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Research Article

Variation in oil content and fatty acid composition of linseed (*Linum usitatissimum* L.) cultivars and their hybrids in sub-mountainous Himalayan region of India

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Abstract

The study assessed the level of variation in oil content and fatty acid composition of linseed cultivars, elite lines and hybrids in sub-mountainous Himalayan region of India. The oil content ranged from 34.60 to 41.14 % in the experimental material. Yellow seeded cultivars had higher oil content in comparison to brown seeded genotypes. Two saturated (Palmitic and steric acid) and three unsaturated fatty acids (Oleic, linoleic and linolenic acid) identified by gas chromatography represented 8.81% and 90.89% of the total oil, respectively. Widest range of variation was observed for linolenic acid followed by oleic acid, while the highest GCV was recorded for linoleic acid. Cross combinations exhibited wider range of variation for linolenic acid in comparison to the parents. All the traits exhibited low heritability and low genetic advance except 1000-seed weight. Oil content showed weak positive association with seed weight. Palmitic acid also showed positive association with oleic acid however, linoleic acid had negative association with linoleic, oleic and stearic acid. Results indicated that induced mutagenesis coupled with recombination breeding will be more beneficial for the development of low linolenic acid lines in linseed.

Keywords: Linseed, Oil content, Fatty acids, Linolenic acid, Correlation

Introduction

Linseed (*Linum usitatissimum* L.) is a multi-purpose crop. It is such a valuable crop that every part of the plant has specific economic importance. The most important linseed producing countries are Canada, Argentina, USA, China, India and Europe (Lidefelt, 2007; Wang *et al.,* 2007). Its seeds when crushed yield oil. Linseed occupies an important position in world market because of its technical grade oil. The quality and utilization of linseed oil is determined by its fatty acid composition. In general, linseed oil has a high level of linolenic acid (35-66%), which imparts it the property of drying oil, suitable for manufacturing paints, stains, inks, varnishes and linoleum etc (Gill, 1987). In India, 25 per cent of total linseed oil is still consumed for edible purpose. High levels of linolenic acid render it unfit for consumption as edible oil due to undesirable odors and flavour reversion associated with the auto-oxidation (Green, 1986a; Graef *et al.,* 1988). But linseed oil is the richest 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, such as soya oil, sunflower oil, rape oil, olive oil, etc. The linolenic acid in linseed oil is 5.5 times more than the sources containing the highest level (Bloedon and Szapary, 2004). This essential fatty acid can be metabolized to eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) by several enzymes in human intestine system (Chen *et al.*, 2002). It is well known that linolenic acid increases the absorption of long chain-polyunsaturated fatty acids (LCPUFA), especially EPA and DHA, and decreases the several disease risks (Visentainer *et al.,* 2005). Recently, there has been a growing interest in the probiotic properties of linseed and its beneficial effects on coronary heart disease, some kinds of cancer and neurological and hormonal disorders (Huang and Milles, 1996; Huang and Ziboh, 2001; Simopoulos, 2002), colon tumor (Dwivedi *et al.,* 2005), breast cancer (Chen *et al.,* 2006; Thompson *et al.,* 1996) and atherosclerosis (Wang *et al.,* 2005; Prasad, 1997). There are very

few published reports of oil content and fatty acid composition of linseed cultivars grown in India which has become essential nowaday to market the produce in terms of nutritional value and quality. Further, variability in oil content and fatty acid composition helps in selection of parents for modified oil breeding programs. Therefore, the present study was conducted to determine the oil content and fatty acid composition (especially omega fatty acids) of linseed genotypes grown in sub-mountainous Himalayan region of India.

Material and Methods

The experimental material comprised 14 cultivars (Table 1) including improved genotypes and 24 cross-combinations (F_1's) of linseed. All these genotypes were grown in the field conditions in 2003/2004 at Plant Breeding Experimental Farm (1290 meter amsl), CSK Himachal Pradesh Agricultural University, Palampur, India. Seed samples were taken at maturity and were used for determination of oil content and fatty acid analysis. Oil content was determined by a wide line nuclear magnetic resonance (NMR). About 2g of oven dried seeds were analyzed by NMR (Newport analyser) with reference to a standard of extracted linseed oil at oilseed section department of Plant Breeding, PAU, Ludhiana. Fatty acids were analyzed by GC equipped with capillary column. The operating conditions for gas chromatography were as follows: injector temperature 250^0C, carrier gas helium at 1.2ml/minute, column temperature 50^0C for 4 minutes, 150^0C for 4 minutes and held at 250^0C until the chromatogram was completed. Identification of fatty acid constituents was carried out by comparing retention times with corresponding fatty acid methyl ester standards. The coefficients of variability were calculated following Burton and Devane (1953) and heritability and genetic advance were estimated according to Lush (1949). Correlation coefficients were estimated according to method given by Searle (1961).

Results and discussion

Oil Content: Large variation was observed for oil content in linseed genotypes under study (Table 2). The oil content ranged from 34.60 to 41.14 % with a mean value of 37.53 % (Table 3). The highest oil content was recorded by KL 178 (41.14%) followed by LCK 9826 (40.82%), KL 221 (40.63%) and KL 210 (40.07%) (Table 2). It was clearly evident that yellow seeded genotypes yielded high oil in comparison to brown seeded genotypes as has been reported earlier also in linseed (Diederichsen and Raney, 2006). Most of dual purpose lines recorded good oil content in seeds except B-509 which is clearly sufficient to their breeding objective. All fiber flax type parental genotypes had low oil content. The trend observed in parental lines was also carried forward to next generation. In general, all cross combinations showed low oil content in comparison to the best parental genotypes due to the involvement of one low performing parent in all the crosses. Therefore, all the crosses having B 509 as one of the parent had low oil content in comparison to LC 2323 combinations (Table 2). The parental lines and hybrids were plotted separately and it was observed that parental lines had higher variation in comparison to hybrids for oil content (Figure 1a). Intermediate values of all cross combinations for oil content suggested lack of heterosis. Linseed has been reported to contain about 40 % oil in the seed (Choo *et al.,* 2007). However several workers have reported variation ranging from 23.28 to 46 % in seeds of different linseed cultivars and under different agro-climatic zones (Bayrak *et al.,* 2010; El-Beltagi *et al.,* 2007; El-Beltagi *et al.,* 2011; Green and Marshall, 1981). Diederichsen and Fu (2008) analyzed world collection of 2934 lines from 72 countries and observed a mean of 38.3 % with wide range of variation from 31.4 to 45.7 for oil content in linseed. Our results are in agreement with Diederichsen and Fu (2008) for mean oil content however, we observed narrow range of variation due to small number of genotypes under evaluation.

Fatty acid composition: The composition of fatty acids was cultivar dependent (Table 2). The amount of total unsaturated fatty acids in studied genotypes was 88.12 to 92.41 % while the amount of total saturated fatty acids ranged from 7.47 to 11.58 % of the total oil. There are several reports of total unsaturated and saturated fatty acids in linseed cultivars varying from 87 to 91 % and 9 to 12 %, respectively (Choo *et al.,* 2007; El Beltagi *et al.,* 2007; El Beltagi *et al.,* 2011; Bhatty, 1995).

The unique feature of linseed is the accumulation of large amounts of linolenic acid (Omega 3), the final product of three desaturation steps. Fatty acid analysis of the linseed cultivars grown in sub-mountainous Himalayan region of India in our study showed 42.61 to 53.51 % linolenic acid whereas the mean values of linoleic, oleic, stearic and palmitic acid were 12.95, 29.77, 4.13 and 5.01 %, respectively (Table 3). The results are in agreement with Diederichsen and Fu (2008) for linoleic, stearic and palmitic acid but, we observed higher values for oleic acid and lower values for linolenic acid. Green (1986b) also observed high oleic acid content in segregating generations of mutant lines. The genotype Belinka (53.51%) showed the highest

linolenic acid followed by its cross combination with LC 2323 (53.22%). Similarly, the cross combination, B 509 x Belinka (16.78%) had the highest linoleic acid followed by Flak-1 (15.78%). Lowest linolenic acid was observed in the cross combination LC-2323 x Aoyogi (42.61%) followed by parental line KL 187 (43.50%). There are several earlier studies with similar results in different regions of world (Bhatty, 1995; Krist *et al.*, 2006; Van Ruth *et al.,* 2001; Baydar and Turgut, 1999; Bean and Leeson, 2002; Robbelen *et al.*, 1989).

Auto-oxidation of high linolenic acid is believed to be the principal cause of off flavours during storage of edible vegetable oils such as soybean and rapeseed (Smouse, 1979). Genetic modification is the only permanent and satisfactory method of lowering the linolenic acid in linseed oil. Hybrids exhibited broader range of linoleic and linolenic acid in comparison to the parents (Figure1b). This could be due to complementation of genes affecting the trait. This offers the opportunity of negative selection to develop low linolenic high linoleic acid lines in segregating generations. However, induced mutagenesis along with recombination breeding will be more beneficial for the development of low linolenic acid lines as has been achieved by Green (1986b).

Genetic parameters and association analysis: Both genotypic (GCV) and phenotypic coefficients of variation (PCV) were low for oil content and fatty acid components. Linoleic and stearic acid exhibited highest GCV (9.53) and PCV (16.50), respectively. The values of genotypic coefficient of variation for all the traits were low in comparison to phenotypic coefficient of variation which clearly indicated low variability and complex nature of oil content and fatty acid components. Further, low heritability coupled with low genetic advance values for these traits showed high environmental influence (Table 3). This means that selection *per se* will not be rewarding for these traits and combination breeding could be useful. These results also suggest the use of induced mutagenesis for creating new genetic variability.

The correlation values between 1000-seed weight, oil content and fatty acid components are presented in Table 3. The results revealed weak positive association (r = 0.176) of 1000-seed weight with oil content. Lower values of seed weight along with oil content were observed in fiber flax genotypes and both the trait values increased in dual purpose as well as seed type genotypes. Diederichsen and Fu (2008) also reported increase in seed weight, oil concentration and oil amount per seed in the order: fibre flax (convar. *elongatum*), intermediate flax (convar. *usitatissimum*) and large-seeded flax (convar. *mediterraneum*). They also observed weak, positive association of higher oil concentration with higher seed weight, and higher seed weight and oil concentration in yellow seeded than brown-seeded genotypes. The results suggested that indirect selection for increased seed oil concentration in linseed is possible by selection for higher seed weight and yellow seed colour.

Among all the fatty acids, palmitic acid showed significant negative association whereas, stearic acid and linoleic acid had weak non significant positive association with oil content. However, Bayark *et al.* (2010) observed positive association between palmitic acid and oil content. Linolenic acid had negative non significant association with oil content. In addition significant positive association was observed between oleic, palimitic (r = 0.385) acid and linoleic, stearic acid (r = 0.342). Linolenic acid observed significant negative association with stearic (r = -0.548), oleic (r = -0.573) and linoleic acid (r = -0.565). These results explained that high oil content will result in low palmitic acid and increase in linolenic acid will result in decrease in stearic, oleic and linoleic acids. Bayrak *et al.* (2010) and Bhatty (1995) also observed negative association of linoleic, stearic and oleic acid with linolenic acid. The association analysis results suggested that selection for increased oil content will not affect the quality of linseed oil except decrease in palmitic acid content and selection for high oleic, linoleic acid will automatically reduce linolenic acid.

The results of present study demonstrated that the oil content and fatty acid composition of linseed genotypes grown in sub-mountainous Himalayan region of India were quite similar to studies conducted in other regions. Yellow seeded genotypes had high oil content in comparison to brown seeded genotypes. Hybrids observed intermediate values for oil content while broader range of variation was exhibited for linolenic and linoleic acid by hybrids in comparison to parents. All oil and fatty acid components expressed low genetic variability, heritability and genetic advance. Association analyses showed high oil content in bold seeded genotypes and strong negative association of linolenic acid with linoleic and oleic acid. These results suggested induced mutagenesis along with combination breeding followed by indirect selection for modified fatty acid composition in linseed.

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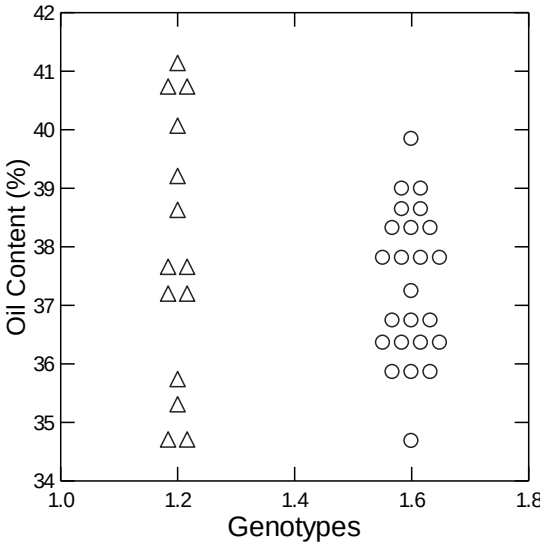

Fig. 1a Oil content

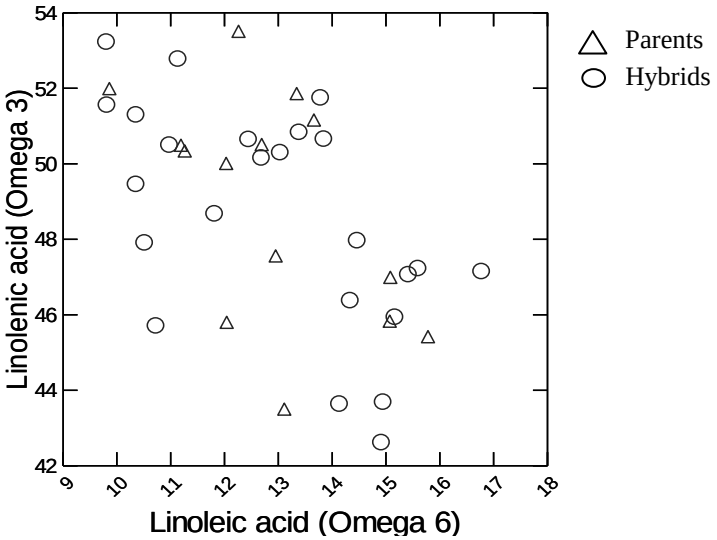

Fig. 1b Omega fatty acids content

Figure 1. Comparison of parents and hybrids for oil and omega fatty acids content

Table 1. Parentage/ source of genotypes along with utilization purpose

S. No.	Genotype	Seed Colour	Parentage/ Source	Use
1	Ariane	Brown	Exotic line from Belgium	Fibre flax
2	Belinka	Brown	Exotic line from Belgium	Fibre flax
3	Aoyagi	Brown	Selection from exotic Ayogi	Fibre flax
4	Flak-1	Brown	Selection from exotic flax line	Fibre flax
5	LMH-62	Brown	(EC-41628 x EC-77959) x (DPL-20 x Neelum)	Dual-purpose
6	LCK-9826	Brown	LCK-88062 x KL-168	Dual-purpose
7	KL-187	Yellow	K-2 x TLP-1	Seed flax
8	KL-178	Yellow	LC-36 x LC-255	Dual-purpose
9	KL-233	Brown	Flax purple x Gaurav	Dual-purpose
10	Janaki	Brown	New River x LC-216	Seed flax
11	KL-221	Yellow	89D-2B/ 5 x SPS 47/ 7-10-3	Seed flax
12	KL-210	Yellow	Flak-1 x SPS 47/ 7-10-3	Seed flax
13	B-509	Brown	Birsa Agricultural University (Bihar)	Dual-purpose
14	LC-2323	Brown	Punjab Agricultural University (Punjab)	Seed flax

Fibre flax: mainly used for fibre extraction from the stem; Seed flax: used for oil & cake extraction from the seeds; Dual-purpose flax: used for both fibre and oil extraction

Table 2 Oil percentage and fatty acid composition of parents and their hybrids in linseed

Genotypes	TW (G)	OC (%)	PA	SA	TS	OA	LA	LLA	TUS
Aoyagi	6.04	35.74	4.73	3.35	8.08	30.40	15.07	45.83	91.3
Flak-1	6.67	37.20	5.09	4.10	9.19	29.37	15.78	45.42	90.57
B-509	6.60	34.87	4.15	5.09	9.24	29.43	12.95	47.56	89.94
Janaki	6.72	39.21	5.02	3.09	8.11	29.80	12.03	50.01	91.84
Belinka	5.69	35.31	5.26	2.98	8.24	25.70	12.26	53.51	91.47
KL-178	5.72	41.14	4.87	3.33	8.2	26.70	13.34	51.86	91.9
KL-210	6.84	40.07	6.02	3.11	9.13	32.98	12.04	45.8	90.82
LMH-62	7.22	37.55	5.22	3.03	8.25	28.34	12.69	50.51	91.54
Ariane	4.85	34.60	4.79	2.99	7.78	30.58	11.19	50.49	92.26
KL-233	4.79	38.63	5.14	2.50	7.64	30.58	11.26	50.34	92.18
LCK-9826	7.17	40.82	4.95	4.16	9.11	25.32	13.66	51.16	90.14
KL-221	7.53	40.63	5.19	2.71	7.9	30.27	9.86	51.99	92.12
LC-2323	6.23	37.26	5.16	4.33	9.49	29.29	15.08	46.99	91.36
KL-187	6.78	37.70	5.57	3.67	9.24	33.19	13.11	43.50	89.8
B-509 x LMH-62	6.62	36.25	4.82	3.59	8.41	28.28	14.47	47.96	90.71
B-509 x Flak-1	6.74	36.63	4.92	2.55	7.47	26.54	13.79	51.74	92.07
B-509 x Belinka	5.69	35.75	4.36	5.34	9.7	26.42	16.78	47.14	90.34
B-509 x Aoyagi	5.55	35.97	5.31	5.01	10.32	30.39	14.14	43.63	88.16
B-509 x KL-221	6.60	36.38	5.64	2.06	7.7	29.87	15.42	47.06	92.35
B-509 x KL-178	6.70	36.40	5.09	4.96	10.05	31.17	14.95	43.68	89.8
B-509 x KL-187	7.44	36.93	4.03	4.56	8.59	27.26	13.39	50.83	91.48
B-509 x KL-210	7.65	36.76	5.28	2.32	7.6	29.08	13.04	50.29	92.41
B-509 x Janaki	7.40	37.73	4.74	5.67	10.41	27.69	15.17	45.93	88.79
B-509 x Ariane	7.55	34.68	4.81	3.87	8.68	28.28	15.6	47.22	91.1
B-509 x LCK-9826	7.35	37.24	5.10	3.59	8.69	30.75	14.34	46.37	91.46
B-509 x KL-233	7.32	37.70	5.42	3.03	8.45	28.73	12.69	50.15	91.57
LC-2323 x LMH-62	7.75	37.77	5.27	3.42	8.69	29.55	9.82	51.55	90.92
LC-2323 x Flak-1	8.30	38.25	5.17	3.84	9.01	31.27	10.36	49.45	91.08
LC-2323 x Belinka	6.77	36.57	5.54	2.55	8.09	28.42	9.81	53.22	91.45
LC-2323 x Aoyagi	6.77	37.85	5.89	5.69	11.58	30.59	14.92	42.61	88.12
LC-2323 x KL-221	7.06	38.64	4.99	3.66	8.65	26.49	13.85	50.65	90.99
LC-2323 x KL-178	7.17	38.35	4.45	3.97	8.42	27.66	11.14	52.77	91.57
LC-2323 x KL-187	5.84	38.21	4.85	3.23	8.08	28.47	12.45	50.64	91.56
LC-2323 x KL-210	5.49	38.31	5.28	3.87	9.15	28.46	10.36	51.29	90.11
LC-2323 x Janaki	6.73	38.99	5.02	5.10	10.12	32.66	10.73	45.70	89.09
LC-2323 x Ariane	6.92	35.94	4.60	4.09	8.69	29.65	10.98	50.49	91.12
LC-2323 x LCK-9826	6.74	39.84	4.79	3.40	8.19	32.53	10.52	47.90	90.95
LC-2323 x KL-233	7.10	38.28	5.36	4.89	10.25	29.07	11.82	48.67	89.56

TW: 1000 seed weight; OC: Oil content; PA: Palmitic acid (C16:0); SA: Stearic acid (C18:0); TS: Total saturated fatty acids; OA: Oleic acid (C18:1); LA: Linoleic acid (C18:2); LLA: Linolenic acid (C18:3); TUS: Total unsaturated fatty acids

Table 3. Mean, range and coefficient of variation of oil content and fatty acid components in linseed

	Character	Mean ± S.E.	Range	Phenotypic coefficient of variation (PCV)	Genotypic coefficient of variation (GCV)	Heritability (h^2_{bs}) (%)	Genetic advance
1	Seed Weight (g)	6.68 ± 0.21	4.79-8.30	14.71	14.38	95.50	1.94
2	Oil content (%)	37.53 ± 0.59	34.60-41.14	4.72	1.08	5.20	0.19
3	Palmitic acid(%)	5.01 ± 0.24	4.03-6.02	11. 90	2.18	3.40	0.04
4	Stearic acid(%)	4.13 ± 0.56	2.06-5.69	16.50	6.11	26.64	0.76
5	Oleic acid(%)	29.77 ± 1.48	25.32-33.19	12.18	0.11	0.00	0.00
6	Linoleic acid(%)	12.95 ± 0.71	9.81-15.78	16.48	9.53	33.40	1.47
7	Linolenic acid(%)	48.55 ± 2.22	42.61-53.51	11.22	0.07	0.00	0.00

Table 4. Correlation coefficients between oil content and fatty acids components in linseed

Characters	Seed Weight	Oil content(g)	Palmitic acid **(%)**	Stearic acid **(%)**	Oleic acid **(%)**	Linoleic acid **(%)**
Oil content	0.176					
Palmitic acid	0.051	-0.360*				
Stearic acid	0.060	0.183	-0.289*			
Oleic acid	0.033	-0.030	0.385**	0.006		
Linoleic acid	-0.077	0.050	-0.124	0.342*	-0.258	
Linolenic acid	0.020	-0.041	-0.222	-0.548**	-0.573**	-0.565**

*P<0.05; **P<0.01