



GENETIC DIVERSITY OF FIELD MUSTARD (*BRASSICA RAPA* L.) AND THEIR SATURATED AND UNSATURATED FATTY ACIDS ASSOCIATION

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SUMMARY

The oilseed crop improvement for *Brassica rapa* L. (field mustard) is considered to be important in respect of yield production coupled with unsaturated fatty acids of the existing cultivars. Hence, the genetic diversity, and nutritional components of 14 *B. rapa* cultivars were evaluated based on their yield attributes and fatty acid profiles at the Sher-e-Bangla Agricultural University, Dhaka, Bangladesh. These 14 *B. rapa* cultivars differed significantly and were grouped into five diversified clusters. The highest intra-cluster distance was found in cluster II (2.43), while the highest inter-cluster distance was found between clusters II and V (20.41). In the fatty acid profile, the maximum threshold acceptable level (<7%) of saturated fatty acids for human consumption was found in Sonali Sarisha and BARI Sarisha-14. The total monounsaturated fatty acids viz., oleic, eicosenoic, and erucic acid ranged from 62.74% in BARI Sarisha-15 to 69.51% in Sonali Sarisha. The highest oleic acid and eicosenoic acid were obtained from the local variety, Maghi (21.06%) and Improved Tori (9.08%). The highest essential linoleic and linolenic acids were found in BARI Sarisha-15 (17.10%) and Improved Tori (8.16%), respectively. However, the local cultivar Maghi, containing the highest oleic acid (21.06%) has the lowest erucic acid (35.53%). On the other hand, BARI Sarisha-14 contained the lowest oleic (17.22%) and the highest erucic (44.02%) acids. The negative and strong correlation also reinforced the reverse relationship between oleic and erucic acids. Similarly, both linoleic (-0.497) and linolenic (-0.443) acids also showed a negative correlation with erucic acid. As a whole, BARI Sarisha-14, BARI Sarisha-15, Maghi, Sonali Sarisha, and Improved Tori could be selected as potential parents for a hybridization program to breed potential recombinant lines with higher oil content and healthier unsaturated oleic and linoleic fatty acids.

Keywords: *Brassica rapa* L., principal component analysis, fatty acids, erucic acid, correlation of fatty acids, oil stability index

Key findings: The maximum threshold level (<7%) of saturated fatty acids was found in the *Brassica rapa* cultivars viz., Sonali Sarisha and BARI Sarisha-14. The highest linoleic and linolenic acids were found in the genotypes, BARI Sarisha-15 and Improved Tori, respectively. The negative and strong correlation reinforces the reverse relationship between oleic and erucic acids.

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INTRODUCTION

On a global scale, *Brassicaceae* oilseeds are major contributors to vegetable oil for daily living and nutritional purposes. Among the *Brassica* species, *B. napus*, *B. juncea*, and *B. rapa* are the most common and widely cultivated as oilseed crops (Choudhary *et al.*, 2015; Singh *et al.*, 2016). Although *B. napus*, known as rapeseed, has become the main oilseed crop, in late-frost areas like China, Northern Europe, and Canada, short-duration and dwarf stature *B. rapa* cultivars are still used as ideal spring cultivars (Quijada *et al.*, 2007; Rahman *et al.*, 2011).

B. rapa is widely cultivated as an oilseed crop in Bangladesh and India. *B. rapa* is the most common and important oilseed crop in Bangladesh, accounting for 70% of total oil crop hectareage (BBS, 2016). It ranks first (70% of total oilseed crop cultivated area) with a cultivated area of 0.308 million ha, producing 351,537 MT of oilseeds during the 2017–2018 fiscal year (BBS, 2019).

B. rapa species is more popular among Bangladeshi farmers than *B. napus* and *B. juncea* because of its short generation cycle (75–80 days) and suitability in cropping patterns, e.g. rice (transplanted aman)–mustard–rice (boro) and/or rice (transplanted aman)–mustard–maize (Sultana *et al.*, 2021). In Bangladesh, the field-level yield of the existing *B. rapa* cultivars is 1.44 MT ha⁻¹ (BBS, 2018), which is still less than half the target of 3 MT ha⁻¹ (Sultana *et al.*, 2021). For this reason, the Bangladesh government has set the oilseed crop improvement research as a national priority.

In Bangladesh, edible oil consumption is increasing every year. According to the US Department of Agriculture (USDA, 2019), Bangladesh's annual edible oil consumption was at 2.85 million MT in 2019, which is 11% higher than the annual consumption in 2018 (Sultana *et al.*, 2021). With insufficient domestic production, almost 90% of the annual requirement of oils and fats are met through importation, indicating a big market deficit and high demands for edible oil in the country (Quaiyum *et al.*, 2015). According to the USDA data, Bangladesh imported about 2.8 million MT of edible oil in the year 2019–2020 and spent two billion US dollars in the fiscal year 2017–2018 for importing both soybean and

palm oil to meet the domestic demand. Unless measures are taken to increase the domestic production of edible oil, the country will continue to lose billions to the foreign market. Moreover, the winter crops in Bangladesh competes with one another due to the limited planting area. Hence, to reduce the import of oil, the only alternative is to increase the yield potential of oil crops. Furthermore, the nutritional quality of oil favorable for human health and consumption is also a major concern. Based on the above-mentioned concerns, steps should be taken to develop high-yielding and better-quality mustard cultivars to satisfy the country's demand for edible oils.

Vegetable oil is an important dietary component that has a substantial impact on human health. In comparison with other major vegetable oils, brassica oil has a higher genetic variability in the fatty acid profile. The seven primary fatty acids found in the *Brassica* genus are palmitic (C16:0), stearic (C18:0), oleic (C18:1), linoleic (C18:2), linolenic (C18:3), eicosanoic (C22:0), and erucic (C22:1) (Singh *et al.*, 2012; Islam *et al.*, 2020). Palmitic and stearic acids are the two most common saturated fatty acids, while the major unsaturated fatty acids are oleic, linoleic, linolenic, eicosanoic, and erucic. Oils containing high erucic acid are suitable for industrial purposes but unsuitable for human edible purposes (Nieschlag and Wolff, 1971; Singh *et al.*, 2014). As a result, developing cultivars with both commercial-free erucic acid and high erucic acid contents are both promising goals for brassica oilseed crop improvement programs. Other important goals include increasing oleic and linoleic acid content and decreasing linolenic acid content.

Oleic acid has been discovered as one of the most important monounsaturated fatty acids in edible oil. Oils with high oleic acid content are heat and oxidation resistant and suitable for a wide range of applications. Brassica seed oil's nutritional properties, like those of other fats and oils, are determined by the fatty acid's compositions, especially the content of oleic, linolenic, and erucic acids. High unsaturated oleic acid oil lowers blood cholesterol, whereas high saturated (palmitic and stearic) fatty acids raise blood cholesterol (Sharafi *et al.*, 2015).

The oil content of the *B. rapa* seed is 42% and the protein content is 25% (Khaleque, 1985). However, the oil content of currently cultivated *B. rapa* cultivars in Bangladesh contains high erucic acid (40%–45%) and high pungency due to high amounts of glucosinolates (300 parts per million or ppm) (Mortuza *et al.*, 2006; Sultana *et al.*, 2021). Consumption of erucic acid has been linked to myocardial lipidosis and heart lesions (Gopalan, 1974). Hence, the erucic acid-rich seed oil is thought unsafe for human consumption (Charlton *et al.*, 1975). Likewise, the protein-rich cake after extraction of this mustard oil has glucosinolates, a sulfur-containing compound that is known to cause iodine deficiency at high concentrations (Alexander *et al.*, 2008). Although mustard oil is very popular in Bangladesh and widely consumed as cooking oil, however, there is no 'canola grade' (<2% erucic acid and <30 ppm glucosinolates) mustard variety in Bangladesh.

Keeping the research priority of breeding with early maturity, high yielding, and nutritionally healthy quality oil in

Bangladesh, the present study investigated the genetic diversity and oil nutritional components of popular field mustard (*B. rapa*) cultivars in Bangladesh focusing on yield attributing traits and fatty acids profile. We investigated the genetic diversity and showed in detail how the major saturated and unsaturated fatty acids correlated with each other and how the biosynthesis of a fatty acid directly affects the biosynthesis of other saturated and unsaturated fatty acids.

MATERIALS AND METHODS

Plant material

Fourteen most popular cultivars of *Brassica rapa* were used as plant material (Figure 1). These 14 cultivars were procured from a) the Bangladesh Agricultural Research Institute (BARI) in Joydebpur, b) the Bangladesh Institute of Nuclear Agriculture (BINA) in Mymensingh, and c) the local cultivators of Bangladesh.

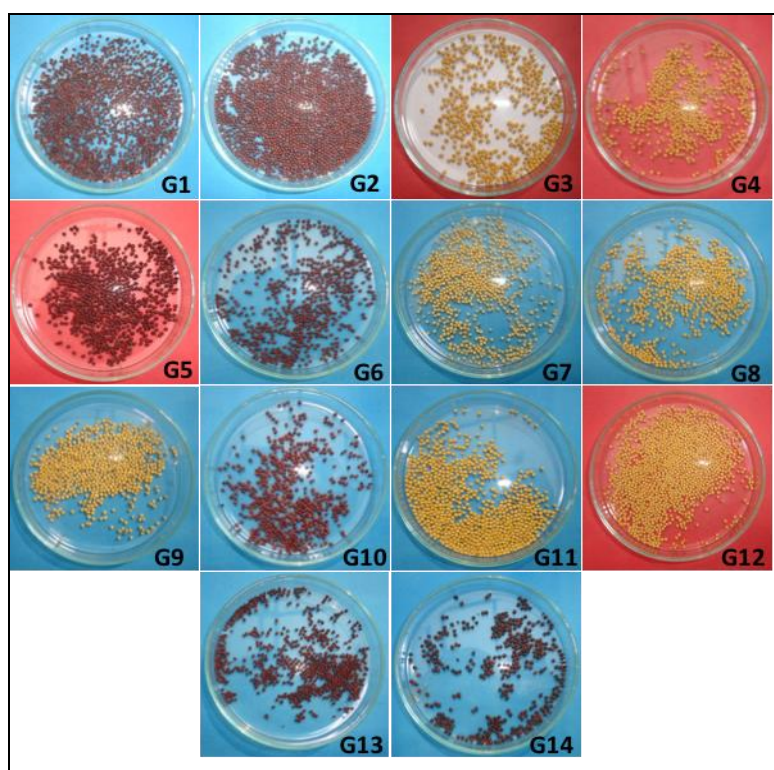


Figure 1. Photographs of seeds of 14 *B. rapa* cultivars used in the experiment. (Legend: G1 = Tori-7, G2 = BARI Sarisha-3, G3 = Sonali Sarisha, G4 = BARI Sarisha-6, G5 = BARI Sarisha-9, G6 = BARI Sarisha-12, G7 = BARI Sarisha-14, G8 = BARI Sarisha-15, G9 = BARI Sarisha-17, G10 = Improved Tori, G11 = Sonali Tori, G12 = Torsha, G13 = Maghi, and G14 = BINA-10).

Field experiment and experimental design

The experiment was carried out in the research field of Sher-e-Bangla Agricultural University, Dhaka, Bangladesh. The experiment was set up in a 300 m² plot area with three replications in a randomized complete block design. The seeds of 14 cultivars were assigned randomly in each block following the line-sowing method. The spacing of line to line and plant to plant was at 30 cm and 8 cm, respectively. Standard agronomic packages e.g., thinning, irrigation, and weeding were practiced as per the technology handbook manual of BARI (2018).

Observation of yield attributing morphological parameters

The data on 11 yield attributing morphological parameters were recorded from first flowering to harvesting of the crop. When 80% of the plants showed maturity symptoms, the crop was harvested on different days. Ten individual plants from each variety were chosen randomly and were used for recording the parameters based on 11 yield attributing morphological characters *viz.*, plant height (cm); first and 50% flowering (days); time of maturity (days); primary and secondary branches plant⁻¹, siliqua plant⁻¹, and siliquae length (cm), and seeds siliqua⁻¹, thousand-seed weight, and seed yield plant⁻¹ (g).

Oil content and fatty acid profile

The oil content, and saturated and unsaturated fatty acids were estimated. The oil contents of seed samples were extracted and estimated following the method described by Hughes (1965). Dried mustard seed flour samples were extracted by ethyl ether using soxhlet flasks. On a dry weight basis, the total oil content was calculated in percentage (%). The composition of fatty acids was calculated using a gas chromatographic process (Cocks and Rede, 1966). Fatty acid methyl esters were prepared using the method defined by Were, *et al.* (2006), with some modifications. For the fatty acid profile, esterified fatty acids were injected into gas chromatography. The standard was obtained from Sigma-Aldrich [Supelco 37 components of mixed FAME (fatty acid methyl ester) in dichloromethane], 595 North Harrison Road, Bellefonte, USA. We used mixed standard but not individual fatty acid methyl ester, C4-C24. Using the standard, the 18 major FAME were detected out of 37 FAME mix standards. Each fatty acid was detected by comparing the standard-sample retention time.

First, we ran mixed standard (C4-C24) in the gas chromatography (column: Thermo 30m x 0.25mm; Detector: FID, 260°C; injection volume: sample and solvent 10mg/ml (2µl), and with the resulting sample we ran the same condition, and finally identified individual peak of respective fatty acid by retention time. Each fatty acid was detected by comparing the standard-sample retention time. Comparing the retention times with those of standard individual peaks of fatty acids, methyl esters were identified. The fatty acid composition of the sample was determined by dividing the individual peak areas of each fatty acid in the chromatogram by the total peak areas of all fatty acids in the oilseed samples (Cocks and Rede, 1966).

Statistical analysis

Analysis of the variance was performed, and error variance was tested for homogeneity (Cochran and Cox, 1957). The 14 mustard cultivars and replications were considered fixed factors and random factors, respectively. Statistical software packages, Statistix10, were used. The significance levels were calculated at 5% and 1% using F statistics. The replication mean values over the three replications for all characters were calculated and separated by DMRT (Duncan Multiple Range Test) at a 5% level of probability (Gomez and Gomez, 1984). The genetic diversity was analyzed following Mahalanobis (1936) generalized distance (D2) (Rao, 1952). Multivariate analyses, e.g., principal component analysis, cluster analysis, and canonical variate analysis were performed using GENSTAT 5.13 software. The correlation coefficients among the fatty acids compositions were measured using SPSS Statistics (version 26). The heatmaps were produced using the heatmapmer (www.heatmapmer.ca).

RESULTS AND DISCUSSION

Phenotypic variations in growth and yield-related traits

The mean performance of 11 phenotypic traits over three replications with the mean sum of square (MSS) of the 14 mustard genotypes is presented in Table 1. Days to first flowering ranged from 22 to 38 days after sowing with a mean value of 28.98. The highest duration for flowering was recorded in G12 (38.00), which was statistically similar to G3 (36.67) and the lowest was found in both G11 and G13 (22.00), which were statistically similar to G10

Table 1. Mean performance of *B. rapa* cultivars for yield attributing traits.

Variety	DFF	D50%F	DM	PH	PB	SB	SP	LS	SS	TSW	SYP
G1	23.33f	28.00d	86.00b	76.02e	4.80abc	6.44a	135.1a-d	5.01de	13.60cd	2.20h	3.90d
G2	29.00cd	33.67b	90.67a	98.50ab	5.30abc	9.21a	153.2ab	6.00a	16.93cd	3.21bc	7.30ab
G3	36.67ab	42.00a	92.00a	104.1ab	3.97c	1.23b	50.17f	5.64a-c	24.00b	3.83a	4.17d
G4	31.33c	34.67b	86.00b	107.12a	4.43c	7.89a	155.9ab	5.73ab	17.57c	2.93de	7.40ab
G5	28.67de	32.00bc	86.00b	97.47ab	5.50abc	8.35a	170.2ab	5.00de	12.33d	3.08cd	6.48bc
G6	28.67de	32.00bc	86.00b	95.35bc	5.73abc	7.71a	152.5ab	5.54a-d	14.97cd	2.93de	6.68bc
G7	30.67cd	33.67b	79.00c	84.86de	6.36ab	1.10b	73.80d-f	4.92e	31.67a	3.32b	7.25ab
G8	35.00b	41.33a	82.00bc	99.03ab	5.66abc	1.01b	84.53c-f	4.97de	24.23b	3.37b	6.85b
G9	31.00cd	34.00b	80.67c	86.83cd	4.70bc	1.44b	74.46ef	4.97de	34.60a	3.10cd	9.09a
G10	23.00f	29.00cd	86.00b	87.00cd	5.06abc	8.71a	180.9a	5.23b-e	16.30cd	2.88e	7.54ab
G11	22.00f	27.00d	86.00b	79.37de	4.16c	8.22a	140.7abc	5.09c-e	14.00cd	2.66fg	4.97cd
G12	38.00a	43.00a	86.00b	102.8ab	6.56a	1.00b	114.1b-e	5.14c-e	22.80b	2.82ef	7.49ab
G13	22.00f	26.67d	81.00c	78.17de	5.36abc	6.05a	157.1ab	4.86e	16.07cd	2.84ef	7.31ab
G14	26.33e	28.33d	82.67bc	83.07de	5.16abc	6.77a	181.9a	4.87e	13.23cd	2.61g	6.27bc
MSS	83.4**	91.2**	40.0**	325.0**	1.71 ^{ns}	33.9**	5636**	0.40**	147.26**	0.45**	5.99**
Mean	28.98	33.24	85.00	91.41	5.20	5.37	130.32	5.22	19.45	2.99	6.62
CV (%)	4.80	5.89	2.68	5.65	17.99	42.35	25.44	5.89	13.19	3.51	15.22
SD	1.39	1.96	2.27	5.16	0.94	2.27	33.15	0.31	2.57	0.11	1.01

Legend: MSS = Mean sum of square, DFF = Days to first flowering, D50%F = Days to 50% flowering, DM = Days to maturity, PH = plant height (cm), PB = Primary branches plant⁻¹, SB = Secondary branches plant⁻¹, SP = Siliqua plant⁻¹, LS = Length of siliqua (cm), SS = Seeds siliqua⁻¹, TSW = 1000 seed weight (g), SYP = seed yield plant⁻¹ (g).

** Significant at $P \leq 0.01$

(23.00) and G1 (23.33). The average days to maturity were recorded as 85.00 with a range of 79.00 to 92.00 days. The variety, G7, required the least number of days to mature (79.00 days), which was statistically similar to G9 (80.67) and G13 (81.00). Meanwhile, the maximum maturity date was recorded in the genotype G3 (92.00 days), which was statistically similar to G2 (90.67).

Plant height, as well as the number of secondary branches plant⁻¹, varied significantly ranging from 76.02 cm in G1 to 107.12 cm in G4; and 1.01 in G8 to 9.21 in G2, respectively. Interestingly, a huge difference was noticed in silique plant⁻¹, which ranged from 50.17 in G3 to 181.9 in G14. The highest number of silique plant⁻¹ was found in the variety G14, which was statistically similar to cultivars G10 and G5.

Again, thousand seed weight, as well as seed yield plant⁻¹, showed significant differences among the 14 mustard cultivars which were recorded from 2.20 g (G1) to 3.83 g (G3) and 3.9 g (G1) to 9.09 g (G9), respectively. The 1000-seed weight represents the boldness of the grain which is an important yield contributing trait. The boldest grain size was observed in the cultivars G3 (3.83 g), followed by G8 (3.37), and G7 (3.32), while the smallest grains were found in cultivars G1 (2.20 g), G14 (2.61 g), and G11 (2.66 g). The variety G9 (9.09 g) had the highest seed yield plant⁻¹, which was found statistically similar to the cultivars G10 (7.54 g), G12 (7.49 g), and G4 (7.40 g). The G1 variety, on the contrary, had the lowest seed yield plant⁻¹ (3.90 g), which was statistically similar to G3 (4.17 g).

The results of the 11 morphological traits of 14 *B. rapa* cultivars showed that the genotypes are diversified. The genotypes G7, G9, and G13 showed early flowering and early maturity indicating that these genotypes might have the gene pools for early maturity. Therefore, G7, G9, and G13 genotypes could be further explored as suitable parental lines for breeding programs to develop new lines with early flowering and maturity traits.

Being a complex and polygenic trait, yield is determined by several minor contributing factors (Marjanovic-Jeromela *et al.*, 2008). In the breeding programs of *B. rapa*, the traits *viz.*, secondary branches, silique, and thousand seed weight are considered major yield contributors as these traits are reported to be directly and positively correlated with the yield performance (Malek *et al.*, 2000; Uddin *et al.*, 2013; Begum *et al.*, 2018; Pal *et al.*, 2019). Given that seed yield performance is directly related to the yield performance of a crop, individual plant

selection (IPS) based on seed yield plant⁻¹ is a general practice for crop improvement in most breeding programs. The present study suggested that based on characters *viz.*, secondary branches plant⁻¹, silique plant⁻¹, and thousand seed weight and seed yield plant⁻¹, the genotypes, G2 (BARI Sarisha-3), G14 (BINA-10), G3 (Sonali Sarisha), G7 (BARI Sarisha-14), and G9 (BARI Sarisha-17) could be selected as promising parents in brassica breeding program to generate the transgressive segregated lines. The desirable high-yielding lines could be selected from the segregated populations and the selected lines could be used as valuable genetic resources for *B. rapa* improvement.

Genetic diversity

In a breeding program, genetic diversity gives a rational basis for selecting parental lines. Based on the 11 traits listed below, the genetic divergence and grouping of the 14 mustard types were investigated in the study.

Principal component analysis (PCA)

The eigenvalues and percent of the variance of 11 component characters *viz.*, plant height (cm), first and 50% flowering (day), time of maturity (day), primary and secondary branches plant⁻¹ (number), silique plant⁻¹ (number), siliquae length (cm), seeds silique⁻¹ (number), thousand-seed weight (g), and seed yield plant⁻¹ (g) of 14 *B. rapa* cultivars were calculated using PCA (Table 2). According to the PCA, only the first three principal components had eigenvalues greater than one, and they were jointly responsible for 84.32% of the overall variation among the genotypes assessed for 11 traits. This finding is coherent with previous reports where they reported that the first three axes accounted for 71.84% (Rauf and Rahim, 2018) and 94.00% (Zaman *et al.*, 2010) of the total variation. Component I contributed the most with the value of 61.72% among the 11 characteristics tested, followed by component II (13.14%), component III (9.46%), and component IV (6.36%).

Clustering of genotypes based upon genetic diversity

Using Tocher's approach, the 14 genotypes were classified into five clusters (Table 3) based on the relative magnitude of values using the 11 yield attributing traits (Rao, 1952). Cluster II, with six genotypes, was the

Table 2. Eigenvalues and percent variation contribution of 11 yield attributing traits of *B. rapa* cultivars.

Principle axis	component	Eigenvalues	Percent variation	Cumulative percent variation
I		8.0233	61.72	61.72
II		1.7077	13.14	74.86
III		1.2299	9.46	84.32
IV		0.8265	6.36	90.68
V		0.4293	3.30	93.98
VI		0.3204	2.46	96.44
VII		0.2205	1.70	98.14
VIII		0.1114	0.86	99
IX		0.0828	0.62	99.66
X		0.0288	0.23	99.91
XI		0.0106	0.09	100.00

Table 3. Distribution of 14 genotypes of *B. rapa* in five clusters.

Cluster	Given name	Genotypes	Number of Genotypes
I	G3	Sonali Sarisha	1
II	G2, G4, G5, G6, G10, G14	BARI Sarisha-3, BARI Sarisha-6, BARI Sarisha-9, BARI Sarisha-12, Improved Tori, BINA-10	6
III	G7, G8, G9	BARI Sarisha-14, BARI Sarisha-15, BARI Sarisha-17	3
IV	G1, G11, G13	Tori-7, Sonali Tori, Maghi	3
V	G12	Torsha	1

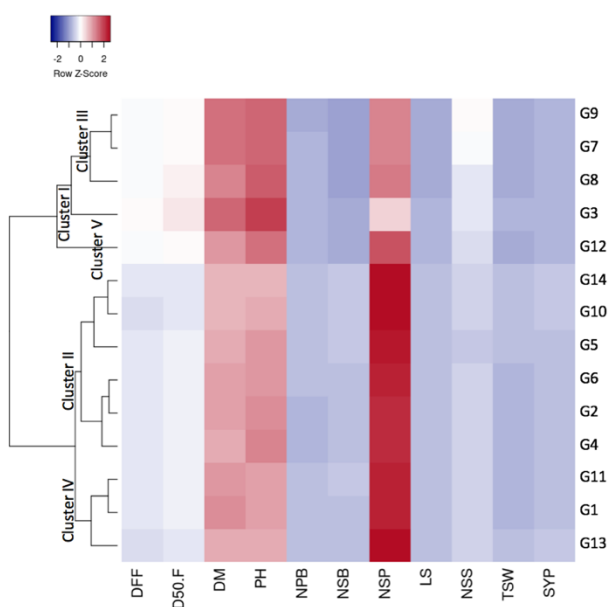


Figure 2. The heatmap represents 14 mustard cultivars groups into five clusters in respect of 11 traits. The red and blue colors in the legend indicate the highest and lowest values, respectively. DFF = Days to first flowering, D50%F = Days to 50% flowering, DM = Days to maturity, PH = plant height (cm), NPB = primary branches plant⁻¹, NSB = secondary branches plant⁻¹, NSP = siliqua plant⁻¹, LS = length of siliqua (cm), NSS = seeds siliqua⁻¹, TSW = 1000 seed weight (g), and SYP = seed yield plant⁻¹ (g).

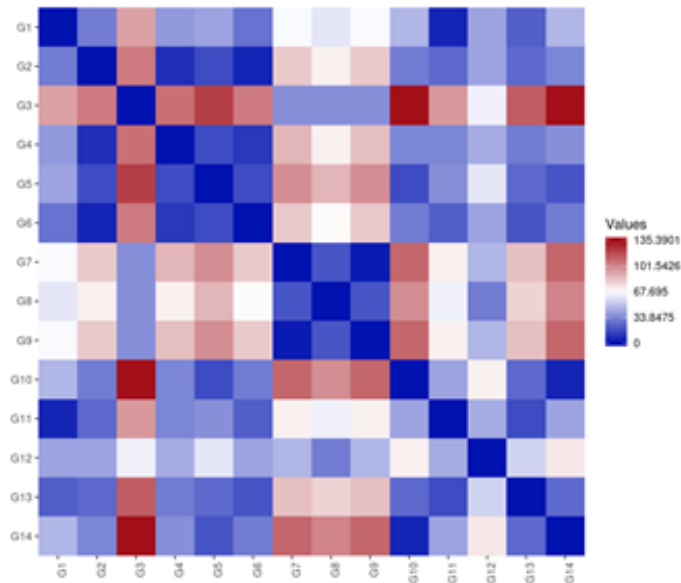


Figure 3. The distance matrix represents the pairwise comparisons between mustard cultivars in respect of 11 traits. The red and blues colors in the legend indicate the highest and lowest values, respectively. DFF = Days to first flowering, D50%F = Days to 50% flowering, DM = Days to maturity, PH = plant height (cm), NPB = primary branches plant⁻¹, NSB = secondary branches plant⁻¹, NSP = siliqua plant⁻¹, LS = length of siliqua (cm), NSS = seeds silique⁻¹, TSW = 1000 seed weight (g), and SYP = seed yield plant⁻¹ (g).

largest of the five clusters, followed by cluster III and cluster IV, each with three genotypes, and cluster I and cluster V, which are solitary clusters. The distribution of the 14 genotypes shown in the heatmap (Figure 2) and in the distance matrix (Figure 3) indicated that significant genetic diversity exists among the genotypes. Jahan *et al.* (2013), Rauf and Rahim (2018), Kumari and Kumari (2018), and Akter *et al.* (2019) found four diverse clusters in *Brassica*, while Khan *et al.* (2013) grouped 32 genotypes into six clusters. In another study, Sandhu *et al.* (2017) grouped 38 genotypes into three clusters.

The inter-cluster distances were consistently greater than the intra-cluster distances, showing that the genotypes of various clusters had more genetic variation (Table 4). Cluster II (2.43) had the greatest intra-cluster distance, indicating greater genetic variance among the genotypes in this cluster, whereas clusters III (1.43) and IV (1.67) had the smallest intra-cluster distance, indicating close genetic ties among these genotypes. Cluster IV and V (20.414) had the largest inter-cluster distance, followed by cluster I and IV (16.181), cluster II and V (15.642), cluster I and II (15.40), and cluster III and IV (13.80).

In a breeding program, it is thought that hybrids involving genotypes from more varied origins produce more heterosis than genotypes from less divergent origins (Falconer, 1981). In the current study, the genotypes belonging to clusters IV and V had the highest distance, indicating that the genotypes may produce a wider spectrum of heterosis and transgressive segregants. The selection of genetically diverse parents is one of the key factors for a successful hybridization program. The findings of this study suggest that when choosing parental lines, genotypes from clusters IV and V should be prioritized to achieve higher heterosis and a wider range of diversity in segregants with novel recombination of desired traits.

Cluster means analysis

Cluster means analysis revealed that mustard genotypes from cluster IV had an early flowering phenotype (22.44 days) (Table 5), whereas genotypes from cluster III had an early maturity phenotype (80.56 days). Cluster I had the highest average plant height (104.13 cm), whereas cluster IV had the lowest average plant height (77.86 cm). The genotypes of cluster V showed the highest

Table 4. Intra (bold) and inter-cluster distances (D^2) for *B. rapa* genotypes.

Cluster	I	II	III	IV	V
I	0.00	15.404	9.374	16.181	15.628
II		2.43	13.640	9.166	15.642
III			1.43	13.802	11.761
IV				1.67	20.414
V					0.00

Table 5. Mean performance of different traits in each cluster.

Variables	Cluster				
	I	II	III	IV	V
Days to first flowering	36.67	27.83	32.22	22.44	38.00
Days to 50% flowering	42.00	31.61	36.33	27.22	43.00
Days to maturity	92.00	86.22	80.56	84.33	86.00
Plant height (cm)	104.13	94.75	90.24	77.86	102.77
Primary branches plant ⁻¹	3.98	5.20	5.58	4.78	6.57
Secondary branches plant ⁻¹	1.23	8.11	1.18	6.91	1.00
Siliqua plant ⁻¹	50.17	165.76	77.60	144.29	114.07
Length of siliqua (cm)	5.65	5.40	4.96	4.99	5.14
Seeds siliqua ⁻¹	24.00	15.22	30.17	14.56	22.80
1000 seed weight (g)	3.83	2.94	3.27	2.57	2.83
Seed yield plant ⁻¹ (g)	4.18	6.95	7.73	5.39	7.50

mean performance for primary branches plant⁻¹ (6.57), as well as, seed yield plant⁻¹ (7.50 g), while the genotypes of cluster I had the highest mean performance for the length of siliqua (5.65 cm) and 1000 seed weight (3.83 g). The maximum number of siliqua plant⁻¹ was found in cluster II (165.76) whereas the minimum number of siliqua plant⁻¹ was found in clusters I (50.17) and cluster III (77.60).

The mean of different clusters varied, implying greater variation among genotypes for all 11 traits. The mean performance of the genotypes in the clusters inferred that the genotypes of cluster III showed early maturing, while the genotypes of clusters V and I showed high yield and yield contributing traits. Taken together, these results inferred that the genotypes from clusters III, V, and I could create novel recombination for early maturing and yield potential lines upon hybridization among the genotypes belonging to these clusters. Previous studies by Zaman *et al.* (2010), Bhuiyan *et al.* (2013), and Kumari and Kumar (2018) supported this notion.

Contribution of characters towards divergence of the genotypes

Table 6 shows the contribution of characters to divergence determined by canonical variate analysis (CVA). Vector I obtained from PCA demonstrated that 50% flowering (0.1158),

secondary branches plant⁻¹ (0.5753), length of siliqua (3.8963), and siliqua plant⁻¹ (0.0294) are the major contributing factors to the genetic divergence. In vector II, the length of siliqua (2.4547), seed yield plant⁻¹ (1.5743), 1000 seed weight (0.4918), and secondary branches plant⁻¹ (0.0535) showed their importance toward genetic diversity.

Vectors I and II had positive values for the length of siliqua, secondary branches plant⁻¹, and seed weight, indicating that these traits contribute significantly to the divergence. In contrast, negative values in both vectors for flowering, maturity, and seeds siliqua⁻¹ suggest that these traits have lower contributions towards the divergence. Similar conclusions were drawn in earlier research (Zaman *et al.*, 2010; Jahan *et al.*, 2013; Akter *et al.*, 2019)

Selection of genotypes based on genetic diversity

The distance between clusters IV and V (20.414) and clusters I and IV (16.181) were the highest, whereas the distance among the other clusters was more or less intermediate (Table 4). The genotypes in clusters IV and V had the greatest distance, implying that the genotypes might form a greater range of transgressive segregants in a later generation. This is due to the hybrids involving the parents from the most diverse clusters have the

Table 6. Relative contributions of the yield attributing traits of *B. rapa* genotypes to the total divergence.

Characters	Vector -1	Vector -2
Days to first flowering	-0.5943	-0.4339
Days to 50% flowering	0.1158	-0.1513
Days to maturity	-0.4784	-0.6466
Plant height (cm)	0.0563	-0.0975
Primary branches plant ⁻¹	-0.7671	-1.5532
Secondary branches plant ⁻¹	0.5753	0.0535
Siliqua plant ⁻¹	0.0294	-0.2387
Length of siliqua (cm)	3.8963	2.4547
Seeds siliqua ⁻¹	-0.2358	-1.0806
1000 seed weight (g)	0.0259	0.4918
Seed yield plant ⁻¹ (g)	-0.5764	1.5743

Table 7. The highest 10 and the lowest 10 inter-genotypic distances among the *B. rapa* genotypes.

Highest distance			Lowest distance		
Genotype		Distance	Genotype		Distance
Sonali Sarisha	Improved Tori	2.149	BARI Sarisha-9	BARI Sarisha-12	0.247
Sonali Sarisha	BARI Sarisha- 9	2.071	BARI Sarisha-3	BARI Sarisha-12	0.254
Sonali Sarisha	BINA-10	2.017	BARI Sarisha-3	BARI Sarisha-6	0.255
BARI Sarisha-3	Sonali Sarisha	2.01	BARI Sarisha-6	BARI Sarisha-12	0.325
Sonali Sarisha	Sonali Tori	1.982	BARI Sarisha-9	BINA-10	0.331
BARI Sarisha-9	BARI Sarisha-14	1.944	Maghi	BINA-10	0.351
BARI Sarisha-14	Improved Tori	1.923	Tori-7	Sonali Tori	0.353
Sonali Sarisha	BARI Sarisha-12	1.921	BARI Sarisha-12	BINA-10	0.357
Sonali Sarisha	Maghi	1.906	BARI Sarisha-12	Improved Tori	0.366
BARI Sarisha-14	Sonali Tori	1.904	BARI Sarisha-14	BARI Sarisha-17	0.375

greatest amount of heterosis. (Hochholdinger and Baldauf, 2018). Selection of genetically diverse parents is a crucial step for a successful breeding program, hence we propose that genotypes from clusters IV and V be given more attention to achieve higher heterosis and a wider range of diversity in segregated populations, as well as a higher possibility of novel recombinations of desired traits. On the other hand, intermediately diversified parents have a greater possibility of contributing heterosis in the subsequent generations. The intermediate inter-clusters distance was found between III and V (11.761), and II and III (13.640) clusters (Table 4). Based on this notion, the genotypes of these clusters could produce transgressive segregates in later generations.

Cluster II had the highest cluster mean of secondary branches (8.11) and siliqua plant⁻¹ (165.76). These traits are considered the most important yield contributing characteristics (Table 5). Further, cluster III

comprised the maximum cluster mean for seeds siliqua⁻¹ (30.17), seed yield plant⁻¹ (7.73), and minimum days to mature (80.56), while cluster IV manifested the earliest flowering trait. In addition, the PCA exhibited the highest inter genotypic distance between Sonali Sarisha (G3) and Improved Tori (G10) at 2.149, followed by Sonali Sarisha (G3) and BARI Sarisha-9 (G5) at 2.071, Sonali Sarisha (G3) and BINA-10 (G14) at 2.017. and BARI Sarisha-3 (G2) and Sonali Sarisha (G3) at 2.01 (Table 7). Therefore, based on the cluster mean, PCA, and inter genotypic distance, BARI Sarisha-14 (G7) and BARI Sarisha-17 (G9) from cluster III, Maghi (G13) from cluster IV, Torsha (G12) from cluster V, Improved Tori (G10) and BINA-10 (G14) from cluster II, and Sonali Sarisha (G3) from cluster I could be selected for a breeding program as their cross combinations may manifest maximum heterosis and create wide genetic variability in their offspring.

Fatty acids composition analysis

Oil content

Considering the genetic diversity and agronomic performances of the 14 mustard cultivars, we selected six cultivars for oil and fatty acids analysis. The results from the analysis of the variance of oil content of the selected six cultivars revealed significant differences among the six cultivars of *B. rapa*. The seed oil content of the cultivars ranged from 36.52% to 42.42% (Figure 4). The highest oil content was recorded in G7 (BARI Sarisha-14, 42.42%) and the lowest in G13, Maghi (36.52%). These findings are consistent with previous research by Kumar and Tsunoda (1980), Mukherjee and Kiewitt (1984), and Velasco *et al.* (1998).

The fatty acid profiles of six mustard cultivars were analyzed by Gas Chromatography and the total fatty acids were categorized into saturated and unsaturated fatty acids (Table 8 and Figure 5). Chromatograms of 14 fatty acids content of six mustard cultivars are shown in Figure 6.

Saturated fatty acids

Total saturated fatty acids (TSFAs) compositions of the selected mustard cultivars are presented in Table 8. The contents of TSFAs ranged from 6.12% (BARI Sarisha-14) to 8.62% (BARI Sarisha-15) (Figure 5). In general, less than 7% TSFAs in edible oil has been considered the maximum acceptable threshold level for human consumption (Wilson, 2004), this result indicates that the oil of two mustard cultivars, Sonali Sarisha and BARI Sarisha-14 had TSFA below the threshold level. A larger amount of TSFAs (>10%) in edible oil induces hypercholesterolemia, which is harmful to human health (Sun *et al.*, 2015). Oil with a high TSFA concentration, on the other hand, is important for the soap and oleochemical industries. In the present investigation, among the TSFAs, the highest percentage of palmitic acid (C16:0) was found in BARI Sarisha-6 (5.19%), followed by Maghi (5.18%), and BARI Sarisha-15 (4.69%), whereas the lowest percentage of palmitic acid was found in Sonali Sarisha (2.58%) (Table 8). In the case of stearic acid (C18:0), the highest value was observed in BARI Sarisha-15 (2.02%) and the lowest in Improved Tori (1.14%). The highest percentage of arachidic acid (C20:0) was found in Sonali Sarisha

(1.67%) and the lowest in Improved Tori (1.34%). The highest percentage of behenic acid (C22:0) was found in Sonali Sarisha (0.55%), whereas the lowest was in Maghi (0.34%).

Unsaturated fatty acids

The total unsaturated fatty acids (TUSFAs) of seed oil of six selected *B. rapa* cultivars ranged from 90.92% (BARI Sarisha-15) to 93.30% (Sonali Sarisha) (Figure 5). TUSFAs consist of monounsaturated fatty acids (MUFAs) and polyunsaturated fatty acids (PUFAs).

Monounsaturated fatty acids (MUFAs)

Total MUFAs varied from 62.74% in BARI Sarisha-15 to 69.51% in Sonali Sarisha, as measured by the total sum of the concentrations of oleic (C18:1), eicosenoic (C20:1), and erucic (C22:1) acids in the seed oils. Local variety Maghi had the maximum oleic acid (21.06%) content, while Improved Tori had the maximum content of eicosenoic (9.08%) (Table 8). The seed oil of Maghi (35.52%) and BARI Sarisha-14 (44.02%) had the lowest and highest percentages of erucic acid, respectively (Table 8 and Figure 6).

Seed oils with a high oleic acid content are preferred for both nutritional and industrial applications (Liu *et al.*, 2002). The seed oil's high oleic acid concentration improves its heat stability, making it more suitable for cooking (Appelqvist, 1980). Furthermore, the high oleic acid content is beneficial for human consumption because oleic acid boosts the high-density lipoproteins (HDLs) in the blood while decreasing the low-density lipoproteins (LDLs) (Chang and Huang, 1998). On the other hand, high erucic acid levels in oil are considered to be anti-nutritional, as it has been linked to lipidosis in children and myocardial fibrosis in monkeys (Ackman *et al.*, 1977). Edible oil high in erucic acid is not advised for human consumption because it raises blood cholesterol levels. However, erucic acid derivatives can be utilized in the plastics, tannery, and cosmetic sectors as chemical additives (Bozzini *et al.*, 2007). The present study shows that the popular mustard cultivars of Bangladesh contain a high level of erucic acid (35.52%–44.02%), which is undesirable for edible purposes, so canola grade mustard cultivars should be bred for human consumption.

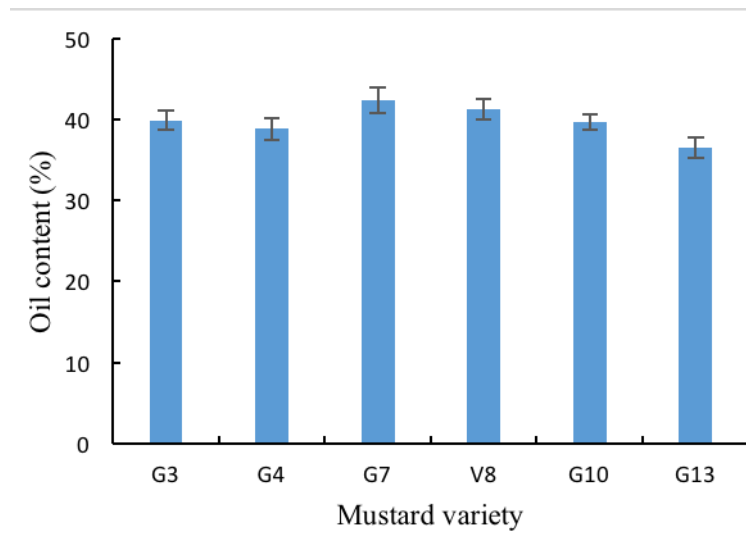


Figure 4. Oil content of six cultivars of *B. rapa*. (Legend: G3 = Sonali Sarisha, G4 = BARI Sarisha-6, G7 = BARI Sarisha-14, G8 = BARI Sarisha-15, G10 = Improved Tori, and G13 = Maghi)

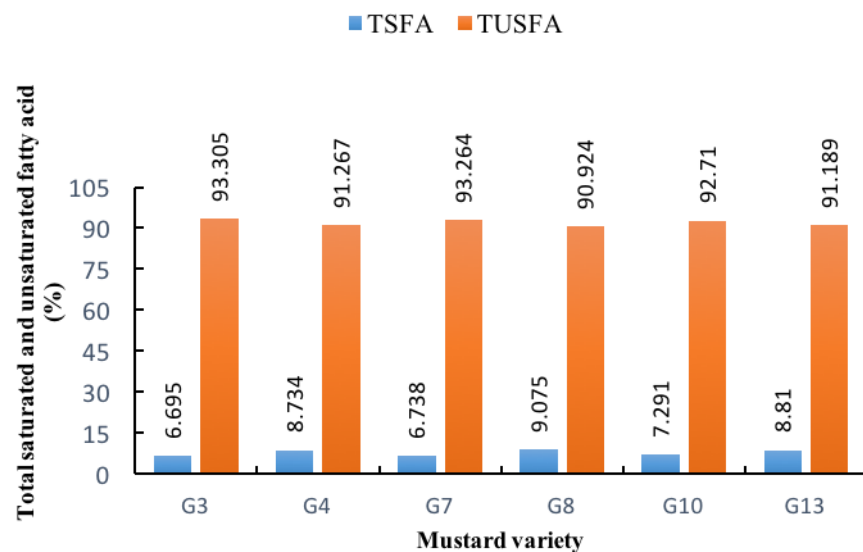


Figure 5. Percentage of total saturated (TSFA) and unsaturated (TUSFA) fatty acid in oil of six cultivars of *B. rapa*. (Legend: G3 = Sonali Sarisha, G4 = BARI Sarisha-6, G7 = BARI Sarisha-14, G8 = BARI Sarisha-15, G10 = Improved Tori, and G13 = Maghi).

Polyunsaturated fatty acids (PUFAs)

The major polyunsaturated fatty acids (PUFAs), linoleic (C18:2) and linolenic (C18:3) acids detected in seed oils of the mustard cultivars varied from 13.24% to 17.10% and 6.06% to 8.16%, respectively (Table 8). The seed oil of BARI Sarisha-15 had the highest concentration (17.10%) of linoleic acid, whereas Sonali Sarisha had the lowest (13.24%) of linoleic

acid. Again, Improved Tori seed oil had the highest linolenic acid concentration (8.16%), whereas Sonali Sarisha seed oil had the lowest (6.06%). BARI Sarisha-15 and Improved Tori seed oils have a high level of linoleic and linolenic acids. Peiretti and Meineri (2007) reported that higher content of linoleic and linolenic acids outperforms other vegetable oils in terms of PUFA proportions.

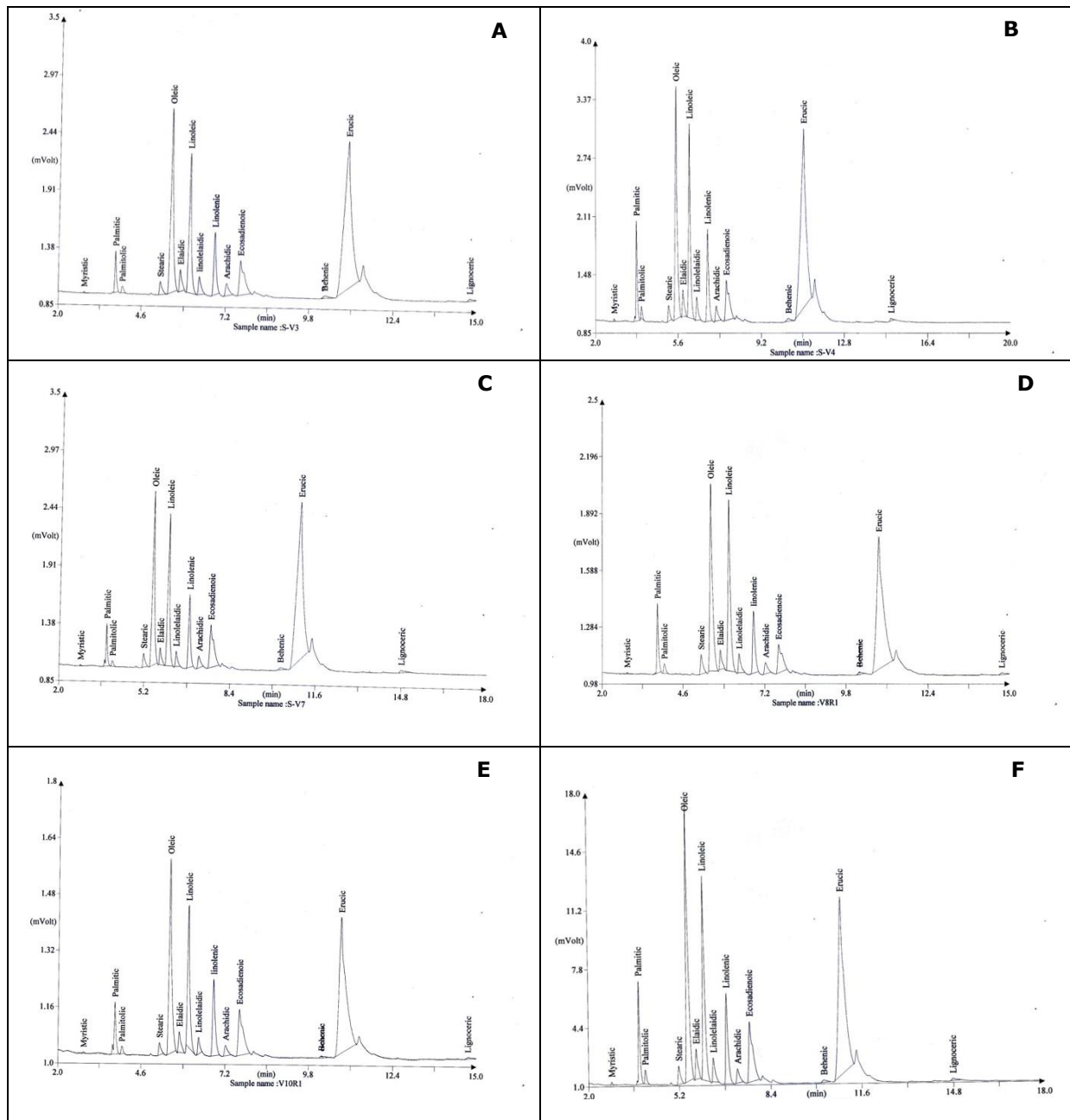


Figure 6. Chromatogram of different fatty acids of (A) Sonali Sarisha, (B) BARI Sarisha-6, (C) BARI Sarisha-14, (D) BARI Sarisha-15, (E) Improved Tori, and (F) Maghi.

Table 8. Major saturated and unsaturated fatty acids content and their ratio in the seed oil of *B. rapa*.

Variety	Saturated fatty acid (%)				Monounsaturated fatty acid (%)			Polyunsaturated fatty acid (%)		Oleic/ linoleic ratio	ω -6/ ω -3 ratio
	Palmitic (C16:0)	Stearic (C18:0)	Arachidic (C20:0)	Behenic (C22:0)	Oleic (C18:1)	Eicosenoic (C20:1)	Erucic (C22:1)	Linoleic (C18:2)	Linolenic (C18:3)		
G3	2.575	1.413	1.668	0.545	19.525	6.826	43.157	13.239	6.058	1.475	2.185
G4	5.190	1.194	1.526	0.453	18.839	5.954	41.081	14.066	6.898	1.339	2.039
G7	2.934	1.329	1.415	0.438	17.225	7.728	44.022	13.957	6.794	1.234	2.054
G8	4.686	2.023	1.498	0.407	20.335	5.420	36.984	17.098	6.366	1.189	2.686
G10	3.991	1.139	1.338	0.406	20.909	9.083	36.732	13.502	8.163	1.549	1.654
G13	5.181	1.411	1.390	0.338	21.058	8.334	35.525	14.319	6.970	1.471	2.054
Min	2.575	1.139	1.338	0.338	17.225	5.42	35.525	13.239	6.058	1.189	1.654
Max	5.19	2.023	1.668	0.545	21.058	9.083	44.022	17.098	8.163	1.549	2.686
Mean	4.093	1.418	1.473	0.431	19.65	7.23	39.59	14.36	6.87	1.38	2.11

Legend: G3 = Sonali Sarisha, G4 = BARI Sarisha-6, G7= BARI Sarisha-14, G8 = BARI Sarisha-15, G10 = Improved Tori and G13 = Maghi

Table 9. Correlation coefficients among the major saturated and unsaturated fatty acids of the seed oil of *B. rapa*.

Fatty acids	Palmitic	Arachidic	Behenic	Lignoceric	Stearic	Palmitoleic	Oleic	Elaidic	Linoleic	Linolelaidic	Linolenic	Eicosadienoic	Erucic
Myristic	0.389	0.157	-0.193	-0.500	-0.051	0.396	-0.059	0.179	-0.011	0.322	-0.327	-0.177	-0.043
Palmitic		-0.387	-0.690	-0.721	0.115	0.797*	0.504	0.359	0.540	0.404	0.337	-0.202	-0.764*
Arachidic			0.803*	-0.086	0.339	-0.186	-0.182	-0.097	0.015	0.522	-0.827*	-0.694	0.563
Behenic				0.103	-0.122	-0.711	-0.455	-0.205	-0.415	-0.043	-0.470	-0.357	0.845*
Lignoceric					0.245	-0.440	-0.419	-0.625	-0.044	-0.419	-0.203	0.275	0.350
Stearic						0.527	0.205	-0.304	0.862*	0.710	-0.637	-0.624	-0.238
Palmitoleic							0.717	0.411	0.698	0.728	-0.032	-0.218	-0.857*
Oleic								0.817*	0.281	0.568	0.282	0.131	-0.843*
Elaidic									-0.250	0.326	0.372	0.325	-0.552
Linoleic										0.632	-0.285	-0.621	-0.497
Linolelaidic											-0.550	-0.657	-0.391
Linolenic												0.669	-0.443
Ecosadienoic													-0.185

Note: ** and * indicates significant 1% and 5% levels, respectively.

Linoleic and linolenic acids are important fatty acids that act as precursors of bioactive long-chain (>20-carbon) fatty acids, which play a role in a range of metabolic functions in the body, including prostaglandin formation (Adam *et al.*, 1986). Moreover, oils containing about 50.00% total PUFAs (this study 23.46% in BARI Sarisha-15) may be useful for a variety of industrial uses, including the production of oil-based paints.

Stability index analysis

The stability index of edible oil, which is the proportions of oleic to linoleic fatty acids, ranged from 1.189 in BARI Sarisha-15 to 1.549 in Improved Tori. The proportions of linoleic (ω -6) and linolenic (ω -3) acids varied from 1.65% in Improved Tori to 2.69% in BARI Sarisha-15 (Table 8). These proportions of oleic, linoleic, and linolenic are crucial for human nutrition (Zhang *et al.*, 2004). In general, it is recommended that we should aim for a 2:1 oleic/linoleic ratio and a 4:1 ω -6/ ω -3 ratio in our diets (Yehuda and Carasso, 1993). Interestingly, in this investigation, seed oil from none of the cultivars studied here met the necessary specifications for oleic and linoleic acid ratios. The stability index analysis of six mustard cultivars revealed that the ratios of ω -6: ω -3 and oleic: linoleic were lower than the required values (Singh and Singh, 2008). With the majority of the currently used vegetable oils fall short of these recommendation values, commercial companies are marketing blended edible oil by mixing more than one type of edible seed oil (Hashempour *et al.*, 2016).

Correlation of saturated and unsaturated fatty acid components

Correlation coefficients and correlation matrix among the main fatty acids in *B. rapa* are shown in Table 9. Palmitic acid, a main saturated fatty acid, had a positive correlation with palmitoleic (0.797), linoleic (0.540), oleic (0.504), linolelaidic (0.404), and linolenic (0.337), and a negative correlation with arachidic (-0.387), behenic (-0.690), lignoceric (-0.721), and erucic (-0.764) acids among the saturated fatty acids, while stearic acid had a significant and positive correlation (0.862) with linoleic acid. Mortuza *et al.*, (2006) also found a positive correlation of palmitic acid with gadoleic, linolenic, oleic, and linoleic acids, and a reverse correlation with erucic acid.

The MUFA, oleic acid had a significant negative association with erucic acid (-0.843) and a significant positive association with

elaidic acid (0.817). Moreover, linoleic (-0.497) and linolenic (-0.443) fatty acids had also negative associations with erucic acid. Sharafi *et al.* (2015) and Mortuza *et al.* (2006) also reported the significant and negative correlations of erucic acid with linolenic, linoleic, and oleic acids. Again, the correlation studies indicated positive but non-significant associations of oleic acid with linoleic and linolenic acids. These results are in contrast to the earlier report by Singh and Singh (2008), where they stated a highly positive and significant association of oleic acid with linoleic and linolenic fatty acids.

The biosynthesis of plant fatty acids is a primary metabolic pathway in plants as they are essentially required for growth and development (Ohlrogge and Browse, 1995). In plants, fatty acids biosynthesis is mostly carried out in the plastids starting the synthesis of malonyl CoA from acetyl CoA through ATP-dependent carboxylation of acetyl CoA catalyzed by the enzyme, acetyl CoA carboxylase (Aid, 2019). The long-chain fatty acids (LCFA) (C16-18), palmitic (C16:0), stearic (C18:0), and oleic (C18:0) acids are synthesized in the chloroplast stroma (Rahman, 2014). On the other hand, biosynthesis of very-long-chain fatty acids (VLCFA) (>18C), e.g., arachidic (C20:0), behenic (C22:0), erucic acid (C22:0) occurs in the endoplasmic reticulum. The biosynthesis of plant fatty acids is correlated with each other (Mortuza *et al.*, 2006; Sharafi *et al.*, 2015). In correlation analysis, we found positive associations of palmitic with palmitoleic, oleic, linoleic, and linolelaidic acids, and negative associations with behenic, lignoceric, and erucic acids (Table 9). Our results revealed that the biosynthesis of saturated and unsaturated fatty acids is interrelated, and the synthesis of saturated fatty acids may regulate the biosynthesis of unsaturated fatty acids.

The VLCFA, erucic acid is synthesized by elongating oleic acid, but more complexity is not ruled out (Sharafi *et al.*, 2015). Eicosenoic acid and then erucic acid is formed through chain elongation of oleic acids. In our study, we found that oleic acid had a significant and negative association with erucic acid (Table 9). The data support the idea that a significant amount of oleic acid is converted to erucic acid in *B. rapa* and other mustard seed oils (Mortuza *et al.*, 2006). The negative relationship between the syntheses of the oleic and erucic acids was reported in *B. carinata* and *B. napus* (Sharafi *et al.*, 2015). In the present investigation, the highest concentration of erucic acid (44.02%) and the

lowest concentration of oleic acid (17.22%) were found in BARI Sarisha-14 (G7). In contrast, the lowest concentration of erucic acid (35.52%) and the highest concentration of oleic acid (21.05%) were found in the local variety Maghi (G13). Therefore, the data directly support that oleic acid and erucic are inversely correlated with each other. The correlations analysis substantiated the phenotypic content of fatty acids in *B. rapa* in the present research.

The low and insignificant associations were observed between oleic and linoleic acids and between oleic and linolenic acids (Table 9). These results are probably due to the involvement of two separate biosynthetic pathways. The first where erucic acid would be made from oleic acid by a chain elongation process, and the second where linoleic and linolenic would be made from oleic acid through consecutive desaturation. As such, genetic disruption of erucic acid may lead to sharp augmentation of oleic acid formation. Again, increment of oleic acid formation might be also a barrier to sharp augmentation of linoleic and linolenic acids in mustard.

CONCLUSIONS

To develop new lines of *B. rapa* with higher yield potential and short duration, the cultivars Maghi, BARI Sarisha-14, BARI Sarisha-15, Sonali Sarisha, and Improved Tori could be selected as potential parental lines for the hybridization program to breed potential recombinant lines with higher oil content and healthier unsaturated oleic and linoleic fatty acids. Further, the development of low erucic but high oleic acid and linoleic acid-containing *B. rapa* genotypes may be possible through genetic hybridization with a canola grade mustard variety followed by appropriate selection cycles.

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