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Fatty Acid Composition and Correlation Analysis of Linseed (*Linum Usitatissimum L.*) Genotypes

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Abstract

The experiment was carried out to evaluate the fatty acid composition of 17 linseeds genotypes and determine the magnitude of correlations of individual fatty acid, oil content and seed yield. Linolenic followed by oleic acids were the predominant fatty acid compositions in all genotypes; ranged from 51.78 to 59.22 % and 15.4 to 23.59 % respectively. The Saturated Fatty Acid (SFA) varied from 7.97 for genotype kassa-2 and 10.84 for CI 1652 X R12-D33C/SPS105. Whereas Unsaturated Fatty Acid (UFA) value ranges from 88.14 to 90.42 for CI 1652 X R12-D33C/SPS105 and CI 1652 X CDC 1747/SPS2 respectively. The highest Oleic Desaturation Ratio (ODR) 0.82 and linoleic to Linolenic Desaturation Ratio (LDR) 0.081 value was recorded by genotype Biltistar. Whereas the lowest ODR and LDR value was exhibited by genotype Jeldu (0.73) and Chilalo X R12-N27G/SPS1 (0.77). The highest SFA/UFA ration was observed in genotype CI 1652 X R12-D33C/SPS105 (0.12), and Bekoji-14 (0.12). Significant (P<0.01) positive correlations were observed between linolenic and yield (r=0.68); oleic and stearic (r=0.63) acids. Negative and significant (p<0.01) correlation were identified between linoleic (-0.52), linolenic (-0.91) and seed yield (-0.49) affected by oleic acid adversely. Hence, plant breeding deals with the management of genetic variability; the presence of fatty acid variation and association of traits in the investigated genotypes has a huge value to design selection procedure and to identify superior genotypes. It is, therefore, necessary to classify and utilize this variability systematically for linseed quality improvement.

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Introduction

Oilseed crops are major sources of lipids for human nutrition as well as for several industrial purposes. The most commonly known oilseeds are groundnut, soybean, palm kernel, cottonseed, olive, sunflower, rapeseed, sesame, linseed, safflower seed, etc. Linseed (*Linum usitatissimum L.*) is one of the ancient oilseeds originated from Mesopotamia cultivated since 5, 000 B.C. for oil, food and fiber [1]. It is a multi-purpose crop, and its importance is showed by the fact that every part of the plant has specific economic use [2].

Linseed oil is used in the manufacturing of paints and varnishes and other wood treatments, linoleum, soap, oil cloth, printers' ink, patent leather, putty and pharmaceuticals. The drying property or the property of absorbing oxygen when exposed in thin layers, which is caused by its high level of linolenic acid, imparts it the property of drying and makes linseed oil valuable in the industry. Due to less oxidation stability, linseed oils having higher linolenic content are not preferred to be used as edible oil [3], with easily combustibility, use of them are increasing more and more as industrial purpose like biodiesel fuels [4].

However linseed oil is the richest abundant plant source of linoleic (Omega-6) and linolenic (Omega-3) Polyunsaturated Fatty Acids (PUFA), which are essential for humans since they cannot be synthesized in the organism and must be ingested in food but its oil is qualitatively different from the more common vegetable oils with high PUFA proportions [5]. High concentrations of α~linolenic acid (C18:3) promote oxidation processes which negatively affect the shelf life of oil and secondary products thereof [6].

Linseed plays a part in functional foods from the point of view of its nutrition and pharmaceutical value. Nutritious components of linseed include oil, protein, lignin, resolvable fiber, mineral and vitamins [7]. Alpha-linolenic acid (Omega-3) fatty acid increases the absorption of Long Chain-Polyunsaturated Fatty Acids (LCPUFA) and have been associated with prevention and treatment of heart disease, arthritis, inflammatory and autoimmune diseases, and cancer [8]. Epidemiologic studies have shown that populations that consume high amounts of oils containing omega-3 fatty acids have lower rates of various types of cancers, including lung, breast, prostate, and colon. Alpha-linolenic was reported to be anti-hyper cholestrolemic and anti-carcinogenic effects and is important for the normal growth and development of brain and retinal tissues of infants [9,10].

The studies on fatty acid composition of several germplasm collections of crop plants have revealed wide variation, offering possibilities of developing superior quality edible oils and specialized industrial oils. Ethiopia is considered as the secondary center of origin for linseed and it has been growing by Ethiopian farmers for many years under wide range of environmental conditions. Hence, considerable variation in fatty acid composition, oil content and seed yield will be expected in Ethiopian linseed germplasms. It is, therefore, commanding to study the fatty acid compositions of the Ethiopian linseed germplasm to identify genotypes for improvement of varieties with superior quality of edible and industrial oil. The present investigation was carried out to evaluate fatty acid composition of Ethiopian linseeds germplasm, correlate the value of individual fatty acid with oil content, and seed yield. Furthermore, the relative efficiency of the desaturation pathways were estimated based on the fatty acid ratios.

Material and methods

Experimental materials

The experimental materials used in the study include 11 cultivated varieties of linseed and 6 advanced lines derived from crossing program. The seeds were collected from the plants grown under rain feed conditions in the field of Holetta Agricultural Research Center.

Six seed oil quality traits, which comprises of the seed oil content and the major fatty acid composition were examined. The seed oil was analyzed using Nuclear Magnetic Resonance (NMR) for oil content and Near Infrared Reflectance Spectroscopy (NIRS) for fatty acids. For oil content measurement, 22 g of seed of each genotype was dried in an oven for 2½ hrs. at 78°C and cooled for 30 minutes prior to examination. It was then, measured following the procedure of Robbelen et al, [11]. The fatty acid composition of the seed was determined using 3 g of seed from each genotype using Foss NIRS 5000 (Weltech Enterprises, Inc, Maryland, USA) in the 1108-2492 ranges with an 8 nm step. The spectrum of each sample was taken by scanning (Win Scan) version 1.5 international, L.L.C (Famatech. Corp, Virginia, USA). In addition, the seed yield was used for analysis in order to determine the variability of genotypes and correlation of seed yield with the fatty acid compositions and oil content.

Statistical data analysis

Fatty acid ratios

It is difficult to evaluate the potential of different phenotypes for plant breeders by comparing individual fatty acid values because they are intercorrelated and any breeding modification will affect the whole system [12]. For this reason, two ratios were additionally used i.e, Oleic Desaturation Ratio (ODR) and Linoleic Desaturation Ratio (LDR). The ODR and LDR are calculated following [13] and they estimate, within the desaturation pathway, the efficiency of the desaturation from Oleic to Linoleic (ODR) and from linoleic to Linolenic Acid (LDR) were calculated as follows:

$$ORD = \frac{\%C18{:}2 + \%C18{:}3}{\%C18{:}1 + \%C18{:}2 + \%C18{:}3}$$

$$ORD = \frac{\%C18{:}2 + \%C18{:}3}{\%C18{:}1 + \%C18{:}2 + \%C18{:}3}$$

The correlation between individual fatty acids, oil percentage, and seed yield were calculated using R software version 4.1.

Result and discussion

Oil content

The oil content ranged from 35.2 to 38.6 %, and 37.08 % was the average value of the studied materials (Table 1). Genotype CI-1652 showed the highest value in the study (Table 2). Worku et al, 2015[14] evaluated 198 Ethiopian linseed accessions, and 30.5 % up to 43.57 % oil content variation was reported. Abebe et al, [15] also conducted a multi-location trial on 20 linseed genotypes for three consecutive years and reported 36.5 to 39.1 % with 38.3 % mean value which is a little bit higher value than the present study. Such variation may be observed by genotypic potential and environmental conditions of the genotypes [16]. Although the genotypes showed narrow range of variation, the result placed the evaluated genotypes from low to medium position compared to international varieties, which is agreement with the above reports.

Table 1: Mean and range of oil content, fatty acid compositions and seed yield of 17 Linseed genotypes.

Fatty acids	Mean	Minimum	Maximum	Std, Dev
Palmitic	5.45	3.96	5.97	0.49
Stearic	4.52	3.39	5.22	0.49
Oleic	19.93	15.74	23.59	1.96
Linoleic	14.22	13.35	16.93	0.90
Linolenic	55.06	51.78	59.22	1.87
Oil %	37.08	35.20	38.60	0.90
yield	646.03	12.59	1708.40	785.44

Fatty acid profile

The fatty acid compositions and their range of variation in different genotypes are shown in Table 1 and 2. Five major fatty acids were identified and quantified. Linolenic followed by oleic acids were found to be the predominant fatty acids compositions in all germplasm investigated in the present study. Similar results were obtained in the previous reports on linseed germplasm [17].

The percentage of Palmitic acid ranges from 3.96 to 5.97 % with a mean value of 5.45 %. Stearic acid varied between 3.39 to 5.22 % with a mean value of 4.52 %. Oleic acid ranges from 15.74 to 23.59 % with average mean value of 19.93. Based on the investigation linoleic and linolenic acid ranges from 13.35 to 16.93 % and 51.78 to 59.22 % with mean value of 14.22 and 55.06 respectively. The accumulation of large amount of linolenic acid is a unique feature of linseed, which is the final product of three desaturation steps or caused by the desaturating processes from palmitic to linolenic fatty acids during their biosynthesis (i.e. palmitic to stearic to oleic to linoleic to linolenic), as reported by several scholars[18-20].

Furthermore, the saturated fatty acid varied from 7.97 for genotype kassa-2 and 10.84 for genotype CI 1652 X R12-D33C/SPS105. Whereas unsaturated fatty acid value ranges from 88.14 to 90.42 for genotypes CI 1652 X R12-D33C/SPS105 and CI 1652 X CDC 1747/SPS2 respectively. Based on the result unsaturated fatty acids are the dominant composition in the studied genotypes. The finding was in agreement with earlier reports [21]. Whereas, the highest SFA/UFA ration was observed in genotype CI 1652 X R12-D33C/SPS105 (0.12), and cultivated variety Bekoji-14 (0.12). Hence plant breeding deals with the management of genetic variability; the presence of fatty acid variation in the investigated genotypes has a huge value to design selection procedure and to identify superior genotypes. It is, therefore, necessary to classify and utilize this variability systematically for quality improvement of linseed.

When we compared the present results with earlier reports some variations for fatty acids were observed, which can be attributed by several factors, such as growth conditions, genetic factors, geographical variations and analytical procedures [22].

The long-chain Linolenic (Poly unsaturated fatty acid) cannot be readily synthesized by the human body and are mostly obtained through the diet. For proper health nutritionist suggest to incorporate linolenic acid in a day-to-day human diet. However, the ratio of linolenic to linoleic is a determinant factor for health. The availability of linolenic acid will be improved by an increase in linolenic to linoleic acid proportion, the ratio is the determinant factor [23]. Therefore, during linseed variety improvement developing varieties with suitable fatty acid composition is important, which has been achieved in many oilseed crops.

Table 2: Fatty acid compositions, oil content, seed yield and their range of variation in 17 Linseed genotypes.

Treatments	Palmitic	Stearic	Oleic	Linoleic	Linolenic	Oil %	Seed yield	ORD	LDR	% of SFA	% of UFA	Ratio of SFA/UFA
CDC 1747 X CI 1652/SPS8	5.66	3.85	18.53	15.40	55.95	36.93	1671.70	0.79	0.78	9.51	89.88	0.11
CI 1652 X CDC 1747/SPS2	5.64	4.26	19.60	14.73	56.09	38.08	1708.40	0.78	0.79	9.90	90.42	0.11
Chilalo X R12-N27G/SPS1	5.35	3.39	15.74	16.93	56.90	37.13	1597.60	0.82	0.77	8.74	89.57	0.10
CI 1652 X R12-D33C/SPS105	5.96	4.88	19.65	14.04	54.46	36.50	1306.90	0.78	0.80	10.84	88.15	0.12
CI 1652 X R12-N27G/SPS5	5.21	4.35	21.66	14.94	52.66	37.20	1233.00	0.76	0.78	9.57	89.25	0.11
R12-100 X CI 1525/SPS1	5.83	4.30	19.70	14.13	56.23	37.96	1599.90	0.78	0.80	10.12	90.06	0.11
Bekoji-14	5.69	4.95	21.01	13.68	54.17	37.5	1705.80	0.76	0.80	10.64	88.87	0.12
kassa-2	3.96	4.00	22.44	14.48	52.18	37.45	1259.0	0.75	0.78	7.97	89.10	0.09
Berene	5.74	4.77	19.50	13.47	56.34	37.00	1617.0	0.78	0.81	10.51	89.31	0.12
Tole	5.86	4.62	19.01	13.79	56.28	36.00	1690.0	0.79	0.80	10.48	89.09	0.12
CI-1525	5.47	5.02	20.68	13.61	54.14	38.5	1430.0	0.77	0.80	10.48	88.43	0.12
CI-1652	5.29	4.51	20.24	14.23	54.98	38.6	1360.0	0.77	0.79	9.79	89.45	0.11
Chilalo	5.70	4.81	20.06	13.89	54.86	35.2	16.70	0.77	0.80	10.51	88.82	0.12
Belay-96	5.16	5.12	21.46	13.35	53.97	36.3	16.80	0.76	0.80	10.27	88.78	0.12
Jeldu	5.10	5.22	23.59	13.44	51.78	37.00	15.40	0.73	0.79	10.32	88.81	0.12
Biltistar	5.06	4.13	16.20	13.97	59.22	37.00	19.00	0.82	0.81	9.19	89.39	0.10
Kulumsa-1	5.97	4.67	19.71	13.70	55.82	36.10	17.80	0.78	0.80	10.65	89.23	0.12
Mean	5.45	4.52	19.93	14.22	55.06	37.08	1573.5	0.78	0.79	9.97	89.21	0.11
SD	0.49	0.49	1.96	0.90	1.87	0.90	191.25	0.02	0.01	0.79	0.56	0.01

SD: Standard Deviation; % of SFA: Percent of Saturated Fatty Acid; % of UFA: Percent of Unsaturated Fatty Acid.

Association of individual fatty acids

Pearson correlation

The average data for bilateral relationships between seed yield, oil content and fatty acid composition of linseed genotypes investigated were subjected to correlation analysis (Table 3). The results revealed that significant (P<0.01) positive correlations were observed between linolenic and yield (r=0.68); oleic acid had also a significant (P<0.01) positive effect on stearic acids (r=0.63). The positive value of one variable indicates the value of one variable increase linearly with increase in another variable. Therefore, this indicates that these characters could be considered as usefully for linseed improvement through selection or hybridization.

On the other hand, negative and significant (p<0.01) correlation between characters were observed, hence linoleic (-0.52), linolenic (-0.91) and seed yield (-0.49) affected oleic acid adversely. These indicate as the value of oleic acid increase the value of linoleic, linolenic and seed yield decrease and vis verse. Strong and negatively significant association was also found between linoleic and stearic acids (-0.88) at 0.01 level of significance.

Correlation test measures the strength and direction of linear correlation. It has no unit of measurement but the value can be any number between -1 and 1. The positive sign shows positive correlation whereas, negative sign indicates negative correlation. Based on the present finding oil content negatively correlated with seed yield.

Besides the direction of the association of characters, several approaches have been suggested to translate the strength of correlation coefficient into descriptors like “weak,” “moderate,” or “strong” relationship. Even though cutoff points are inconsistence for scientific interpretations researchers suggest 0 to 0.1 no correlation, 0.10 to 0.39 weak correlation, 0.4 to 0.69 as moderate correlation, 0.7 to 0.89 strong correlation and 0.9 to 1 very strong correlation [24-26]. Based on these cutoff points' linolenic and linoleic, linoleic and stearic have a very strong correlation. Similar results have been previously reported by other authors for strong correlation of linolenic and linoleic fatty acid [27]. However weak correlation was reported by Batta et al, [18] and Green [19]. The genetic make of the genotypes, environmental effect and its interaction with the genotype and method and precision of fatty acid profile analysis may be the cause for the variation of the reports. Hence, it is very crucial to take attention all the factors that affect linseed fatty acid composition. On the other hand, oleic and stearic, linolenic and seed yield had moderate correlation. But the relation between linolenic and oil content; yield and stearic acid was considered as negligible or no correlation. Meanwhile, yield is dependent on a number of related traits and environmental factors, understanding of quality traits interrelationship is essential in designing linseed-breeding strategy.

In general, the magnitude of trait associations showed a variation in oil content, seed yield and fatty acid profile. The positive and significant association of linolenic acid and seed yield implies simultaneously improvement of both traits may be possible. On contrary, oleic acid and linolenic acid showed a significant negative correlation. Linseed oil with high linolenic acid has highly susceptible to oxidation. Therefore, in order to improve the shelf life and quality of linseed oil for cooking focusing on the improvement of oleic acid may come up with an excellent outcome. This inverse relationship between oleic and linolenic acids found in this study was also reported by Wakjira, et al, [20]. The descending order of oleic, linoleic and linolenic acids synthesis maybe the reason for the result.

Figure 3: Correlation coefficients between seed yield, oil content and major fatty acids of Ethiopian linseed genotypes.

Variable	Palmitic	Stearic	Oleic	Linoleic	Linolenic	Oil Content	Yield
Palmitic	1						
Stearic	0.25ns	1					
Oleic	-0.32ns	0.63**	1				
Linoleic	-0.13ns	-0.88**	-0.52*	1			
Linolenic	0.41ns	-0.44ns	-0.91**	0.22ns	1		
Oil Content	-0.24ns	-0.18ns	0.09ns	0.15ns	-0.07ns	1	
Yield	0.39ns	-0.02ns	-0.49*	-0.14ns	0.68**	-0.34ns	1

*: Significant at 5 per cent level; **: Significant at 1: per cent level; ns: non-significant.

Conclusion and recommendations

The aim of this study is to determine the variation and association of fatty-acid compositions, oil content and seed yield of linseed genotypes. The present study revealed significantly useful variability in oil content, seed yield and fatty acid profile, indicating the possibility of improving genotypes for these characteristics in the current breeding program. Moreover, high correlation coefficients between studied fatty acid (linolenic and linoleic, linoleic and stearic acid) were found. The report finds out the availability of highest amount of linolenic acid in all genotypes which favors their importance for industrial application. However, it is doubtful that the quality requirement for cooking oil can be obtained from the current available genotypes. Therefore, it is worthwhile if Ethiopian linseed breeding program will practice either modern breeding techniques like gene editing, mutation, genetic engineering, etc. or introducing gene pool from other countries.

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