Journal of Oilseed Brassica, 15 (1) :43-51, January 2024

Nutritional profiling and genetic relatedness among Indian mustard (*Brassica juncea* L) genotypes

Priyamedha[1,2]*, VV Singh[2], Bhagirath Ram[2], HK Sharma[2], MS Sujith Kumar[2], Pradeep Kumar[2], Monika Dubey[2] and PK Rai[2]

[1]*Central Rainfed Upland Rice Research Station (ICAR-National Rice Research Institute), Hazaribag 825301, Jharakhand, India*
[2]*ICAR-Directorate of Rapeseed-Mustard Research, Bharatpur 321303, Rajasthan, India*
**Corresponding author: priyamedha.pb@gmail.com*
(Received: 15 September 2023; Revised: 24 November2023; Accepted: 05 December 2023)

Abstract

Development of genotypes with improved nutritional qualities with high antioxidants is highly desirable in Indian mustard (*Brassica juncea* L.). A set of forty-four advanced breeding lines of Indian mustard derived from cross [EC564648×(Rajat×NUDHYJ3)], [NRCHB101×NUDHYJ5], [EC552573×(Varuna×NUDHYJ3)] and [NRCDR02×NUDHYJ5] were undergo nutritional profiling for oil and seed meal and molecular marker analysis using SSR markers for genetic relatedness among themselves. The genetic parameters for the nutritional traits have also been studied. The double zero advanced breeding lines, DRMRQ4-7-23 (high oleic acid), DRMRQ1-11-32 (moderate linoleic acid with high oleic/linoleic ratio), DRMRQ4-5-25 (low linolenic acid with high ω-6/ω-3 ratio), DRMRQ4-1-58 (high β-carotene content), DRMRQ 2-3-17 (high flavonoid content) and DRMRQ1-16-27 (high antioxidant) were reported to be nutritionally improved lines. Trait flavonoid content exhibited highest GCV (34.74%) and oil content exhibited the lowest (0.83%) one. Traits like oleic acid, linoleic acid, linolenic acid, oleic/linoleic ratio, ω-6/ω-3 ratio, antioxidant and β-carotene exhibited more than 90% heritability. Thirty polymorphic primers out of 135 SSR primers generating 76 alleles with two to four alleles per primer and PIC value ranging from 0.129 to 0.678 were used to calculate Jaccard's dissimilarity coefficients ranging from 0.09 to 0.74 with an average of 0.40. The UPGMA based dendrogram representing genetic dissimilarity among different genotypes grouped 44 double low advanced breeding lines into four clusters with fourth cluster divided into three sub-clusters. SSR based clustering showed that although these genotypes are derived from different crosses, yet they are genetically related to each other probably due to common double zero parentage. These nutritionally enriched double zero genotypes are important genetic resources for the mustard breeders.

***Keywords*:** *Double zero, genetic diversity, nutritional parameter, SSR markers*

Introduction

Indian mustard (*Brassica juncea* L.) is the most important source of oilseed in India among other cultivated oleiferous brassicas like *B. rapa* and *B. napus*. The seven major fatty acids extracted from oleiferous brassicas are palmitic (C16:0), stearic (C18:0), oleic (C18:1), linoleic (C18:2), linolenic (C18:3), eicosanoic (C22:0), and erucic (C22:1) acids. Palmitic and stearic acid are saturated fatty acids composes less than 7% of brassica oil, while unsaturated fatty acids are its main component making the oil more desirable for human consumption. Further, high oleic acid and low linolenic acid impart longer shelf life to the oil. High ratio of oleic to linoleic fatty acid and linoleic (ω-6) to linolenic (ω-3) fatty acid increases the quality of oil. However, seed oil of *B. juncea* is characterized by significant amount of long-chain monounsaturated fatty acids, mainly erucic acid (C22:1) that is nutritionally undesirable for human consumption (Renard and Mcgregor, 1992; Mortuza *et al.*, 2006). Brassica oil has also been found to possess β-carotene content higher than hemp and flax seed oils (Teh and Birch, 2013). β-carotene being a precursor of vitamin-A needs to be added as an important nutritional parameter while developing genotypes with high oil quality. Seed meal remains after extraction of oil is another valuable product containing about 40% protein with a favorable composition of essential sulphuric amino acids like methionine and cysteine (Downey and Bell, 1990) and also rich in minerals (Ca, Mg and P) and vitamins (B4 and E). Mustard seed meal is also known to be a rich source of antioxidants (Yoshie-Stark *et al.*, 2008; Szydowska Czerniak *et al.*, 2010) in which the antioxidant potential is mainly contributed by phenolic compounds with other constituents like flavonoids, tocopherols, ascorbic acid etc. However, in comparison to the other popular sources of meal, the meal of *B. juncea* 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 (Griffiths *et al.*, 1998; Walker and Booth, 2001). Several studies have been carried out to investigate the antioxidant activity in rapeseed-mustard seed meal (Ildikó, 2006; Das, 2009) because of an increasing interest in determining relevant dietary sources of antioxidant compounds for food industries during past few years. Development of Indian mustard genotypes with improved nutritional qualities in oil (<2 % erucic acid, >40% oleic acid, <12% linolenic acid and a ratio of 2:1 for ω-6 to ω-3) and in seed meal (<30 micromoles glucosinolate per gram of defatted seed meal with high antioxidants) is highly desirable. Further, establishment of genetic relatedness among developed genotypes using molecular markers serves the purpose to select genetically diverse genotypes that can be used in creating selectable variation for the desired traits.

Therefore, the present study was undertaken to study the nutritional parameters of advanced breeding lines of Indian mustard, association among the nutritional parameters and establishment of genetic relatedness among these genotypes using molecular markers.

Materials and Methods

Experimental materials and biochemical evaluation for nutritional parameters

A set of forty-four advanced breeding lines namely DRMRQ1-16-27, DRMRQ1-17-26, DRMRQ1-22, DRMRQ1-23, DRMRQ1-8-34, DRMRQ1-12-31, DRMRQ1-7-35, DRMRQ1-11-32, DRMRQ1-20-24, DRMRQ1-15-28, DRMRQ1-13-30, DRMRQ1-18-25, DRMRQ1-2-37 and DRMRQ1-10-33 derived from cross [EC564648×(Rajat×NUDHYJ3)], DRMRQ2-1-1, DRMRQ2-11-10, DRMRQ2-1-2-7, DRMRQ2-1-7, DRMRQ2-2-4, DRMRQ2-2-3-8, DRMRQ2-2-7, DRMRQ2-2-16, DRMRQ2-1-6-3, DRMRQ2-11-2, DRMRQ2-1-8-6 and DRMRQ2-3-17 derived from cross [NRCHB101×NUDHYJ5], DRMRQ4-14-48, DRMRQ4-11-50, DRMRQ4-7-55, DRMRQ4-5-26, DRMRQ4-17-4, DRMRQ4-17-6, DRMRQ4-7-24, DRMRQ4-7-23, DRMRQ4-5-25, DRMRQ4-17-46, DRMRQ4-6-54, DRMRQ4-10-51, DRMRQ4-1-58, DRMRQ4-9-53, DRMRQ4-5-56, DRMRQ4-16-47 and DRMRQ4-3-57 derived from cross [EC552573×(Varuna×NUDHYJ3)] and DRMRQ5-4 derived from cross [NRCDR02×NUDHYJ5], were developed through pedigree method of selection (Priyamedha *et al.*, 2021), and evaluated biochemically for three consecutive years (2017 to 2019). The nutritional parameters included, saturated fatty acid (%); oleic acid (%); linoleic acid (%); linolenic acid (%); erucic acid (%), beta-carotene (ppm) content in oil and percent oil content; glucosinolate (μM/g), antioxidant (AAE, ascorbic acid equivalent; mg/g) and flavonoid (QE, quercetin equivalent; mg/g) in defatted seed meal.

Determination of nutritional parameters of oil

Percentage of different fatty acids in oil i.e., saturated fatty acid; oleic acid; linoleic acid; linolenic acid and erucic acid in oil were analyzed by gas-liquid chromatograph (Nucon model 5765) using a flame ionization detector with SP 2300 + 2310 SS column, through methyl esters of oil samples of the 44 genotypes under study prepared by transesterification. The conditions maintained were column temperature: 240°C, injector temperature: 230°C and detector temperature: 250°C. Nitrogen gas was used as carrier gas with a flow rate of 40-50 ml/min. Peaks of the fatty acid methyl esters were identified by comparing their retention time with that of the known standards, run under similar separation conditions. β-carotene content in oil was estimated by spectrophotometric method as provided by AACC (1995).

Determination of oil content and nutritional parameters of seed meal

To determine the percent oil content in the genotypes, seeds were thoroughly ground in a pestle and mortar and 10.0 g triplicates of ground seeds were extracted with hexane for 24 h in a Soxhlet apparatus. Subsequently, hexane was removed from the oil by rotary evaporator under reduced pressure and the weights of the residual oils were calculated. The seed meal remaining after the extraction of total oil was preserved for estimation of glucosinolate, antioxidant and flavonoids. Estimation of total glucosinolate in seed meal was done by complex formation between glucosinolate and sodium tetra-chloropalladate solution as described by Thies (1982) and modified by Mawlong *et al.* (2017). The intensity of the color produced was measured by using a spectrophotometer at 425 nm wavelength. Total flavonoid content and total antioxidant activity were estimated through methanolic extracts of seed meal in spectrophotometer as described by Zhishen *et al.* (1999) and Prieto *et al.* (1999) respectively.

DNA extraction and marker analysis

Genomic DNA was isolated from young and healthy leaves using the standard cetyl trimethyl ammonium bromide protocol (Doyle and Doyle, 1990). Purification of DNA was carried out by adding 10mg/100ml of RNase to the sample vial containing crude DNA at a proportion of 3μl/100ml. DNA quantification was done by using agarose at a concentration of 0.8%. A total of 30 polymorphic out of 135 simple sequence repeat (SSR) markers were used for genetic diversity analysis. PCR assay was carried out in 96-well fast thermal cycler (PE

Applied Biosystems, USA) in a total volume of 10 µl reaction mixture. Each reaction mixture contained 25 ng of genomic DNA, 1.0 unit Taq DNA polymerase (Dream Taq, Thermo Scientific), 20 ng each forward and reverse primer, 10X Dream Taq PCR assay buffer with 1.5 mM $MgCl_2$, 0.2 µl dNTPs mix and reaction volume was made up to 10 µl by adding nuclease free water. Amplification was carried out according to the following set up: the cycle was repeated 35 times after initial denaturation at 94°C for 5 min with each cycle consisted of cyclic denaturation at 94°C for 45 s, annealing temperature at 55-58°C for 50 s, primer extension at 72°C for 1 min and final extension of 72°C for 7 min followed by incubation at 4°C. The PCR product bands resolution was done electrophoretically on 2.5% agarose gel containing 0.01% ethidium bromide prepared in 1× TAE (Tris-Acetic acid-EDTA) buffer. 50bp and 100bp DNA Ladder (Thermo Scientific) was used as standard to compare PCR amplicon band sizes. The gel was run for 2.5 h at 120V. The gel picture was visualized in gel documentation system (IG/LHR, Syngene, UK).

Statistical analysis

The mean data were analyzed for analysis of variance (ANOVA), genetic parameters and Spearman's correlation coefficient using SAS 9.1 software. Genetic distance was calculated by using the binary (0/1) matrix generated from SSR markers based on Jaccard's dissimilarity Index. Genetic distance matrix was further used to construct UPGMA dendrogram using DARwin 6.0 software (Perrier *et al.*, 2006).

Results and Discussion

The present study uses a set of forty-four advanced breeding lines (genotypes) developed from four different crosses namely, EC564648×(PCR7×NUDHYJ3), NRCHB101×NUDHYJ5, EC552573 × (Varuna × NUDHYJ3) and (NRCDR02 × NUDHYJ5). These genotypes were evaluated biochemically for nutritional parameters of oil and seed meal. The association among the nutritional traits was also studied by calculating correlation coefficient for them. Genetic relatedness among these 44 genotypes have been studied using SSR markers.

Evaluation of genotypes for nutritional parameters

Biochemical analysis for nutritional parameters of oil and seed meal (Table 1) reflected that ranges for oil content varied from 41.68% (DRMRQ2-1-8-6) to 43.08% (DRMRQ4-7-23), saturated fatty acid from 3.53% (DRMRQ2-1-7) to 5.70% (DRMRQ2-2-7), oleic acid from 23.78% (DRMRQ4-16-47) to 41.37% (DRMRQ4-7-23), linoleic acid from 26.34% (DRMRQ1-11-32) to 39.41% (DRMRQ4-9-53), linolenic acid from 14.14% (DRMRQ4-5-25) to 29.41% (DRMRQ1-2-37), oleic/linoleic ratio from 0.62 (DRMRQ4-16-27) and 1.28 (DRMRQ1-11-32), ω-6/ω-3 ratio from 1.21 (DRMRQ1-2-37) to 2.54 (DRMRQ4-5-25), erucic acid from 1.08% (DRMRQ1-22) to 1.93% (DRMRQ4-3-57), β-carotene from 1.65 ppm (DRMRQ4-9-53) to 4.64 ppm (DRMRQ4-1-58), glucosinolate from 18.88 µg/g (DRMRQ4-14-48) to 28.98 µg/g (DRMRQ2-1-6-3), flavonoid from 0.38 QE mg/g (DRMRQ4-10-51) to 1.56 QE mg/g (DRMRQ 2-3-17) and antioxidant from 22.72 AAE mg/g (DRMRQ4-14-48) to 46.65 AAE mg/g (DRMRQ1-16-27) in the genotypes. These genotypes were also nutritionally enriched in comparison to the range of different nutritional parameters (0.06-0.98 ppm beta carotene, 0.25-0.80 QE mg/g flavonoid and 11.87-21.95 AAE mg/g antioxidant) reported in non-quality varieties of Indian mustard by Bala *et al.* (2011) and Kumar *et al.* (2017). The genotypes namely, DRMRQ4-7-23, DRMRQ1-11-32, DRMRQ4-5-25, DRMRQ4-1-58, DRMRQ 2-3-17 and DRMRQ1-16-27 possessed high oleic acid, moderate linoleic acid with high oleic/linoleic ratio, low linolenic acid with high ω-6/ω-3 ratio, high -carotene content, high flavonoid content and high antioxidant respectively. These genotypes had been identified as most promising in nutritional point of view among all the studied genotypes. The biochemical evaluation of these advanced genotypes indicated their nutritional superiority and these can be exploited as valuable genetic resources in breeding for nutritional enhancement of Indian mustard genotypes.

Genetic parameters and trait association

Analysis of variance indicated significant difference among the genotypes for oil content and other nutritional parameters of oil and meal. The genetic parameters that include phenotypic coefficient of variation (PCV), genotypic coefficient of variation (GCV), heritability (broad sense) and genetic gain were studied to measure the range of variability and to determine the magnitude of heritable variation present in the genotypes under study (Table 2).

Among all the nutritional traits (parameters) studied the flavonoid content exhibited highest GCV (34.74%) and oil content exhibited the lowest (0.83%) one. The PCV values were slightly higher than their respective GCV values for all the traits studied, depicting little to moderate influence of environmental factors on their expression. Heritability ranged from 33.4% for erucic acid content in oil to 99.7% for antioxidant. Traits like oleic acid, linoleic acid, linolenic acid, oleic/linoleic ratio, ω-6/ω-3 ratio, antioxidant and β-carotene also exhibited more than 90% heritability, indicating that selection for these traits is easy because of close correspondence between genotype and phenotype. Further, highest genetic gain of 56.25% was estimated

Table 1: Nutritional parameters of oil and seed meal of Indian mustard genotypes

Genotype	oil content (%)	saturated Fatty Acid (%)	oleic acid (%)	Linoleic acid (%)	Linolenic acid (%)	Erucic acid (%)	Gluco-sinlate (µg/g defatted seed-meal)	Antioxi-dants (AAE mg/g)	Elavonoid (QE mg/g)	Beta-carotene (ppm)
DRMRQ 1-16-27	42.52	4.50	35.91	34.75	18.37	1.11	28.03	38.76	0.39	3.87
DRMRQ 1-17-26	42.65	4.57	40.26	39.37	24.37	1.16	26.99	31.42	0.49	3.44
DRMRQ 1-22	42.77	5.43	34.73	35.58	20.29	1.08	23.70	23.72	0.96	4.44
DRMRQ 1-23	42.25	5.23	34.34	36.34	20.26	1.14	22.65	29.49	0.33	4.19
DRMRQ 1-8-34	42.63	5.60	32.40	36.87	24.78	1.57	22.60	31.36	0.44	4.39
DRMRQ 1-12-31	42.89	4.27	33.41	37.72	23.87	1.14	28.97	32.77	0.72	4.20
DRMRQ 1-7-35	42.88	4.53	36.56	37.49	21.48	1.47	26.51	29.57	1.27	3.45
DRMRQ 1-11-32	42.81	4.57	33.75	26.34	15.35	1.17	27.77	41.81	0.68	3.42
DRMRQ 1-20-24	42.83	4.63	30.59	35.34	25.46	1.49	27.40	36.70	1.17	3.55
DRMRQ 1-15-28	42.83	5.23	35.22	34.48	20.39	1.88	28.34	29.36	0.48	2.82
DRMRQ 1-13-30	43.04	4.33	28.53	37.36	16.41	1.82	27.59	30.54	0.41	2.95
DRMRQ 1-18-25	42.69	4.53	35.88	37.48	25.40	1.91	23.47	26.48	1.07	3.50
DRMRQ 1-2-37	42.02	4.23	39.47	35.55	29.41	1.91	26.61	32.51	0.61	4.06
DRMRQ 1-10-33	42.78	5.63	34.47	35.36	27.52	1.76	28.54	38.58	0.27	3.55
DRMRQ 2-1-1	41.70	4.37	41.07	32.25	18.51	1.28	23.23	38.50	0.93	2.84
DRMRQ 2-11-10	42.27	4.50	35.87	36.53	24.84	1.52	26.23	45.13	1.16	3.33
DRMRQ 2-1-2-7	42.42	4.57	34.26	33.66	19.35	1.28	28.03	28.52	0.59	3.55
DRMR Q 2-1-7	42.34	3.53	34.48	39.24	22.59	1.24	21.52	34.40	0.68	3.66
DRMRQ 2-2-4	42.23	4.50	33.34	38.60	23.51	1.10	28.88	34.61	0.65	3.17
DRMRQ 2-2-3-8	41.87	4.57	33.28	37.72	20.27	1.25	27.67	46.65	0.75	3.85
DRMRQ 2-2-7	42.26	5.70	40.17	39.16	20.48	1.68	22.54	27.52	0.99	3.51
DRMRQ 2-2-16	42.66	5.50	35.92	33.68	18.49	1.08	28.07	46.57	0.68	3.20
DRMRQ 2-1-6-3	42.12	3.80	28.91	34.50	16.53	1.09	28.98	32.80	0.86	3.44
DRMRQ 2-11-2	41.90	5.40	36.13	32.37	20.21	1.45	25.82	32.42	0.78	3.72
DRMRQ 2-1-8-6	41.68	5.40	34.88	37.62	19.38	1.87	28.28	26.80	0.55	2.54
DRMRQ 2-3-17	42.81	4.43	30.43	32.38	25.39	1.54	23.14	35.69	1.56	3.18
DRMRQ 4-14-48	42.45	5.43	33.57	39.35	20.26	1.88	18.88	22.72	0.54	2.06
DRMRQ 4-11-50	42.49	5.13	31.48	36.00	23.37	1.91	23.64	36.67	1.55	2.79
DRMRQ 4-7-55	41.69	5.53	32.45	35.61	24.58	1.90	27.62	25.51	0.60	2.94
DRMRQ 4-5-26	41.71	4.60	36.68	33.66	19.66	1.09	27.43	38.64	0.58	4.59
DRMRQ 4-17-4	42.09	4.40	40.63	33.33	16.23	1.09	23.07	32.31	0.40	4.24
DRMRQ 4-17-6	42.02	4.43	39.95	33.67	20.16	1.09	28.17	31.20	0.52	3.82
DRMRQ 4-7-24	41.76	5.10	35.25	35.93	20.38	1.15	26.06	42.54	0.33	3.27
DRMRQ 4-7-23	41.75	4.57	41.37	34.58	15.25	1.80	20.90	35.45	0.68	2.91
DRMRQ 4-5-25	42.01	4.70	35.55	35.85	14.14	1.10	24.73	31.30	0.59	3.93
DRMRQ 4-17-46	42.13	5.01	36.72	38.34	20.18	1.66	26.60	28.12	0.46	3.50
DRMRQ 4-6-54	41.99	5.47	34.06	36.91	24.93	1.13	26.21	38.66	0.48	4.37
DRMRQ 4-10-51	42.08	4.30	34.43	38.66	18.06	1.09	26.35	28.42	0.38	3.85
DRMRQ 4-1-58	42.00	4.57	36.31	37.62	18.33	1.08	25.69	37.63	0.57	4.64
DRMRQ 4-9-53	41.93	4.70	33.72	39.41	21.74	1.19	26.82	34.61	1.26	1.65
DRMRQ 4-5-56	41.99	4.27	32.38	38.51	19.26	1.44	27.41	29.53	0.54	3.76
DRMRQ 4-16-47	41.69	4.20	23.78	38.39	26.59	1.76	28.21	28.60	0.42	2.88
DRMRQ 4-3-57	42.07	4.33	36.44	36.34	27.36	1.93	25.57	27.82	0.58	2.80
DRMRQ 5-4	42.62	4.70	34.96	32.58	20.34	1.78	28.63	26.48	1.15	3.10
NRCHB 101 (non-quality check)	41.33	3.801	1.55	19.30	35.69	39.53	134.73	47.38	0.65	3.28
PDZ1 (quality check)	42.52	3.37	29.31	33.62	24.18	1.83	28.37	29.44	0.43	2.79

Table 2: Genetic parameters for oil and seed meal nutritional traits in Indian mustard genotypes

Trait	Mean ± SEM	PCV (%)	GCV (%)	Heritability (%)	Genetic gain as % of mean at 5%
Oil content (%)	42.32±0.26	1.34	0.83	38.3	1.06
Saturated fatty acid (%)	4.75±0.16	11.95	10.42	76.1	18.72
Oleic acid (%)	34.86±0.24	10.01	9.93	98.6	20.32
Linoleic acid (%)	35.96±0.17	7.25	7.20	98.7	14.72
Linolenic acid (%)	21.22±0.15	17.04	16.99	99.5	34.91
Oleic/ linoleic ratio	0.97±0.008	13.52	13.45	98.9	27.54
ω-6/ ω-3 ratio	1.74±0.02	16.49	16.42	99.1	33.67
Erucic acid (%)	1.85±0.19	22.00	12.72	33.4	15.15
Glucosinolate (µg/g)	25.78±0.72	9.83	8.55	75.8	15.34
Antioxidant (AAE mg/g)	33.16±0.18	17.79	17.76	99.7	36.54
Flavanoid (QE mg/g)	0.79±0.12	45.79	34.74	61.8	56.25
Beta-carotene (ppm)	3.48±0.06	18.62	18.42	97.6	37.48

Table 3: Spearman's correlation among nutritional trait in Indian mustard

Trait	Oil content	Saturated fatty acid	Oleic acid	Linoleic acid	Linolenic acid	oleic/ linoleic ratio	ω-6/ ω-3 ratio	Erucic acid	Glucosi -nolate	Antio- xidant	Flava- noid	Beta- carotene
Oil content	1.00	-0.11	-0.14	0.02	0.20	-0.03	-0.18	-0.14	0.02	-0.08	0.13	0.13
Saturated fatty acid		1.00	0.04	-0.06	0.07	0.11	-0.03	-0.11	-0.02	-0.07	0.10	-0.04
Oleic acid			1.00	-0.19	-0.21	0.81	0.15	0.20	-0.26	0.01	-0.23	0.15
Linoleic acid				1.00	0.25	-0.63	0.18	-0.15	-0.11	-0.26	-0.16	-0.07
Linolenic acid					1.00	-0.27	-0.86	-0.13	0.00	-0.06	0.31	-0.11
Oleic/ linoleic ratio						1.00	-0.02	0.18	-0.07	0.16	-0.12	0.15
ω-6/ ω-3 ratio							1.00	0.05	-0.18	-0.09	-0.27	0.06
Erucic acid								1.00	0.05	-0.08	-0.11	0.15
Glucosinolate									1.00	0.13	-0.28	-0.15
Antioxidant										1.00	0.15	0.16
Flavanoid											1.00	-0.15
Beta-carotene												1.00

for flavonoid content, which indicate that simple selection methods can be used for improvement of this trait. Chauhan *et al.* (2002) and Kumar *et al.* (2013) reported similar kind of findings while studying some of the oil and meal nutritional parameters in Indian mustard. The association study among nutritional trait (Table 3) showed significant positive association between linoleic and linolenic acid that was also reported by Priyamedha *et al.* (2018), while studying different crosses among quality and high yielding varieties of *B. juncea*.

The association between oleic/linoleic ratio and oleic acid as well as between ω-6/ω-3 ratio and linolenic acid (ω-3) was found to be highly significant positive and negative respectively, which are bit obvious. Significant negative correlation between glucosinolate and oleic acid was observed, which is further supported by Chauhan *et al.* (2007) while studying quality trait in rapeseed-mustard varieties. In the study, it was observed that flavonoid showed positive correlation with antioxidant (0.15) because the flavonoid in oilseeds correlates positively with antioxidant due to presence of radical scavengers (Nieto *et al.*, 1993). A positive correlation between β-carotene and antioxidant was also observed.

SSR marker analysis and clustering of the genotypes

A total of 76 alleles generated from 30 polymorphic SSR markers (Table 4) out of 135 were used for studying genetic relatedness among the genotypes. The number of alleles per primer was varied from two to four, while the size of the fragments ranged from 100bp to 550bp. Allelic differentiation measured in terms of PIC

(Polymorphic information content) value ranged from 0.129 to 0.678. The SSR primer BRMS-042 was found to have highest PIC value of 0.678 making it most informative marker among all SSR primers used in the study. Jaccard's dissimilarity coefficients based on SSR data ranged from 0.09 to 0.74 with an average of 0.40.

The UPGMA based dendrogram representing genetic dissimilarity, grouped the 44 genotypes into four clusters with fourth cluster divided into three sub-clusters (Fig.1). First cluster comprised of three genotypes of which two were derived from the same cross (EC564648 ×[Rajat × NUDHYJ3]) showed 90% similarity and one derived from cross (EC552573 × [Varuna×NUDHYJ3]) showed about 80% similarity with both other genotypes. All the three genotypes clustered in one group were having NUDHYJ3 common in their parentage. Cluster II had 15 genotypes which included 11genotypes derived from cross having NUDHYJ5 as common parent, while 4 genotypes were having NUDHYJ3 as common parent. As reported, the double low donor parents, NUDHYJ3 and NUDHYJ5 are having same parent i.e., Heera in their origin (Barve *et al.*, 2009), which may be a probable reason for grouping of these genotypes in one cluster. Moreover, two genotypes namely, DRMRQ2-2-4 and DRMRQ2-2-3-8 showed 90% similarity derived from the same cross. Again, three genotypes grouped into third cluster with two derived from the cross (NRCHB 101×NUDHYJ5) and single genotype derived from cross (NRCDR02×NUDHYJ5). Cluster IV comprised of 23 genotypes distributed into three sub-clusters. Seven genotypes derivative of same cross (EC564648×[Rajat×NUDHYJ3]) fall into sub-cluster IV(a). Another set of seven genotypes with four derived from the cross (EC552573 × [Varuna × NUDHYJ3]) and three from the cross (EC564648 × [Rajat × NUDHYJ3]) grouped into sub-cluster IV(b). Nine genotypes with seven derived from same cross i.e., EC552573 × (Varuna × NUDHYJ3) and two from EC564648 × [Rajat × NUDHYJ3] were grouped into sub-cluster IV(c). The results indicated that the genotypes, although derived from crosses with exotic lines (EC564648 and EC552573) and high yielding varieties (NRCHB101 and NRCDR02) as parents, yet they are more or less genetically related to each other as they all are having common quality parents i.e., NUDHYJ3 and NUDHYJ5 in their parentage. Grouping of genotypes of Indian mustard derived from similar or distantly related parents into same cluster by using molecular markers were also reported by Ghosh *et al.* (2019).

Table 4: Detail of polymorphic SSR markers used in genetic diversity study among Indian mustard genotypes

Primer	Forward sequence	Reverse sequence	No. of alleles	Tm (ºC)	PIC Value
Ra2 F09	AGCCGTTATTATCGTCGTGG	TCATTGCATCAGATTGTCGG	3	55	0.39
KBRH043E02	ATGCAAGCTTCATGGTGTCA	CATCAGCAAAATTTCATTTGTGT	3	53	0.5
5Ra3-H01	TCGCGTCCCTCTTTTGATAC	CACAAACCACACATGGATGC	2	54	0.15
ENA20	GATGGAGGAAGAAGACAAGAC	TCTGAACTACCAAAGCCAAC	2	56	0.43
BRMS-011	GAACGCGCAACAACAAATAGTG	CGCGTCACAATCGTAGAGAATC	3	55	0.60
KBRH138G23	TTTGACATCGTGCAATGCTA	TTGGGCTGGTCCTGAAGATA	3	55	0.41
BRMS-042	GGATCAGTTATCTGCACCACAA	TCGGAATTGGATAAGAATTCAA	4	58	0.68
ENA8	ACTGAGAGCAACAACAACAAC	GTAGAGACGGAACCCTGA	3	55	0.66
Ni2B07	AGAGATTCAAACCGAGTGCC	GGGGCTAGCTTCATCATCC	2	55	0.48
BRMS-001	GGTGGCTCTAATTCCTCTGA	ATCTTTCTCTCACCAACCCC	2	51	0.43
Ra2-A10	CCAGTGTGTGTGTGTGTGTG	TTTAACAGATAGCGCAGTGGTC	3	55	0.67
Ra3-C04	CTAACCTCAGACGGAGACGG	CTTTAAACTCCGACCAACCG	2	56	0.50
EJU1	GGTGAAAGAGGAAGATTGGT	AGGAGATACAGTTGAAGGGTC	2	55	0.50
KBRH143H15	TCTGCATCAAAATGCTAAAATGA	TGATCTTTTAGAAACAAAGATCGAG	3	58	0.53
nia_m026a	AATGAGTAATGTCCCACACGA	TGAAATTGCGGATTCTTTAGC	2	55	0.15
BRMS-017	GGAAAGGGAAGCTTCATATC	CTGGAAAGCATACACTTTGG	3	54	0.57
cnu_m626a	TCTCTGCGTGTGATCAGTGAC	AACCGAAGGATTTTCCAACC	4	55	0.38
Ra2-G10	GAGACTCTCTCTCTCTCTCTCT	AATACGTGTGTGCCACCAAA	2	55	0.42
nia_m091a	TGGTTCTGCTATTGCTGTCA	GAAGTTTGTGAGCCAGGAAA	2	52	0.38
KBRH048O11	GCCTCTACCTGGCTTCAGCA	TCATTTGGCGCATACTTCCA	3	53	0.65
ENA20	GATGGAGGAAGAAGACAAGAC	TCTGAACTACCAAAGCCAAC	3	55	0.43
cnu_m587a	CATCATTGGCTTTGGGAGTT	CGAGTGGGAAAGAAAAACACA	2	56	0.49
BRMS-043	GCGATGTTTTTTCTTCAGTGTC	TTAATCCCTACCCACAATTTCC	2	56	0.15
nia_m043a	CCATTCGAGGTGGTCGTAAA	AGAAAACGGACCTCGATTCA	2	52	0.47
nia_m062	CGACGGCACATAAAGGAGAT	AGGATTTAGCTGACGGCTTT	2	55	0.50
Ra2-F11	TGAAACTAGGGTTTCCAGCC	CTTCACCATGGTTTTGTCCC	3	53	0.48
Ra2-G08	ATGTCCGGATAACCGAATCC	GAAGCTTTTCAATTTTTAAGTTCTCTC	2	55	0.45
BRMS-005	ACCTCCTGCAGATTCGTGTCGCT	GACCTTTCTTACCGCTC	2	53	0.13
BRMS-036	GGTCCATTCCTTTTTGCATCTG	CATGGCAAGGGGTAACAAACAT	2	55	0.49
KBRH139B23	ATCTCATGGTTGGTTCACCG	ATTTCCAAAACACACACGCA	3	57	0.58

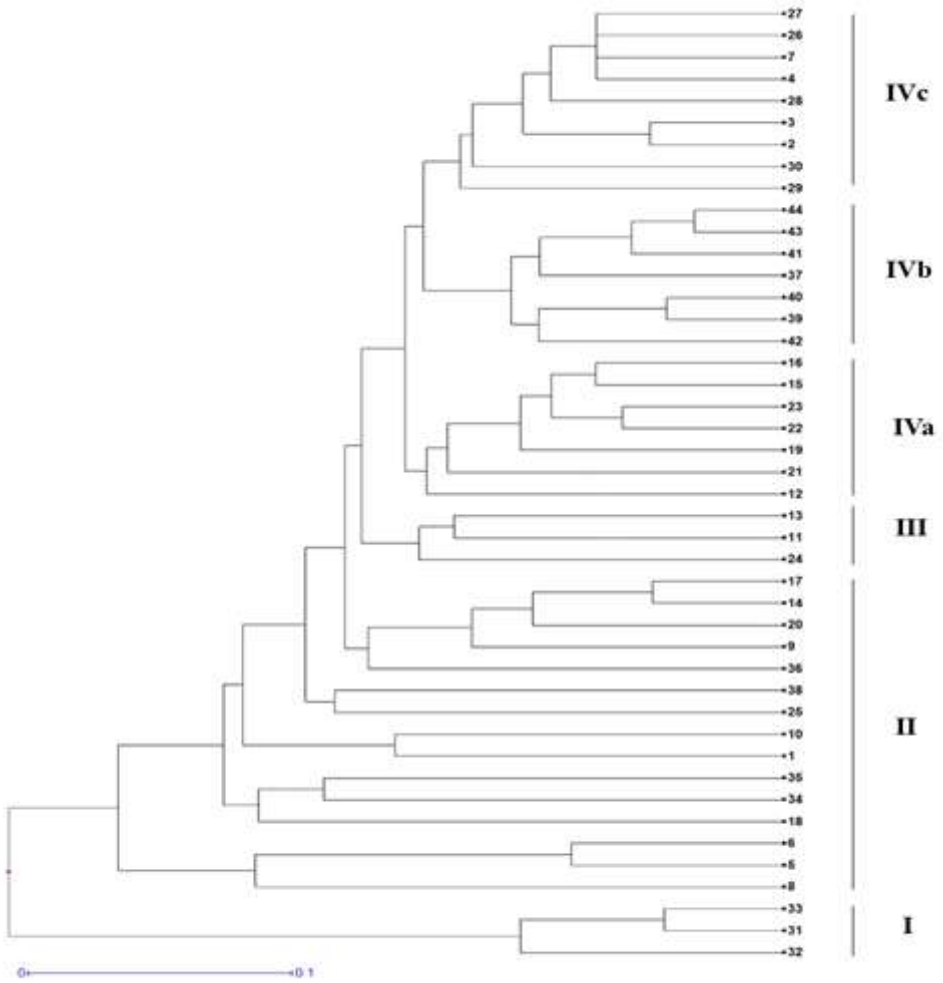

Fig.1: Dendrogram representing dissimilarity index among 44 advanced breeding lines of Indian mustard

Where; 1. DRMRQ2-1-1, 2. DRMRQ4-5-26, 3. DRMRQ4-17-4, 4. DRMRQ1-16-27, 5. DRMRQ 2-3-17, 6. DRMRQ2-1-6-3, 7. DRMRQ4-7-23, 8. DRMRQ4-5-25, 9. DRMRQ4-17-46, 10. DRMRQ2-11-10, 11. DRMRQ2-1-2-7, 12. DRMRQ1-8-34, 13. DRMRQ4-6-54, 14. DRMRQ2-2-4, 15. DRMRQ1-12-31, 16. DRMRQ1-18-25, 17. DRMRQ2-2-3-8, 18. DRMRQ2-2-7, 19. DRMRQ1-17-26, 20. DRMRQ4-10-51, 21. DRMRQ1-13-30, 22. DRMRQ1-22, 23. DRMRQ1-23, 24. DRMRQ5-4, 25. DRMRQ4-9-53, 26. DRMRQ1-11-32, 27. DRMRQ4-5-56, 28. DRMRQ4-14-48, 29. DRMRQ4-7-24, 30. DRMRQ4-3-57, 31. DRMRQ1-20-24, 32. DRMRQ4-16-47, 33. DRMRQ1-15-28, 34. DRMRQ2-1-8-6, 35. DRMRQ2-11-2, 36. DRMRQ2-1-7, 37. DRMRQ4-1-58, 38. DRMRQ2-2-16, 39. DRMRQ4-17-6, 40. DRMRQ4-11-50, 41. DRMRQ1-7-35, 42. DRMRQ4-7-55, 43. DRMRQ1-2-37, 44. DRMRQ1-10-33

Note: Parentage [DRMRQ1= EC564648 × (Rajat×NUDHYJ3), DRMRQ2= NRCHB101 × NUDHYJ5, DRMRQ4= (EC552573 × (Varuna × NUDHYJ3), DRMRQ5 = NRCDR02 × NUDHYJ5]

Conclusion

Nutritional profiling of the 44 advanced genotypes established their importance as a stable genetic resource for the mustard quality breeders. Some promising genotypes can be used as a potential donor for different nutritional traits of oil and seed meal. The distantly related genotypes as revealed by SSR marker analysis can be used for creating selectable variation for specific nutritional trait in *B. juncea*.

References

Bala M, Kumar S and Singh L. 2011. Antioxidant potential of rapeseed-mustard seed meal extracts. *Ind J Agric Biochem* **24**: 55-59.

Barve YY, Gupta RK, Bhadauria SS, Thakre RP and Pawar SE. 2009. Induced mutations for development of *B. juncea* canola quality varieties suitable for Indian agro-climatic conditions. In: Shu QY. (ed) Induced plant mutations in the genomics era. Food and Agriculture Organization of the United Nations, Rome, pp. 373-375.

Chauhan JS, Bhadauria VPS, Singh M, Singh KH and Kumar A. 2007. Quality characteristics and their interrelationships in Indian rapeseed-mustard (*Brassica sp*.) varieties. *Ind J Agric Sci* **77**: 616-620.

Chauhan JS, Tyagi MK and Tyagi P. 2002. Genetic analysis of oleic and linoleic acid content in Indian mustard (*B. juncea*) *SABRAO J Breed Genet* **34**: 73-82.

Das R, Bhattacherje C and Ghosh S. 2009. Preparation of mustard (*B. juncea*) protein isolate and recovery of phenolic compounds by ultrafiltration. *Ind Eng Chem Res* **48**: 4939-4947.

Downey RK and Bell JM. 1990. New developments in canola research. In: Shahidi F. (ed) Canola and rapeseed production, chemistry, nutrition and processing technology. New York, Van Nostrand Reinhold Publication, pp. 37-46.

Doyle JJ and Doyle JL. 1990. Isolation of plant DNA from fresh tissue. *Focus* **12**: 13-15**.**

Ghosh S, Mazumder M, Mondal B, Mukherjee A, De A, Bose R, Das S, Bhattacharyya S and Basu D. 2019. Morphological and SSR marker-based genetic diversity analysis of Indian mustard (*B. juncea*) differing in *Alternaria brassicicola* tolerance. *Euphytica* **215**: 206.

Griffiths DW, Birch ANE and Hillman JR. 1998. Antinutritional compounds in the Brassicaceae: analysis, biosynthesis, chemistry and dietary effects. *J Hort Sci Biotech* **73**: 1-18.

Ildikó SG, Klára KA, Marianna TM, Ágnes B, Zsuzsanna MB and Bálint C. 2006. The effect of radio frequency heat treatment on nutritional and colloid-chemical properties of different white mustard (*Sinapis alba*) varieties. *Innov Food Sci Emerg Technol* **7**: 74-79.

Kumar B, Pandey A and Singh KR. 2013. Genetic diversity for agro-morphological and oil quality traits in Indian mustard (*B. juncea*). *Bioscan* **8**: 771-775.

Kumar Sujith MS, Mawlong I, Nanjundan J, Aravind J and Singh D. 2017. Variation in -Carotene and other antioxidants among different species of oilseed brassica. *Ind J Agric Biochem* **30**: 129-134.

Mawlong I, Sujith Kumar MS, Gurung B, Singh K and Singh D. 2017. A simple spectrophotometric method for estimating total glucosinolates in mustard de-oiled cake. *Int J Food Prop* **20**: 3274-3281.

Mortuza GM, Dutta P and Das ML. 2006. Erucic acid content in some rapeseed-mustard cultivars developed in Bangladesh. J *Sci Food Agric* **86**: 135-139.

Nieto LM, Hoyos SG, Rubio FC, Pareja MG and Cormenzana AR. 1993. The biological purification of waste products from olive oil extraction. *Bioresour Technol* **43**: 215.

Perrier X and Jacquemoud-Collet J P. 2006. DARwin Software.

Prieto P, Pineda M and Aguilar M. 1999. Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of vitamin E. *Analyt Biochem* **269**: 337-341.

Priyamedha, Haider ZA, Kumar A, Ram B, Kumar A, Singh VV and Rai PK. 2018. Analysis of genetic parameters and correlation for yield and quality traits in Indian mustard (*B. juncea*). *J Oilseed Brassica* **9**: 146-150.

Priyamedha, Singh VV, Dubey M, Kumar P, Ram B, Kumar MSS and Rai PK. 2021. Deployment of double zero and white rust resistance trait and validation of known linked molecular markers in Indian mustard (*B. juncea*) *Ind J Genet Plant Breed* **81**: 422-430.

Renard S and Mcgregor S. 1992. Antithrombogenic effect of erucic acid poor rapeseed oil in the rats. *Rev Fr Crop Cros* **23**: 393-396.

Szydłowska-Czerniak A, Amarowicz R and Szłyk E. 2010. Antioxidant capacity of rapeseed meal and rapeseed oils enriched with meal extract. *Eur J Lipid Sci Technol* **112**: 750-760.

Teh SS and Birch J. 2013. Physicochemical and quality characteristics of cold-pressed hemp, flax and canola seed oils. *J Food Compost Anal* **30**: 26-31.

Thies W. 1982. Complex formation between glucosinolate and tetra chloro palladate (II) and its utilization in plant breeding. *Eur J Lipid Sci Technol* **84**: 338-342.

Verma U, Thakral NK and Neeru. 2016. Genetic diversity analysis in Indian mustard (*B. juncea*). *Int J Appl Math Statis Sci* **5**: 2319-3980.

Walker KC and Booth EJ. 2001. Agricultural aspects of

rape and other Brassica products. *Eur J Lipid Sci Technol* **103**: 441-446.

Wanasundara JPD. 2011. Proteins of brassicaceae oilseeds and their potential as a plant protein source. *Crit Rev Food Sci Nutr* **51**: 635-677.

Yoshie-Stark Y, Wada Y and Wasche A. 2008. Chemical composition, functional properties, and bioactivities of rapeseed protein isolates. *Food Chem* **107**: 32-39.

Zhishen J, Mengcheng T and Jianming W. 1999. The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. *Food Chem* **64**: 555-559.