Research Article

RACHNA CANOLA (RBN-13016): A HIGH-YIELDING RAPESEED CULTIVAR ENRICHED WITH OMEGA-3 AND OMEGA-9 FATTY ACIDS

Hafiz Saad Bin Mustafa[1]*, Misbah Zulfqar[1], Ejaz -ul-Hasan[1], Tariq Mahmood[1], Huma Qamar[1], Mariam Hassan[1], Sundas Shahzad[1], Muhammad Riaz[1], Ahsan Mohyo-ud-Din[1], Ahmad Nawaz Gill[1], Qamar Ansar Tufail[1], Muhammad Altaf[1], Idrees Ahmad[1], Muhammad Rizwan Bashir[1], Ijaz Haider[1], Ghulam Sarwar[2], Hafiz Saeed-ur-Rehman[3], Amir Hameed[4]

[1]*Directorate of Oilseeds, Ayub Agricultural Research Institute, Faisalabad, Pakistan.*
[2]*Cotton Research Institute, Multan, Pakistan.*
[3]*Ayub Agricultural Research Institute, Faisalabad, Pakistan.*
[4]*Statistical Section, Ayub Agricultural Research Institute, Faisalabad, Pakistan.*
**Correspondence: saadpbg@gmail.com*

ABSTRACT

Rapeseed oil is the prime source of two essential fatty acids, Omega-3 (alpha-linolenic acid), and Omega-6 (linoleic acid). Oleic acid is a mono-unsaturated omega-9 fatty acid that is also available in rapeseed oil in good ratio. To improve quality of edible oil and seed yield in rapeseed, comprehensive research was conducted at Oilseeds Research Institute Faisalabad (Pakistan) to evolve rapeseed cultivars requiring qualitative and quantitative characteristics with broader adaptability. The pedigree method of selection was used after hybridization (Legend x RBN-03052) during 2006-07, and homozygous progenies of 6016-B from F6 were bulked during 2012-13 for seed yield evaluation in different agroclimatic regions. Its performance was evaluated in the station and sixteen outstation seed yield trials from 2014 to 2020. Additive main effect and multiplication interaction analysis (AMMI) was performed to investigate the performance of promising line RBN-13016 along with checking Super Canola and both parents RBN-03052 (female parent) and Legend-1 (male parent) at sixteen ecological environments of Pakistan. Additive main effect and multiplication interaction analysis was done to estimate the responses of genotype, environment, and genotype x environment interactions based on seed yield. It has proved its worth in seed yield trials, and its performance was significantly better than the check variety and its parents. Rachna Canola (RBN-13016) was specifically bred for high Omega-3 (alpha-linolenic acid) and Omega-9 (Oleic acid), which were found to be significantly higher than the check variety as well as its parents. Other quantitative traits included medium maturing period, bold seed, long silique, lodging tolerance, Alternaria blight resistance, white rust resistance, high seed yield potential, good oil protein content, and broader adaptability. Based on high seed yield, high Omega-3 (9.14%) and Omega-9 (72.17%), the Punjab Seed Council (Pakistan) approved RBN-13016 as the variety "Rachna Canola" for general cultivation. Due to versatility in characteristics, this rapeseed cultivar can be effectively grown in different regions and countries with the same prevailing climatic conditions as in Pakistan.

Keywords: *Breeding, Cultivar, Rapeseed, Omega-3, Omega-9 and Productivity*

INTRODUCTION

The Brassica genus has 37 species, four commonly cultivated worldwide; including *B. juncea, B. napus, B. rapa and B. carinata*. The fatty acid composition of the rapeseed oil, obtained from traditional oilseed crops (*B. juncea, B. napus and B. rapa*) is 1% steric, 5% palmitic, 9% linolenic, 14% linoleic, 15% oleic acid and 45% erucic acid [1, 16, 4]. Due to premium quality oil for food and non-food purposes, the demand for rapeseed oil has increased daily. Rapeseed oil is comprised of good-quality fatty acids and antioxidants. Naturally, rapeseed oil and other brassica species contain high erucic acid, limiting food use. This limitation has been overcome by the development of 00 varieties (free of erucic acid and low glucosinolates). According to the Canola Council of Canada, Canola oil derived from brassica species is low in erucic acid (<2%) and glucosinolates (<30 µmol/g in meal) [13].

Oil and fatty acids are vital for all living being's growth and development. Plants, animals, and oleaginous organisms are known natural resources for

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oil and fatty acids. Plant sources primarily come from rapeseed, mustard, sunflower, soybean, olive, and palm [2]. Edible oilseed crops contain mainly five fatty acids, out of which two saturated fatty acids are stearic acid (18:0), palmitic acid (16:0), and three unsaturated fatty acids are α-linolenic acid (ALA, ω-3, 18:3), linoleic acid (ω-6, 18:2) and oleic acid (18:1), in their seed oil. Alpha-linolenic acid and linoleic acid are polyunsaturated fatty acids (PUFA), also known as essential fatty acids. Humans and animals cannot synthesize essential fatty acids, so they must be acquired through diet. On the other hand, oleic acid is a mono-unsaturated fatty acid (MUFA) necessary to enhance the stability of edible oil [15].

Omega 3 fatty acid is an unsaturated fatty acid with a double bond between the second and third carbon atoms from the end of the hydrocarbon chain. Omega-3 fatty acids (ALA, DHA, and EPA) are mostly found in seafood, such as fish. It is also obtained from rapeseed, soybean, chia, and walnut. Omega-3 fatty acid protects against various diseases. Omega-3 fatty acid helps lower blood pressure and reduce the chance of heart attack, abnormal heart rhythm and stroke, sudden cardiac death in heart disease patients, triglycerides, and plaque development in the arteries. The potential to make the longer-chain omega-3 fatty acids from ALA can be lessened as you age [8]. Omega-3 fatty acid also plays an anti-inflammatory role in building cell membranes in the brain [18]. Deficiency of ω-3 fatty acid results in heart problems, poor circulation, fatigue, skin dryness, depression, and low frustration control.

Rapeseed breeders have a big challenge in developing rapeseed cultivars with desirable fatty acid profiles to overcome the issue of malnourishment. Oilseeds Research Institute, Faisalabad, Pakistan is working on the development of high-yielding rapeseed varieties with high omega-3 and omega-9 content through conventional breeding.

MATERIALS AND METHODS

Breeding for Cultivar Development: Research was initiated to develop high yielding 00 Rapeseed cultivar having high omega-3 and omega-9 during 2006-07 at Directorate of Oilseeds, Ayub Agricultural Research Institute Faisalabad (73.0512° East, 31.4040° North) Pakistan. Based on quality analysis and high seed yield potential, legend and RBN-03052 were selected as parental lines. The parental line RBN-03052 (♀) was a locally developed high-yielding strain, while Legend (♂) was an Australian variety of rapeseed with low Erucic acid (1.5%) and Glucosinolates (< 30 µ moles/g). The cross was attempted to create genetic variability for seed yield, Omega-3, and Omega-9. The F_1 plants were grown and self-pollinated during 2007-08. The Pedigree method was used to select single plants and progeny rows based on seed yield potential, 00 quality, Omega-3, Omega-9, lodging tolerance, disease resistance, along good plant vigor [11, 12]. In the F_2 generation, 275 single plants with better plant vigor and tolerance to lodging were selected, and their seeds were collected for yield evaluation and quality analysis; homogenization selections and rejections were made until F_6 generation based on seed yield and quality analysis. In the F_6 generation, 20 plants to progeny rows were grown, and ten best lines were selected for quality analysis. Based on quality analysis, six selected lines were bulked as RBN-13016 and evaluated for seed yield in station seed yield trials.

Adaptability Study: The promising line RBN-13016 was evaluated in Multilocation seed yield trials for adaptability/stability studies in sixteen different agro-climatic zones across the country during 2018-19 and 2019-20 [22, 10, 21, 13]. Average annual rainfall of sixteen experimental locations in different agroecological zones is given in Fig. 1. Latitude, Longitude, and Altitude of sixteen diverse experimental locations are also given in Table 1.

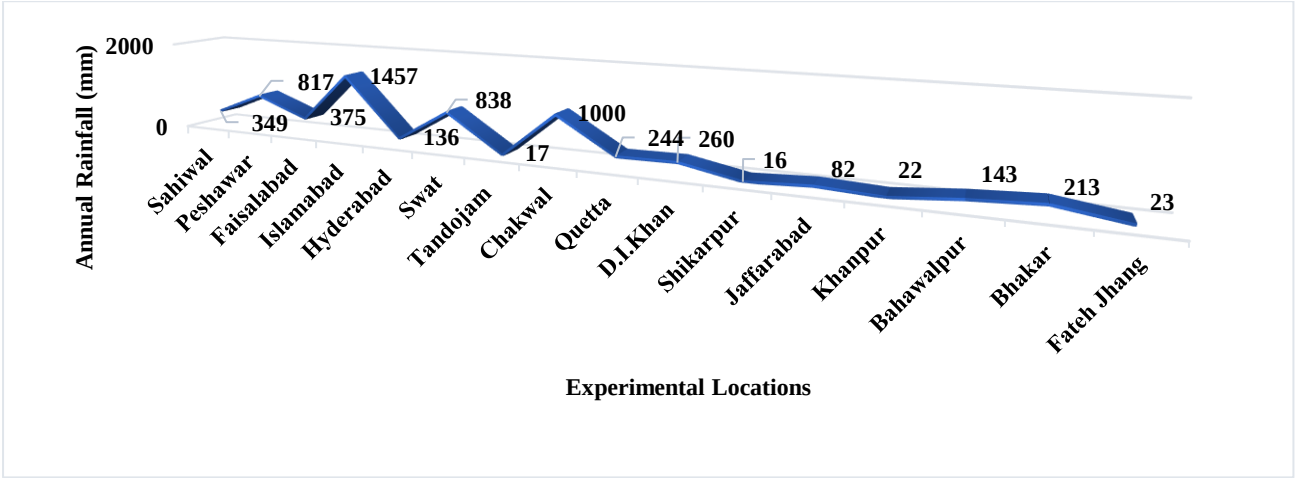

Figure 1. Average annual rainfall of sixteen experimental locations

Table-1. Latitude, Longitude and Altitude of sixteen experimental locations

Locations	Latitude and Longitude	Altitude (m)
Sahiwal	30.6682° N, 73.1114° E	152
Peshawar	34.0151° N, 71.5249° E	331
Faisalabad	31.4504° N, 73.1350° E	186
Islamabad	33.6844° N, 73.0479° E	507
Hyderabad	25.3960° N, 68.3578° E	13
Swat	35.2227° N, 72.4258° E	980
Tandojam	25.4281° N, 68.5307° E	23
Chakwal	32.9328° N, 72.8630° E	498
Quetta	30.1798° N, 66.9750° E	1680
D.I. Khan	31.8626° N, 70.9019° E	165
Shikarpur	27.9570° N, 68.6380° E	13
Jaffar Abad	28.3009° N, 68.1908° E	53
Khanpur	28.6769° N, 70.5741° E	88
Bahawalpur	28.5062° N, 71.5724° E	118
Bhakkar	31.6082° N, 71.0854° E	159
Fateh Jhang	33.5673° N, 72.6506° E	354

Oil Quality Analysis: Oilseeds calibrated Near-Infrared Reflectance Spectroscopy (Model; Perten DA 7250 Oilseeds) determined the oil content and fatty acid profile at each step to find high Omega-3 and Omega-9 lines. Gas Chromatograph also verified the oil quality of the promising line RBN-13016 at the Pakistan Council of Scientific and Industrial Research, Lahore, Pakistan.

Statistical Analysis: Statistical analyses were carried out with the statistical software Statistics 8.1 at a 5% probability level of least significant difference [14]. Data were further subjected to AMMI biplot analysis to test the adaptability and stability.

RESULTS AND DISCUSSION

Adaptability and Stability Studies in Different Agro-ecological Regions: Additive main effect and multiplication interaction analysis (AMMI) was performed to investigate the performance of promising line RBN-13016 along with check Super Canola and both parents RBN-03052 (female parent) and Legend-1 (male parent) at sixteen ecological environments of Pakistan. Additive main effect and multiplication interaction analysis was done to estimate the responses of genotype, environment, and genotype by environment interactions based on seed yield as discussed in Table-2.

Table 2. Mean sum of square data of Stability trials 2018-19 and 2019-20

Source of Variation	Degree of freedom	Seed Yield
Environment	14	3754514**
Year	1	117ns
Env * Year	15	4597**
Replication		
Error Env* Year*Rep	64	675

Gen	3	4027231**
Env*Gen	45	15312**
Year*Gen	3	629ns
Env*Year*Gen	45	3782**
Error Env*Year*Rep*Gen	192	834
Total	383	

Grain Yield Stability of Ethiopian Mustard Genotypes is estimated by Using AMMI Analysis in the Highlands of Bale, Southeastern Ethiopia [20]. Multivariate analysis for adaptability and yield stability studies of rapeseed strains in different agro-climatic zones are also used in Pakistan [5]. The differential response of genotypes, environments, and genotype × environments for seed yield have been shown and described in Figure 2.

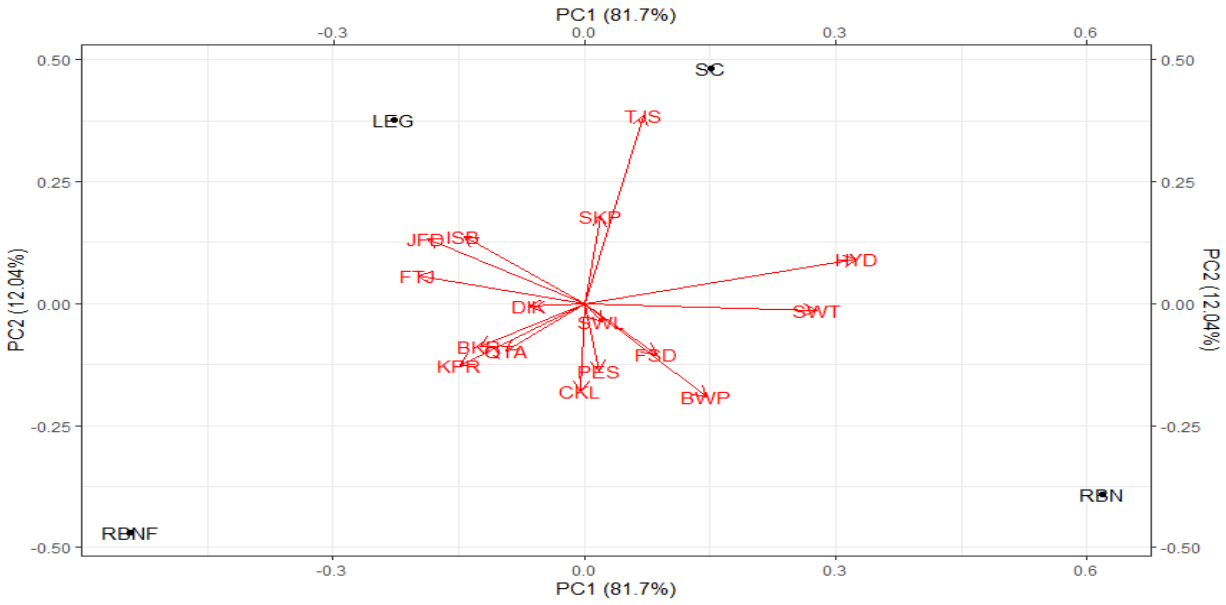

Figure 2. Description of AMMI Biplot Analysis

AMMI Biplot Analysis for Seed Yield: In the present study, the AMMI biplot graphic analysis of promising line RBN-13016 along with check Super Canola and both parents RBN-03052 (female parent) and Legend-1 (male parent) tested at sixteen different locations. This graph showed that the first two principal components explained 93.74% of the total variance (Fig. 2). AMMI biplot tells us that the ideal genotype must have a high yield and more stability. So, based on a biplot analysis of two years of data, two mega-environments were identified. The first mega-environment contains locations Faisalabad, Sahiwal, Islamabad, Chakwal, Bahawalpur, Khanpur, Bhakkar, Fateh Jhang, D.I. Khan, Quetta, Peshawar and Jaffarabad, and the second mega-environment contains locations Swat, Tandojam, Hyderabad and Shikarpur. The present study exhibited that RBN-13016 performed best in all the locations of the first mega-environment (Table-3). The other genotypes had the shortest vectors, showing that they were less responsive to interaction with the environments. This graph exhibited that experimental sites have environmental diversity, and genotypes showed different genetic behavior, which is evident in the PC

graph. An ideal environment should be highly differentiating of the genotypes and representative of the target environment. So, in the current study, locations Tandojam, Hyderabad, and Swat were the most discriminating of the entries, as exhibited by the longer distance between their markers and origin (Table-4 and Fig. 2). AMMI analysis is used to evaluate brassica strains in different agro-climatic regions [14].

Table 3. LSD all pairwise mean comparison test of Seed Yield for Genotypes

Genotypes	Seed Yield
RBN-13016	2304.7^{A}
Super Canola (C)	2019.9^{A}
Legend (M)	1881.6^{C}
RBN-03052 (F)	1859.8^{D}

Table 4. LSD all pairwise mean comparison test of Seed Yield for Genotypes

Genotypes	Seed Yield
Sahiwal	2906.3^{A}
Peshawar	2906.0^{A}
Faisalabad	2817.7^{B}
Islamabad	2665.1^{C}
Hyderabad	2480.7^{D}
Swat	2422.8^{E}
Tandojam	1967.7^{F}
Chakwal	1898.4^{G}
Quetta	1876.9^{H}
D.I. Khan	1861.0^{I}
Shikarpur	1845.4^{J}
Jaffarabad	1758.1^{K}

Khanpur	1574.8^{L}
Bahawalpur	1271.6^{M}
Bhakkar	1268.5^{M}
Fatehjang	743.0^{N}

Twenty-two spring oilseed rape genotypes were studied at five experimental sites, and (AMMI) model was used to determine the genotype, environment, and GE effects [3]. They found mean seed yield was highly affected by environment, genotype-by-environment interaction, and genotype effects, respectively. The AMMI model could distinguish and recommend genotypes with narrow and broad adaptations. The response and yield stability of canola genotypes to multi-environments is studied by using GGE biplot analysis [17]. The GGE biplot model effectively interpreted this study's G, E, and GEI variation and best revealed the relationships between both genotypes and environments. Genotype-Environment interaction in Rapeseed (*Brassica napus* L.) in two normal and stress conditions were studied by following the AMMI Model [6]. It was found that the treatment and site's main effects and treatment by sites interaction effect were significant for the seed yield of rapeseed cultivars.

AMMI model was used to study Genotypes × Environment interaction analysis for Ethiopian mustard (*Brassica carinata* L.) genotypes [19]. Usefulness of AMMI model was demonstrated along with biplot analyses in the interpretation of seed yield data from a multi-environment experiment in identifying stable genotypes. The AMMI model analysis provided estimates of the magnitude and significance of the effects of GE interaction and its interaction principal components relative to G and E effects. The stability and adaptability of genotypes were estimated through AMMI biplots.

Quality Characteristics: Quality analysis of RBN-13016 and check varieties showed that the strain RBN-13016 has the erucic acid 0.2% and glucosinolates 23 µ mole/g in oil and meal. It has 41-42 % edible oil. In addition, RBN-13016 contains significantly high omega-3 (9.14%) and omega-9 (72.17%) in its oil. It has a good balance between omega-3 (9.14%) and omega-6 (22.66%), which is why it is a healthy edible oil. The most important in nature is oleic acid (omega-9) monounsaturated fatty acid, also present 72.17% in RBN-13016. The fatty acid proofing is showed in Figure 3.

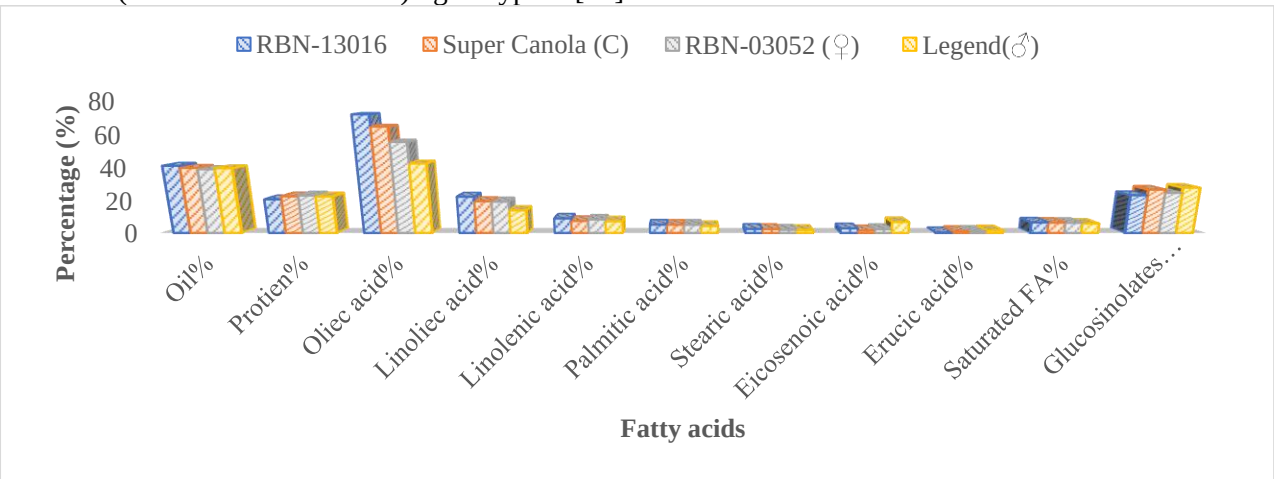

Figure 3. Quality analysis of RBN-13016 in comparison with check variety and parents

Experiment was conducted for Comprehensive evaluation of high-oleic acid using Gas Chromatography to identify high-oleic acid rapeseed lines rapidly (7). Near-infrared analysis for protein estimation wa used. Analyzation of 26 Rapeseed and Mustard genotypes was done through Gas Chromatography to determine fatty acid profile [15]. They observed significant differences among the genotypes for oil content and fatty acids content. A comparative study of 262 samples of winter oilseed

rape with different content of fatty acids in oil was carried out through Near Infrared Reflectance Spectroscopy (NIR) [9]. The reference method was Gas Chromatography (GC). Verifying a validation equation in 50 randomly selected samples proved high correlation coefficients between NIRS analysis and GC values. They concluded that non-destructive NIRS analysis enables rapid and reliable selection of materials with different compositions of fatty acids in Brassica seeds.

The salient morphological characteristics of the newly developed variety Rachna Canola (RBN-13016) are listed in Table 5.

Table 5. Salient Characteristics of Rachna Canola (RBN-13016) Vs Check Variety Super Canola

Parentage	Legend × RBN-03060	
Pedigree	F1, 2024, 3035, 4044, 5025, 6016-B (RBN-13016)	
Plant Characteristics	**Rachna Canola**	**Super Canola (C)**
Botanical name	*Brassica napus* L.	*Brassica napus* L.
Days to maturity (days)	130 -140	170 -180
Plant height (cm)	180 - 200	200 - 230
Stem thickness (mm)	19-21	22-23
Days to flower initiation (days)	50-60	70 – 80
Siliqua length (cm)	6-7	4-5
1000 seed weight (g)	3.20 - 4.00	3.00 -4.00
Oil (%)	41-43	40 – 42
Protein (%)	21-22	20-21
Erucic acid (%)	0.2	0.3
Glucosinolates μ m/g oil free meal	23	24
Oleic Acid (Omega-9) %	72.17	54.2
Linoleic Acid (Omega-6) %	22	23
Alpha Linolenic Acid (Omega-3) %	9.14	8.0

Figure 4. Rachna Canola (RBN-13016) at seed development stage

CONCLUSION

Rachna Canola (RBN-13016) is a new high-yielding double-zero rapeseed variety with good quality oil and meal. Rachna Canola contains more omega-3 and omega-9 fatty acids than all other prevailing rapeseed cultivars. This variety has been approved for general cultivation based on wider adaptability in agroecological zones. It is concluded that Rachna Canola will help curb undernourishment and ensure food security.

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