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# Genetic variability studies for quality traits in rapeseed-mustard

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## Abstract

Brassica oil is the world's third most important sources of edible vegetable oils diet, since they provide energy, improve taste, palatability of food. The fatty acid composition of oil is extremely important as the presence or absence of different fatty acids and their relative amounts determine the nutritional quality of the oil. The present study was carried out in the biochemistry laboratory of ICAR-DRMR, Bharartpur during 2015- 16 for the fatty acid profiling with the wield of Gas Chromatography using twenty five advanced breeding lines of Gobhi Sarson (*Brassica napus* L.) and three released varieties of brown sarson (*B. rapa* var. *Brown Sarson*). All the genotypes were genetically maintained at MRCFC, Khudwani, SKUAST-Kashmir, India. The results have revealed that the oleic acid in the 28 genotypes ranged from 22.58 to 56.67%, while as the linoleic & linolenic acid ranged from 16.63 to 29.05 % and 4.58 to 26.76 respectively. The erucic acid an anti-nutritional factor also showed wide range and the study has identified genotypes KGS-8, KGS-10, KGS-36 and KGS-40 for low erucic acid (less than 2 percent). KGS-10 was identified for the canola type as the said genotype contained the erucic acid of 1.99% & glucosinolate content of 26.3 ìmole per gram in the defatted meal (double zero).The study has further revealed that the erucic acid showed significantly high, but negative correlation with oleic acid (-0.92), linoleic acid (-0.83) and linolenic acid (-0.52), whileas significant and positive correlation was observed with Palmitic+Stearic acid (0.03) and glucosinolate content (0.16).The considerable genetic variation and high heritability for different fatty acid composition suggested that selection for improving some of the fatty acid composition would be promising in the future breeding programme.

***Kew words:*** *Fatty acid composition, genetic variability, rapeseed-mustard*

## Introduction

Oilseed rape has been cultivated for thousands of years in Asia andthe Indian subcontinent and then later in Europe (Asif *et al.*, 2015). Among the oilseeds, 7.98 MT of Rapeseed-Mustard was produced in India during 2016-17 from an area of 6.0 Mha with a productivity of 13.24 q/ha (Anonymous, 2018). In state of Jammu and Kashmir, the crop occupied an area of 54.52 thousand hectares and the production of 326 thousand quintals with an average productivity of 5.98 q/ha (Anonymous, 2015-16). In the rapeseed-mustard group, brown sarson (*Brassica rapa* var. *Brown Sarson*) is the major oilseed crop of Kashmir valley grown in *Rabi* season and is the only crop which fits well in the oilseed– paddy rotation, because of having great buffering capacity to withstand frost conditions and comparatively early maturing than any other *rabi* cereal crop. Lot of research efforts have been focussedtowards rapeseed-mustard breeding programme, with the objective to increase production, productivity and sustainability of oilseed-based cropping systems and to raise the farm income of the poor farmers of the valley.

The rapeseed breeding strategies are mostly dealing with developing varieties characterized by high and stable seed and oil yield , as well as by low content of glucosinolates and erucic acids (Ali *et al*., 2003). The fatty acid composition controls functional and nutritional values of different vegetable oils, varying considerably depending on the plant species (Sharafi *et al.*, 2015). The saturated fatty acids (SFAs) includes Palmitic acid whereas, the unsaturated fatty acids are either monounsaturated (MUPAs) i.e. erucic acid and oleic acid or polyunsaturated fatty acids (PUFAs) such as omega-3- alpha- linolenic acid and omega-6- linoleic acid which are nutritionally important (Rai *et al*., 2018). The presence and absence of these fatty acids determine the nutritional quality of the edible oils (Bhattacharya *et al.,* 2012). This has attracted researchers to seek new sources of oil or new fatty acids composition within wide varieties of plant species. The presence of genetic variation for fatty acid composition and its utilization has been found to be essential for genetical improvement of the oil quality and subsequently developing new cultivars (Murphy, 1995). A diverse germplasm repository of rapeseed-mustard,

both of ghobi sarson and brown sarson procured from different national and, international institutes and from local sources are continuously being maintained at Mountain research Centre for field Crops Khudwani, Ananatnag. Therefore, the present research work was aimedto evaluate the oil fatty acid composition of germplasm lines pertaining to Gobhi sarson (*B. napus* L.) and Brown sarson (*B. rapa var. Brown Sarson* L.) to identify the desirable genotypes for initiating breeding programme for improved fatty acid composition.

## Material and Methods

### Estimation of Fatty Acid Composition

Twenty five advanced breeding lines of Gobhi Sarson (*B. napus* L.) and three released varieties of Brown Sarson (*B. rapa var. Brown Sarson*) of SKUAST-K were used in the present study (Annexure-1) for fatty acid profiling and glucosinolate estimation (ìmol/g). All the lines were genetically maintained at MRCFC, Khudwani Centre. The 28 genotypes were analysed for fatty acid profiling in the biochemistry laboratory of ICAR-DRMR, Bharartpur during 2015- 16. The protocol and methodology of Paquot and Hautfenne, (1987) for Gas Chromatography was used for fatty acid profiling. 10-15 seeds from each genotype were crushed and then oven dried. Then the powder was transferred to theair tight screw capped vial. 0.5 ml petroleum ether was added to it (40-60 $^{o}$C) and kept at room temperature for 1.5 hrs. The meal was discarded and transferred the solvent extract to another vial. After then 0.5 ml 0.8 % NaOH in methanol was added and vortexed and then kept for 45 min at room temperature. 0.75 ml 8 % NaCl was added to it and vortexed 0.2-2.5 µl of extract was injected to upper layer to Gas Chromatography column. Then the retention time was compared with standard methyl esters to identify individual fatty acids. The detailed programme in GC (Nucon model 5765) is described below.

Column: SP-2300 (2%) + SP-2310 (3%)

$N_2$ flow rate: 30 ml/min

$H_2$ flow rate: 30 ml/min

Zero air flow rate: 300 ml/min

Injector temp: 240 $^{o}$C

Detector temp: 250 $^{o}$C

### Estimation of Total Glucosinolates

The ground seeds of each sample were defatted by homogenizing with n-hexane 3-4 times until oil was completely removed. The defatted seed meal was allowed to dry and then used for total glucosinolate estimation (ìmol/g). The methanolic extract was prepared by homogenizing 0.1 g defatted seed meal with 80 % methanol in a 2 ml vial. The homogenate was centrifuged at 3000g for 4 min after keeping in room temperature overnight. The supernatant was collected and made up to 2 ml with 80 % methanol. 100 µl of this extract was mixed with 0.3 ml distilled water and 3 ml 2 mM sodium tetrachloropalladate solution (58.8 mg sodium tetrachloropalladate + 170 µl conc. HCl + 100 ml distilled water). After incubating at room temperature for 1hr, absorbance was measured at 425 nm using a spectrophotometer (Labomed UV-VIS Double beam UVD-3500). 100 µl distilled water was used in place of methanolic extract in case of blank. Total glucosinolates was calculated using the formula:

$$Y = 1.40 + 118.86 \times A_{425}$$

The Phenotypic coefficients of variation (PCV) and genotypic coefficients of variation (GCV) were calculated using the following models

$$PCV = \acute{o}p/\grave{i} \; 100$$

$$GCV = \acute{o}g/\grave{i} \; 100$$

where, *ó*p, *ó*g, and ì represent phenotypic variance, genotypic variance, and mean of the traits, respectively.

## Results and Discussion

The nutriment properties of *Brassica* seed oil, like other fats and oils, are dependent on its fatty acids composition, particularly the amount of oleic, linoleic, linolenic, and erucic acid contents. The analysis of variancerevealed

Table 1. Analysis of variance for main fatty acids (%) and glucosinolate content(ì mol/g) of 28 genotypes of rapeseed-mustard

| Source of Variation | df | Palmatic + stearic acid (%) | Oleic acid (%) | Linoleic acid (%) | Linolenic acid (%) | Ecosenoic acid (%) | Erucic acid (%) | Glucosinolate (ì mol/g) |
|---|---|---|---|---|---|---|---|---|
| Replication | 2 | 0.07 | 21.08 | 17.52 | 0.06 | 0.99 | 14.37 | 46.89 |
| Treatment | 27 | 46.61** | 460.67* | 38.35* | 45.57* | 32.61* | 431.33* | 800.23* |
| Error | 27 | 0.12 | 2.93 | 2.30 | 0.74 | 0.20 | 0.73 | 2.95 |

* & **Significant at 5% and 1% levels

Table 2. Estimates of phenotypic variation ($\sigma^2 p$), genotypic variation ($\sigma^2 g$) , phenotypic coefficient of variation (PVC) and genotypic coefficient of variation (GCV) for fatty acids(%)and glucosinolate content (ì mol/g)of 28 genotypes of rapeseed-mustard

| | Mean | Range | $\sigma^2 g$ | p | GCV | PCV | $h^2_b$ |
|---|---|---|---|---|---|---|---|
| Palmatic + Stearic acid (%) | 5.11 | 3.07-7.70 | 1.38 | 1.39 | 23.04 | 23.07 | 99.76 |
| Oleic acid (%) | 34.13 | 20.54-56.60 | 121.21 | 122.49 | 32.25 | 32.42 | 98.95 |
| Linoleic acid (%) | 22.77 | 16.63-31.12 | 18.40 | 19.15 | 19.07 | 19.46 | 96.07 |
| Linolenic acid (%) | 9.73 | 4.58-26.76 | 14.96 | 15.24 | 39.72 | 40.10 | 98.13 |
| Ecosenoic acid (%) | 6.64 | 1.01 -13.76 | 13.03 | 13.14 | 53.5 | 53.78 | 99.23 |
| Erucic acid (%) | 20.56 | 1.23-39.31 | 177.28 | 178.54 | 64.92 | 65.15 | 99.29 |
| GLS(ì mol/g) | 64.67 | 26.32-96.18 | 263.87 | 264.85 | 25.11 | 25.15 | 99.63 |

$\sigma^2$g=Genotypic variance;p= Phenotypic variance; GCV= Genotypic coefficient of variation; PCV= Phenotypic coefficient of variation; = heritability in broad sense: GLS=Glucosinolate

significant differences among Brassica populations (P < 0.05) with respect to Palmitic + Stearic acid, Oleic acid , Linoleic acid, Linolenic acid, Ecosenoic acid, Erucic acid and Glucosinolate content .

The oleic acid is of one of the main unsaturated fatty acids which plays an important role in human nutrition. The oleic acid content in the present set of genotypes ranged from 20.54 to 56.6 with average value of 34.13 (Table 2, 4).The high oleic acid oil has cholesterol lowering properties (Rakow, 2003) and furthermore the high oleic acid contained oils are greatly resistant to heating on oxidation and are, therefore, suitable substitute to low oleic acid genotypes in commercial food-service applications entailing for long-life stability. The Palmitic+Stearic acid acid showed a range from 3.07 to 7.7 (average value 5.11) while as, linoleic acid content ranged from 16.63 to 31.12 (mean value 22.77). Similarly, the linolenic acid content ranged from 4.58 to 26.76 (average value 9.73) and ecosenoic acid content from1.01 to 13.76 (mean value 6.64).

The erucic acid is one of the important fatty acids found mostly in genus*Brassica*. This fatty acid is harmful to the human health, however high erucic acid oil is quite useful for industrial applications and generally is valuable raw material for manufacture of industrial products such as plasticizers, detergents, and surfactants and also in the synthesis of nylon and in the lubricant and emollient industries (Murphy 1996). Keeping in view the nutritional health requirement, the occurrence of erucic acid is considered as antinutritional factor for human consumption, therefore the development of genotypes with low erucic content is a priority in *Brassica* breeding. In the current study, some genotypes had high erucic content and some had very low erucic content. The erucic acid content in the present set of genotypes ranged from 1.23 to 39.31 % with mean value of 20.56 %. The erucic acid content in genotypes KGS-8, KGS-10, KGS-36 and KS-40 was observed to be less than 2% and are considered as single zero genotypes. Similar study carried out by Sharafi *et al* (2015) in *B. napus* identified some genotypes with high as well as with very low erucic acid erucic acid and some cultivarslike Okapi and Opera (from *B. napus*) as free-erucic acid genotypes.

Glucosinolates are major sulphur components in crucifers and are secondary plant metabolites that occur naturally in Brassicaceae, derived from glucose and amino acid that contain S and N. They causes different problems in human beings and animals, especially too high concentrations in the oilcake affect thyroid function and causes goiters in monogastric animals. Hence, reducing the glucosinolate content in seed meal has remained worldwide a prime objective of rapeseed-mustard quality improvement programmes (Mawlong *et al*., 2017). The present data related to glucosinolate content ranged from 26.32 to 96.18 ìmol/g with an overall mean of 64.67 ìmol/g (Table 4). The lowest glucosinolate content of 26.32 ìmol/g was observed in KGS-10, whereas the highest was observed in KGS-35 (92.0 ìmol/g). Thus KGS-10 was identified as the double zero (Canola quality) genotype , having erucic acid less than 2% and glucosinolate content less than 30 ìmol/g in defatted meal. Similar trends in results in glucosinate content were observed in their respective studiesby Chaudhary *et al* (1999) and Khulbe *et al* (2000).The range of genetic variability present in the particular character is measured by genotypic coefficient of variation (GCV) (Shekhawat *et al*., 2014). The phenotypic variance were found higher than corresponding genotypic variance for all the quality traits under study (Table 2), which is in relation with the other

Table 3. *Pearson correlation* for different fatty acids (%) and glucusinolate content (ìmol/g) of 28 genotypes of rapeseed-mustard

| | GLS (ì mol/g) | Palmatic + stearic (%) | Oleic acid (%) | Linoleic acid (%) | Linolenic acid (%) | Ecosenoic acid (%) | Erucic acid (%) |
|---|---|---|---|---|---|---|---|
| GLS (ìmol/g) | - | -0.094 | 0.096 | -0.096 | -0.163 | -0.148 | 0.034 |
| Palmatic + Stearic acid (%) | | - | -0.239 | -0.126 | -0.341 | 0.290 | 0.167 |
| Oleic acid (%) | | | - | 0.681** | 0.281 | -0.685** | -0.921** |
| Linoleic acid (%) | | | | - | 0.613* | -0.721** | -0.839** |
| Linolenic acid (%) | | | | | - | -0.521* | -0.525* |
| Ecosenoic acid (%) | | | | | | - | 0.660** |
| Erucic acid (%) | | | | | | | - |

GLS: Glucosinolate.
* & **Significant at 5% and 1% levels

Table 4. Mean values for different fatty acids (%) and glucusinolate content (ì mol/g) of 28 genotypes of rapeseed-mustard

| Genotypes | Palmetic + Stearic acid (%) | Oleic acid (%) | Linoleic acid (%) | Linolenic acid (%) | Ecosenoic acid (%) | Erucic acid (%) | Glucosinolate (ìmol/g) |
|---|---|---|---|---|---|---|---|
| KGS-5 | 6.14 | 29.64 | 24.97 | 8.85 | 9.28 | 21.53 | 53.59 |
| KGS-6 | 5.49 | 30.32 | 21.29 | 10.41 | 9.14 | 21.84 | 65.71 |
| KGS-7 | 4.11 | 46.72 | 26.10 | 10.68 | 5.54 | 6.06 | 60.20 |
| KGS-8 | 4.55 | 52.22 | 24.05 | 11.39 | 3.58 | 1.64 | 66.56 |
| KGS-10 | 5.05 | 49.77 | 27.30 | 11.26 | 3.44 | 1.99 | 26.32 |
| KGS-11 | 6.17 | 27.44 | 22.81 | 6.15 | 7.99 | 27.34 | 52.34 |
| KGS-12 | 5.79 | 25.18 | 20.96 | 7.61 | 7.85 | 25.96 | 45.20 |
| KGS-24 | 4.76 | 48.56 | 25.41 | 11.59 | 4.45 | 2.99 | 69.69 |
| KGS-25 | 4.83 | 52.29 | 25.93 | 11.83 | 2.14 | 2.24 | 59.85 |
| KGS-27 | 3.81 | 20.55 | 20.16 | 8.53 | 8.51 | 39.32 | 65.00 |
| KGS-28 | 4.43 | 30.50 | 20.73 | 13.32 | 5.35 | 23.52 | 82.67 |
| KGS-29 | 5.73 | 37.39 | 27.43 | 10.46 | 5.99 | 11.27 | 88.25 |
| KGS-30 | 6.55 | 30.34 | 20.91 | 6.69 | 8.16 | 27.09 | 56.73 |
| KGS-32 | 6.52 | 26.83 | 16.63 | 4.58 | 11.43 | 31.83 | 60.39 |
| KGS-35 | 4.48 | 45.60 | 25.26 | 7.39 | 3.98 | 12.21 | 92.00 |
| KGS-36 | 5.16 | 49.74 | 29.51 | 9.76 | 2.13 | 1.24 | 72.04 |
| KGS-37 | 5.63 | 25.45 | 18.41 | 7.53 | 7.06 | 34.32 | 82.23 |
| KGS-38 | 3.36 | 56.67 | 24.01 | 10.88 | 2.15 | 2.54 | 86.13 |
| KGS-39 | 6.96 | 28.38 | 21.31 | 8.02 | 10.27 | 24.80 | 48.58 |
| KGS-40 | 4.28 | 34.26 | 31.12 | 6.77 | 1.02 | 1.73 | 50.06 |
| KGS-41 | 7.70 | 30.04 | 25.11 | 8.51 | 2.34 | 25.54 | 71.72 |
| KGS-42 | 3.88 | 27.06 | 23.06 | 9.53 | 12.21 | 22.22 | 65.70 |
| KGS-43 | 6.99 | 24.56 | 17.82 | 9.23 | 13.76 | 31.19 | 58.45 |
| KGS-44 | 4.50 | 29.08 | 18.81 | 6.65 | 10.72 | 27.99 | 66.86 |
| KGS-45 | 5.06 | 22.58 | 17.36 | 6.51 | 7.97 | 39.11 | 96.19 |
| Gulchin | 3.69 | 29.81 | 21.85 | 9.49 | 6.51 | 32.82 | 50.38 |
| *Shalimar Sarson-1* | 3.07 | 21.18 | 19.04 | 10.55 | 7.35 | 37.13 | 41.77 |
| *Shalimar Sarson-2* | 4.43 | 23.62 | 20.47 | 8.46 | 5.67 | 38.37 | 76.43 |
| CD (0.05%) | 0.51 | 3.20 | 2.47 | 1.51 | 0.88 | 3.20 | 3.11 |
| CV | 6.19 | 5.72 | 6.62 | 10.19 | 8.13 | 9.49 | 2.93 |

findings in *B. napus*(Ali *et al*., 2006). The genetic variances for most of the traits were generally 3 to 15 times greater than the environmental variance indicating significant genetic control over expression of quality traits in brassica (Khan *et al*., 2008). The estimates of GCV was observed to be higher in oleic acid, linolenic acid, ecosenoic and erucic acid (>30),whileas moderate GCV was recorded for Palmitic + Stearic acid and glucosinolate content (20-30). The low values of GCV (<20) were observed in linoleic acid.

The measures of heritability i.e. the contribution of genotypic and phenotypic variance coupled with the expected genetic gain during selection provides useful information regarding the performance of selective population in comparison to base population and measuring effect of environment and its interaction component (Comstock E, Robinson, 1952). High genetic advance may be expected whenhigh heritability is attributed to additive gene action. High values of heritability have been recorded for all the traits revealing that major portion of phenotypic variance for these traits could be attributed to genotypic variance (Table 2).

Correlation analysis of important plant characters leads to a directional model for quality response. The results ofassociation studies among the traits studied are shown in table-3. Erucic acid an antinutritional component showed significantly high, but negative correlation with oleic acid (-0.92), linoleic acid (-0.83) and linolenic acid (-0.52), whileassignificant and positive correlation was observed with Palmitic + Stearic acid (0.03) and glucosinolate content (0.16). On the other hand, glucosinolate content showednon significantthough positive correlation with oleic acid (0.09) and erucic acid (0.03), and negative correlation with Palmatic + Stearic acid (-0.09), linoleic (-0.09), linolenic (-0.16), and ecosenoic acid (-0.14). Linoleic acid showed significant but positive association with oleic acid and linolenic acid. On the other hand ecosenoic acid showed significant but negative association with oleic acid,linoleic acid and linolenic acid and therest of the traits amongst each other showed non significant associations.The Significant but negative correlation among oleic acid with erucic and linolenic acidswere observed by Katavic*et al*., 2001 and Sasongko and Mollers, 2005.The considerable genetic variation and high heritability for different fatty acid composition suggested that selection for improving some of the fatty acid composition would be promising in the future breeding programme.

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Annexure-1: List of 28 genotypes of rapeseed-mustard evaluated for fatty acid (%) profiling and glucosinolate content (ì mol/g)

| Code | Species | Sub species | Origin |
|---|---|---|---|
| KGS-5 | *B. napus* | - | IPK Germany |
| KGS-6 | *B. napus* | - | IPK Germany |
| KGS-7 | *B. napus* | - | IPK Germany |
| KGS-8 | *B. napus* | - | IPK Germany |
| KGS-10 | *B. napus* | - | IPK Germany |
| KGS-11 | *B. napus* | - | IPK Germany |
| KGS-12 | *B. napus* | - | IPK Germany |
| KGS-24 | *B. napus* | - | IPK Germany |
| KGS-25 | *B. napus* | - | IPK Germany |
| KGS-27 | *B. napus* | - | IPK Germany |
| KGS-28 | *B. napus* | - | IPK Germany |
| KGS-29 | *B. napus* | - | IPK Germany |
| KGS-30 | *B. napus* | - | IPK Germany |
| KGS-32 | *B. napus* | - | IPK Germany |
| KGS-35 | *B. napus* | - | IPK Germany |
| KGS-36 | *B. napus* | - | IPK Germany |
| KGS-37 | *B. napus* | - | IPK Germany |
| KGS-38 | *B. napus* | - | IPK Germany |
| KGS-39 | *B. napus* | - | IPK Germany |
| KGS-40 | *B. napus* | - | IPK Germany |
| KGS-41 | *B. napus* | - | IPK Germany |
| KGS-42 | *B. napus* | - | IPK Germany |
| KGS-43 | *B. napus* | - | IPK Germany |
| KGS-44 | *B. napus* | - | IPK Germany |
| KGS-45 | *B. napus* | - | IPK Germany |
| Gulchin | *B. rapa* | *var. brown sarson* | Released VarietySKUAST-K |
| *Shalimar Sarson-1* | *B. rapa* | *var. brown sarson* | Released Variety SKUAST-K |
| *Shalimar Sarson-2* | *B. rapa* | *var. brown sarson* | Released Variety, SKUAST-K |