Breeding for oil quality in Rapeseed and Mustard : A Review

ABSTRACT

Varieties having oil with < 2% erucic acid and glucosinolates <30 u moles/g defatted seed meal are termed as "Canola" (00) and fetch premium in the International market. Indian cultivars possess high erucic acid (About 50 %) and high glucosinolates (100-280 u moles/g defatted seed meal). Indian rapeseed-mustard breeding programme was also reoriented to accommodate quality parameters and lay emphasis to develop "Canola" varieties. (Chauhan *et al.,* 2002). Work is in progress and efforts have been underway to improve the agronomic base of low yielding zero erucic lines and to recombine low erucic acid and low glucosinolate to develop '00' varieties. From the review of this topic it can be inferred that zero erucic, low linolenic, high oleic and high lenoleic fatty acid and double zero genotypes are available and some double zero varieties are also under cultivation in India and western countries. This indicates that there is a possibility of development of '0' and '00' cultivars in our country. There for more emphasis is required for improvement of oil and meal quality in Rapeseed-mustard through extensive breeding work.

Key word: Rapessed mustard, erucic acid, glucosinolate, fatty acid.

Introduction

The Brassicaceae, contains about 3500 species and 350 genera, is one of the 10 most economically important plant families. It is distinguished on the basis of the presence of conduplicate cotyledons (i.e. the cotyledons are longitudinally folded around the radical) and/ or two-segmented fruits (siliquae), which contain seeds in one or both segments, and only simple hairs, if present. Oilseed Brassicas also referred to as rapeseed-mustard, an important group of oilseed crops in the world, comprise eight cultivated crops of tribe Brassiceae within the family Cruciferae (Brassicaceae) (Willis, 1973). The word 'rape' and 'mustard' have been derived from the word *rapum* meaning turnip and European practice of mixing the sweet 'must' of old wine with crushed seeds of black mustard [*Brassica nigra* (L.) Koch] to form a hot paste, respectively (Hemingway, 1976).

Classification of Rapeseed & Mustard

Rapeseed-mustard crops in India comprise traditionally grown indigenous species, namely toria [*Brassica campestris* syn. *B. rapa* L. var. toria, 2n (AA) = 20], brown sarson [*B.campestris* syn. *Brassica rapa* L. var. brown sarson, 2n (AA) = 20], yellow sarson [*B. campestris* syn. *Brassica rapa* L. var. yellow sarson, 2n (AA) = 20], Indian mustard [*B. juncea* (L.) Czernj & Cosson, 2n (AABB) = 36], black mustard [*B.nigra* (L.) Koch 2n(BB) = 16] and taramira [*Eruca sativa/ vesicaria* Mill., 2n (EE) = 22] which have been grown since about 3,500 BC along with non-traditional species like gobhi sarson [*B. napus* L. ssp *oleifera* DC var. *annua* L., 2n (AACC) = 38], white mustard [*Sinapis alba* L. 2n (SS) = 24] and Ethiopian mustard or karan rai [*B. carinata* A. Braun, 2n (BBCC) = 34]. Toxopeus and Oost (1985) suggested that the name *B. campestris* be changed to *B. rapa* since floral parts of two are indistinguishable and both have the same chromosome number (2n = 20) and are fully inter-fertile.

Rapeseed & Mustard scenario

India is the fourth largest oilseed economy in the world. Among the seven edible oilseeds cultivated in India, rapeseed-mustard contributes 28.6% in the total oilseeds production and ranks second after groundnut sharing 27.8% of oilseed economy. Rapeseed-mustard group of oilseed crop is valued in terms of quantity and quality of the oil and protein of seed. Increasing oil content and improving the quality of oil and seed meal has been the major objectives of rapeseed mustard breeding programme in India as well as in world since long. Oil quality is determined by the nature and content of fatty acid. Erucic acid in oil of the Indian rapeseed mustard varieties is quite high (Chauhan *et al.*, 2011).

The area, production and productivity of rapeseed-mustard in the world was 33.62 million ha, 61.4 million tons and 1815 kg/ha respectively during 2013–14. In India, production of 7.4 million tons from an area 6.3 million ha. with an average productivity of 1176kg/ha during 2012-13. In Chhattisgarh area, production and productivity was 0.05 million ha., 0.02 million tons 464 kg/ha, respectively during 2012-13(www.drmr.res.in).

Chemical Composition

Rapeseed-mustard seeds, in general, consist of 35-45 per cent oil, 17-25 per cent proteins, 8-10 per cent fibres, 6-10 per cent moisture and 10-12 per cent extractable substances. Oil is predominantly (92-98%) triacylglycerol of fatty acids (Ct6-C22). The remaining portion of the oil is composed of a number of lipid compounds including unsaponifiable hydrocarbons, tarpins, sterols, tocopherols, glycolipids and phospholipids. Of all the fatty acids, erucic acid (C22) comprises nearly 50 per cent. In comparison to Indian varieties of mustard (8. *juncea)* and rape (8. *campestriS)* European varieties of gobhi sarson *(8. napuS)* and turnip rape (8. *campestriS)* had .low erucic acid. The seed meal of rapeseed-mustard mainly consists of protein (35-40%), carbohydrates (14-15%), fibre (10-12%), moisture (68%), ash (4-6%), minerals and vitamins (11.5%), glucosinolates (2-3%), tanin (1.6-3.1%), sinapin (1-1.5%), and phytic acid (3-6%). Among all the constituents fibre, tanin, phytic acid, glucosinolate and sinapin lower the feed, value of seed meal, otherwise could bean useful feed for animals as it possesses well balanced amino acids composition of proteins comparable to that of milk.

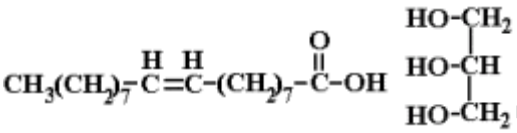
Fats and oil

Oils are fats that are liquid at room temperature, like the vegetable oils used in cooking. Oils come from many different plants and from fish. Oils are NOT a food group, but they provide essential nutrients. Fats are short, medium and long chain molecules comprised of many essential fatty acids connected together. Just like protein is comprised of amino acids, so fats are comprised of fatty acids. Some fatty acids are essential to our diet, without which life cannot be sustained. This is to say that the essential fatty acid (EFA) cannot be manufactured in the body in sufficient amounts to supply the needs of the body and so is considered essential to diet for health and well being (Gurr and Harwood, 1991).

Fats and oils improve texture, taste and palatability of food and good source of higher calories to the density (9 Kcal/g) as compared to cereals and pulses (4.9 Kcal energy /g).They are also essential for absorption and transportation of fat soluble vitamins (Vitmin A, E and K) fat soluble antioxidants and nutrients. Dietary intake of fats plays an important role in nutritional and metabolic process vegetable oils are the only source of essential fatty acid to the body. EFA are precursor of a group of chemically related compounds called prostaglandins.

Prostaglandins play a key role in regulating physiological process like lowering the blood pressure, preventing blood clot in the arteries, vascular damage in the brain and heart, becoming the newest “Miracle Drugs” due to their anti proliferative activities.

Triglycerides are the main constituents of vegetable oils and animal fats. Triglycerides have lower densities than water (they float on water), and at normal room temperatures may be solid or liquid. When solid, they are called "fats" or "butters" and when liquid they are called "oils". A **triglyceride**, also called triacylglycerol (TAG), is a chemical compound formed from one molecule of glycerol and three fatty acids.



Oleic Acid

Glycerol or Glycerin

Glycerol is a trihydric alcohol (containing three **-OH** hydroxyl groups) that can combine with up to three fatty acids to form monoglycerides, diglycerides, and triglycerides. Fatty acids may combine with any of the three hydroxyl groups to create a wide diversity of compounds. Monoglycerides, diglycerides, and triglycerides are classified as *esters* which are compounds created by the reaction between acids and alcohols that release water (**H_2O**) as a by-product.

The main biosynthetic pathway of fattyacid synthsis is as follows:

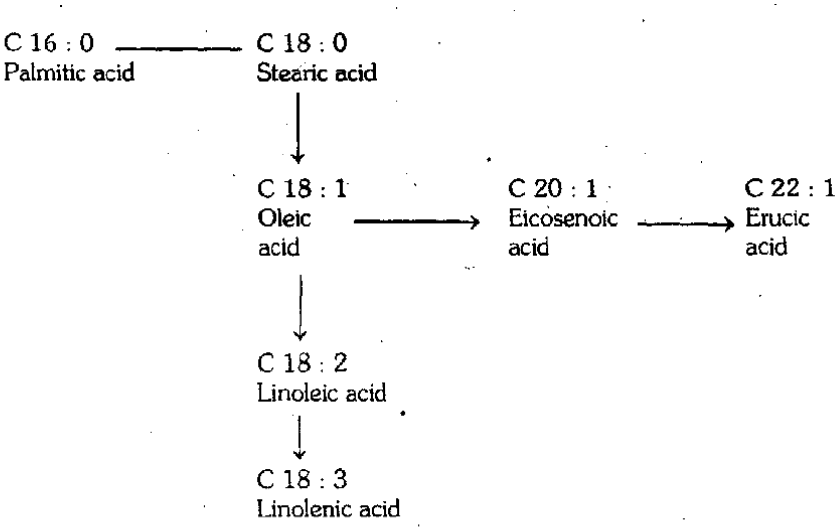


Fig. 1. Biosynthetic pathway of main fatty acids (Jonsson, 1977)

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 biosynthetic desaturation pathway, selection for high linoleic acid has resulted in increased levels of linolenic acid, while selection for low linoleic acid levels.

Table No. 1 Fatty acid composition of rapeseed-mustard seed oil

Fatty acids		**Per cent**
SFA	**Palmitic (16:0)**	**1-3**
	Stearic (18:0)	**1-3**
MUFA/ PUFA	**Oleic (18:1)**	**8-40**
	Linoleic (18:2)	**10-29**
	Linolenic (18:3)	**5-18**

	Erucic (22:1)	**42-57**

QUALITY IN RAPESEED & MUSTARD

Oil Quality

May be defined as the types and the proportion of fatty acids present in the oil of rapeseed & mustard.

Linoleic acid (LA) is a carboxylic acid with an 18-carbon chain and two *cis* double bonds; the first double bond is located at the sixth carbon from the methyl end. It is a colorless liquid at room temperature.

Linolenic acid (18:3) although polyunsaturated and an essential dietary fatty acid, is undesirable in edible oil because of its three double bond structure which are prone to auto-oxidation resulting in off-flavours and reduced shelf-life of the oil.

Erucic acid

Erucic acid is a monounsaturated omega-9 fatty acid. It is known as monounsaturated because it has only one double-bonded carbon atom in its fatty acid chain.

The term omega-9 refers to a group of fatty acids, including erucic and oleic acid, which have their double carbon bond occurring at the ninth position from the end of their acid chains. This is known as the n-9 position.

Glucosinolate

The glucosinolates are a class of organic compounds that contain sulfur and nitrogen and are derived from glucose and an amino acid.

Classification of quality based on the presence of fatty acid.

- **Single Zero (Single Low):-** Varieties having low erucic acid (< 2%) but high percentage of glucosinolate in seed meal.
- **Double Zero (Double Low or Canola): -** Variety with less than 2% of erucic acid and less than the 30 u moles/g of glucosinolate in seed meal.
- **Triple Zero(Bell 1984):-** Double low yellow coated variety as also low in fibre it is referred as triple varieties.

Table:2 Diversified usage of oil with modified fatty acid composition.

Type	**Use**
Zero erucic acid < 2%	**Nutritionally superior**

High erucic acid (40-55%) (>80%)	**Industrial polymers, lubricants, plastic industry Cosmetics, pharmaceuticals.**
High stearic acid (20-40%)	**Margarines**
Epoxy fatty acids	**Polymers**
Wax *esters*	**Resins**
High petroselinic acid	**Cosmetics, lubricants**
Very low linolenic acids(<3%)	**Prolonged shelf life. Margarines**
High linoleic acid (40-50%)	**Nutritionally superior**
Oleic acid (upto 70%)	**Nutritionaly superior**

Breeding for Quality:-

Development of canola or '00' varieties and their cultivation in India would have many fold advantages:

- 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.

Quality breeding in India started in 1970's. Efforts during this phase confined to evaluation of existing variability available in the indigenous germplasm. However, neither zero erucic acid nor glucosinolate types could be identified. In indigenous selection/ collections, glucosinolate ranged from 63 to 102 u moles/g on seed basis. The achievement of this phase was the identification of SM 1, a low erucic acid (10%) accession. This genetic stock, however, had poor agronomic base and could not be exploited either as commercial variety or donor source for quality improvement. The slow pace of research got strengthened with the launching of the Indo-Swedish collaborative project in 1975. Several 'o' erucic strains were identified during this phase.

In India, the rapeseed oil and seed meal quality improvement programme has the following objectives:

- Evaluation of available low erucic acid/low glucosinolate lines of *B. juncea* and *B. napos* for yield and quality parameters.
- Development of low erucic and/or low glucosinolate rapeseed-mustard varieties.
- Basic studiesto understand the genetics and breeding behaviour of erucic acid and glucosinolate content.

- Generation of information on the reaction '0/00' types to endemic pests and diseases.

The concerted efforts on the evaluation of germplasm indigenous and exotic, revealed wide range of variation for different fatty acids in Indian mustard. Breeding efforts have been underway in India since 1970 to reduce glucosinolate content in the seed of rapeseed mustard varieties up to 30 micro moles/g defatted seed meal (low or 0) and erucic acid up to 2% (low or 0) as well as combining both to develop double zero or double low varieties to meet the internationally acceptable standard of oil and seed meal (Chauhan *et al.,* 2002). reviewed the quality improvement programme of rapeseed-mustard in India. First low erucic acid variety, Pusa Karishma of Indian mustard and first double low variety, GSC 5 of gobhi sarson was released in 2004 and 2005, respectively. Presently, five low erucic varieties have been released in *Brassica juncea*. In gobhi sarson (*B.napus*), 5 double low (low erucic and low glucosinolate) varieties have been released.

The current efforts are to recombine low erucic acid with low glucosinolate content in Indian mustard and refining the agronomic base to improve yield potential of double low gobhi sarson strains (Chauhan *et al.,* 2011).

Source of quality traits:

Table 3. Identified traits

Genotypes having glucosinolate content less than 30micro mole/g defatted meal			
B. juncea	**NUDH –YJ-1 NUDH-YJ-2**	***B. napus***	**HNS99(OE)3,NUDB-09, NUDB-26-11**
Genotypes having low erucic acid (Single low)			
B. juncea	**LES -17-1, LES 21, LES 38, YSRL 9-18-23, TERI (OE) M 21**	***B. napus***	**NUDB-26-11, Phaguni [TERI (OE) R 03], Shyamali [TERI (OE) R 09],**
Genotypes having low erucic acid (<2%) and low glucosinolate i.e. Canola type (<30µ moles/ g fat free meal)			
B. juncea	**Heera, NUDHYJ-5, TERI GZ-05**	***B. napus***	**BCN 14, CAN 138, GSC 5 (GSC3A), TERI (00) R 985, TERI (00) R 986,TERI (00) R**

			9903,

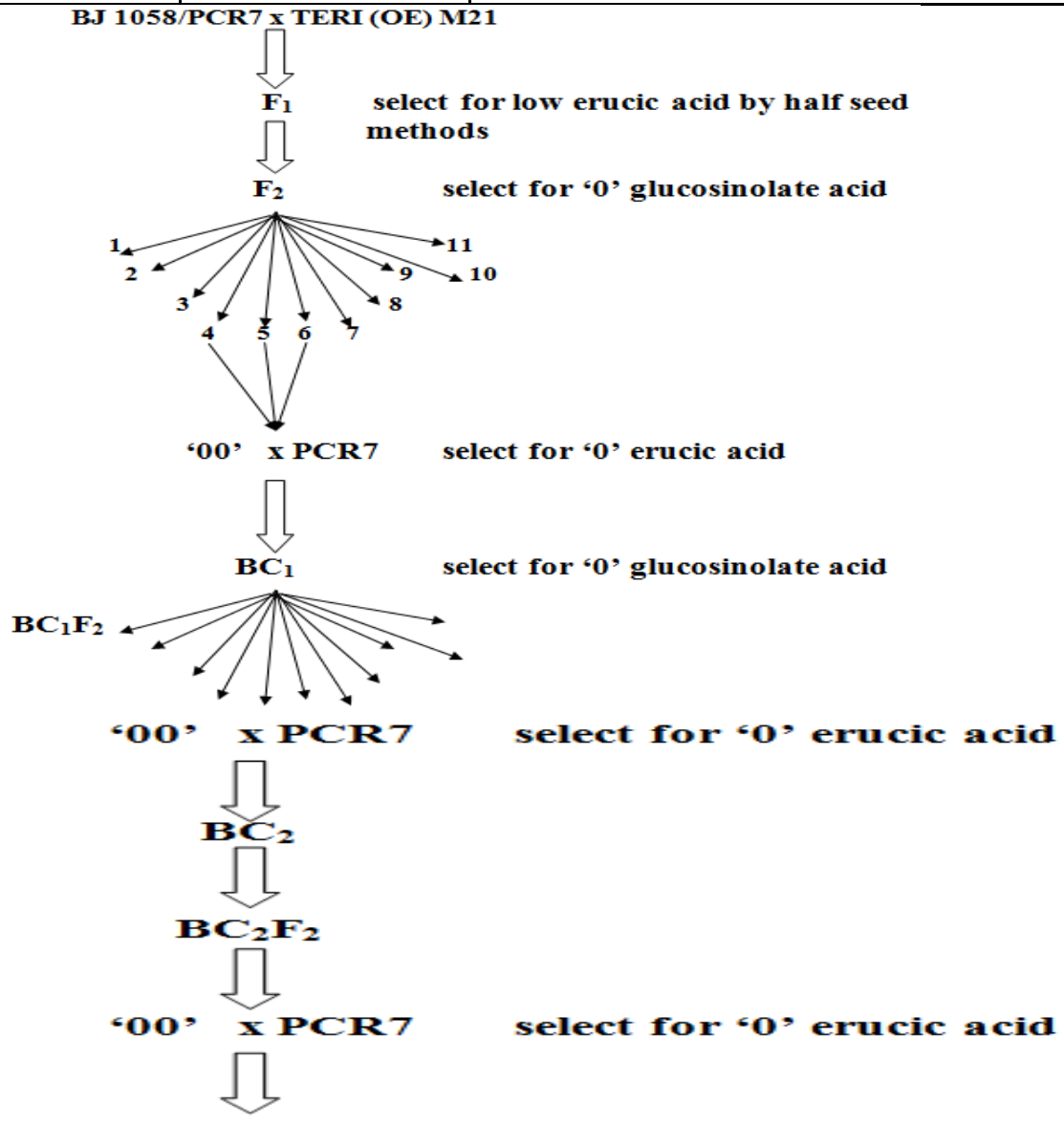
Table 4. The enhanced quality rapeseed-mustard strains registered at ICAR

TERI GZ-05 - INGR 04078 [TERI-Uphaar]	**High oleic and linoleic acid, yellow seeded, double low *B. juncea***
TERI (OO) R9903 - INGR 04077 [TERI-Uttam]	**High oil content(43%), canola quality, early maturing *B. napus***
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***

Table 5. Donor and their unique features

National identity	**Donor/other identity**	**Novelty /unique feature(s)**
Brassica juncea		
IC 296501	**Heera**	**Low glucosinolate content (16.96 µ moles/g of seed) and low erucic acid in oil (0.1%)**
IC 296507	**NUDHYJ 5**	**Low glucosinolate content (9.3 µ moles/g of seed) and low erucic acid (0.1%)**
IC 296689	**NDYR 8**	**Yellow seeded mustard with high oil content (45.7%)**
IC 296690	**NDYR 10**	**Yellow seeded mustard with high oil content (45.8%)**
IC 405233	**TERIGZ 05**	**High oleic and linoleic acid, yellow seed and double low material**
IC 546947	**PRQ-2005-1**	**Low erucic acid with yellow seed**
IC 296827	**NUDB 38**	**Early maturing double low gobhi sarson**

		B. napus.
IC 296828	**NUDB 42**	**Early maturing double low gobhi sarson B. napus.**
***B. compestris* (*toria*)**		
IC 552726	**PHOP 2-2**	**High oleic acid (70.1%), low erucic acid (0.2%) and brown seeded**
IC 552727	**PHOT 8-2-11**	**Low linolenic acid (3.63%) and brown seeded**



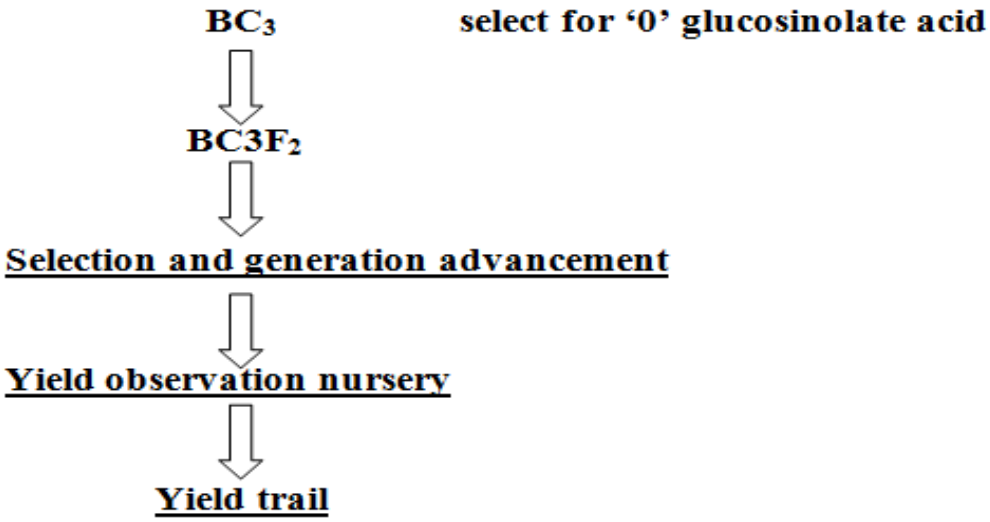


Fig 2. Backcross Method

Quality Improvement through Backcross Method

Half seed technique

- To breed rapeseed cultivars without erucic acid
- Seeds are allowed to germinate on wet filter paper untill the radicle has a length of 10 mm.
- Outer cotyledons are removed and analysed for fatty acid composition by gas liquid chromatography or paper chromatography.
- Only those test embryo lacking erucic acid are planted and grown to maturity.
- Synthesis of eicosenoic and erucic acids is controlled by two multiple allelic loci.

Table 6. Breeding methods and distributions

S.No.	Breeding methods	Species	Name of varieties/lines	Remarks
01	Selection	*B. compestris*	Span	Zero Erucic acid
		B. napus	oro	Zero Erucic acid
02	Mutation	*B. juncia*		3.6% linolenic acid
03	Hybridization	*B. juncia*	EC287711,Shiva 1, QM 13, QM14, QM39 RW 21-61	Low erucic acid

			Zem-1 **Zem-2**	**Zero & low Erucic acid respectively**
Biotechnological Approach				
04	**Somaclonal Variation**	***B. juncia***	**Pusa jai kisan**	**Isolated from Varuna**
05	**Anther culture**	***B. napus***	**Winfield**	**Canola type**
06	**Transgenic**	***B. compestris***	**Laurical**	**Lauric acid content**
07	**Transgene(s) Antisense fae 1**	***B. juncea* and *B. napus***		**Low eruvic acid**

Status of quality improvement programme in India.

Brassica juncea

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

B. napus

- Fortyone low erucic acid and/or low glucosinolate strains evaluated under AICRP R&M till 2010–11 cropping season.
- Hyola 401, first double low erucic gobhi sarson hybrid was released during 1996; five having low erucic and low glucosinolate have been identified for released up to December, 2010.Six strains (two having low erucic and four having low erucic and low glucosinolate) have been registered with National Bureau of Plant Genetic Resources (ICAR), New Delhi.

***B. rapa* var. toria**

- Two strains of toria, viz PHOP 2-2 (INGR 07033) having high oleic (70.1%) and low erucic acid and PHOT 8-2-11 (INGR 07034) with low linolenic acid (3.03%) have been registered with National Bureau of Plant Genetic Resources (ICAR), New Delhi.

Table 7. Varieties recommended for specific conditions/ possessing particular trait.

Crop Species	Traits	Name of variety/varieties /trait
Indian mustard	**High oil content**	**Narendra Swarna Rai 8, NRCDR 02, Rohini**
	Quality trait (low erucic acid)	**Pusa Karishma, Pusa Mustard 21, (low erucic acid) Pusa Mustard 22, Pusa Mustard 24, ELM 079 (RLC 1)**
Gobhi sarson	**Quality traits Low erucic acid and glucosinolate**	**GSC 5, Hyola 401, NUDB 26-11, OCN 3 (GSC 6), TERI Uttam Jawahar**

Problems in Quality Breeding

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

Future Strategies

- Diversification of sources of low glucosinolates and their utilization in the breeding programmes
- Development of inexpensive and rapid method for mass screening for fatty acids and glucosinolates to facilitate early generation selection
- Use of biotechnological tools like dihaploid breeding to improve the selection efficiency and marker aided selection

References:

Banga, S. S. 2008. Breeding for improved quality in Brassica oilseeds. *Sustainable production of oilseeds rapeseed - mustard technology. Agrotech Publishing Academy* Udaipur-313 002:118-130.

Bell, J. M. 1984. Nutrients and toxicants in rapeseed meal: a review. *J. Ani. Sci*. 58: 996– 1010.

Chauhan, J. S., Singh, K. H., Singh, V. V. and Kumar, S. 2011. Hundred Years of Rapeseed- Mustard Breeding in India: Accomplishments and Future Strategies**.** *I. J. Agril. Sci.* 81 (12): 1093–1109.

Chauhan, J. S., Tyagi, M. K., Kumar, P. R., Tyagi, P., Singh, M., and Kumar, S. 2002. Breeding for oil and seed meal quality in Rapeseed - mustard in india – A review. *Agric. Rev.* 23(2):71-92.

Gurr, M.I. and Harwood, J.L. 1991. Fatty acid structure and metabolism.In: Gurr MI and Harwood J L (eds) *Lipid Biochemistry, An Introduction*. London: Chapman and Hall:21-27.

Hemingway, J. S. 1976. Mustard: *Brassica* spp. and *Sinapis alba* (Cruciferae).*Evolution of Crop Plants*.Simmonds (Ed.). Longmans, London: 56–69.

Kumar, S. and Chauhan, J. S. 2010. *Rapeseed-mustard: diatatry and nutritional aspect.* Directorate of Rapeseed-Mustard Research, Sewar, Rajasthan Bharatpur-321 303:1-3.

NBPGR. 2011. *Annual Report 2010–11*. National Bureau of Plant Genetic Resources, Pusa, New Delhi: 109.

USDA. 2010. United States Department of Agriculture - Rapeseed area, yield and production Table No.15. http://www. fas. usda. gov/psd online/psd report. Asps.

Willis, J.C. 1973. *A Dictionary of the Flowering Plants and Ferns. Eighth Edition*. Cambridge University Press, Cambridge et alibi. 1245pp.