



(A)



(B) (C)

Fig 2: Structure of (A) erucic acid (B) linoleic acid (C) linolenic acid

The following is the major biosynthetic process for fatty acid production:

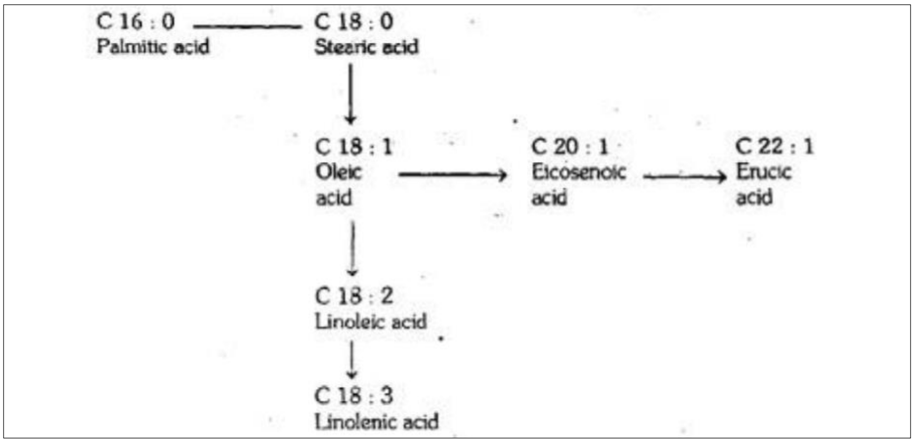


Fig 3: Biosynthetic pathway of main fatty acids (Jonsson, 1977)

Erucic and linolenic acids are the end products of the biosynthetic pathway in which oleic acid is converted to eicosenoic and subsequently erucic acid by decreasing saturation or chain elongation. Chauhan *et al*. (2002) [10] [10] investigated the production of fatty acids in oil seed Brassicas. It follows similar pathway like many other oilseed crops. The stages of carbon chain elongation and desaturation are controlled by enzymes and can be manipulated genetically. Canadian breeders were able to achieve the genetic blocks in the chain elongation phase of stearic acid that control the production of erucic acid from oleic, linolenic, and linoleic acids.

Table 1: Fatty acid composition of rapeseed and mustard seed oil Chauhan *et al.* (2002) [10]

Fait	adds	Pan
SFA	Ptak (16:0)	1 - 3
	Stark (In)	04 - 3.5
MUFA /PUPA	Mit (18:11	12 - 24
	Lesokie(18:21	12 -16
	Lboksic (18:3)	7-10
	Esucic I22:11	40- 55

Seed meal quality

Another useful product obtained from rapeseed-mustard seeds is the meal that remains as a by-product after oil extraction. It includes approximately 40% protein and has a good amino acid profile, with a relatively high proportion of essential sulfuric amino acids, methionine, and cysteine (Downey and Bell, 1990) [11]. Protein (35-40%), carbs (14-15%), fibre (10-12%), moisture (6-8%), ash (4-6%), minerals and vitamins (1-1.5%), glucosinolates (2-3%), tanin (1.6-3.1%), sinapin (1-1.5%), and phytic acid (3-6%) are the primary components of rapeseed-mustard seed meal. It is also high in minerals (Ca, Mg, and P), as well as vitamins B4 and E. However, rapeseed-mustard meal has a large amount of anti-nutritional chemicals called glucosinolate as compared to other common sources such as soybean (Wanasundara, 2011) [12]. Rapeseed meals include high amount of glucosinolates, tannins, erucic acid, sinapine, phytic acid, mucilage, and other antinutrients because of which symptoms of of reduced intake, modest liver damage, and reduced volatile fatty acid synthesis are observed when the toxins are supplied orally. Cleavage products from glucosinolate hydrolysis diminish feed palatability by decreasing thyroid gland iodine uptake, notably in non-ruminants like pigs and poultry (Bell, 1984) [13]. As a result, melioration of nutritional qualities by producing new varieties with different oil and meal properties has been a major goal in rapeseed-mustard quality breeding.

Glucosinolate: In addition to erucic acid, rapeseed-mustard seed meal also contains glucosinolate which is considered as another anti-nutritional factor in seed meal. The glucosinolates are a category of sulphur and nitrogen-containing chemical compounds produced from glucose and an amino acid found mainly in the members of brassicae family. The seeds and vegetative tissues of brassicas contain over 120 known glucosinolates. Thiohydroximate-O-sulfonate groups linked to glucose and an alkyl, aralkyl, or indolyl side chain are the chemical components of glucosinolates (R) (Agerbirk *et al*., 2012) [14]. In brassicas, glucosinolates are accompanied by myrosinase, a thioglucosidase (EC 3.2.3.1) that catalyses the hydrolysis of glucosinolates to a number of breakdown products after cellular damage (Fenwick *et al*., 1983) [15]. Most of the known glucosinolates are abundantly present in most Indian cultivars (120–280 moles/g defatted oil). Mustard samples obtained from many countries include entirely or mostly sinigrin, however those from India and Pakistan have gluconapin as a substantial component in addition to sinigrin (Chauhan *et al*. 2002) [10]. According to Chauhan *et al*. 2002 [10], the enzyme thioglucoside glucohydrolase, also known as myrosinase, breaks down glucosinolates to produce sulphate, glucose, and other glucon products. Cleavage products from glucosinolate hydrolysis, such as isothiocyanates, oxazolidimethiones, and nitriles, are extremely poisonous to nonruminants like pigs and poultry, and represent a significant non-tariff barrier.

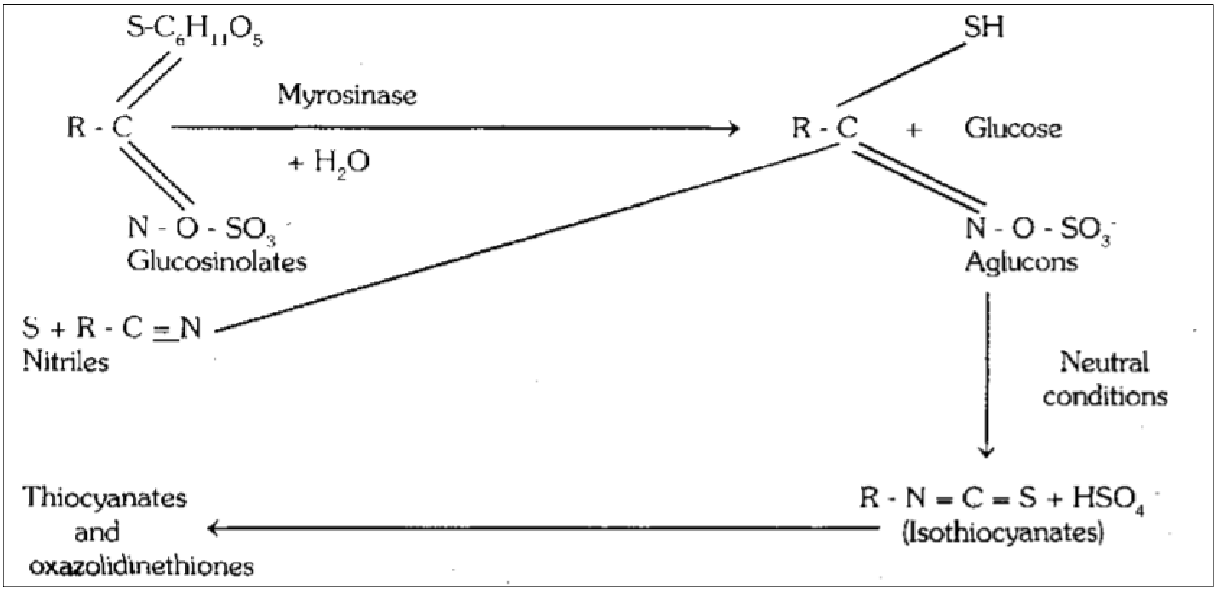


Fig 4: Enzymatic hydrolysis of glucosinolates and the by-products (Chauhan *et al*. 2002) [10]

Table 2: Major Glucosinolates present in different species of Brassicae family (Ugolini *et al*., 2008)

Crop	Botanical name	Majorglucosinolates (%)
Indian mustard	*Brassica juncea*	Sinigrin (~90)
Karan rai (Ethiopian mustard)	*Brassica carinata*	Sinigrin (~97)
Black mustard	*Brassica nigra*	Sinigrin (~95)
Gobhi sarson	*Brassica napus*	Progoitrin (~70)
Taramira	*Eruca sativa*	Glucoerucin (~95)
Yellow / white mustard	*Sinapis alba*	Glucosinalbin (~100)
Cabbage	*Brassica oleracea*	Progoitrin

Based on presence of fatty acids quality can be categorized as

Single Zero (Single Low): Varieties with low erucic acid content (2%), but high glucosinolate content in seed meal.

Double Zero (Double Low or Canola): Varieties with less than 2% erucic acid and less than 30µ moles/g glucosinolate in seed meal

Triple Zero (Bell 1984) [13] A triple variety is a double low yellow coated variety that is also low in fiber.

Canola or '00' varieties

Varieties containing oil with less than 2% erucic acid and the solid component of the seed less than 30mol of any one or combination of 3-butenyl glucosinolate, 4-pentenyl glucosinolate, 2-hydroxy-3-butenyl glucosinolate, and 2-

hydroxy-4-pentenyl glucosinolate per gram of air-dry, oil-free solid in defatted seed meal (00) is considered as canola or '00' varieties. The first zero erucic acid strains of *Brassica napus* were established in 1961, according to the National Oilseeds and Vegetable Oils Development Board. These cultivars are often known as 'double low' or 'double zero' ('00'). After the 1970s, canola quality rapeseed oil gained popularity, and it has since been widely acknowledged as a healthy food oil. The increasing popularity of canola quality oil led to the introduction of Brassica campestris strains with no erucic acid. In 1967, the cultivar 'Bronowski' of B. napus was identified, with a glucosinolate concentration of roughly 12 mol/g oil-free meal. It also has a low level of erucic acid in the seed oil (7–10 percent). As a result, the 'Bronowski' gene is the source of low glucosinolate content, which has been used to develop high-quality canola cultivars. India's potential demand for canola oil has been estimated at around 0.85 million tonnes, roughly equal to 10% of the country's current edible oil imports (Commodity online, 2012). Thus, expanding the area and production of canola type rapeseed-mustard types is essential in order to meet current and projected domestic demand for canola quality rapeseed-mustard oil, as well as to capitalize on the commodity's growing trade potential.

Breeding for quality

Canola or '00' varieties development and cultivation in India would provide substantial advantages.

1. To boost the market value of oil and seed meal, as well as their versatility of use.
2. Oil and seed meal's nutritional value should be enhanced.
3. To obtain a market price that is remunerative.
4. Canola meal is a high-protein animal feed supplement made from the dry matter left over after the oil is collected.
5. To increase seed meal's export potential.
6. Canola oil is a versatile vegetable oil that may be used in a lot of different things.

The quality improvement initiative for rapeseed oil and seed meal in India has the following goals:

1. The assessment of indigenous and exotic germplasm revealed a large range of diversity for different fatty acids in Indian mustard after a determined effort.
2. For yield and quality metrics, B. juncea and B. napus low erucic acid/low glucosinolate lines have to be evaluated.
3. Producing information about the reaction types '0' and '00' to endemic pests and diseases.
4. Basic research into erucic acid and glucosinolate content, its genetics and breeding behavior.

In India, quality breeding began in the 1970s. During this period, efforts were restricted to assessing the existing variability in indigenous germplasm (Anand, 1974; Kumar, 1978; Ahuja and Banga, 1992). But there were no zero erucic acid or glucosinolate forms discovered back then. On a seed basis, glucosinolate levels in indigenous selections/collections ranged from 63 to 102 µ moles/g. The identification of SM 1, a low erucic acid (10%) accession, marked the end of this phase. However, due to its poor agronomic background, this genetic stock could not be used as a commercial variety or a donor source for quality enhancement. With the commencement of the Indo-Swedish collaborative effort in 1975, the slow pace of research was accelerated. During this phase, several '0' erucic strains were discovered. Since 1970, breeding efforts in India have been in progress to reduce glucosinolate content in the seed of rapeseed mustard varieties to 30 µ moles/g defatted seed meal (low or 0) and erucic acid to 2% (low or 0), along with incorporating the two to develop double zero or double low varieties to meet the internationally acceptable standard of oil and seed meal (Chauhan *et al*., 2002) [10]. Pusa karishma was the first low erucic acid variety released in India in 2004 after which in 2005 first double low variety GSC5 of gobhi sarson was released in India. In *Brassica juncea* five low erucic cultivars and in gobhi sarson (*B.napus*) five double low cultivars (low erucic and low glucosinolate) have been released till the year 2005.

Kumar *et al*. (2009) [20] demonstrated that in *Brassica juncea*, single low variants with low erucic acid content (2%) have been produced. Under several crop improvement and quality improvement programmes, an effort is being made to establish real canola type cultivars (Double low) in *B. juncea*. Hybridization along with pedigree selection was the most common approach for transforming traditional varieties into canola kinds in the beginning. Several economically important traits, including double low, high oil content, shattering tolerance in *B.napus*, and low erucic/high oleic acid, yellow seed coat colour, double low, and resistance/tolerance to fungal diseases, *Albugo candida* and *Alternaria brassicae* in *B.juncea*, were transferred via wide hybridization aided by embryo rescue. Later, interspecific and intergeneric crosses were made, allowing breeders to quickly develop a large number of new gene combinations with desirable traits (Agnihotri *et al*., 1998) [22]. Following that, the emergence of the half-seed method gave breeders complete control over their material. Efforts are currently being made to recombine low erucic acid with low glucosinolate content in Indian mustard, as well as refine the agronomic base to boost yield potential of double low gobhi sarson strains (Chauhan *et al*., 2011) [2]. More advanced technologies such as mutagenesis (Barve *et al*., 2009), marker-assisted selection (MAS), and genetic engineering (transgenic) have transformed the way quality breeding is done in recent years (Agnihotri, 2010) [23].

Table 3: The enhanced quality traits registered as ICAR (Agnihotri *et al*., 2008) [21]

S. No.	Name of genotypes	Quality of genotypes
1	TERI GZ-05 - INGR 04078 (TERI-Uphaar)	High oleic and linoleic acid, yellow seeded, double low *B. juncea*
2	TERI (OO) R9903 - INGR 04077 (TERI-Uttam)	High oil content(43%), canola quality, early maturing *B. napus*
3	TERI (OO) R986-INGR 99007 (TERI-Gaurav)	Early maturing, dwarf double low *B. napus*
4	TERI (OO) R985-INGR 99008 (TERI-Garima)	High oleic acid, double low *B. napus*
5	TERI (OE) R09- INGR 98005 (TERI- Shyamali)	Low erucic acid, high oleic *B. napus*
6	TERI (OE) R03- INGR98002 (TERI- Phaguni)	Low erucic-acid, early maturing *B. napus*
7	TERI (OE) M21-INGR 98001 (TERI-Swarna)	Low erucic acid, yellow seeded, early maturing *B. juncea*

Status of quality improvement programme in India

***Brassica juncea* (Mustard)**

- During the 2010–11 cropping season, 124 low erucic acid/glucosinolate content strains were investigated under AICRP R&M.
- The first low erucic acid mustard, Pusa Karishma (LES 39), was released for Delhi state in 2004, and five low erucic acid varieties have been discovered for release up to December 2010.
- The National Bureau of Plant Genetic Resources (ICAR), New Delhi, has registered four strains (one each of low erucic and low glucosinolate and two low erucic and low glucosinolate).

***Brassica napus* (Rapeseed)**

AICRP investigated forty one low erucic acid and/or low glucosinolate strains of *Brassica napus*.

The genes and quantitative trait loci (QTLs) governing erucic acid and glucosinolate concentration in Brassicas have been mapped and cloned using a variety of molecular markers. They have also been used to manipulate erucic acid and glucosinolate profiles in Brassicas using MAS for double low genotypes derived from segregating generations of crosses involving high and low erucic acid and/or glucosinolate parents (Gupta *et al*.,2004; Ramchiary *et al*., 2007; Hasan *et al*., 2008 and Bisht *et al*., 2009) [25, 27, 26, 24].

Constraints in Quality Breeding

1. Because the majority of quality features are difficult to assess and evaluate, quality breeding places a significant demand on resources, time, especially money.
2. The majority of quality attributes are polygenic. As an outcome, quality trait selection during segregating generations is extremely challenging.
3. Many desirable characteristics have a low heredity and are strongly influenced by the environment. This slows down the choosing process.

Future strategies for development

- Use of biotechnological technologies such as dihaploid breeding and marker- assisted selection to improve selection efficiency.
- Rapid cycling facilities are being developed to speed up the generation and replication of high-quality materials.
- To assist early generation selection, a low-cost and quick approach for mass screening for fatty acids and glucosinolates has been developed.
- In the development and evaluation of breeding materials, shuttle breeding can be utilized.
- Diversification of sources of low glucosinolates (since BJ 1058 is generated from an interspecific source in *B. juncea*) and their application in breeding programmes.
- To extend the genetic foundation, bi-parental mating and interse crossing among the selected low erucic/low glucosinolates lines should be pursued.
- To facilitate early generation selection, a low-cost and quick approach for mass screening for fatty acids and glucosinolates have to be developed.
- Understanding the genetics of Indian mustard fatty acid profiles and glucosinolate levels.

Conclusion

- In the rapeseed-mustard industry, the back cross method is the most extensively utilized method for quality enhancement. The use of doubled haploidy and whole genome selection based on AFLP markers during backcrossing facilitated the transfer of the low glucosinolate phenotype.
- Backcross breeding can also be used to generate cultivars with low erucic acid levels by introducing two recessive genes.
- To minimize erucic acid and glucosinolate, selection pressure can be used effectively.
- Major fatty acids in oil are simply inherited, while glucosinolate content is controlled by complex genetic mechanism and is also maternally influenced.
- The main emphasis is on recombining low erucic acid with low glucosinolate content in Indian mustard, as well as refining the agronomic base to boost production potential of double low Gobhi Sarson strains.

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