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Status and perspective of canola quality rapeseed-mustard cultivation in India : a review

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Abstract

Rapeseed-mustard (*Brassica* spp.) constitutes an important source of edible oil and meal. However, traditional rapeseed-mustard varieties accumulate high amount of erucic acid and glucosinolate in their seeds. These quantitatively inherited anti-nutritional factors drastically reduce the quality of rapeseed-mustard seed oil and meal for consumption purposes. Development of rapeseed-mustard varieties with low erucic acid and glucosinolate content, therefore, has been an important breeding objective worldwide. Breeding programmes for developing canola quality (<2% erucic acid in oil and <30 µmoles of glucosinolate/g of oil-free seed meal) rapeseed began in Canada as early as 1956. In India, however, the major efforts were made in the 1970's with the launch of Indo-Swedish and Indo-Canadian collaborative projects (1979–94) to improve rapeseed-mustard oil and meal quality and consequently varieties low in erucic acid and/or glucosinolate were developed. However, the existence of a significant yield gap between potential yield and the yield obtained in frontline demonstrations indicates the need for further refinement in technology packages for these varieties. In addition, it is also important to educate the masses about the health benefits of canola quality oil. This paper reviews the status and perspective of breeding and cultivation of canola quality rapeseed-mustard varieties, besides highlighting the impact of technology on profitability, strategies, policies, and future outlook for canola quality oil promotion in India.

Keywords**:** *Canola, erucic acid, glucosinolate, impact of technology, rapeseed-mustard*

Introduction

Rapeseed-mustard (*Brassica* spp.) is a major group among the oilseed crops in the world. It constitutes the world's third important source of edible oil. Nutritional and functional properties of oils are determined by their fatty acid composition and the distribution pattern of fatty acids within the triacylglycerol molecule (Pham and Pham, 2012). A good percentage of essential fatty acids (linoleic acid; C18:2 and linolenic acid; C18:3) in rapeseed-mustard oil makes it desirable from nutritional point of view, but high amount of erucic acid; C22:1 (40-57%) lowers its utility as edible oil (Agnihotri *et al*., 2007 and Singh *et al.*, 2014). Consumption of oil with >20% erucic acid in the diet causes myocardial fibrosis and lipidosis in monkeys (Ackman *et al*., 1977). The meal remaining as by-product after extraction of oil is another valuable product obtained from the rapeseed-mustard seeds. It contains about 40.0% protein with a favorable composition of amino acids, including comparatively high content of essential sulphuric amino acids, methionine and cysteine (Downey and Bell, 1990). In addition, it is also rich in minerals (Ca, Mg and P) and contains vitamin B_4 and E. However, in comparison to the other popular sources such as soybean, rapeseed-mustard meal contains high amounts of anti-nutritional compounds called glucosinolate (Wanasundara, 2011). Cleavage products from hydrolysis of glucosinolate reduce the feed palatability by affecting the iodine uptake by the thyroid glands, especially in non-ruminants including pigs and poultry (Bell, 1984). Therefore, the amelioration of nutritional qualities by developing new varieties having alternative oil and meal characteristics has been an important objective in quality breeding of rapeseed-mustard.

Breeding programmes for developing rapeseed varieties low in erucic acid and glucosinolate began in Canada as early as 1956. Many improved varieties were developed and registered under the trademark 'Canola' by the Canola Council of Canada. Subsequently, the term 'Canola' became a generic name referring to rapeseed varieties containing < 2% erucic acid in oil and < 30 µmoles of glucosinolate/g of oil-free seed meal. These varieties are also referred as 'double low' or double zero ('00'). The acceptance for canola quality rapeseed oil gained momentum after 1970's and since then it has been widely recognized as healthy edible oil. In countries like Canada, Japan, USA and Australia canola is the preferred cooking oil. The trade in canola oil and seed over the last few decades indicates that the demand for these commodities is steadily rising (Fig 1). In India, however, the share of canola oil is very low in the total vegetable oil consumption. Nevertheless, the scenario is changing with the rise of an economically empowered middle class and the spread of health consciousness among the population (Agnihotri and Kaushik, 2002). The domestic production and import of canola oil has been on the rise in the recent years. During the year ending October 2012, India imported more than 16,000 tonnes of canola oil which commands a price premium over other edible oils in the Indian market. The potential demand for canola oil in India has been estimated to be nearly 0.85 million tonnes which is nearly equivalent to 10% of current edible oil import by India (Commodity online, 2012). Therefore, to meet the existing and potential domestic demand for canola quality rapeseed-mustard oil and to exploit the growing trade potential for the commodity, expansion in area and production of canola type rapeseed-mustard varieties is very important.

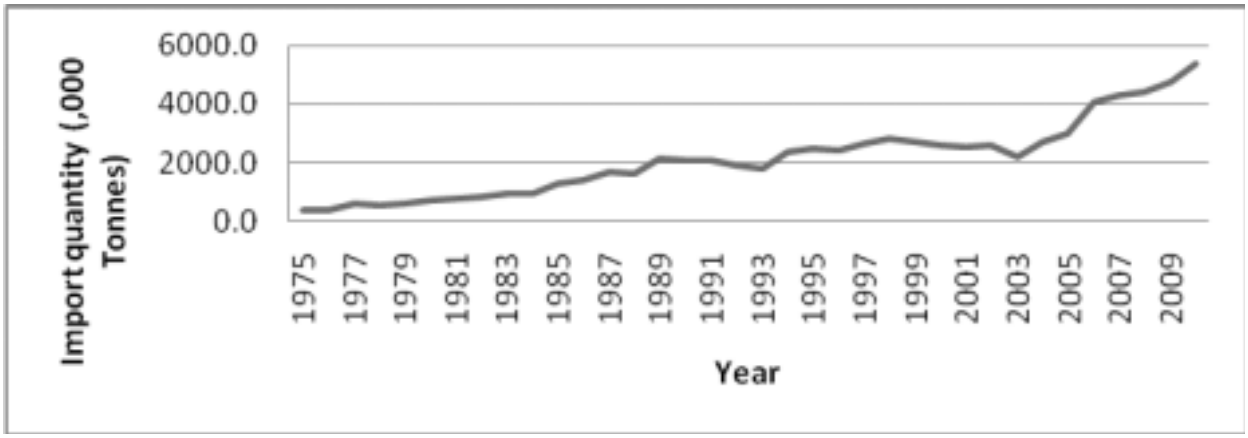

Fig 1 : Trend of global import of rapeseed oil

The objective of this paper is to critically evaluate the prospects and challenges for breeding and cultivation of canola quality rapeseed-mustard varieties in India, and to assess its potential as a strategic component in enhancing edible oil availability in the country.

Breeding for canola quality rapeseed-mustard

In the beginning, hybridization coupled with pedigree selection was the predominant method to transform traditional varieties into canola types. Later, interspecific and intergeneric crosses were made which allowed breeders to rapidly create many new combinations of genes with desirable characteristics (Agnihotri and Kaushik, 1998). Subsequently, the introduction of half-seed method equipped the breeders with a perfect control of their material. Selection by means of half-seed technique considerably reduces the amount of operational breeding material (Downey and Harvey, 1963). In recent years, more specialized tools like mutagenesis (Barve *et al*., 2009), marker-assisted selection (MAS), and genetic engineering (transgenic) have revolutionized the way in which quality breeding was undertaken (Agnihotri, 2010). An array of molecular markers has been used for mapping and cloning of the genes and quantitative trait loci (QTLs) controlling erucic acid and glucosinolate content in Brassicas.

They have also been employed for the manipulation of erucic acid and glucosinolate profiles in Brassicas through MAS for double low genotypes 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).

Genetics of erucic acid and glucosinolates content in rapeseed-mustard

Inheritance of erucic acid

The erucic acid content has been shown to be under the control of the embryonic genotype and governed by a single non-dominant gene in diploid species, *Brassica rapa* (AA, 2n = 20) (Dorrell and Downey, 1964) and *B. oleracea* (CC, 2n = 18) (Chen and Heneen, 1989). However, in amphidiploid species; *B. napus* (AACC, 2n = 38), *B. juncea* (AABB, 2n = 36) and *B. carinata* (BBCC, 2n = 36), it is governed by two additive genes (Harvey and Downey, 1964; Kirk and Hurlstone, 1983; Getinet *et al*., 1997). Of the two genes in the amphidiploid species, one occurs in each respective genome (Anand and Downey, 1981; Fernandez-Escobar *et al*., 1988; Bhat *et al*., 2002).

Ecke *et al*. (1995) and Jourdren *et al*. (1996) mapped the two loci *viz. E1* (*Bn-FAE1.1*) and *E2* (*Bn-FAE1.2*) determining erucic acid content in *B. napus* population using random fragment length polymorphism (RFLP) and random amplified polymorphic DNA (RAPD) markers, respectively. These two loci were later assigned to two independent linkage groups by Thormann *et al*. (1996).

Studies on *Arabidopsis thaliana* mutants deficient in very long chain fatty acids revealed that the fatty acid elongase (*FAE1*) coding for ß ketoacyl-CoA synthase (KCS) is the key gene required in the seeds for the elongation from oleic acid (C18:1) to erucic acid (C22:1) (Kunst *et al*., 1992; James *et al*., 1995; Lassner *et al*., 1996). The functional role of the *FAE1* gene was ascertained by genetic transformation of a zero erucic acid *B. napus* genotype (Lassner *et al*., 1996). In 1998, two cDNAs of gene-encoding KCS were isolated from a *B. napus* genotype by using sequence information from the *Arabidopsis FAE1* gene. Subsequently, two *FAE1* genes (*FAE1.1* and *FAE1.2*) in *B. napus* were mapped by detecting polymorphism through polyacrylamide gel electrophoresis from the amplification products of the partial *FAE1* genes (Fourmann *et al*., 1998). These two genes were found to co-segregate with two QTLs, *E1* and *E2*, controlling erucic acid content (Jourdren *et al*., 1996). Bhat *et al*. (2002) reported that the gene E_2 associated with the A genome contributes greater to the total erucic acid content in *B. juncea* than the gene E_1 located on the B genome and confirmed the unequal contributions of the two genes (E_1= 12%, E_2= 20%) to high erucic acid content in conventional digenomic *Brassica*. Gupta *et al*. (2004) isolated two full-length *FAE1* genes in *B. juncea* from one high and one low erucic acid lines and detected single nucleotide polymorphisms (SNPs) in the two genes by sequence comparison. These two genes were mapped to *B. juncea* genome and their association with the erucic acid trait was established.

Inheritance of glucosinolate

Glucosinolate biosynthesis in Brassicas has quantitative and sporophytic inheritance and is regulated by complex genetic factors (Kondra and Stefansson, 1970; Halkier and Gershenzon, 2006). It has been extensively studied through QTL mapping and gene cloning in *Arabidopsis* (Compos de Quiros *et al*., 2000; Li *et al*., 2008). Aliphatic glucosinolates are reported to be controlled by two different sets of genes, *GSL-ELONG* and *GSL-PRO*, controlling side chain elongation and *GSL-OXID*, *GSL-ALK* and *GSL-OH* controlling the modification of side-chain carbons (Halkier and Gershenzon, 2006). In *B. napus*, three to five QTLs regulating glucosinolate content are reported by several workers (Toroser *et al*., 1995; Howell *et al*., 2003). In *B. juncea*, genetic studies on aliphatic glucosinolates reported two to eight genes (Love *et al*., 1990b; Stringam and Thiagarajah, 1995; Sodhi *et al*., 2002; Chauhan *et al*., 2007). Two QTLs for 2-propenyl, three QTLs for 3-butenyl glucosinolates, and five QTLs for total seed aliphatic glucosinolates were detected by Cheung *et al*. (1998) and Mahmood *et al*. (2003). Ramchiary *et al*. (2007) reported six QTLs for seed glucosinolate content in *B. juncea*. These large effect QTLs were fine

mapped using a candidate gene approach and comparative sequence analyses of *Arabidopsis* and *B. oleracea* (Bisht *et al*., 2009). The results revealed the existence of epistasis and additive effects of glucosinolate genes in different genetic backgrounds in *B. juncea*.

Development of canola quality rapeseed-mustard: historical perspective

Genetic improvement in rapeseed for low erucic acid content was initiated firstly in Canada. A mutant with low level of erucic acid was identified from German spring type *B. napus* forage cultivar 'Liho' in 1959 (Stefansson *et al*., 1961). In 1968, the first low erucic acid *B. napus* 'Oro' was selected from crosses between Nugget (*B. napus* selection) and Liho. In 1971, another low erucic *B. napus* 'Zephyr' was selected from a cross between 'Oro' and 'Target' (*B. napus* selection), with improved oil and protein content. In the same year, the first low erucic acid *B. rapa* 'Span' was developed from low erucic *B. rapa* selections and Arlo (Canada's first *B. rapa* variety). In 1973, another low erucic *B. rapa* variety 'Torch' was released as a selection from 'Span' with improved yield. Initial breakthrough for low glucosinolate breeding came with the development of *B. napus* cultivar 'Bronowski' (Kondra and Stefannson, 1970). It is regarded as an only donor source of low glucosinolate trait to canola quality *B. napus* and *B. rapa* cultivar. World's first double low *B. napus* and *B. rapa* cultivars, 'Tower' and 'Candle', respectively, were developed by pedigree selection of interspecific crosses in 1970s (Stefansson and Downey, 1995; McVetty *et al*., 2009).

First low erucic acid lines (Zem-1 and Zem-2) of *B. juncea* were discovered from commercial mustard samples in Australia (Kirk and Oram, 1981). A single BC_1F_3 plant of *B. juncea* named 'BJ-1058' developed from interspecific cross between an Indian type 3-butenyl glucosinolate containing *B. juncea* selection and a 'Bronowski-gene(s)' containing low glucosinolate *B. rapa* (Love *et al*., 1990a). The first canola quality *B. juncea* 'Arid' and 'Amulate' was released in Canada in the year 2002. The 'National *Brassica* Improvement Program (NBIP)' was initiated in Australia in 1992 which developed more productive canola quality *B. juncea* lines (JN004, JR033, JR042, JR046, JR048, JR049, JR050, JR055, JR136, JO6019 etc). 'Dune' was the first canola *B. juncea* cultivar released in Australia in the year 2007. In *B. carinata*, the first low erucic line was developed in 1988 by interspecific hybridization between low erucic *B. napus* genotype 'Duplo' and *B. carinata* germplasm 'C101' followed by recurrent backcrossing to *B. carinata* parent (Fernandez-Escobar *et al*., 1988).

In India, traditional rapeseed-mustard seed oil accumulates high amounts of erucic acid comprising 40-57% of total fatty acids and 80-160 µmole glucosinolate/g of oil-free seed meal (Agnihotri and Kaushik, 2002). In India, although breeding efforts to develop double low varieties in *Brassica* cultivars have been underway since 1970, the research was strengthened with the launch of Indo-Swedish collaborative project in 1975. Several low erucic strains were identified during this phase. Thirty exotic low/double low lines including Zem-1 and Zem-2 of *B. juncea*; Torch, Tobin, Candle and Span of *B. rapa*; Tower, Altex and Westar of *B. napus* were introduced for testing in India. This collaborative project remained operative until 1988. However, the introduced strains were low yielding due to their non-adaptability to Indian growing conditions. Objective of the Indo-Canadian collaborative project from 1979-94 was also to improve rapeseed-mustard oil and meal quality. Indo-Australian project (2004–10) between Indian Council of Agricultural Research (ICAR) and Australian Centre for International Agricultural Research (ACIAR) was specially aimed for developing double low quality genotypes (Chauhan *et al*., 2011).

In 1996-97, a 'National Network project for Improvement of Oilseed *Brassica* quality' was established by ICAR to transfer improved quality traits from exotic germplasm into agronomically suitable cultivars of *B. juncea* (Banga *et al*., 1988 and Chauhan *et al*., 2002a). Efforts were also made to improve the oil quality through interspecific hybridizations (Agnihotri *et al*., 1995), mutagenesis (Barve *et al*., 2009), and genetic engineering

Table 1: Low erucic acid and/or low glucosinolate rapeseed-mustard germplasm registered in India

Germplasm	Unique features	Acc. No.
B. *juncea*		
Heera	Low glucosinolate (16.96 µmoles/g oil-free seed meal) and erucic acid (0.1%) content	IC 296501
NUDH-YJ- 5	Low glucosinolate (9.3 µmoles/g oil-free seed meal) and erucic acid (0.1%) content	IC 296507
TERI-Swarna	Zero erucic acid content, yellow seeded, early maturing	IC 296684
TERI-Uphaar	High oleic and linoleic acid content, low glucosinolate and erucic acid content, yellow seeded	IC 405233
PRQ-2005-1	Low erucic acid content, yellow seeded	IC 546947
B. *napus*		
TERI-Phaguni	Zero erucic acid content, early maturing	IC 296685
TERI-Shyamali	Zero erucic acid and high oleic acid (70.1%) content	IC 296688
TERI-Gaurav	Zero erucic acid and low glucosinolate (15.3 µmoles/g oil-free seed meal) content, early maturing	IC 296731
TERI-Garima	Zero erucic acid, low glucosinolate (12.2 µmoles/g oil-free seed meal) and high oleic acid (57%) content	IC 296732
NUDB-38	Double low, early maturing	IC 296827
NUDB-42	Double low, early maturing	IC 296828
TERI-Uttam	Double low, high oil content (> 43%), early maturing	IC 405232

Source: Chauhan *et al*., 2011

(Sivaraman *et al*., 2004). Initial efforts were concentrated on the development of genetic stocks for low erucic acid and low glucosinolate in the indigenous cultivars using exotic donor sources. As a result, two zero erucic acid germplasm: TERI-Swarna, and PRQ-2005-1, and three double low germplasm: Heera, TERI-GZ-05 and NUDH-YJ-5, were registered in *B. juncea*. In addition, seven double low germplasm of *B. napus*: TERI-Phaguni, TERI-Shyamali, TERI-Gaurav, TERI-Garima, NUDB-38, NUDB-42, and TERI-Uttam were, also registered (Table 1). To expedite the development of low erucic acid/double low varieties, crop improvement programmes were initiated in a coordinated network made under the umbrella of 'All India Coordinated Research Project on Rapeseed-Mustard (AICRP-RM)'. Under the AICRP-RM quality improvement programmes, Australian (JR042, JN010, JN033, JN031, JN049, JN009, JN004, JM016 and JM006) and Chinese (CBJ001, CBJ002, CBJ003 CBJ004 and XINYOU5) double low lines were used as donors. These efforts led to the development and release of first double low variety 'GSC-5' of *B. napus*, and first low erucic acid variety 'Pusa Karishma' of *B. juncea* in 2003 and 2004, respectively. Presently, eight low erucic varieties of *B. juncea* and six double low varieties of *B. napus* are released in India. Fatty acid profile and glucosinolate content in low erucic acid and double low rapeseed-mustard varieties released in India is presented in Table 2.

Status of technology and its impact on profitability of canola cultivation in India

The resource allocation for canola cultivation is dependent on a number of factors including the level of technology, price policy, and relative profitability (Stiglitz, 1996). The rapeseed-mustard in India is mainly grown in fragile and high risk rainfed regions (Nanwal *et al*., 2012). The data from frontline demonstrations (47 demonstration trials) conducted under the AICRP-RM, during the period 2007-08 to 2011-12 on *B. napus* varieties with canola quality, were analyzed to quantify the impact of technology on different parameters presented in Table 3. An additional net monetary returns of INR 4385/ha and

Table 2: Fatty acid profile and glucosinolate content in low erucic acid and double low rapeseed-mustard varieties released in India

Varieties	Pedigree	Fatty acid (%)						Glucosinolate (¼moles/g oil free seed meal)	Year of release
		SFA	Oleic (C18:1)	Linoleic (C18:2)	Linolenic (C18:3)	Eicosenoic (C20:1)	Erucic (C22:1)		
B. *juncea*									
Pusa Karishma	Pusa Basanti x Zem-1	4.3	42.3	32.6	18.5	0.05	0.85	55.4	2004
Pusa Mustard-22	Pusa Barani x Zem-2	6.2	42.6	35.3	13.9	0.98	1.1	62.8	2006
Pusa Mustard-21	Pusa bold x Zem-2	4.6	42.1	38.2	13.3	1.2	0.7	70.5	2006
RLC-1	QM4 x Pusa Bold	7.6	33.4	40.4	16.9	1.0	0.8	60.1	2007
Pusa Mustard-24	(Pusa Bold x LEB-15) x LES-29	8.4	39.8	36.3	13.4	3.7	0.11	57.2	2007
RLC-2	QM-4 x Pusa Bold	4.3	35.7	35.8	22.7	0.77	0.77	58.9	2012
B. *napus*									
Hyola-401 (Hybrid)	44002A x 4154R	4.7	60.2	21.1	10.8	2.3	0.9	17.7	2000
GSC-5	Hyola-401 x (Agat x GSL-8888)	4.3	61.1	23.9	6.5	2.7	1.5	21.5	2003
TERI-Uttam-Jawahar	ISN706 x *Raphnobrassica*	5.3	54.0	25.5	9.0	4.6	1.6	12.2	2004
GSC-6	(NECN-13 x Tribute) x NECN-13	4.6	70.0	15.8	5.5	2.5	1.4	19.0	2007
NUDB 26-11	Derived from mutation of Westar	1.5	68.7	14.0	6.1	2.9	1.3	27.5	2007

*SFA denotes ‘saturated fatty acids’ (Palmitic & Stearic)

Table 3: Impact of improved technology on canola cultivation

Description	Value
Yield Increase Over Farmers Practice (%)	12.4
Yield Increase (kg/ha)	208.3
Average Additional Net Monetary Returns (Rs/ha)	4385
Increase in Cost of cultivation (%)	10.5
Incremental Benefit Cost Ratio	4.9

***** Annual reports of AICRP-RM (1997-2010)

12.4% yield improvement over farmers practice were offered on adoption of improved technology package recommended for canola varieties (Table 3). Assuming the potential yield of 2685 kg/ha for the variety TERI-Uttam-Jawahar as a bench mark potential yield (Chauhan *et al*., 2012), the yield gap between potential yield and the yield obtained in frontline demonstration during the last five cropping seasons was 30.1%. The high level of yield gap indicates the need for further refinement in technology package for canola varieties. However, the adoption of the recommended technologies can reduce this yield gap significantly. For each additional rupee invested in improved technology package over the existing farmers practice, a return of 4.9 rupees can be expected. The demonstrated high returns to the additional capital invested will increase the level of investment in technology and its adoption by the farmers.

Future outlook

Enhancement of area and productivity of canola varieties in India are facing policy, technological, and environmental constraints. Because both low erucic acid and low glucosinolate traits are inherited independently, a large number of segregating plant populations need to be screened which require precise and efficient screening techniques. Combining double low traits with good yielding capability is also difficult because they are quantitative traits governed by multiple recessive genes. Conventional breeding methods, therefore, must be coupled with biotechnological tools to transfer double low characters in high yielding cultivars. Marker-assisted selection would be helpful in enhancing precision and selection efficiency for double low traits. Genetic engineering methods to transfer double low traits in high yielding cultivars of *B. juncea* are also quite helpful, but still at nascent stage. Development and use of non-destructive analytical method will make the analysis easier, less cumbersome and less time consuming. The diverse challenges in policy and environmental domains also need critical evaluation. The geographic spread of the *B. napus* canola varieties is limited mainly in the states of Punjab, Himachal Pradesh and Haryana. At present, the cultivation of canola crop accounts for less than 1.0 % of the total area under rapeseed-mustard cultivation in India. The rain-fed and low input intensive cultivation practice of rapeseed in high risk production environment is a major factor in low yield realization. Plant breeding programmes with varietal development objective in canola should be given priority in research resource allocation considering the potential quantum of economic benefits. Raising the share of canola crops in total rapeseed-mustard cultivation is important for increasing the quality of edible oil available to the consumers. In India, since 80% of the area under rapeseed-mustard is planted with *B. juncea* varieties, greater emphasis has been given in developing low erucic acid *B. juncea* varieties. The traditional preference for qualities like pungency in India can make the low erucic acid *B. juncea* varieties (with medium to high glucosinolate content in seed meal) more popular among consumers. Use of improved exotic donors in canola variety development programmes could improve the agronomic potential of existing low erucic/double low varieties. Cultivation of *B. napus* in areas vacated late (upto mid-December) by the previous Kharif crops such as cotton and paddy, can increase the acerage under canola varieties and give more economic returns than the late sown crops including barley and wheat. Demand for the low erucic oil/canola oil can be increased through awareness for its health benefits. Promotion of contract farming and price support schemes are necessary for enhancement of low erucic/canola oil production. Ensuring availability of high quality low erucic acid edible oil can play a crucial role in the economy, because India could become a key contributor to the world's healthy rapeseed-mustard oil production.

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