

DEVELOPING GENETICALLY ENHANCED ETHIOPIAN MUSTARD (*Brassica carinata*) LINES FOR UTILIZATION AS POTENTIAL BIOFUEL SOURCE

Surinder K Sandhu¹, K H Singh², Indu Rialch^{1*}, Sanjula Sharma¹ and Amrit Pal Brar¹

¹Department of Plant Breeding and Genetics, Punjab Agricultural University, Ludhiana-141004, Punjab

²ICAR- Directorate of Rapeseed-Mustard Research, Bharatpur-321 303, Rajasthan

ABSTRACT

The present investigation was carried out to develop derived *Brassica carinata* (BBCC) lines from *Brassica juncea* and *Brassica napus*. *Brassica juncea* was crossed with *B. napus* and a variable gene pool was generated. *B. carinata* type variants were selected followed by intraspecific hybridization with high yield potential *B. carinata* lines/varieties viz., MCR-1-6, MCR-1-19, Kiran and Pusa-swarnim. Interspecific hybridization and population improvement were initiated to enrich *B. carinata* gene pool for desired traits and documentation of potential lines which can be further exploited for commercial breeding. Among all these lines, PDC 37-5, PDC 37-2, PDC 82-2, PDC 37-3, PDC 32-1 and PDC 36-1 having low level of SFA and high erucic acid are desirable as the former prevents crystal formation under colder climates, whereas, the latter with longer carbon chains allows more efficient conversion into aviation fuel and emission of fewer particulates than conventional oil. For seed yield, PDC 64-1, PDC 96-1 and PDC 55-1 were found promising lines. The developed lines infused with desired agronomic traits presented genetic stock for rainfed oilseed crop as well as environmentally sustainable source of renewable fuel.

Keywords: Determinate plant type, Ethiopian mustard, Fatty acids, Interspecific hybridization

Brassica carinata A. Braun (Ethiopian mustard) is a natural amphidiploid ($2n=34$, BBCC), thought to be derived from interspecific hybridization of two diploid species. The B genome is from *B. nigra* (BB, $2n = 16$) and the C genome is from *B. oleracea* (CC, $2n = 18$). The Triangle of U (Nagaharu, 1935) describes the close genetic relationship between amphidiploid species *B. carinata*, *B. juncea* and *B. napus*, and diploid species *B. nigra*, *B. rapa*, and *B. oleracea*. It is considered to have originated in Ethiopia, where it is used both as an oilseed as well as a leafy vegetable with little differentiation into various crop types. *B. carinata* has been evaluated as a potential oilseed crop in the United States, Canada, India, Italy and Spain. The ability to adapt in arid and semi-arid conditions, resistance to abiotic and biotic stresses and high yield levels make this crop appreciated in terms of agronomic and energy balances. It is low in oil (31.5- 34.9%) and fiber (5.1-9.4%) while high in protein content (47.1-51.3%) than that of *B. rapa* and *B. napus* for the livestock industry. Seed oil from *B. carinata* has industrial applications where oils with high erucic or linolenic acid contents are required. Recently, the use of *B. carinata* as biodiesel is being explored. Its seed oil is high in erucic acid (>40%) content, rendering it well suited for the production of biofuel and other bio-based applications (Roslinsky *et al.*, 2021).

Presently, Indian mustard occupies almost 90%

of total acreage under rapeseed-mustard cultivation in India and is highly vulnerable to biotic and abiotic stresses result in severe yield losses every year and is responsible for unstable production trends. Indian mustard crop is mainly cultivated by marginal and small-farmers, wherein about 25% of the rapeseed-mustard area is rainfed, causing critical yield losses particularly in the drought-prone areas of eastern and western parts of the country (Sharma *et al.*, 2018). In rainfed growing areas, moisture stress is one of the main constraints for sustainable and efficient mustard productivity during the crop growth and development. Brassica species with better ability to access soil moisture and improved water use efficiency could increase yields in an economic and environmentally sustainable way (Sahoo *et al.*, 2015). *B. carinata* could be a potential oilseed species for the semiarid tropics or moisture stress area, but no work has been done to explore the agronomic potential of the crop (Lal *et al.*, 2019). In case of interspecific hybridization, this crop can be used as potential donor for transfer of useful traits into other oilseed Brassicas, as this crop possesses various agronomically desirable traits, but this species is also associated with some inherent undesirable attributes like long maturity duration, tall stature, poor harvest index, small seed size and low oil content (Thakur *et al.*, 2019). To harness the potential of this crop, there is a need for genetic enhancement for desired traits including short maturity duration, short plant height, bold seed size and oil content. Siliquae of this species generally don't shatter, except in severe weather, and consequently can be directly combined

*Corresponding author : indudehal10@gmail.com

Date of receipt: 27.06.2022, Date of acceptance: 31.07.2023

when the seed has reached maturity and seed moisture is less than 9 per cent.

B. carinata is self-compatible species and has been estimated to outcross 30% of the time in the absence of pre-pollination barriers (Labana *et al.*, 1987). It can hybridize with *B. juncea* with 0.02 (11 F_1 seeds harvested from 375 pollinations) seeds per pollination (Getinet *et al.*, 1997). The high level of pairing observed in the hybrids might be due to auto/allosyndesis pairing within and among the genomes. This might be attributed to structural similarities and duplications in some of the chromosomes of species of Brassica genus (Robbelen, 1960). In present study, with the intention to introgress traits of short stature, earliness, enhancement in oil content and seed size from *B. juncea* to *B. carinata*, interspecific hybridization cycles with *B. juncea* followed by population improvement were initiated to enrich the *B. carinata* gene pool. It was expected that extant of variability shall increase in both species and the genetic stocks will be generated for desired traits with earliness, high oil content, bold seed size and short plant height from *B. juncea* and simultaneously, tolerance against abiotic and biotic stresses, profused branching, siliqua bearing upto shoot tip and more siliquae on branches from *B. carinata*. From the generated stocks, *B. carinata* type (on phenotypic basis) variants were selected and were subjected to intraspecific hybridization with *B. carinata* elite lines/varieties. This paper reports the characterization of stable novel variants of *B. carinata* type for agronomical and biochemical traits

MATERIALS AND METHODS

Two approaches viz., interspecific hybridization and population improvement were followed to enrich the *B. carinata* gene pool at ICAR-Directorate of Rapeseed Mustard Research, Bharatpur. In interspecific hybridization; Pusa Swarnim (a variety of *B. carinata*) was crossed with Indian mustard variety NRCHB 101. Resulting F_1 (ABC) were grown and diploidization of genome was realized through colchicines treatment. Subsequent generations (F_2 - F_5) were advanced through pedigree selection and named MCR. In population improvement, one *B. carinata* population "MCB 1", was grown in vicinity to *B. juncea* gene pool and open pollination was allowed. Seed set on *B. carinata* genotype was harvested and random bulk was drawn to raise next population in vicinity of *B. juncea* gene pool. Three cycles were repeated and subsequent generations were advanced through pedigree selection and selected plants were designated as MCB 1. Hybridization was further practiced among derivatives of these two populations and segregating generations as well as advanced lines were grown at PAU Ludhiana. Promising genotypes were selected at PAU Ludhiana and these were evaluated for seed yield and yield

related traits. In 2015-2016, single plant selections on the basis of desired plant type (short stature, silique till tip of branch, profuse branching and early maturity) were made in F_2 generation. F_3 seed was sown in 2016 at off season nursery at Keylong for generation advancement. The selfed F_4 seed was sown in the plot size of two rows of 5m row length at PAU, Ludhiana, Punjab state; 30.90° N, 75.86°E, during *rabi* season 2016-2017. Second cycle of single plant selections was conducted and a set of 175 lines were selected. F_5 seeds were sown at PAU, Ludhiana during *rabi* season 2017-2018 in preliminary yield evaluation trial in plot size of three rows of 3m length along with check PC-6 (*B. carinata* variety, released for commercial cultivation in Punjab state in 2015).

Based on plant architecture, seed size and colour, 106 lines were selected. These lines, at F_6 generation, were sown in confirmatory yield evaluation trial at PAU, Ludhiana during 2018-19 *rabi* season in plot size of three rows of 5m length at row to row spacing of 30cm. Data were recorded at physiological maturity for seed yield and yield components traits and were analysed statistically SAS 9.3 (SAS Institute 2011). Seed yield (g) was recorded on plot basis after manual threshing and drying of seed of each genotype along with check PC-6. For biochemical analysis, oil contents of 5g dried intact *B. carinata* seeds were determined using non-destructive bench top nuclear magnetic resonance (NMR) spectrometer (MQC23, Oxford Instruments, UK). The fatty acid composition of the *B. carinata* seed oil was analyzed using gas chromatography (GC) (model 7820A series, Agilent Technologies, Palo Alto, CA, USA) equipped with a flame ionization detector (FID). Esterification of the fatty acids was performed using the method given by Appelqvist (1968). Separations of fatty acids were carried out on an Agilent CP-Sil 88 (25m x 0.25mm x 0.20mm) FAME column. One microlitre of sample was injected at a split rate of 80:1. Individual fatty acids were expressed as percentages of the total fatty acids.

k-means clustering, a method of vector quantization, was used for clustering the 106 test genotypes which aims to partition the points (n observations) into k groups such that the sum of squares from points to the assigned cluster centers is minimized and hence partition into k clusters in which each observation belongs to the cluster with the nearest mean, serving as a prototype of the cluster. This results in a partitioning of the data space into Voronoi cells (the set of data points which are nearest to the cluster centre). The algorithm of Hartigan and Wong (1979) is used.

RESULTS AND DISCUSSION

The 106 selected lines were derived from ten different intra-specific combinations, which involved

crossing of desired *B. carinata* lines with improved *B. carinata* lines/varieties during their developmental process. Variables number of lines were selected from intra-specific cross combinations viz., 50 lines from MCB1-1-4/MCR-1-19(Set-1); 8 lines from MCB1-1-4/MCR1-6 (Set-2), 9 lines from MCB1-1-4/Kiran (Set-3), 8 lines from MCB1-1-6/MCR1-6 (Set-4), 3 lines from MCB1-2-3-7/Kiran (Set-5), 15 lines from MCB 1-2-3-13/Kiran (Set-6), 5 lines from MCB1-2-3-14/MCR1-6 (Set-7), 3 lines from MCB1-2-3-14/Kiran (Set-8), 2 lines from MCB1-2-7-2/MCR1-19 (Set-9) and 3 lines from MCB1-3-4/ Pusa swarnim (Set-10). The characterization of test genotypes (106) for seed yield revealed wide variation ranged from 0.279 to 4.54 kg/plot. Ranking of genotypes led to identification of top ten high yielding genotypes including check PC-6 (ranked 8th). The seed yield of these 10 lines ranged from 3.186 (PDC 33-3; MCB1-1-4/MCR1-19) to 4.536 kg/plot (PDC 64-1; MCB1-1-4/Kiran) with three lines viz., PDC 64-1 ; PDC 96-1 and PDC 55-1 originated from parentage MCB 1-1-4/Kiran, MCB1-2-7-2/MCR 1-19 and MCB 1-1-4/MCR 1-6, respectively yielding significantly higher than check PC-6 (Table 1). The oil content in these lines varied from 32.15 (PDC 49-2 from MCB1-1-4/MCR1-19) to 39.59% (PDC 52-2 from MCB-1-1-4/MCR-1-6). The genotype, PDC 52-2 was recorded with 39.59 % oil content which is higher (1.69%) than the commercial *B. carinata* variety PC-6 (38.93 %). The plant height, primary branches and secondary branches of the three genotypes viz., MCB 1-1-4/Kiran, MCB1-2-7-2/MCR 1-19 and MCB 1-1-4/MCR 1-6 were also higher than check variety PC-6. Amongst high yielding derived lines, out of nine selectants, five were from MCB1-1-4/

MCR1-19 (Set 1) intraspecific cross combination.

In *B. carinata* species, one of the major market acceptability hurdles is its smaller seed size than Indian mustard. So, one of the thrusts in present study was to improve seed size. Top 10 genotypes with bold seed size were recorded as PDC 82-2, PDC 14-1, PDC 33-3, PDC 82-3, PDC 80-1, PDC 31-1, PDC 4-2, PDC 12-3, PDC 83-2 and PDC 84-4. Amongst these, PDC 33-3 was also coupled with high seed yield and hence, suggested for commercial utility. In oilseed Brassicas, genetic enhancement for high oil content is one of the most important breeding objectives. Amongst oilseed amphiploids, *B. carinata* species has been recognized as low oil content species in comparison to *B. juncea* and *B. napus*; oil content among the accessions varied from 38 to 56% in *B. napus*, 20 to 31% in *B. juncea*, 25 to 29% in *B. carinata* (Sharafi *et al.* 2015). Genetic enhancement through interspecific hybridization with *B. juncea* led to remarkable improvement in derived *B. carinata* lines; for oil content, exhibiting variation from 31.26 to 41.66% in 106 lines. For oil content too, top 10 lines were demarcated as PDC 41-1, PDC 21-1, PDC 99-1, PDC 21-3, PDC 66-3, PDC 12-4, PDC 12-6, PDC 25-2, PDC 12-3, PDC 21-2. The oil content in these lines ranged from 39.96 to 41.66 % (41-1; MCB1-1-4/MCR1-19). These lines may serve as high value genetic stock in commercial breeding. Though the seed yield in these lines was relatively low but intraspecific crossing with high yield lines followed by stringent selection for both the traits can lead to significant improvement.

The nutritional properties of *Brassica* seed oil, like other fats and oils, are dependent on its fatty acids

Table 1. Seed yield, its component traits and oil content in promising derived *B. carinata* lines

Derived <i>B. carinata</i> lines	Cross combination	Seed yield (Kg/plot)	Plant height (cm)	Number of Primary branches	Number of Secondary branches	Oil content (%)
PDC 64-1	MCB 1-1-4/Kiran	4.456*	186	15	52	35.79
PDC 96-1	MCB1-2-7-2/MCR 1-19	4.086*	180	16	51	36.68
PDC 55-1	MCB 1-1-4/MCR 1-6	4.073*	111	10	49	37.43
PDC 52-1	MCB 1-1-4/MCR 1-6	3.495	150	14	34	36.67
PDC 37-1	MCB1-1-4/MCR1-19	3.364	200	13	37	35.54
PDC 33-1	MCB1-1-4/MCR1-19	3.311	125	5	20	34.14
PDC 49-2	MCB1-1-4/MCR1-19	3.331	123	6	24	32.15
PC-6 ¹	-	3.418	101	6	19	38.93
PDC 52-2	MCB 1-1-4/MCR 1-6	3.304	158	8	29	39.59
PDC 33-3	MCB1-1-4/MCR1-19	3.088	134	3	13	37.44
	Grand mean	1.920				
	CD (5%)	0.103				
	CV (%)	1.661				

¹PC-6 is commercial variety of *B. carinata* released in Punjab state, 2016

Table 2. Erucic acid content in selected derived *B. carinata* lines‡ along with their fatty acid profile

Derived <i>B. carinata</i> lines	Cross combination	Saturated fatty acids	Oleic acid	Linoleic acid	Linolenic acid	Eicosenoic acid	Erucic acid
PDC 50-2	MCB1-1-4/MCR1-19	5.05	6.7	19.05	19.59	1.03	48.59
PDC 37-2	MCB1-1-4/MCR1-19	3.49	10.75	21.29	15.64	0.56	48.37
PDC 59-1	MCB 1-1-4/Kiran	4.9	9.2	18.7	18.5	0.75	47.9
PDC 80-2	MCB 1-2-3-13/Kiran	4.49	8.2	17.22	21.73	0.77	47.59
PDC 12-3	MCB1-1-4/MCR1-19	5.19	9.39	17.01	20.18	0.76	47.46
PDC 81-2	MCB 1-2-3-13/Kiran	4.14	8.1	16.92	22.78	0.94	47.12
PDC 91-1	MCB 1-2-3-14/Kiran	4.85	8.56	15.28	23.27	0.92	47.12
PDC 12-4	MCB1-1-4/MCR1-19	5.22	9.16	16.66	21.15	0.83	46.98
PDC 10-1	MCB1-1-4/MCR1-19	4.08	10.07	18.07	20.56	0.75	46.74
PDC 12-5	MCB1-1-4/MCR1-19	5.06	9.55	17.19	20.61	0.85	46.74

‡Erucic acid content of top 10 derived *B. carinata* lines out of 106 test lines

composition, particularly the amount of oleic, linoleic, linolenic, and erucic acid. The seven major fatty acids extracted from members of the genus *Brassica* are found to be palmitic ($C_{16:0}$), stearic ($C_{18:0}$), oleic ($C_{18:1}$), linoleic ($C_{18:2}$), linolenic ($C_{18:3}$), eicosanoic ($C_{22:0}$), and erucic ($C_{22:1}$) acids. The improved 106 lines were profiled for these fatty acids. The characterization of *B. carinata* accessions revealed that oleic acid content ranged from 8.42 to 10.69 (Sharafi *et al.* 2015) but in our improved lines, oleic acid ranged from 6.7 to 16.73 (MCB1-1-6/MCR1-6). High oleic acid in edible oils is of high value as its anti-oxidative ability provides a longer shelf life to oil, eliminating the need for a hydrogenation process (Przybylski *et al.* 2013). It is well proven that it can decrease low-density lipoprotein levels in humans and then decrease the risk of cardiovascular disease. High oleic acid oils can be heated to a higher

temperature without smoking, so that the cooking time can be reduced and as a result, food takes up less oil. Amongst top 10 ranked lines (viz., PDC 67-1, PDC 41-1, PDC 97-1, PDC 64-1, PDC 37-1, PDC 69-2, PDC 80-1, PDC 49-2, PDC 60-1 and PDC 96-1); PDC 41-1, derived from MCB 1-1-4/MCR1-19, recorded with 16.34 oleic acid and 41.66 % oil content. Such lines could be of high significance as donors in commercial breeding.

Recently, very high erucic content has emerged as priority in *Brassica* breeding. Erucic acid and its derivatives represent important industrial feedstock compounds, and there is an increasing demand to produce high erucate oils in this regard. High erucic acid oil is valuable raw material for manufacture of industrial products such as plasticizers, detergents, surfactants, polyesters, film-processing agents, coatings, cosmetics, and pharmaceuticals. The major

Table 3. Saturated fatty acids content in selected derived *B. carinata* lines‡ along with their fatty acid profile

Derived <i>B. carinata</i> lines	Cross combination	Saturated fatty acids	Oleic acid	Linoleic acid	Linolenic acid	Eicosenoic acid	Erucic acid
PDC 36-1	MCB1-1-4/MCR1-19	3.31	12.77	18.57	20.11	0.8	44.44
PDC 37-5	MCB1-1-4/MCR1-19	3.37	11.18	21.87	16.77	0.75	46.06
PDC 37-2	MCB1-1-4/MCR1-19	3.49	10.75	21.29	15.64	0.56	48.37
PDC 82-2	MCB 1-2-3-13/Kiran	3.54	10.03	17.06	22.28	0.87	46.23
PDC 58-1	MCB 1-1-4/MCR 1-6	3.61	10.89	18.56	22.47	0.88	43.58
PDC 37-3	MCB1-1-4/MCR1-19	3.61	11.52	24.99	14.92	0.73	45.22
PDC 32-1	MCB1-1-4/MCR1-19	3.63	9.93	14.99	25.76	0.8	44.84
PDC 83-1	MCB 1-2-3-13/Kiran	3.63	11.77	20.64	21.29	0.72	41.94
PDC 21-2	MCB1-1-4/MCR1-19	3.64	11.33	17.76	21.98	0.79	44.5
PDC 21-3	MCB1-1-4/MCR1-19	3.68	12.87	17.41	23.2	0.77	42.06

‡Saturated fatty acids of top 10 derived *B. carinata* lines out of 106 test lines

Table 4. K-means clustering of 106 test genotypes: cluster center means and sum of squares (within and between clusters)

Cluster	No. of genotypes	Values of cluster centers							within sum of squares
		Seed yield	Oil Content	Saturated fatty acids	Oleic acid	Linoleic acid	Linolenic acid	Erucic acid	
1	7	2.36	32.32	3.79	11.18	23.49	15.25	0.71	15.786
2	37	1.92	38.53	4.36	10.30	16.95	22.38	0.81	158.849
3	28	2.32	36.20	4.53	13.41	20.63	20.08	0.77	180.233
4	35	1.55	35.48	4.94	9.81	19.89	20.39	0.89	133.525
Total of within sum of squares									488.39
Between sum of squares									359.61
Total sum of squares									848

derivative of erucic acid is erucamide, which is used as a surface-active additive in coatings and as an anti-block agent. A comparison of the performance of *B. carinata* oil-derived biodiesel with a commercial rapeseed oil-derived biodiesel and petroleum diesel fuel led to conclusion that *B. carinata* can be exploited as commercial biodiesel promising oil crop and offers the possibility of exploiting the Mediterranean marginal areas for energetic purposes. *B. carinata* lines exhibited high variation for erucic acid ranging from 33.73 (PC-6) to 48.59% (MCB1-1-4/MCR1-19). Derived *B. carinata* lines viz., PDC 10-1, 12-3, 12-4, 12-5, 37-2 and 50-2 derived from MCB1-1-4/MCR 1-19; PDC 59-1 and 80-2 derived from MCB 1-2-3-13/Kiran and PDC 81-2 and 91-1 derived from MCB1-1-4/Kiran were sorted as top 10 genotypes among 106 lines developed. The erucic acid in these lines ranged from 46.74 (PDC 12-5) to 48.59 (PDC 50-2) (Table 2). But for human consumption, it is not desired as higher erucic acid in cooking oil hampers the myocardial conductance in humans and leads to increased blood cholesterol levels. The saturated fatty acids (SFAs) include palmitic and stearic acids. The

presence and absence of these fatty acids determine the nutritional quality of the edible oils (Bhattacharya *et al.* 2012). It has been reported that an ideal edible oil should comprise of low saturated fats (less than 7%), high oleic acid (50%), moderate amounts of linoleic (<40%) and low linolenic acid (<14%). Low SFAs is also desired for *B. carinata* as biodiesel crop as saturated fats are responsible for crystal formation that leads to clouding and filter plugging in cold temperatures, making carinata oil a preferred feedstock for biodiesel in colder climates. In test lines, SFA assorted from 3.31 (PDC 36-1) to 5.71 % ((PDC 12-2), much lower than that generally found in other Brassica species viz., *B. juncea* and *B. napus*. As for other traits, test lines were also sorted for SFA content and in top rankers, SFA ranged from 3.31 to 3.68% (Table 3). Among these lines, PDC 37-2 with seed yield of 3.02 kg/plot displayed good agronomic potential coupled with low SFA (3.49) and high erucic acid (48.37). Such lines, after further evaluation, may serve as potential source for biofuels production especially in marginal areas as *B. carinata* is well suited in arid and semi-arid (Hagos *et al.*, 2020;

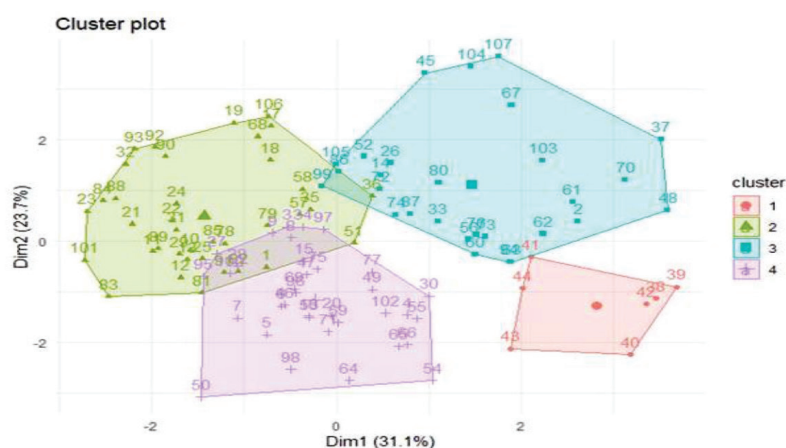


Fig. 1. K-means clustering of 106 derived *B. carinata* lines into four groups

Seepaul *et al.*, 2021) At biochemical level, *B. carinata* contain less saturated fatty acids and high erucic and linoleic acids makes this crop as desirable oilseed that can be processed into biofuel (Mulvaney *et al.*, 2019, Seepaul *et al.*, 2019)

The polyunsaturated fatty acids (PUFAs) i.e., linoleic and linolenic acids are essential fatty acids; not synthesized by human body and hence must be obtained from diet. The high linoleic acid levels in edible oil reduce blood cholesterol and prevents atherosclerosis. Linolenic acid, though essential fatty acid, but should be in moderate amount as its presence in the oil may lead to rancidity and off flavor (Sharafi *et al.*, 2015). In our lines, linoleic acid ranged from 25.69 to 11.95 whereas linolenic acid from 25.76 to 14.16. PDC 5-2 with linoleic acid 25.02 coupled with high seed yield of 4.28 kg/plot; oil content 36.67%; SFA 4.93 %; oleic acid 12.67 and erucic acid 38.1 % can be exploited in commercial breeding.

On the basis of correlation studies, it was found that oleic acid has negative correlation with erucic acid. Highly significant positive correlation of oil content with oleic acid and negative with erucic acid is of significance from breeding perspective. Oleic acid showed positive significant correlation with linoleic acid and negative significant association with erucic acid whereas linoleic acid showed negative association with erucic acid. Two genetically independent biosynthetic pathways operate in fatty acid synthesis- one which converts oleic acid to linoleic and the other which converts oleic to eicosenoic to erucic acid. SFA (Palmitic acid + Stearic acid) found negatively correlated to linolenic and erucic acids. Erucic acid is formed from oleic acid via chain lengthening process and linoleic and linolenic acids are formed by successive desaturation of oleic acid. To assess the diversity in derived lines (106) along with check, 107 lines were grouped in four clusters based on seed yield and biochemical traits. Cluster wise genotypes and within and between cluster means sum of squares are given in Table 4. K-means clustering grouped genotypes into four clusters (Fig.1), thereby exhibiting diversity in derived lines. Cluster I with high seed yield harbored seven genotypes, distinctly separated from rest of three and were derived from a single cross combination (MCB1-1-4/MCP1-19). Rest of three clusters exhibited overlapping for some lines and were not recorded any relatedness to their pedigree.

Developed *B. carinata* genotypes exhibited significant genetic gain for important yield traits over check variety of *B. carinata* (PC-6). Derived *B. carinata* lines exhibited profuse branching, silique bearing upto shoot tip and more siliques on branches along with introgressed traits of *B. juncea* viz., short stature, high oil content, high seed size and seed yield. *B. carinata*, by

virtue of its biotic and abiotic stress tolerance, low input requirements, non-pod shattering trait appended with genetic enhancements to rectify its agronomic limitations, can emerge as high value resilient oilseed crop.

Authors' contribution

Conceptualization and designing of research work (SKS, KH); Execution of field/lab experiments and data collection (IR, SS); Analysis of data and interpretation (SKS, IR, SS, APB); Preparation of manuscript (SKS, IR, SS).

LITERATURE CITED

- Appelqvist L A 1968. Rapid methods of lipid extraction and fatty acid methyl ester preparation for seed and leaf tissue with special remarks on preventing the accumulation of lipid contaminants. *Arkiv Kemi* **28**: 551–70.
- Bhattacharya S, Sinha S, Dey P, Das M and Maiti M K 2012. Production of nutritionally desirable fatty acids in seed oil of Indian mustard (*Brassica juncea* L.) by metabolic engineering. *Phytochem Rev* **11**: 197–209.
- Getinet A, Rakow G, Raney J P and Downey R K 1997. Glucosinolate content in interspecific crosses of *Brassica carinata* with *B. juncea* and *B. napus*. *Plant Breeding* **116**: 39-46.
- Hagos R, Shaibu A S, Zhang L, Cai X, Liang J, Wu J, Lin R and Wang X 2020. Ethiopian Mustard (*Brassica carinata* A. Braun) as an alternative energy source and sustainable crop. *Sustainability* **12**: 7492. doi:10.3390/su12187492.
- Hartigan J A and Wong M A 1979. Algorithm AS 136: A k-means clustering algorithm. In *Applied Statistics* **28**.1, pp. 100–108.
- Labana K S, Ahuja K L and Banga S S 1987. Evaluation of some Ethiopian mustard (*Brassica carinata*) genotypes under Indian conditions. Proceedings of the 7th International Rapeseed Congress, Poznan, Poland, May 11–14, pp.115.
- Lal B, Rana K S, Rana D S, Shivay Y S, Sharma D K, Meena B P and Gautam P 2019. Biomass, yield, quality and moisture use of *Brassica carinata* as influenced by intercropping with chickpea under semiarid tropics. *J Saudi Soc Agric Sci* **18**: 61-71.
- Mulvaney M J, Leon R G, Seepaul R, Wright D L and Homan T L 2019. *Brassica carinata* seeding rate and row spacing effects on morphology, yield and oil. *Agron J* **111**: 528-35.
- Nagaharu U 1935. Genome analysis in *Brassica* with special reference to the experimental formation of *B. napus* and peculiar mode of fertilization. *J Jap Bot* **7**: 389–452.
- Przybylski R, Gruczynska E and Aladedunye F 2013. Performance of regular and modified canola and soybean oils in rotational frying. *J Am Oil Chemists' Soc* **90**: 1271-80. doi: 10.1007/s11746-013-2278-0.
- Robbelen G 1960. Beitrage zur Analyse des Brassica Genomes. *Chromosoma* **11**: 205-28.

- Roslinsky V, Falk K C, Gaebelein R, Mason A S and Eynck C 2021. Development of *B. carinata* with super-high erucic acid content through interspecific hybridization. *Theor Appl Genet* **134**:3167–81 <https://doi.org/10.1007/s00122-021-03883-2>.
- Sahoo C R, Dash M and Acharya N 2015. Effect of water stress on nutrient content and their uptake in Indian mustard [*Brassica juncea* (L.) czern and coss] genotypes. *G J B A H S* **4**: 110-16.
- SAS Institute Inc. (2011) Base SAS® 9.3 procedure guide. SAS Institute Inc, Cary
- Seepaul R, Bliss C M, Wright D L, Marois J J, Leon R, Dufault N and Olson S M (2019). Carinata, the Jet Fuel Cover Crop: Production Recommendations for the Southeastern United States. Available online: <http://edis.ifas.ufl.edu>.
- Seepaul R, Kumar S, Iboyi J E, Bashyal M, Stansly T L, Bennett R, Boote K J, Mulvaney M J, Small I M, George S and Wright D L 2021. *Brassica carinata*: Biology and agronomy as a biofuel crop. *GCB Bioenergy* **13**: 582–99. <https://doi.org/10.1111/gcbb.12804>
- Sharafi Y, Majidi M M, Goli S A H and Rashidi F 2015. Oil content and fatty acids composition in Brassica species. *Int J Food Prop* **18**: 2145-54.
- Sharma P, Sharma H O and Rai P K 2018. Strategies and technologies for enhancing rapeseed-mustard production and farmer income. *Indian Farming* **68**: 44–48.
- Thakur A K, Singh K H, Sharma D, Parmar N and Nanjundan J 2019. Breeding and genomics interventions in Ethiopian mustard (*Brassica carinata* A. Braun) improvement – A mini review. *S Afr J Bot* **125**: 457-65.