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Characterization of Rapeseed Germplasm for Various Quality Parameters

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Abstract: Pakistan is facing with a chronic deficit in edible oil production with better quality characteristics for value added products. Biochemical characterization was evaluated in 20 rapeseed (10 entries each of *Brassica napus* and *Brassica campestris)* genotypes. These lines were characterized for major long-chain fatty acids, protein contents, glucosinolates, moisture % age, oil contents using NIRS, Spectrophotometer and NMR respectively. The biochemical assessment of *Brassica napus* genotypes revealed the range of 6.5 to 7.8% moisture, 43.3 to 47.4 % oil contents, 23.2 to 30.4 % protein contents, 49.0 to 53.8 % oleic acid and 7.9 to 9.6 % linolenic acid, while that of *Brassica campestris* genotypes showed the range of 5.5 to 7.6 % moisture, 44.1 to 48.1 % oil contents, 22.8 to 26.0 % protein contents, 38.5 to 47.9 % oleic acid and 8.0 to 10.2 % linolenic acid. Most of the lines were found to have a high percentage of oil and protein contents and oleic acid that can further be utilized in objective specific *Brassica* breeding program. Almost all the lines were high in erucic acid and glucosinolates except *Brassica* campestris line 399596 which was low in glucosinolates (28.8 µmol g^{-1}). Further, highly significant negative correlation was observed for protein and oil contents among all the genotypes studied. The genotypes/lines with better quality characteristics should be exploited to improve the quality characteristics of rapeseed.

Key words: Moisture content · Oil content · Protein content · Oleic acid · Linolenic acid · Erucic acid Glucosinolates · Rape seed and Germplasm

INTRODUCTION

The improvement of chemical composition and quality of *Brassica* is one of the breeding priorities. Previously, efforts were mainly directed towards increase in seed oil yield. During recent years, due to growing awareness about the nutritional quality of the oil and meal, the emphasis has shifted towards breeding for quality traits in rapeseed. The local rapeseed cultivars have high amounts of nutritionally undesired components such as erucic acid and glucosinolates and low amount of the desired components like protein, oil and essential fatty acids. The monounsaturated oleic acid is important from nutritional standpoint because it lowers the undesirable LDL cholesterol level and also confers high stability required for healthy cooking [1] and monounsaturated fatty acids more effectively prevent arteriosclerosis than polyunsaturated fatty acids [2]. The high oleic oil can be heated to a higher temperature without smoking, so that the cooking time is reduced and less oil is absorbed [3]. Genotypes with increased oleic acid contents have been reported for several food crops, such as, *Brassica rapa* L. [4] *Brassica carinata* A. Braun [5] and *Brassica napus* [6]. The development of low C18:2 and C18:3 canola types indirectly raises the C18:1 levels and produces a canola oil with greater heat stability (High CI8:1) and reduced

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potential to go rancid (Low CI8:3). This new novel fatty acid profile of *Brassica napus* would be >80% CI8:1, <10% CI8:2, <2% CI8:3. Fatty acid composition of oil of the zero erucic acid *Brassica napus* L. is rich in oleic acid and contains moderate levels of linoleic and linolenic acid, which is advantageous primarily for human nutrition to obtain the highest possible content of oleic acid and to maintain the 2: 1 ratio of linoleic to linolenic acid, while preserving the average total content of saturated acids [7]. Polyunsaturated fatty acids are highly susceptible to auto-oxidation, which involves the production of free radicals, implicated in a number of diseases, tissue injuries and in the process of aging [8]. As a consequence, the reduction of the levels of polyunsaturated fatty acids and their substitution by the monounsaturated oleic acid is an important goal for the development of higher quality oil [9].

Linolenic acid is recognized as an essential fatty acid and has a role in reducing plasma cholesterol levels [10]. The ideal rapeseed oil for human diet uses would contain less than 3 % of C18:3 [11]. Rajcan *et al*. [12] studied that the undesirable characteristic of rapeseed oil is a relatively high level of linolenic acid (18:3), which is easily oxidized leading to rancidity and a shortened shelf life of the oil.

Kaushik [13] studied that erucic acid and glucosinolates are the two toxic substances found in rapeseed/mustard. The erucic acid not only affects the taste and flavour but also cause heart problem in human by increasing cholesterol level. Jourdren *et al*. [14] reported that rapeseed oil rich in erucic acid have led to increased interest in the improvement of the high-erucic-acid (50-60%) varieties which has many industrial applications. Glucosinolates cause the nutritional disorder and adversely affects growth and reproduction of animals as well if fed at significant levels in diet [15]. At high levels glucosinolates can adversely affect thyroid function by competing with the iodine due to similar structure and cause goiter problem in animals [16]. The presence of some glucosinolates in agricultural crop plants, such as oilseed rape *(*Brassica napus*)* and Brassica vegetables has been reported to create a good defense system against various pathogens [17].

The present study was undertaken to assess the rapeseed diversity for quality characters and to evaluate for glucosinolates, erucic acid and modified fatty acid profile thus providing necessary information to the Brassica breeders for further strengthening the Brassica breeding program.

MATERIALS AND METHODS

Twenty genotypes/lines of rapeseed (10 entries each of *Brassica napus* and *Brassica campestris*) were assessed biochemically during the present study. The diversity, provided by the Institute of Biotechnology & Genetic Engineering, The University Agricultural Peshawar was planted in the IBGE green houses. The following rapeseed genotypes were used in the research study Table 1.

Rapeseed diversity was assessed for quality parameters, including oil and moisture content by Nuclear Magnetic Resonance (NMR), protein and fatty acids using Near Infrared Reflectance Spectroscopy (NIRS) and glucosinolates by Spectrophotometer.

NMR analysis is a non destructive technique which does not require any sample preparation or chemicals. Oil and moisture contents were determined in whole seed scanned on NMR system (Oxford instrument MQA 7005).

First of all a few standards (Sample with known oil and moisture content) were run on NMR in order to standardize the NMR apparatus. Afterwards each sample was run accordingly and readings were obtained.

Near infrared reflectance spectroscopy (NIRS) is the measurement of the wavelength and intensity of the absorption of near infrared light by a sample. Near infrared light spans the 800 nm to 2.5 μm (12, 500 to 4000 cm^{-1}) range and is energetic enough to excite overtones and combinations of molecular vibrations to higher energy levels. NIR spectroscopy is typically used for quantitative measurement of organic functional groups, especially O-H, N-H and C=O. The components and design on NIR instrumentation are similar to UV - Vis absorption spectrophotometers. The light source is usually a PBS solid state detector. Sample holders can be of glass or quartz and typical solvents are CCl_4 and CS_2. Protein and Fatty acid (oleic acid, linolenic acid and erucic acid) of all 20 rapeseed *(Brassica napus* and *Brassica campestris*) were determined on a NIR system FOSS (6500) [18] at Oilseed Laboratory, Nuclear Institute for Food and Agriculture (NIFA) Tarnab, Peshawar. NIR analysis is a non destructive technique which does not require any sample preparation or chemicals. Protein and Fatty acids (CI8:1, CI8:2, CI8:3) were determined in whole seed scanned on NIR system (FOSS 6500 equipped with ISI version I.02a software program of infra Soft International). The samples of all 20 rapeseed lines were scanned three times to minimize the sampling error.

Table 1: Description of all 20 rapeseed genotypes used in the research study

S.No	*B. napus*	S.No	*B. campestris*
1	Torch	1	T-16
2	Altex	2	2163
3	Maluka	3	2065
4	ww-15171	4	1203
5	Baro	5	366
6	Global	6	399596
7	Puma	7	459976
8	8a111-1	8	TP-57-1
9	Narindra	9	P1-367601
10	366822	10	P1-392029

The observations were recorded. The reference calibration for fatty acids (CI8:1, CI8:2, CI8:3) on NIR System was developed by the estimation of fatty acid through Gas Chromatography (UNICAM 610 capillary column).

Spectrophotometer (Shimadzu UV 1700) was used for assessment of glucosinolates in *Brassica* germplasm. About 1 g seed was taken in a 5 ml of plastic scintillation vial containing the small size steel rods and added 3 ml of myrosinase activation buffer (1 mM Sodium ascorbate, 0.02 M imidazole, pH 5.5) to each vial. The vial containing mixture was vigorously shaken for 10 minutes and an equal volume of 1 ml charcoal, was also added to each vial after shaking. Each mixture was then transferred into 2 eppendorf and aqueous phase was recovered by centrifugation at 10000 rpm for 5 minutes. The supernatant was transferred to a fresh tube containing 2 ml of colour reagent. Also eight glucose standard solutions of known concentration were prepared by serial dilution of the 8 mM glucose solution. The samples and standards were then kept in water bath at 37°C for 15 to 20 minutes. The 8 mM glucose solution is equivalent to 160 µmole glucosinolates per gm seed at absorbance of 546 nm on a spectrophotometer.

A calibration curve was constructed from the standards and the unknown concentration of glucosinolates content was estimated by comparing its intensity with that of the standards.

Data analysis was performed using SPSS 13.0 software for Windows (Chicago, USA). Two-way analyses of variance (ANOVAs) were performed and treatment means were compared using LSD test. Regression analyses were used for protein and oil content.

RESULTS AND DISCUSSION

The 20 rapeseed (Brassica napus and Brassica campestris) genotypes included in the present study were assessed for various quality parameters including moisture and oil content by Nuclear Magnetic Resonance (NMR), Fatty acids and protein by Near Infrared Reflectance Spectroscopy (NIRS) and glucosinolates by UV -spectrophotometer. The statistical analysis of the data showed that all the parameters were significantly different among all the *Brassica napus* and *Brassica campestris* genotypes, studied.

Moisture Content (%): The moisture content among the *Brassica napus* and *Brassica campestris* genotypes aranged from 6.5 to 7.8 % and 5.5 to 7.6 % of fresh seed, respectively (Table 2 and 4). In case of *Brassica napus* the maximum moisture content of 7.8 % was found in Maluka closely followed by Altex, Narindra and Baro respectively, while minimum moisture content of 6.5 % was observed in Global. The mean value of 10 *Brassica napus* genotypes for moisture content was 7.27 ± 0.40 % (Table 2 and 3) where as that of *Brassica campestris* the maximum moisture content of 7.6 % was found in entry 366 closely followed by TP-57-1 and PI-367601, while minimum moisture content of 5.5 % was observed in T-16. The mean value of 10 *Brassica campestris* genotypes for moisture content was 6.89 ± 0.60 % (Table 4 and 5). The seed moisture content (4-9%) of the total seed weight for *Brassica napus,* which was important for commercial value and storage point of view [19]. The amount of moisture in the fresh oil seeds varies from 5.50 to 8.50%, which is somewhat similar to our findings (20).

Oil Content (%): The oil content of the *Brassica napus* genotypes included in the study ranged from43.3 to 47.4 %, with a mean value of 45.44± 1.54 of fresh seed. Maximum oil content (47.4 %) was observed for 8a111-1 closely followed by Altex (47.1 %), while ww-15171 exhibited minimum percentage (43.3 %) of oil. Mean value of 10 *Brassica napus* genotypes for % oil content was 45.44 ± 1.54 of fresh seed (Table 2 and 3). While that of *B. campestris* genotypes ranged from 44.1 to 48.1 % of fresh seed with a mean value of 45.67 ± 1.45 of fresh seed. Maximum oil content (48.1 %) was observed for 2163, while 459976 exhibited minimum percentage (44.1 %) of oil (Table 4 and 5). Similar oil content (44.3 %) was found by Velasco *et al*. [21] using NIRS for screening of quality traits in rapeseed. However, comparatively lower levels of oil content in seed ranging from 38 to 44 % and 36 to 46 % were reported by Novoseloy *et al.* [22] and Si *et al.* [23] respectively. These differences may be due to variation in genotype of diversity and/or environmental influences. Brassica cultivars did not show statistical differences for oil content in both years of 2003 and 2004 [24].

Table 2: Composition of 10 *Brassica napus* lines (Average values) as determined by NMR, NIRS and Spectrophotometer

Parameters	N	Minimum	Maximum	Range	Mean	Std. Error	Std. Deviation	Variance
Moisture content (%)	10	6.5	7.8	1.3	7.27	0.12	0.40	0.16
Protein content (%)	10	23.2	30.4	7.2	26.83	0.68	2.16	4.69
Oil content (%)	10	43.3	47.4	4.1	45.44	0.48	1.54	2.37
Oleic acid (%) 18:1	10	49.0	53.8	4.8	51.13	0.52	1.64	2.70
Linolenic acid (%) 18:3	10	7.9	9.6	1.7	9.00	0.18	0.59	0.35
Erucic acid (%) 22:1	10	34.1	52.5	18.4	44.52	2.19	6.92	48.00
Glucosinolates µmol g^{-1}	10	46.7	111.4	64.7	83.96	7.73	24.44	597.55

Table 3. Composition of individual genotype of *Brassica napus* as determined by NMR, NIRS and spectrophotometer

Genotypes	Moisture content (%)	Protein content (%)	Oil content (%)	Oleic acid (%)	Linolenic acid (%)	Erucic acid (%)	Glucosinolates(µmol g^{-1})
Torch	7.4 BC	29.1 B	43.4 E	49.9 EF	9.3 ABC	47.6 D	46.7 J
Altex	7.6 AB	26.6 E	47.1 AB	49.0 G	9.1 C	51.0 B	51.4 I
Maluka	7.8 A	25.9 F	46.5 C	49.3 FG	8.4 D	52.5 A	106.7 C
ww-15171	7.4 BC	30.4 A	43.3 E	50.8 D	8.3 D	49.5 C	110.9 B
Baro	7.5 AB	28.7 C	45.5 D	53.0 B	7.9 E	52.5 A	85.1 F
Global	6.5 E	27.4 D	43.7 E	53.8 A	9.1 C	37.2 H	94.6 E
Puma	6.8 DE	26.3 E	45.2 D	51.9 C	9.6 A	38.3 G	111.4 A
8a111-1	7.1 CD	23.2 H	47.4 A	50.1 E	9.5 AB	39.5 F	72.5 G
Narindra	7.6 AB	24.4 G	46.8 BC	50.8 D	9.2 BC	43.0 E	62.0 H
366822	7.0 D	26.3 E	45.5 D	52.7 B	9.6 A	34.1 I	98.3 D
LSD at $P \leq 0.01$	0.364	0.356	0.426	0.677	0.356	0.386	0.257

Table 4: Composition of 10 *Brassica compestris* lines (Average values) as determined by NMR, NIRS and Spectropohometer

Parameters	N	Minimum	Maximum	Range	Mean	Std. Error	Std. Deviation	Variance
Moisture content (%)	10	5.5	7.6	2.10	6.89	0.19	0.60	0.36
Protein content (%)	10	22.80	26.00	3.20	24.50	0.35	1.13	1.29
Oil content (%)	10	44.1	48.1	4.00	45.67	0.46	1.45	2.12
Oleic acid (%) 18:1	10	38.5	47.9	9.40	44.00	0.81	2.56	6.58
Linolenic acid (%) 18:3	10	8.0	10.2	2.20	8.81	0.18	0.59	0.35
Erucic acid (%) 22:1	10	39.3	54.8	15.50	48.91	1.40	4.44	19.79
Glucosinolates µmol g^{-1}	10	28.8	118.8	90.00	84.17	8.30	26.27	690.27

Table 5: Composition of individual genotype of *Brassica campestris* as determined by NMR, NIRS and Spectrophotometer

Genotypes	Moisture content (%)	Protein content (%)	Oil content (%)	Oleic acid (%)	Linolenic acid (%)	Erucic acid (%)	Glucosinolates (µmole g^{-1})
T-16	5.5 G	25.8 B	44.2 FG	38.5 I	8.9 BC	54.8 A	118.8 A
2163	6.7 EF	22.8 G	48.1 A	44.4 E	8.4 D	49.0 E	90.4 D
2065	7.0 CDE	23.0 G	44.8 E	45.7 C	8.0 E	45.8 H	115.1 B
1203	7.2 BCD	23.7 F	47.0 BC	45.1 D	8.5 D	47.6 G	78.3 G
366	7.6 A	25.8 B	44.8 E	46.2 B	8.7 CD	48.9 E	82.0 F
399596	6.4 F	24.3 DE	44.5 EF	47.9 A	10.2 A	39.3 I	28.8 J
459976	6.9 DE	26.0 A	44.1 G	43.8 F	8.4 D	47.9 F	63.5 I
TP-57-1	7.4 AB	24.2 E	47.3 B	42.3 H	8.9 BC	53.9 B	88.3 E
P1-367601	7.3 ABC	24.6 CD	46.7 C	43.1 G	8.9 BC	52.6 C	73.5 H
P1-392029	6.9 DE	24.8 C	45.2 D	43.0 G	9.2 B	49.3 D	103.0 C
LSD at $P \leq 0.01$	0.30	0.31	0.37	0.34	0.32	0.28	0.33

Protein Content (%): Protein and major fatty acids (Oleic acid, linolenic acid and erucic acid) were analyzed by Near Infrared Reflectance Spectroscopy (NIRS). The protein content of 10 *B. napus* genotypes ranged from 23.2 to 30.4 % of fresh seed with a mean value of 26.8 % 26.83 ±2.16. ww-15171 had the maximum protein content of 30.4 %, while 8alll-1 yielded the minimum protein content of 23.2 % (Table 2 and 3) where as that of 10 *Brassica campestris* genotypes ranged from 22.8 to 26 % with a mean value of 24.50% ±1.13 of fresh seed. 459976 had the maximum protein content of 26 %, while 2163 yielded the minimum protein content of 22.8 % (Table 4 and 5). These results are in agreement with the findings of Velasco *et al*. [21] and Muhammad *et al*. [25] who reported the protein value ranging from 13.4 to 28.3 % and 23.8 to 25.5 %, respectively in fresh seed of *Brassica* genotypes. Significant variations between cultivars and years could be the combined effect of genetic make of the cultivars tested and climate variations of years.

Oleic Acid Content (%): The monounsaturated oleic acid is important from nutritional standpoint because it lowers the undesirable LDL cholesterol level and also confers high stability required for healthy cooking. The oleic acid in *Brassica napus* and *Brassica campestris* genotypes ranged from 49 to 53.8 % and 38.5 to 47.9 % of the total fatty acids, respectively. In case of *B. napus* maximum oleic acid was found in Global (53.8%), while Altex showed the minimum value of 49 %. Mean oleic acid content for 10 *Brassica napus* genotypes was 51.13 ± 1.54 % (Table 2 and 3). Moreover in *Brassica campestris* maximum oleic acid was found in 399596 (47.9%) whereas T-16 showed the minimum value of 38.5 %. Mean oleic acid content for 10 *Brassica campestris* genotypes was 44 ± 2.56 % (Table 4 and 5). The level of oleic acid found was lower than that of Pallot *et al*. [26] who reported a range of 56 to 74 % of oleic acid in *Brassica* using NIRS. However, Agnihotri and Kaushik [27] reported comparatively lower values of oleic acid content ranging from 40 to 50 % in fresh seed of *Brassica napus.*

Linolenic Acid Content (%): Several studies on recently developed low linolenic acid lines of canola have demonstrated that this type of canola oil has improved storage and frying stability. The polyunsaturated linolenic acid of *Brassica napus* genotypes ranged from 7.9 to 9.6 % of the total fatty acids in our study. Maximum value of 9.6 % for linolenic acid was observed in Puma closely followed by 8all1-1 and Torch, while Baro showed the lowest value of 7.9 %. Mean linolenic acid content for 10 *Brassica napus* genotypes was 9 ± 0.59 % (Table 2 and 3) while that of *10 Brassica campestris* genotypes ranged from 8 to 10.2 %. Maximum value of 10.2 % for linolenic acid was observed in 399596, while 2065 showed the lowest value of 7.9 %. Mean linolenic acid content for 10 *Brassica campestris* genotypes was 8.81 ± 0.59 % (Table 4 and 5). Similar findings were reported by Ishida *et al*. [28] who observed 3.3 to 13.1 % linolenic acid in Brassica napus cultivars.

Erucic Acid Content (%): In the present study erucic acid content among the *Brassica napus* genotypes ranged from 34.1 to 52.5 %. The Maluka had the highest erucic acid content of 52.5 %, while 366822 showed the lowest value for for erucic acid 34.1 % of fresh seed. Mean erucic acid content for 10 *Brassica napus* genotypes was 44.52 % ± 4.4 % (Table 2 and 3) while that of *Brassica campestris* genotypes ranged from 39.3 % to 54.8 %of the total fatty acids. The T-16 had highest erucic acid content of 54.8 % whereas 399596 showed the lowest value for erucic acid 39.3 % (Table 4 and 5). Luhs *et al.* [29] reported that series f alleles have been identified in *Brassica napus* and Brassica rapa which make it possible to breed strains containing almost any level of erucic acid from less than 1 % to about 60 % of total fatty acids. Bhardwaj and Hamama [30] reported significant variation among 455 accessions of *Brassica napus* for erucic acid content.

Glucosinolates Content (%): Glucosinolates, the sulfur containing compounds were analyzed by spectrophotometer. The glucosinolate content of *Brassica napus* genotypes ranged from 46.7 to 111.4 µmol g^{-1} with the mean value of 64.85± 6.92 µmol g^{-1} of fresh seed. The genotype Puma showed the maximum value of 111.4 µmol g^{-1} whereas Torch showed the minimum value of 46.7 µmol g^{-1} (Table 2 and 3). In addition to that *Brassica campestris* genotype ranged from 28.8 to 118.8 µmol g^{-1} of fresh seed with mean value of 84.17± 26.27 µmol g^{-1} (Table 4 and 5). The genotype T-16 showed the maximum value of 118.8 µmol g^{-1} for glucosinolate content while 399596 showed the minimum value of 28.8 µmol g^{-1} that may be used in breeding program for developing canola type varieties/hybrids. Velasco *et al*. [31] reported a lower mean value of 51.2 µmol g^{-1} of rapeseed using NIRS. Bhardwaj and Hamama [30] reported higher glucosinolates content in *Brassica napus* (49.2 µmol g^{-1}) than *Brassica napus* mean (43.8 µmol g^{-1}).

A relationship between protein and oil content was also studied using regression analysis. A strong negative correlation was observed between protein and oil contents among all the tested *Brassica napus* and *Brassica campestris* (Fig. 1 and 2) genotypes studied. A linear decrease in oil content was shown with the increase in protein content and vice versa. Charron *et al.* [32] and Singh *et al*. [33] reported negative correlation between oil and protein content in rapeseed and soybean.

The present quality assessment studies in rapeseed (*Brassica napus* and *Brassica campestris*) lines indicated the existence of a wide variation with respect to various parameters among the genotypes, which can serve a prominent role in designing future breeding programme by the Brassica breeders.

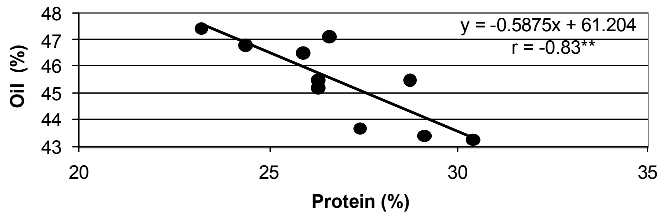

Fig. 1: Protein and oil content (%) among 10 *Brassica napus* genotypes

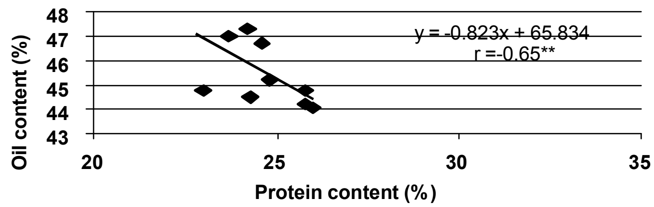

Fig. 2: Protein and oil contents (%) among 10 *Brassica campestris* genotypes

Conclusion and Recommendations

Conclusion: It is concluded that maximum moisture content (6.5-7.8), protein content (23.2-30.4) and oleic acid (49-53.8) were recorded in *Brassica napus*. While higher oil content (44.1-48.1) and linolenic acid (8-10.2) were observed in *Brassica compestris*. Lower glucosinolate (28.8 µmol g^{-1}) were recorded in Brassica compestris line 399596.

Recommendation: It is recommended that for the purpose of oil production *Brassica compestris* genotypes are recommeded for general cultivation in Khyber Pakhtun Khwa Province, Pakistan.

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