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Fatty Acid Profiling in Rapeseed Mustard (*Brassica* species)

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A B S T R A C T

An experiment was conducted during 2014-2015 to study the oil and fatty acids content in 26 genotypes of *Brassica* species. Significant differences ($p \leq 0.05$) were observed among the genotypes for the oil quality, viz. oil content and fatty acids content. Oil content is in the range of 32.67–39.47%, 37.82–40.56% and 40.35–41.43% in *Brassica juncea*, *Brassica napus* and *Brassica rapa* seeds, respectively. The saturated fatty acid (Palmitic acid) content is in the range of 3.08–3.85, 3.70–5.15, 2.75–3.73% in *Brassica juncea*, *Brassica napus* and *Brassica rapa*, respectively and oleic acid content is in the range of 0.80–48.70, 16.15–37.98 and 6.21–16.15 in *Brassica juncea*, *Brassica napus* and *Brassica rapa*, respectively. Significant variation ($p \leq 0.05$) was also observed for Linoleic acid content and linolenic acid. Linoleic acid content varied from 11.00- 45.30, 18.57- 26.93 and 14.08- 18.18% in *Brassica juncea*, *Brassica napus* and *Brassica rapa*, respectively and linolenic acid content varied from 11.10- 26.72, 9.99- 17.23 and 9.82- 26.66% in *Brassica juncea*, *Brassica napus* and *Brassica rapa*, respectively. The Erucic acid, another important trait also differed significantly amongst the *Brassica* species genotypes being 0.80- 49.40, 10.04- 34.96 and 43.77- 49.99%. The minimum Erucic acid content was recorded in *Brassica juncea* genotypes PM-24 (0.80%) and significantly at par with PM-21, PM-22, Pusa Karishma and Nov Gold, whereas maximum Erucic acid content were recorded in Pusa Bold (49.40%), DGS-1(34.96%) and RSPT-02 (49.99%) in *Brassica juncea*, *Brassica napus* and *Brassica rapa*, respectively. Significant variability in fatty acids content were noted in rapeseed mustard. The present study can be utilized in the breeding programme to develop qualitative genotypes with higher oil content and yield

Introduction

Rapeseed mustard is the preferred source of vegetable oil occupies a premier place in the world among all the oilseed crops and is also used as animal fodder, manure, condiment and has various industrial implications. The fatty acid content varies among various members of the *Brassicaceae* family and is determined by factors like the type of species, variety and the environmental conditions to which it is subjected (Mekki, 2013). *Brassica* species are

enriched in various saturated and unsaturated fatty acids. The saturated fatty acids (SFAs) includes Palmitic acid whereas, the unsaturated fatty acids are either monounsaturated (MUPAs) i.e. erucic acid and oleic acid or polyunsaturated fatty acids (PUFAs) such as omega-3- alpha- linolenic acid and omega-6- linoleic acid which are nutritionally important. The presence and absence of these fatty acids determine the nutritional quality of the edible oils (Bhattacharya *et al.,* 2012). Higher amount of Palmitic acid in edible oil leads to increased serum cholesterol (Kumar *et al.,* 2014). Erucic acid which forms about 50% of the fatty acid composition of mustard oil is considered to be anti-nutritional when present <2% in the edible oil, whereas higher amount of erucic acid in mustard oil makes it industrially important (Sharafi *et al.,* 2015). It has been reported that an ideal edible oil should comprise of low saturated fats (<6%), high oleic acid (>50%), moderate amounts of linoleic (<40%) and low linolenic acid (<14%) (Potts *et al.,* 1999). In Indian rapeseed mustard varieties, the oil obtained has higher amounts of erucic acid (35.7–51.4%) and low amounts of oleic acid (10-15%) (Kaushik and Agnihotri, 2000; Chauhan *et al.,* 2007) which out lies the International Standards. Therefore, for meeting the quality standards there is an immediate need to develop *Brassica* varieties with low as well as high levels of erucic acid that can be used for edible and commercial purposes, respectively. Apart from checking the levels of erucic acid, another important objective should be to increase the level of oleic and linoleic acid and to reduce the amount of linolenic acid in the edible oil (Robbelen, 1991). One important approach towards this is to develop *Brassica* germplasm having low undesirable fatty acids by transferring the potential genes, preferably from the wild species into the cultivated ones through efficient breeding programmes (Pospisil *et al.,* 2007). For the development of the improved *Brassica* germplasm, comprehensive knowledge of the fatty acid composition of various *Brassica* species (*B. juncea, B. carinata, B. oleracea, B. nigra, and B. rapa*) becomes a pre-requisite.

Keeping this in view, the present study was carried out with the aim of evaluating various *Brassica juncea, Brassica rapa and Brassica napus* varieties for their fatty acid composition and oil content as well as assessing the variation for these traits within the species. The information thus obtained will prove useful in breeding programmes directed towards improvement of oil content and fatty acid composition in *Brassica.*

Materials and Methods

Plant materials

The seeds of 26 *Brassica* species including *Brassica juncea* (14 varieties), *Brassica napus* (7 varieties) and *Brassica rapa* (5 varieties) were procured from various sources (Table 1). The procured seed material was cultivated at the experimental field of SKUAST- Jammu. Three rows of each genotype were planted and the recommended package practices and plant protection measures were followed. Seeds were harvested when the plants attained complete maturity. Harvested seeds were used for oil content and fatty acid analysis.

Determination of oil content

The oil content from the matured seeds was extracted in the soxhlet apparatus using petroleum ether as solvent for 6 h according to the AOCS method (AOCS, 1993). Seeds of different mustard varieties were dried at 40 °C for 4 h in an oven to reduce the moisture level to 4- 5%. The dried seeds were then thoroughly ground and mixed with ether for extracting the total oil content. Subsequently, ether was removed from the oil by rotary

evaporator under pressure and the oil content was calculated from the weight of oil and seeds using the formula,

$$Oil\ content\ (\%) = \frac{\text{Oil weight}}{\text{Seed weight}} \times 100$$

Fatty acid profiling

The fatty acid profiling of the *Brassica* varieties was carried out using Gas- Liquid Chromatography (GLC). Fatty acid methyl esters (FAME) of oil sample from each variety were developed according to the method described by Goli *et al.,* (Goli *et al.,* 2008). Agilent 6890N gas chromatography equipped with a Flame Ionization Detector (FID) was used for FAME analysis. The oven injector and detector temperatures were regulated at 230 and 250^0C, respectively. Ultra-pure nitrogen gas was used as carrier.

The peaks of FAME were analysed by comparing their retention time to that of the known standards which had been subjected to similar separation conditions. The amount of individual fatty acids was expressed as % of the total fatty acids.

Results and Discussion

Total oil content (%)

The total oil content, range and mean values of 26 *Brassica* varieties is shown in Table 2. Among all the *Brassica* species cultivated in India, *Brassica rapa* is the richest oil bearing species followed by *Brassica napus* and *Brassica juncea.* The seed oil content among the genotypes varied from 32.67- 39.47% in *Brassica juncea,* 37.82- 40.56% in *Brassica napus* and 40.35- 41.43% in *Brassica rapa* (Table 2). The results indicate that sufficient variation exists for oil content within the genotypes of various species studied. Existence of variation for seed oil content is the remarkable feature of *Brassica* oil seeds which makes them a potential resource for exploitation in future plant breeding programmes. The results obtained in the present study are in close agreement with the earlier findings by other researchers (Mukherjee and Kiewitt, 1984; Getinet *et al.,* 1997; Rabiee *et al.,* 2004; Wilson, 2004; Singh *et al.,* 2014; Sharafi *et al.,* 2015). These results indicate that the different genotypes studied can serve as a reservoir of potential genes that can be used for improvement of oil content in different species of *Brassica* genus via various plant breeding programmes.

Fatty acid profiling

Table 3 indicates the fatty acid composition of various *Brassica* genotypes studied. In the present study, the content of saturated fatty acid (SFA) i.e. Palmitic acid (C16:0) ranged between for *Brassica juncea* genotypes, 3.70-5.15% in *Brassica napus* genotypes and 2.75-3.73% in *Brassica rapa* genotypes.

The amount of the SFA within different *Brassica* species was found to be less than 7% which is considered to be suitable for human consumption (Table 3). *Brassica* oilseeds with higher amounts of SFA lead to health disorders like hypercholesterolemia in humans whereas; the species with high palmitic acid have potential applications in soap and chemical industries (Singh *et al.,* 2014).

Various *Brassica* genotypes studied showed huge variation for the amount unsaturated fatty acids i.e. the MUFAs and the PUFAs comprising of oleic (C18:1), eicosenoic (C20:1) erucic (C22:1), linolenic (C18:3) and linoleic (C18:2) acids, respectively. Among the MUFAs, the amount of oleic acid in the *Brassica* genotypes ranged from 0.80- 48.70% in *B. juncea,* 16.15- 37.98% in *B. napus*, 6.21-16.15% in *B. rapa* (Table 3).

Table.1 List of *Brassica* varieties used for evaluation of fatty acid composition and oil content

S. No	Variety	Species	Source
1	RSPR-03	*B. juncea*	Division of Plant Breeding and Genetics, SKUAST-Jammu
2	Kranti	*B. juncea*	Division of Plant Breeding and Genetics, SKUAST-Jammu
3	Pusa Bold	*B. juncea*	Division of Plant Breeding and Genetics, SKUAST-Jammu
4	RL-1359	*B. juncea*	Division of Plant Breeding and Genetics, SKUAST-Jammu
5	RSPR-01	*B. juncea*	Division of Plant Breeding and Genetics, SKUAST-Jammu
6	NPJ-153 (MCN-11-14)	*B. juncea*	Division of Plant Breeding and Genetics, SKUAST-Jammu
7	Nov Gold	*B. juncea*	Division of Plant Breeding and Genetics, SKUAST-Jammu
8	NRCDR-2	*B. juncea*	Division of Plant Breeding and Genetics, SKUAST-Jammu
9	Varuna	*B. juncea*	BHU, Varanasi, UP
10	Pusa Mahak	*B. juncea*	Division of Genetics, IARI, New Delhi
11	PM-21	*B. juncea*	Division of Genetics, IARI, New Delhi
12	PM-22	*B. juncea*	Division of Genetics, IARI, New Delhi
13	PM-24	*B. juncea*	Division of Genetics, IARI, New Delhi
14	Pusa Karishma	*B. juncea*	Division of Genetics, IARI, New Delhi
15	RSPN-28	*B. napus*	Division of Plant Breeding and Genetics, SKUAST-Jammu
16	RSPN-29	*B. napus*	Division of Plant Breeding and Genetics, SKUAST-Jammu
17	DGS-1	*B. napus*	Division of Plant Breeding and Genetics, SKUAST-Jammu
18	GSL-1	*B. napus*	Division of Plant Breeding and Genetics, SKUAST-Jammu
19	RSPN-25	*B. napus*	Division of Plant Breeding and Genetics, SKUAST-Jammu
20	HNS-0901 (NCN-11-2)	*B. napus*	DRMR, Bharatpur, Rajasthan, India
21	GSL-101 (NCN-11-8)	*B. napus*	DRMR, Bharatpur, Rajasthan, India
22	RMT-08-02 (TCN-11-12)	*B. rapa*	DRMR, Bharatpur, Rajasthan, India
23	PT-303 (TCN-11-15)	*B. rapa*	DRMR, Bharatpur, Rajasthan, India
24	RH-0701 (TCN-11-6)	*B. rapa*	DRMR, Bharatpur, Rajasthan, India
25	RSPT-01	*B. rapa*	Division of Plant Breeding and Genetics, SKUAST-Jammu
26	RSPT-02	*B. rapa*	Division of Plant Breeding and Genetics, SKUAST-Jammu

Table.2 Total oil content (%) and glucosinolate content (µmol/g defatted seed meal) in various *Brassica* genotypes

S. No	Variety	Oil Content (%)
***Brassica juncea* genotypes**		
1	RSPR-03	38.48
2	Kranti	37.03
3	Pusa Bold	38.60
4	Varuna	37.43
5	RSPR-01	37.74
6	RL-1359	38.63
7	Pusa Mahak	38.33
8	NPJ-153 (MCN-11-14)	39.18
9	PM-21	32.67
10	PM-22	33.19
11	PM-24	39.47
12	Pusa Karishma	35.20
13	Nov Gold	38.12
14	NRCDR-2	38.96
	Mean	**37.36**
	Range	**32.67- 39.47**
***Brassica napus* genotypes**		
15	RSPN-28	39.70
16	RSPN-29	39.33
17	DGS-1	40.03
18	GSL-1	39.82
19	RSPN-25	39.04
20	HNS-0901 (NCN-11-2)	40.56
21	GSL-101 (NCN-11-8)	37.82
	Mean	**39.47**
	Range	**37.82- 40.56**
***Brassica rapa* genotypes**		
22	RSPT-01	41.42
23	RSPT-02	40.80
24	RMT-08-02 (TCN-11-12)	40.35
25	PT-303 (TCN-11-15)	41.43
26	RH-O701 (TCN-11-6)	40.52
	Mean	**40.90**
	Range	**40.35- 41.43**

Table.3 Fatty acid composition of various *Brassica* varieties

		Saturated Fatty acid (%)	Unsaturated Fatty acids (%)				
S. NO	Varieties	Palmitic acid	Oleic acid	Linoleic acid	Linolenic acid	Eicosenoic acid	Erucic acid
***Brassica juncea* genotypes**							
1	RSPR-03	3.44	10.35	20.77	24.08	1.11	40.21
2	Kranti	3.85	7.34	18.57	24.40	0.00	45.83
3	Pusa Bold	3.68	7.10	16.41	22.32	1.05	49.40
4	Varuna	3.23	10.92	18.50	20.94	1.26	45.13
5	RSPR-01	3.59	8.24	18.17	26.72	0.00	43.24
6	RL-1359	3.47	8.67	17.41	24.03	0.00	46.39
7	Pusa Mahak	3.08	7.60	15.33	24.44	1.04	48.48
8	NPJ-153 (MCN-11-14)	3.30	8.19	18.34	23.06	0.00	47.09
9	PM-21	-	0.90	39.80	13.60	29.50	0.90
10	PM-22	-	1.00	45.30	11.10	34.50	1.00
11	PM-24	-	0.80	34.20	15.20	34.90	0.80
12	Pusa Karishma	-	0.90	37.60	18.80	31.30	0.90
13	Nov Gold	-	48.70	11.00	17.80	14.80	48.70
14	NRCDR-2	-	47.40	11.50	12.00	15.50	47.40
	Mean	-	**12.01**	**23.06**	**19.89**	**11.78**	**33.25**
	Range	**3.08-3.85**	**0.80- 48.70**	**11.00- 45.30**	**11.10- 26.72**	**0.00- 34.90**	**0.80- 49.40**
***Brassica napus* genotypes**							
1	RSPN-28	4.87	19.45	21.67	11.20	14.72	28.05
2	RSPN-29	4.14	24.13	20.71	11.24	15.94	23.80
3	DGS-1	4.32	16.15	18.57	9.99	15.97	34.96
4	GSL-1	4.71	21.06	18.83	10.58	13.64	31.15
5	RSPN-25	5.15	20.76	19.87	10.30	15.58	28.29
6	HNS-0901 (NCN-11-2)	4.36	34.68	23.01	14.31	12.58	10.04
7	GSL-101 (NCN-11-8)	3.70	37.98	26.93	17.23	0.00	15.01
	Mean	**4.46**	**24.89**	**21.37**	**12.12**	**12.63**	**24.47**
	Range	**3.70- 5.15**	**16.15- 37.98**	**18.57- 26.93**	**9.99- 17.23**	**0.00- 15.97**	**10.04- 34.96**
***Brassica rapa* genotypes**							
1	RSPT-01	2.75	12.67	16.82	9.97	10.13	47.63
2	RSPT-02	2.99	10.17	15.18	10.75	10.87	49.99
3	RMT-08-02 (TCN-11-12)	3.73	11.73	18.18	20.89	1.67	43.77
4	PT-303 (TCN-11-15)	3.00	16.15	16.06	9.82	10.85	44.10
5	RH-0701 (TCN-11-6)	3.11	6.21	14.08	26.66	5.13	44.79
	Mean	**3.12**	**11.39**	**16.06**	**15.62**	**7.73**	**46.06**
	Range	**2.75- 3.73**	**6.21- 16.15**	**14.08- 18.18**	**9.82- 26.66**	**1.67- 10.87**	**43.77- 49.99**

The results obtained in the study are in agreement with those obtained by Chhokar *et al.,* 2008; Singh *et al.,* 2014; Sharafi *et al.,* 2015. Higher amounts of oleic acid are considered to be of nutritive value for human consumption as it increases the level of High-density lipoproteins (HDLs) and decreases the level of low- density lipoproteins (LDLs) in blood (Chang and Huang, 1998). Moreover, high oleic acid content in seed oil makes it more thermo stable, thereby making it more suitable for cooking purposes (Appelqvist, 1968). Apart from playing an important role in increasing the efficiency of cooking oil, oleic acid makes the seed oil more suitable for industrial purposes as well (Wilson, 2004). Another important MUFA is the erucic acid which is known to be antinutritional and undesirable for human consumption when present in higher amounts in edible oil.

The erucic acid content in *Brassica* species varied from 0.80- 49.40% in *B. juncea* varieties, 10.04- 34.96% in *B. napus* varieties and 43.77- 49.99% in *B.rapa* varieties (Table 3). Higher erucic acid in cooking oil hampers the myocardial conductance in humans and leads to increased blood cholesterol levels (Bozzini *et al.,* 2007; Sinha *et al.,* 2007). Several genotypes in the present study which showed higher levels of erucic acid will be of utmost importance for various industries. Oil rich in erucic acid is used as raw material in plastic, tannery, cosmetic, polyster and detergent industries (Rakow and Raney, 2003, Coonrod *et al.,* 2008). At present, when the *Brassica* breeding programmes are focused towards the development of zero erucic lines for nutritional purpose the genotypes Pus Karishma, PM- 21 and PM- 24 with low erucic acid will be of huge importance. Various *Brassica rapa* genotypes in the present study have erucic acid <40% which is in context to the previous results reported by Appelqvist, 1971; Velasco *et al.,* 1998; Chhokar *et al.,* 2008; Sharafi *et al.,* 2015.

The PUFAs i.e. linoleic and linolenic acids should be present in lower levels in the cooking oil. The amount of linoleic and linolenic acids varied between 11.00- 45.30%, 11.10- 26.72%; 18.57- 26.93%, 9.99- 17.23% and 14.08- 18.18%, 9.82- 26.66% in *B. juncea, B. napus* and *B. rapa* genotypes, respectively (Table 3). Similar results have been reported by Peiretti and Meineri, 2007; Singh *et al.,* 2014; Sharafi *et al.,* 2015. These PUFAs are known to be the precursors of long chain fatty acids involved in the synthesis of metabolically important compounds like prostaglandins. Linoleic acid which is an essential fatty acid isn't synthesized by human body and hence must be obtained from diet. It has been reported high linoleic acid levels in edible oil reduces blood cholesterol and prevents atherosclerosis (Ghafoorunissa, 1994). Although linolenic acid is another essential fatty acid, yet its presence in the oil may lead to rancidity and off flavor (Sharafi *et al.,* 2015).

According to the present study, the *Brassica* varieties having low levels of erucic acid and high levels of linoleic acids can be used in *Brassica* breeding programmes directed towards enhancing the quality of oil for nutritional and industrial applications.

The present research work was conducted to assess genotypes for qualitative parameters. Most of *Brassica* varieties cultivated in our country containing high level of erucic acid have deleterious effects on human health. Efforts are being made to develop varieties which contain less than 2 per cent erucic acid through breeding programmes. Erucic acid is a mono-unsaturated omega-9 fatty acid, denoted C22: 1 w-9. It is a potent inhibitor of saturated very long chain fatty acids, erucic acid inhibits the synthesis of the long chain fatty acids. Since 1970, there has been a growing awareness about the nutritional quality of the oil and meal and this has shifted

the emphasis towards breeding for high yield and quality traits in rapeseed mustard in order to bridge the gap between production and consumption. This strategy however, proved to be of little help to overcome the deficit of edible oil. The Indian rapeseed and mustard cultivars have high amounts of nutritionally undesired components, erucic acid and glucosinolates. Therefore, there is an urgent need to develop new varieties, containing low levels of erucic acid and glucosinolates. The data revealed that genotypes i.e. PM-21, PM-22, PM-24, Pusa Karishma and Nov Gold have low erucic acid content making them fit for commercial cultivation and use for introgression of low erucic acid traits in higher yielding cultivars. The information related to the significant variability of the fatty acid content in the various *Brassica* species observed in the present study can be utilized in the breeding programmes to develop genotypes with higher qualitative potential.

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