Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2020; 9(1): 1532-1540
Received: 26-11-2019
Accepted: 27-12-2019

Viraj Rathod
Department of Biotechnology, Junagadh Agricultural University, Junagadh, Gujarat, India

Solanki HV
College of Agriculture, Khapat, Porbandar, Gujarat, India

Corresponding Author:
Viraj Rathod
Department of Biotechnology, Junagadh Agricultural University, Junagadh, Gujarat, India

Breeding for oil quality improvement in *Brassica*

Viraj Rathod and Solanki HV

Abstract
Rapeseed-mustard is the third most important source of vegetable oil in the world and is grown in more than 50 countries across the globe. Mustard oil contains high amount of mono-unsaturated fatty acids, a good ratio of polyunsaturated fatty acids, relatively high level of oleic acid and the favorable balance between linolenic and linoleic acids. High erucic acid in oils and glucosinolate in seed meal is an anti-nutritional factor. Canola oil is a useful vegetable oil used in a range of cooking with <2% erucic acid and glucosinolates <30 µ moles/gm. Erucic acid is significantly and positively correlated with glucosinolate content, while both are significantly and negatively correlated with oleic and linoleic acid. Erucic acid can be reduced with increase in other fatty acids. Back cross method is most widely used for quality improvement in this crop. Inter-specific methodology was also successful for the enhancement of yield and seed quality in the development of *B. juncea*.

Keywords: *Brassica*, Breeding, improvement, oil, quality

Introduction
In the current time, increased sustainable production of crop varieties is the most challenging task for plant breeders. Improvement of yield and quality within a limited period of time with deteriorating cultivable land, water scarcity, *etc*. is in demand in present century. Since the increased yield alone may not fulfill the needs of human nutrition, improvement of nutritional quality and value addition for different uses are of prime importance. In India, the oilseeds form the second largest agricultural commodity. Among the nine annual oilseed crops grown in India, oilseed *brassica* rank second in importance contributing about 30% to the total oilseed produced. The Indian cultivars, due to high content of erucic acid (40–50% erucic acid in the seed oil) and glucosinolates (upto 300 µm/g glucosinolates in the deoiled meal), have limited preference in international market. The canola quality exotic rapeseed cultivars, commonly known as double low or 'oo', used in a range of cooking with <2% erucic acid and glucosinolates <30 µ moles/gm. Since, *B. juncea* among the oilseed *Brassica*s acquires the maximum share of cultivated area in our country, the improvement of nutritional quality in *B.juncea* is most desired to suit our needs.

Major Economic uses of Rapeseed and Mustard
1. Rapeseed and mustard provide edible oil which is used as cooking medium in north India.
2. Seed is used as condiment in the preparation of curries.
3. Split seed and oil is used for pickling.
4. The leaves of the young plants are used as vegetable.
5. Oil cake is used as animal feed.
6. Oil is utilized soap making and plastics manufacturing.

Oil Quality
Rapeseed-mustard seeds, generally consists of 35-45% oil, 17-25% proteins, 8-10% fibers, 6-10% moisture. Mustard oil contains high amount of mono-unsaturated fatty acids and a good ratio of polyunsaturated fatty acids, which is good for heart. It contains the least amount of saturated fatty acids *i.e.* less than 10%, making it safe for heart patients. Adults and children should consume a maximum of 10% of their total energy intake in the form of saturated fat to reduce the risk of heart disease (WHO, 2018) [16]. It may be said that it is as good as any other edible oil as it has favorable balance between linolenic and linoleic acids (Chauhan *et al*., 2002) [4].

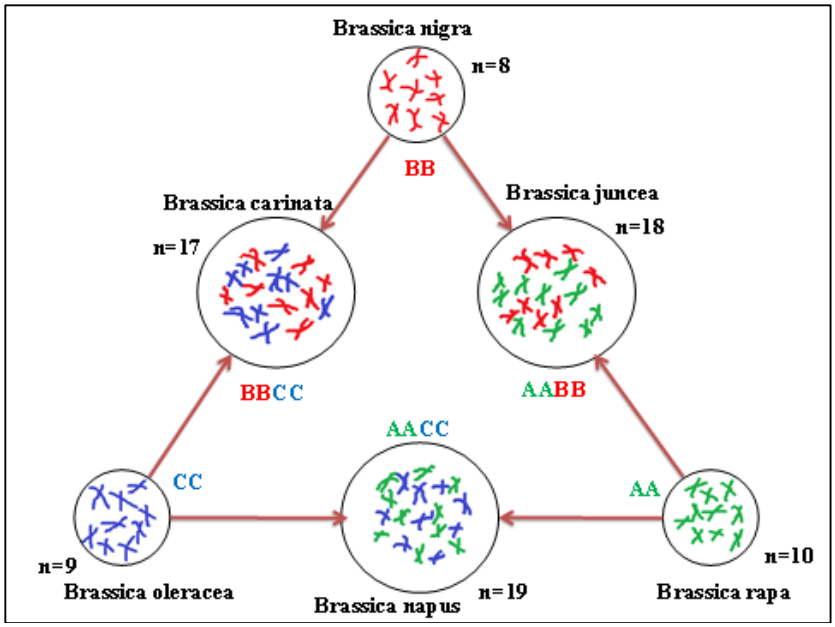


Fig 1: *Brassica* Crop Relationships

Table 1: Classification of mustard-rapeseed

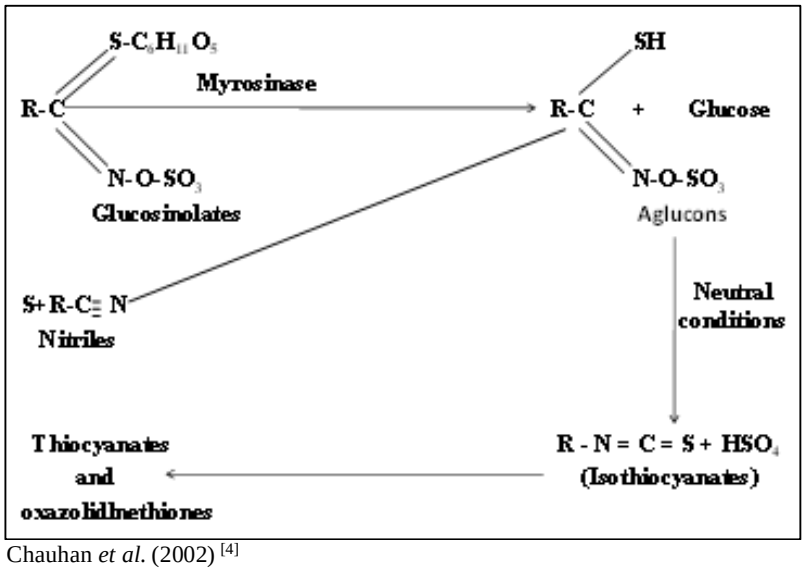
English name	Vernacular name	Botanical name	Characteristics of seeds
Indian mustard / Brown mustard	Rai, ryada, raya, laha, lahta, sasve, herbo	*Brassica juncea*	Seeds are medium sized, round and dark brown or black in colour.
Brown sarson /Rapeseed	Brown-sarson, Bhoori- sarson	*Brassica rapa* var. Brown sarson	Seeds are light reddish, bold and large.
Indian rape / Toria	Toria, tori, lahi	*Brassica rapa* var. toria	Seeds are dark brown, bold and large sized.
Abyssinian mustard/Ethiopian mustard	Karan rai	*Brassica carinata*	Seeds are small, round and reddish brown.
Rocket Salad	Duan, tera, tara, saundh, taramira	*Eruca sativa*	Seeds are light reddish brown in colour and distinctly ovoid in shape.
Rapeseed	Gobhi sarson	*Brassica napus*	Seeds are large and brownish black.
Yellow sarson / Rapeseed	Yellow sarson, Pilli sarson	*Brassica rapa* var. yellow sarson	Seeds are slightly smaller than sarson, ovoid in shape and yellow in colour.

Chauhan *et al.* (2002) [4]

Table 2: Fatty acid composition of rapeseed-mustard seed oil

Fatty acids		Per cent
SFA	Palmitic (16:0)	1 – 3
	Stearic (18:0)	0.4 - 3.5
MUFA / PUFA	Oleic (18:1)	12 – 24
	Linoleic (18:2)	12 – 16
	Linolenic (18:3)	7 – 10
	Erucic (22:1)	40 – 55

Chauhan *et al.* (2002) [4]



Chauhan *et al.* (2002) [4]

Fig 2: Biosynthetic pathway to main fatty acids

Chauhan *et al*. (2002) [4] studied the fatty acid biosynthesis in oil seed *Brassicas*. It follows a pathway similar to that of many other oilseed crops. The carbon chain elongation and desaturation steps are under enzymatic control and amenable to genetic manipulation. Erucic acid is synthesized by the elongation of oleoyl Co A via eicosenoic acid (See figure 2). High erucate strains of rapeseed possessed oleoyl elongase while it is absent in low erucate varieties. Both erucic and linolenic acids are the end products of the biosynthetic pathway in which oleic acid either undergoes decreasing saturation or further chain elongation to form eicosenoic and then erucic acid. The genetic blocks in the chain elongation step of stearic acid controls the biosynthesis of erucic acid from oleic, linolenic and linoleic acids were practically-feasible and achieved by Canadian breeders. A reduction of these fatty acids (linolenic and erucic) is possible if the enzymes for the synthesis of these fatty acids are eliminated. Linoleic and linolenic acids are produced by the same bio-synthetic desaturation pathway, selection for high linoleic acid has resulted in increased levels of linolenic acid, while selection for low linoleic acid tended to result in lower linolenic acid levels.

Table 3: Fatty acid composition of *Brassica* oilseeds

Species	Variety and line	Fatty acid composition (% of total)					
		C16:0	C18:0	C18:1	C18:2	C18:3	Total Sat.
B. napus	Canada com.	3.9	1.9	60.6	19.1	10.6	7.0
	AC Excel	3.6	2.0	65.1	16.6	9.2	6.8
B. juncea	J90-4253	4.3	2.1	43.6	34.6	11.9	7.6
Modified lines							
B. napus	5314-22	3.9	2.0	77.8	8.7	2.8	7.4
	5906-6	2.4	1.1	55.0	29.3	8.5	4.3
	5908-31	3.1	1.2	67.7	16.7	7.8	5.3
B. juncea	J00-6717	3.4	2.8	63.8	16.0	10.6	7.2

Canada com. = Canada commercial *B.napus* canola crop 2002, Canadian Grain Commission harvest survey;
AC Excel and experimental lines=Saskatoon Field Tests.
Rakow *et al.* (2007) [10]

Rakow *et al.* (2007) [10]. Produced yellow-seed forms of *B.napus* from crosses with *B.rapa* and *B.juncea* which have higher seed oil content, and lower meal fiber content to improve the feed value of the meal (See table 3). They also produced germplasm with high oleic/low linolenic acid content to improve the nutritional value of canola oil as well as its technological qualities for use in the production of solid fats without trans-fatty acids. The content of saturated fats in canola oil was reduced to less than 5% of total fatty acids. Inter-specific methodology was also successful in the development of *B. juncea* mustard as an edible oilseed crop with high yield and seed quality.

Table 4: Nature of fatty acid (FA) composition (%) in the seeds of twelve *Brassica juncea* accessions

Accession	Saturated FA		Unsaturated FA			Polyunsaturaed FA	
	Palmitic C16:0	Stearic C18:0	Oleic C18:1	Eicosenoic C20:1	Erucic C22:1	Linoleic C18:2	Linolenic C18:3
AC 00790	2.8	1.3	13.2	7.3	41.2	20.9	9.3
AC 01098	2.8	1.0	17.0	7.8	39.9	19.0	10.3
AC 01244	3.1	0.8	13.1	7.9	41.6	19.7	10.9
AC 01440	3.4	0.8	13.9	7.8	41.1	20.4	9.8
AC 01774	3.2	0.8	12.9	9.7	41.5	18.2	10.3
AC 01847	3.2	0.7	14.5	8.8	37.9	22.2	10.5
AC 02246	2.7	1.2	18.8	8.1	41.3	14.9	7.9
AC 02310	3.1	0.7	11.6	7.9	39.6	19.6	12.3
AC 05041	2.7	0.8	13.3	10.1	43.5	16.3	11.3
AC 05088	3.0	0.9	14.2	10.6	39.4	18.2	10.5
AC 05181	2.8	0.7	12.8	8.1	44.6	16.9	11.8
AC 05184	2.7	1.1	11.4	8.3	45.6	17.1	11.1
Mean	2.9	0.9	13.8	8.5	41.4	18.6	10.5
Standard	0.22	0.19	2.02	0.99	2.12	2.0	1.11

Iqbal *et al.* (2006) [5].

Iqbal *et al*. (2006) [5] observed that FAC in the twelve accessions of *B. juncea* was dominated by the unsaturated erucic acid (41%) (See table 4). The mean monounsaturated oleic acid was 13.8%, which is an undesirable composition for use as cooking oil. Of the polyunsaturated fatty acids, linolenic was below 14% in *B. juncea* which is desired for a better shelf-life and linoleic acids were at comparable levels in both species. The variation within a specific fatty acid was low for all the fatty acids.

Table 5: Fatty acid content (%) of seeds of *Brassica juncea, B. napus* canola cultivars and their F_1 hybrids

Fatty acid	Oleic (C18:1)	Linoleic (C18:2)	Linolenic (C18:3)	Erucic (C22:1)
B. juncea	8	16	11	46
B. napus[1]	47.2 ± 6.0	19.5 ± 1.4	11.0 ± 1.1	0.3 ± 0.2
F_1 hybrids[2]	34.8 ± 3.9	17.1 ± 1.3	10.3 ± 0.9	18.8 ± 1.4

[1]n = 6 cultivars
[2]n = 6 interspecific hybrids Iqbal *et al*. (2006) [5].

Iqbal *et al*. (2006) [5] studied that to stabilize the fatty acid composition and improve the agronomic characteristics of *B. juncea*, a breeding strategy needs to be developed. Interspecific crosses and embryo rescue are a viable method to alter the fatty acid composition of *B. juncea* towards canola quality for human consumption. Both oleic acid (C18:1) and erucic acid (C22:1) show an intermediate value between the parents. Oleic acid showed the most variation from 28 to 41%, while erucic acid varied within a narrow range from 17 to 21% andso did linoleic (C18:2) and linolenic acid (See table 5).

Table 6: Diversified usage of oil with modified fatty acid composition

Zero erucic acid (< 2%)	Nutritionally superior
High linoleic acid (40-50%)	Nutritionally superior
Oleic acid (up to 70%)	Nutritionally superior
Very low linolenic acid (<3%)	Prolonged shelf life, margarines
High erucic acid (40-50%)	Industrial polymers, lubricants, plastic industries, Cosmetics and pharmaceuticals.
High stearic acid (20-40%)	Margarines

Chauhan *et al.* (2002) [4]

Meal quality

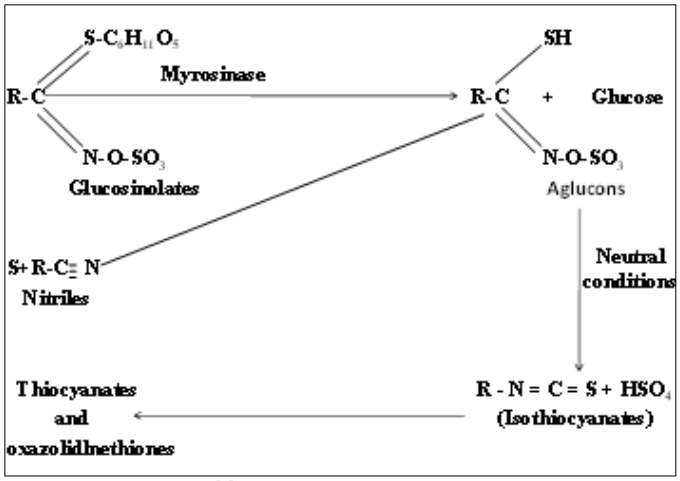
Table 7: Rapeseed-mustard seed meal quality

1	Protein (%)	36-38
2	Carbohydrates (%)	14-15
3	Fiber (%)	10-12
4	Mineral and vitamins (%)	1-1.5
5	Minerals	P, Ca, Mg, K, Mn, Zn
6	Glucosinolate (%)	2-3
7	Tanin (%)	1.6-3.1
8	Sinapine (%)	1-1.5
9	Phyticacid (%)	3-6
10	Crude fiber (%)	12
11	Nutritional properties	Vit. A, Well balance amino acids except methionine, high glucosinolate

Chauhan *et al.* (2002) [4]

Glucosinolate

Besides, high erucic acid in oils, glucosinolate in seed meal is another anti- nutritional factor. Glucosinolates are a group of plant thioglucosides found principally among members of family *Brassicae***.** The vegetative tissue and seed of cruciferous plant contain one or more of over 120 known glucosinolates. Most of the Indian cultivars are rich in total glucosinolates (120 – 280 µmoles/g defatted oil). Indian mustard samples from many countries contain only or mainly sinigrin but those from India and Pakistan have gluconapin as the major component in combination with sinigrin (Chauhan *et al*. 2002) [4].



Chauhan *et al.* (2002) [4]

Fig 3: The general structure of glucosinolates and the end products formed by enzymatic breakdown after cellular disruption

Chauhan *et al*. 2002 explained that the glucosinolates are broken down by the enzyme thioglucoside glucohydrolase commonly known as myrosinase to yield sulphate, glucose and other glucon products (See figure 3). Cleavage products from hydrolysis of glucosinolates like isothiocyanates, oxazolidimethiones and nitriles are very toxic to non-ruminants such as pigs and poultry is a strong non-tariff barrier.

Table 8: Major glucosinolate present in different primary and originating species of oil seed *Brassicas*

Species	Major glucosinolate component
B. juncea	Sinigrin, gluconapin
B. carinata	Sinigrin
B.napus	Progoitrin
B. campestris	Gluconapin, glucobrassicanapin, glucobrassicin
B. nigra	Sinigrin
B. oleracea	Progoitrin

Chauhan *et al.* (2002) [4]

What is "Canola" or "00"?

Varieties having oil with <2% erucic acid and glucosinolates <30 µ moles/gm in defatted seed meal are termed as "Canola" (00).

National Oilseeds and Vegetable Oils Development Board have mentioned that the first zero erucic acid strains of *Brassica napus* were developed in 1961. This was followed by the development of zero erucic acid strains of *Brassica campestris*. *B. napus* cultivar 'Bronowski' was discovered in 1967 containing glucosinolate content of about 12 µ mol/g oil-free meal. It also contained low erucic acid in the seed oil (7%–10%). Therefore, 'Bronowski' gene is the source for low

glucosinolate content, which has been utilized for the development of canola quality cultivars.

Advantages of Canola cultivation (Chauhan *et al.*, 2002) [4]

- To elevate nutritional value of oil and seed meal.
- To fetch remunerative market price.
- To increase market value and versatile usage of oil and seed meal.
- To enhance export potential of seed meal.
- Canola oil is a useful vegetable oil used in a range of cooking.
- Canola meal is the dry matter left after the oil is extracted and is a valuable high protein livestock feed supplement.

Table 9: Varieties with single and double low characteristics in Indian mustard

S. No.	Variety	Year of release	Oil Content (%)	Average Yield (kg/ha)	Special characteristics
1	Pusa Karishma (LES 39)	2004	37-38	1731-2506	Low erucic acid (< 2%)
2	Pusa Mustard -22 (LET 17)	2006	35.5	2070	Suitable for irrigated conditions, low erucic acid.
3	Pusa Mustard-21(LES 1-27)	2006	34-40	2111	Low erucic acid (<2%)
4	ELM-079	2007	38	1600-2000	Suitable for irrigated areas, prone to lodging and shattering, tolerant to Alternaria blight, resistant to white rust, low erucic acid <2%.
5	Pusa Mustard -24 (LET 18)*	2007	36.6	2025	For timely sown irrigated conditions, low in erucic acid.

* Not notified [7].
Kumar *et al.* (2009) [7]

Kumar *et al.* (2009) [7] showed that single low varieties with low erucic acid content (<2%) have been developed in *Brassica juncea*. An effort for developing true canola type varieties (Double low) in *B. juncea* is being taken up under various crop improvement and quality improvement programmes. The list of varieties developed with single and double low characteristics in *Brassica juncea* is given in table 9.

Table 10: Quality strains of rapeseed-mustard registered by TERI, New Delhi

Crop	Strain	INGR no.	Specific traits
B. juneea	Swarna [TERI (OE) M 21]	98001	Zero erucic acid, yellow seeded and early maturity (117 days)
B. napus	Phaguni [TERI (OE) R 03]	98002	Zero erucic acid, early maturity (136 days)
B. napus	Shyamali [TERO (OE) R 09]	98005	Zero erucic acid with high oleic acid (70.1%) and high oil content
B. napus	TERI Gaurav [TERI (00) R 985]	99007	Zero erucic acid, low glucosinolates (15.3 moles/g defatted seed meal), early maturity (125 days) dwarf
B. napus	TERI Garima [TERI (00) R 986]	99008	Zero erucic acid, low glucosinolates

Chauhan *et al.* (2002) [4]

Table 11: Enhanced quality strains registered at ICAR

TERI (OO) R9903 - INGR 04077 [TERI-Uttam]	High oil content, canola quality, early maturing *B. napus*
TERI GZ-05 - INGR 04078 [TERI-Uphaar]	High oleic and linoleic acid, yellow seeded, double low *B. juncea*
TERI (OO) R986-INGR 99007 [TERI-Gaurav]	Early maturing, dwarf double low *B. napus*
TERI (OO) R985-INGR 99008 [TERI-Garima]	High oleic acid, double low *B. napus*
TERI (OE) R09-INGR 98005 [TERI-Shyamali]	Low erucic acid, high oleic *B. napus*
TERI (OE) R03-INGR 98002 [TERI-Phaguni]	Low erucic-acid, early maturing *B. napus*
TERI (OE) M21-INGR 98001 [TERI-Swarna]	Low erucic acid, yellow seeded, early maturing *B. juncea*

Agnihotri *et al.* (2008) [1].

Several economically important traits were transferred via wide hybridization aided with embryo rescue; double low, high oil content, shattering tolerance in *B.napus*, and low erucic/ high oleic acid, yellow seed coat color, double low, and resistance/ tolerance to fungal diseases, *Albugo candida* and *Alternaria brassicae* in *B.juncea*.

In addition to the low erucic acid and low glucosinolate, yellow seed coat colour is another desired characteristic and during recent years the concept of '00' is being expanded to '000' to include this as one of the major breeding objectives.

Advantage of yellow coloured seed coat

Yellow seeds contains 1-2 per cent extra oil in the embryo because the yellow seeds possess a thinner seed coat and higher oil content in the embryo.

Genetic study

Table 12: Performance of '0' and '00' *B.napus* lines

Strain	Yield (kg/ha)	Oil content (%)	Quality status
GSL 6001	1662	40.2	'00' Low erucic and glucosinolate
GSL 6016	1440	41.6	'0' Low erucic acid
GSL 8814	1603	43.7	'0' Low erucic acid
GSL 8884	1492	41.4	'00' Low erucic acid and glucosinolate
GSL 9001	1296	40.8	High erucic acid
GSL 1(check)	1296	40.8	High erucic acid

Chauhan *et al.* (2002) [4]

Chauhan *et al*. (2002) [4] studied that GSL 8814, GSL 6001, GSL 9001, GSL 8884, GSL 6016 of gobhi sarson (See table 12) had yield advantage ranging from 11.1 to 28.3 per cent over GSL 1.

Table 13: Performance of low erucic acid in mustard strains at Pantnagar

Strain	Maturity (days)	Seed yield (kg/ha)	1000 Seed weight (g)	Oil content (%)	Erucic acid (%)
PRQ 9701	135	1319	3.6	41.8	0.0
PRQ 9705	131	1533	3.5	41.8	0.3
PRQ 9707	138	1805	36	41.1	0.6
Varuna	133	978	3.5	40.1	55.6
Kranti	134	1041	3.7	40.7	55.8
CD (0.05)	-	341	-	0.9	-
CV (%)	1.5	10.2	5.5	1.0	-

Chauhan *et al.* (2002) [4]

Chauhan *et al*. (2002) [4] studied that these three strains were significantly out-yielded Kranti whereas PRQ 9505 and PRQ 9707 were significantly superior to both the checks (See table 13).

Table 14: Mean squares, Range, Phenotypic (PCV) and Genotypic (GCV) coefficients of variation, Broad sense heritability and Genetic advance for major fatty acids in oil of Rapeseed –Mustard

Character	Mean+SE	Range	PCV	GCV	h^2	Genetic advance
Palmitic acid	4.222±0.136	2.57-6.18	22.09	21.85	97.9	1.88
Stearic acid	0.914±0.043	0.11-2.98	77.14	67.99	99.6	1.45
Oleic acid	36.31+1.522	7.68-83.86	69.10	68.97	99.6	51.50
Linoleic acid	18.66+0.563	5..89-39.72	43.61	43.51	99.5	16.68
Linolenic acid	9.24±0.325	0.00-20.75	65.83	65.75	99.7	12.49
Ecosinoic acid	6.184+0.406	0.00-14.06	66.19	65.86	99.0	8.35
Erucic acid	23.62±0.738	0.00-47.22	77.33	77.27	99.8	37.57

Singh *et al.* (2002) [13]

Table 15: Correlation coefficients among the fatty acids in oilseed *Brassicas*

Fatty acid	Stearic acid	Oleic acid	Linoleic acid	Linolenic acid	Ecosinoic acid	Erucic acid
Palmitic acid	0.015 0.020	0.531** 0.537**	-0.034 -0.036	-0.362** -0.367**	-0.228 -0.233	-0.577** -0.585**
Stearic acid		-0.016 -0.017	0.463** 0.466**	0.339* 0.341*	-0.334* -0.335*	-0.260* -0.260*
Oleic acid			-0.354** -0.354**	-0.787** 0.789**	-0.563** -0.567**	-0.813** -0.815**
Linoleic acid				0.681** 0.683**	-0.028 -0.026	-0.208 -0.208
Linolenic acid					0.270* 0.274*	0.364** 0.364**
Ecosinoic acid						0.494** 0.497**

* and ** significant at P = 0.05 and P = 0.01 levels, respectively.

Singh *et al.* (2011) [12]

Widest range of variation was observed by Singh *et al.* (2011) [12] for oleic acid followed by erucic acid while highest GCV was recorded for erucic acid followed by stearic and oleic acids (See table 15). High heritability coupled with high genetic advance was exhibited by oleic acid followed by erucic acid. Oleic acid was negatively associated with all fatty acids except palmitic acid, to which it showed positive relation. Erucic acid also exhibited negative correlation with all other fatty acid except ecosinoic and linolenic acids. Stearic acid was positively correlated with linoleic and linolenic acids while it exhibited negative association with ecosinoic and erucic acids. Linoleic acid was positively correlated with lenolenic acid. Results are indicative of the fact that erucic acid can be reduced with increase in other fatty acids.

Correlation and path analysis

Table 16: Path coefficient showing direct (bold) and indirect effects of major fatty acids on oil content in twenty-two *Brassica* genotypes

Fatty acids	Palmitic acid (C16:0)	Stearic acid (C18:0)	Oleic acid (C18:1)	Linoleic acid (C18:2)	Linolenic acid (C18:3)	Eicosenoic acid (C20:1)	Erucic acid (C22:1)	Oil content
C16:0	-0.616	0.067	0.505	0.043	0.181	0.456	-0.428	-0.208
C18:0	-0.188	0.306	0.528	-0.011	-0.263	-0.075	-0.190	-0.107
C18:1	-0.335	0.057	0.970	0.011	-0.472	0.072	-0.843	0.413*
C18:2	-0.146	-0.030	0.269	0.077	0.359	0.242	-0.614	-0.157
C18:3	-0.163	-0.109	-0.287	0.340	0.687	-0.005	-0.081	-0.382
C20:l	-0.341	-0.029	0.155	0.012	-0.304	0.820	-0.675	0.362
C22:1	0.486	-0.122	-0.673	-0.226	-0.031	-0.752	*0.526*	-0.208

Where, *p<0.05

Islam *et al.* (2009) [6]

Islam *et al*. (2009) [6] observed that direct effect of all fatty acids except palmitic acid on oil content was positive (See table 16). Indirect effect of erucic acid through all other fatty acids except palmitic acid on oil content was negative. Indirect effect of palmitic acid via all other fatty acids except erucic acid was positive.

Gene action

Table 17: Genetic of glucosinolate content in rapeseed- mustard

Crop	Gene action
B. juncea	
1. High glucosinolate	Nuclear with additive gene
2. Low glucosinolate	Three partial recessive genes, maternally controlled
B. campestris	
1. Presence of gluconapin and progoitrin	Alleles with partial dominance
B. napus	
1. High glucosinolate	Additive and non-additive
2. Progoitrin	Additive-dominance
3. Aliphatic glucosinolate	Maternally controlled / additive and non- additive gene

Chauhan *et al.* (2002) [4] Glucosinolate content *per se* or specific component(s) is controlled by complex genetic mechanism and also maternally influenced (See table 17).

Table 18: Glucosinolate profile and content (µ mol / g-1 seed) of key Indian mustard cultivars

Genotype	2-Propenyl	3-Buteny	4-Pentenyl	Others	Total
Varuna	22.0±3.2	89.8±2.7	2.3±0.2	1.7±0.2	115.8±4.1
Pusa Bold	37.1±4.5	80.4±3.6	1.9±0.1	1.9±0.2	121.3±5.4
Kranti	21.3±3.5	86.7±4.1	1.5±0.0	3.2±0.1	115.9±5.2
Krishna	27.3±1.2	74.7±2.3	2.5±0.1	2.7±0.3	107.2±3.1
Bio 902	22.7±4.6	81.3±3.9	1.2±0.0	1.9±0.1	107.1±5.9
Sej 2	31.5±3.3	75.4±4.1	0.9±0.0	2.7±0.1	110.5±5.3
RH 30	25.3±3.8	87.1±3.6	1.4±0.2	2.1±0.1	115.9±5.2
RLM 198	30.2±2.1	65.5±3.2	0.4±0.0	1.6±0.1	97.7±3.7
RLM 514	23.1±2.8	71.8±2.3	0.5±0.0	2.0±0.1	97.4±4.1
RLM 619	19.8±3.1	85.6±2.3	1.6±0.0	2.4±0.1	109.4±4.9
RL 1359	21.3±1.5	72.9±1.8	1.8±0.1	2.6±0.2	98.6±3.2
PBR 91	33.3±2.0	83.2±3.1	1.9±0.1	1.7±0.2	120.1±2.1

Sodhi *et al.* (2002) [14]

The glucosinolate content in current varieties of Indian mustard (*Brassica* juncea), the main oilseed *Brassica* type, ranges from 97.4 to 121.3 µ moles g-1 seed (See table 18). Most of the Indian strains contain 3-butenyl as the major aliphatic glucosinolate component, 2-propenyl is another important component. The higher level of 3-butenyl in Indian cultivars is in contrast to Europian *B.juncea* types which have 2-propenyl as the major glucosinolate.

Breeding for low glucosinolate content

Agronomically well-adopted mustard cultivars *i.e.* Varuna, Pusa bold and RL 1359 have been mainly used for developing desired quality cultivars using Heera / BJ 1058 as donors for low meal glucosinolate content at Agricultural Universities in Ludhiana, Hisar, Pantnagar besides, Tata Energy Research Institute, New Delhi and National Research Centre (Rapeseed-Mustard) at Bharatpur. Pedigree breeding and backcross methods have been used to achieve '00' characteristics in good agronomic base. Many experimental strains with low glucosinolate content and superior agronomic backgrounds are still inferior in yield than the conventional mustard cultivars. Attempts to develop improved '00' versions by hybridizing Heera with Pusa bold were not very successful (Malode *et al*. 1995) [9]. Inspite of intensive efforts, only limited progress has been achieved in mustard due mainly to restricted availability of superior low glucosinolate donors. In addition, large population size in initial segregating generations is almost always limited by severe restriction imposed on sample size owing to difficult and time-consuming analytical procedures for glucosinolate content. So it can be said that several experimental stocks with superior agronomic attributes are still inferior in yield than the standard mustard cultivars.

Use of doubled haploidy and whole genome selection, based on AFLP markers, during backcrossing has enabled transfer of low glucosinolate trait to a predominant mustard variety, Varuna (Sodhi *et al*. 2003) [15]. Pending the development of high yielding '00' mustard strains, efforts are being made to commercialize early maturing canola quality *B.napus* strains, especially for cultivation in cooler areas of north-west India. Two canola quality varieties (PAU bred pure line GSC-5 and Adventa hybrid Hyola 401) have been recommended recently by Punjab Agricultural University for general cultivation in Punjab. All these strains possess <25 µ moles glucosinolates g-1 defatted meal. Inspite of these achievements, a significant breakthrough is still awaited in terms '00' mustard genotypes.

Breeding for low erucic acid content

Table 19: Inheritance of erucic acid in two crosses of Indian mustard

Generation	Total plants studied	Observed frequency					Expected ratio	df	χ^2 value	P value
		<2	2-14	15-27	28-40	>40				
Varuna x LES-39										
P1	10	-	-	-	7	3	-	-	-	-
P2	10	10	-	-	-	-	-	-	-	-
F1	20	-	-	18	2	-	-	-	-	-
F2	203	9	52	84	46	12	1:4:6:4:1	4	2.4	0.66
B1	98	-	-	19	58	21	1:2:1	2	3.4	0.18
B2	108	21	64	23	-	-	1:2:1	2	3.8	0.15
Varuna x LES-1-27										
P1	10	-	-	-	4	6	-	-	-	-
P2	10	10	-	-	-	-	-	-	-	-
F1	20	-	-	12	8	-	-	-	-	-
F2	205	8	48	81	57	11	1:4:6:4:1	4	3.1	0.54
B1	100	-	-	23	58	19	1:2:1	2	2.9	0.24
B2	101	19	58	24	-	-	1:2:1	2	2.7	0.26

Singh *et al.* (2015) [11]

Varuna produces very high erucic acid content i.e. >40%, while LES-39 and LES-1-27 possess very low erucic acid content i.e. <2%. The F1 plants of both the crosses, Varuna x LES-39 and Varuna x LES-1-27 showed medium to high erucic acid content comparable to Varuna indicating low erucic acid is a recessive character.

The segregation pattern of the erucic acid trait in the F_2 generation of crosses Varuna x LES-39 and Varuna x LES-1-27 fits well in 1:4:6:4:1 theoretical ratio (χ^2 = 2.4 and 3.1, respectively), indicating digenic inheritance of erucic acid trait with additive gene action. This digenic nature of the low erucic acid trait was confirmed from the results of backcross populations as well (B_1: χ^2 = 3.4 and 2.9, respectively; B_2: χ^2 = 3.8 and 2.7, respectively). These results of digenic recessive nature of low erucic acid content are in agreement with the previous findings (Bhat *et al*. 2002; Chauhan *et al*. 2003) [2, 3]. Non-significant differences for erucic acid content in F_1 of both the crosses and their respective reciprocals indicated the absence of maternal influence, which signifies equal contribution of both the parents in the inheritance of erucic acid trait. These results were in accordance with the earlier findings reported by Liu and Liu (1989) [8] that fatty acids were genetically governed by genotype of embryo without maternal effect in *B.juncea*. It was also observed that the segregants showing low erucic acid (<2%) have also recorded high oleic acid (42-47%). This clearly suggests that breeding for low erucic acid will offer multiple nutritional advantages by increasing the content of desirable fatty acid like oleic acid.

The study showed that low erucic acid varieties can be developed through introgression of the two recessive genes through backcross breeding. The digenic recessive mode of inheritance with additive gene effect also conveyed that varieties with low erucic acid can be developed through pedigree breeding by recognizing transgressive segregants in the early segregating generations.

Problems in Quality Breeding

- Most of the quality traits are polygenic in nature. Therefore, selection for quality traits during the segregating generations is very difficult.
- Most of the quality traits are difficult to estimate and evaluate, therefore, quality breeding imposes considerable demand on resource, and time including money.
- Many quality traits have low heritability and are markedly affected by the environment. This retards the progress under selection.

Conclusion

- Erucic acid in oil and glucosinolate in deoiled cake are two nutritionally toxic undesirable factors in rapeseed-mustard.
- Selection pressure can be profitably applied to reduce erucic acid and glucosinolate.
- Erucic acid is significantly and positively correlated with glucosinolate content, while both are significantly and negatively correlated with oleic and linoleic acid.
- Major fatty acids in oil are simply inherited, while glucosinolate content is controlled by complex genetic mechanism and is also maternally influenced.
- Back cross method is most widely used for quality improvement in rapeseed-mustard. During backcrossing, use of doubled haploidy and whole genome selection, based on AFLP markers has enabled transfer of low glucosinolate trait. Also the varieties with low erucic acid can be developed through introgression of two recessive genes through backcross breeding.

References

1. Agnihotri A, Gupta K, Prem D, Sarkar G, Mehra V, Zargar SM. Genetic enhancement in rapeseed-mustard for quality and disease resistance through *in vitro* techniques, 2008.
2. Bhat MA, Gupta ML, Banga SK, Raheja RK, Banga SS. Erucic acid heredity in *B juncea* - some additional information. Plant Breed. 2002; 121:456-458.
3. Chauhan JS, Tyagi MK, Tyagi P. Genetic analysis of fatty acid profile in a cross of Indian mustard. [*B. juncea* (L.) Czern and Coss]. In 6th Agricultural Science Congress. Bhopal, India, 2003, 13-15.
4. Chauhan JS, Tyagi MK, Kumar PR, Tyagi P, Singh M, Kumar S *et al*. Breeding for oil and seed meal quality in rapeseed-mustard in India. Agric. Rev. 2002; 23:71-92.
5. Iqbal MCM, Weerakoon SR, Peiris PKD. Variability of fatty acid composition in interspecific hybrids of mustard

Brassica juncea and Brassica napus. Cey. J Sci. (Bio. Sci.). 2006; 35:17-23.
6. Islam MS, Rahman L, Alam MS. Correlation and path coefficient analysis in fat and fatty acids of rapeseed and mustard. Bangladesh J Agril. Res. 2006-2009; 34:247-253.
7. Kumar A, Sharma P, Thomas L, Agnihotri A, Banga SS. Canola cultivation in India: scenario and future strategy. 16th Australian Research Assembly on Brassicas. Ballarat Victoria, 2009.
8. Liu DF, Liu HL. Inheritance of erucic and Eicosenoic acids in Brassica juncea. Heriditas-Beizing. 1989; 11:17-20.
9. Malode SS, Swamy RV, Khalatkar AS. Introgression of '00' quality characters in *Brassica juncea* cv. Pusa bold. Proc. 9th Int. Rapeseed Congress., 4-7 July, 1995, Cambridge-UK, 1995, 431-433.
10. Rakow G, Eckstein JA, Raney JP. Rapeseed genetic research to improve its agronomic performance and seed quality. HELIA. 2007; 30:199-206.
11. Singh J, Yadava DK, Vasudev S, Singh N, Muthusamy V, Prabhu KV *et al*. Inheritance of low erucic acid in Indian mustard [*Brassica juncea* (L.) Czern. and Coss.]. Indian J Genet. 2015; 75:264-266.
12. Singh M, Tomar A, Mishra CN, Srivastava SBL. Genetic parameters and character association studies in Indian mustard. J Oilseed Brassica. 2011; 2:35-38.
13. Singh SP, Jeena AS, Kumar R, Sachan JN. Variation for quality parameters and fatty acids in *Brassica* and related species. Agric. Sci. Digest. 2002; 22:205-206.
14. Sodhi YS, Mukhopadhyay A, Arumugam N, Verma JK, Gupta V, Pental D. Genetic analysis of total glucosinolates in crosses involving a high glucosinolate Indian variety and a low glucosinolate line of Brassica juncea. Plant Breed. 2002; 121:508-511.
15. Sodhi YS, Mukhopadhyay A, Arumugam N, Verma JK, Gupta V, Pental D *et al*. Transfer of low glucosinolate trait into an Indian mustard variety of *Brassica juncea*. Proc. 11th Int. Rapeseed Congress., 6-10 July, 2003, Copenhagen-Denmark, 2003, 497-499.
16. WHO. Healthy diet. Available at https://www.who.int/news-room/fact-sheets/detail/healthy-diet. Accessed 26 February, 2018, 2019.
17. Wikipedia contributors Triangle of U. Wikipedia, The Free Encyclopedia. Available at: https://en.wikipedia.org/w/index.php?title=Triangle_of_U&oldid= 859479928. Accessed 2 March, 2018, 2019.