Journal of Agricultural Science; Vol. 6, No. 9; 2014
ISSN 1916-9752 E-ISSN 1916-9760
Published by Canadian Center of Science and Education

Evaluation of Oil Content and Fatty Acid Compositions of Flax (*Linum usitatissimum* L.) Varieties of India

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Received: April 11, 2014 Accepted: June 21, 2014 Online Published: August 15, 2014

doi:10.5539/jas.v6n9p198 URL: http://dx.doi.org/10.5539/jas.v6n9p198

Abstract

Flax (*Linum usitatissimum* L.) is the third largest natural fiber crop and one of the five major oil crops in the world. An experiment was conducted to evaluate the oil content and fatty acid compositions of flax varieties grown in Chhattisgarh state of India. The oil content ranged from 33.97% to 42.27% in the experimental material. The highest oil content was recorded for variety Deepika (42.27%) followed by Indira Alsi-32 (42.12%), Sharda (41.12%) and Kartika (41.11%). Two saturated (Palmatic and stearic acid) and three unsaturated fatty acids (Oleic, linoleic and linolenic acid) were identified by gas chromatography represented 12.34% and 87.65% of the total oil, respectively. Fatty acid analysis of the flax varieties in our studies showed 33.14% to 54.82% of linolenic acid whereas, the mean value of linoleic, oleic, stearic and palmatic acid were 15.88%, 27.76%, 6.26% and 6.07%, respectively. The variety RLC-92 (54.82%) exhibited the highest linolenic acid followed by RLC-134 (53.01%), R-552 (52.41%), R-4140 (51.14%), RLC-122 (51.08%) and GS-64 (50.49%) while the lowest linolenic acid was observed in the variety Kiran (33.14%) followed by Sharda (34.41%) and Neela (36.85%). Both genotypic (GCV) and phenotypic coefficients of variation (PCV) were low for oil content and fatty acid components. Linoleic acid exhibited highest GCV (17.52%) and PCV (17.63%), respectively. High heritability with moderate genetic advance was observed for oil content and all fatty acid components suggest that selection *per se* will be rewarding for these traits. Correlation coefficient revealed weak positive association of 1000 seed weight with oil content. Among all the fatty acids, palmatic acid showed significant positive association while linoleic acid showed significant negative association. Results indicated that higher oil content varieties showed higher linolenic acid in flax.

Keywords: flax, fatty acids, oil content, linolenic acid, correlation

1. Introduction

Plant oils are important macromolecules for both human consumption and for industrial applications. Human consumption accounts for 80% of oil consumption in the world (Luhs & Friedt, 1994). Six percent is used as animal feed, and the remaining is used in industrial applications. Flax (*Linum usitatissimum* L.) is a multi-purpose crop, and its importance is showed by the fact that every part of the plant has specific economic use. The most important flax producing countries are Canada, Argentina, USA, China, India and Europe (Lidefelt, 2007; Wang et al., 2007). Flax occupies an important position in world market because of its technical grade oil, its seeds when crushed yield oil. During the last decade, the interest in flax has been reinvented in modern societies due to increased awareness of omega-3 nutrition, as flax is the richest agricultural source of omega-3 fatty acids. Flax oil is an important nutraceutical and is added in functional foods for helath benefits. The nutritional significance of flax oil is due to the presence of higher levels of α-linolenic acid (ALA) of omega-3 fatty acid (O3FA) family. ALA, an essential fatty acid, acts as a precursor for biologically active longer chain polyunsaturated fatty acids (PUFA) of omega-3 class, mainly the Eicosapentaeonic acid (EPA and Docosahexaeonic acid (DHA) (Rajwade et al., 2010).

The quality and utilization of flax oil is determined by its fatty acid composition. In general, flax 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% of total flax 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 flax 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 flax oil is 5.5 times more than the sources containing the highest level (Bloedon & 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 risk of several diseases (Visentainer et al., 2005). Recently, there has been a growing interest in the probiotic properties of flax and its beneficial effects on coronary heart disease, some kinds of cancer and neurological and hormonal disorders (Huang & Milles, 1996; Huang & 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 flax varieties grown in India which has become essential nowadays 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 evaluate oil content and fatty acid compositions (especially omega-3 fatty acid) of flax varieties grown in Chhattisgarh state of India.

2. Materials and Methods

The experimental material comprised of 48 flax varieties (Table 1). All these varieties were grown in the field condition using a Randomized Complete Block Design with three replications during *rabi season*, 2011-12 at Research cum Instructional Farm, Department of Genetics and Plant Breeding, College of Agriculture, Indira Gandhi Agricultural University, Raipur, India. Seed samples were taken at maturity and 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 flax oil at oilseed section of Department of Biochemistry, Directorate of Oilseed Research, Hyderabad. Fatty acids were analyzed by GC equipped with capillary column. The seeds were oven-dried at 40 ^{o}C for 4 hours, using a hot air oven, up to moisture content of about 5%. Flax seeds were powdered in mortar and pestle and 100 mg powder was esterified using 4 ml methenolic-HCL and extracted in 3 ml hexane. The hexane extracts were dried in argon current and reconstituted in 1:50 volumes of chloroform (Manku et al., 1983). 1 µl of this extract was injected in Autosystem XL GC (Perkin Elmer, USA) with SP-2330 Supelco capillary column, 30 meter long and 0.32 mm diameter. The temperature program was 150 ^{o}C for 10 min, followed by 10 ^{o}C rises per min up to 220 ^{o}C and steady for 10 min. Helium 1 ml per min was used as the carrier gas. The injector port was maintained at 240 ^{o}C and FID detector temperature was 275 ^{o}C. Appropriate fatty acid standards were procured from Sigma Aldrich (USA) and the fatty acid peaks were identified by integrating them with the satndards' profiles. The area under the peak was expressed as percentage fatty acid content. Estimation of each sample was repeated minimum three times.

3. Results and Discussion

3.1 Oil Content

Large variation was observed for oil content in flax varieties under study (Table 1). The oil content ranged from 33.97% to 42.27% with a mean value of 38.41% (Table 2). The highest oil content was recorded for Deepika (42.27%) followed by Indira Alsi-32 (42.12%), Sharda (41.12%) and Kartika (41.11%) while the lowest was observed in Neela (33.97%) and LC-54 (35.81%), respectively. The effect of oil content on linolenic acid is depicted in Figure 1. Flax has been reported to contain about 40% oil in the seed (Choo et al., 2007). However several findings have reported variation ranging from 23.28% to 46% in seeds of different flax varieties and under different agro-climatic zones (Green & Marshall, 1981; El-Beltagi et al., 2007; Bayrak et al., 2010; El-Beltagi et al., 2011). 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 flax. The present study is in agreement with Diederichsen and Fu (2008) for mean oil content however, we observed narrow range of variation due to small number of varieties under evaluation.

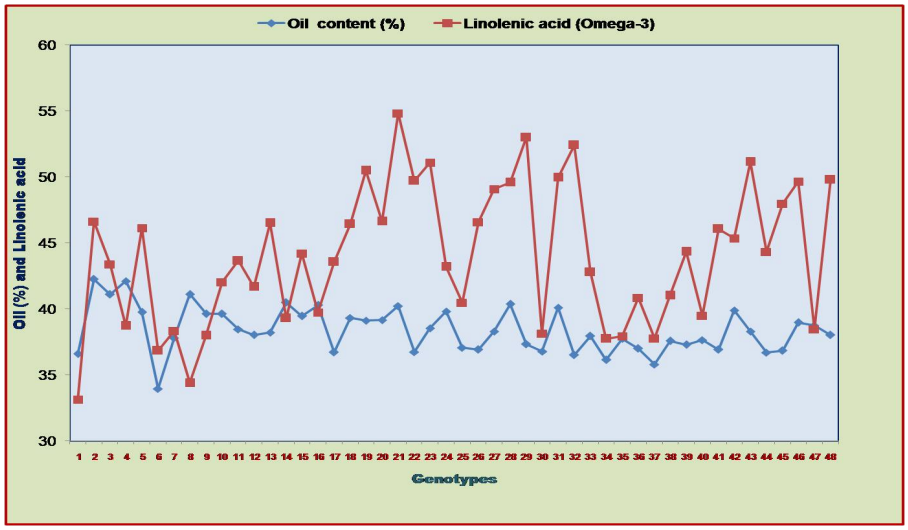

Figure 1. Effect of oil content on linolenic acid (Omega-3)

3.2 Fatty Acid Composition

Fatty acid analysis of the flax seeds showed presence of five major fatty acids with predominance of 18 carbon species, α-linolenic acid (ALA 18:3), linoleic acid (LA 18:2), oleic acid (OA 18:1), steraic acid (SA 18:0) and palmitic acid (PA 16:0), all of which are members of the same pathway catalyzed by the elongase and desaturase enzymes. The comparison of fatty acid was cultivar dependent (Table 1). The amount of total unsaturated fatty acids in selected varieties was 83.78% to 89.97% while the amount of total saturated fatty acids ranged from 10.02% to 16.22% of the total oil. There are several reports of total unsaturated and saturated fatty acids in flax varieties varying from 87% to 91% and 9% to 12%, respectively (Bhatty, 1995; Choo et al., 2007; El-Beltagi et al., 2007; El-Beltagi et al., 2011).

The unique feature of flax is the accumulation of large amounts of linolenic acid (Omega 3), the final product of three desaturation steps. Fatty acid analysis of the flax varieties grown in Chhattisgarh state of India in our study showed 33.14% to 54.82% linolenic acid whereas, the mean value of linoleic, oleic, stearic and palmatic acid were 15.88%, 27.76%, 6.26% and 6.07%, respectively (Table 2). These results are in agreement with Diederichsen and Fu (2008) for linoleic, stearic and palmatic acid but, we observed higher values for oleic acid and lower values for linolenic acid. Green (1986 b) also observed high oleic acid content in segregating generations of mutant lines. The variety RLC-92 (54.82%) exhibited the highest linolenic acid followed by RLC-134 (53.01%), R-552 (52.41%), R-4140 (51.14%), RLC-122 (51.08%) and GS-64 (50.49%) while the lowest linolenic acid was observed in the variety Kiran (33.14%) followed by Sharda (34.41%) and Neela (36.85%). There are several earlier studies with similar results in different regions of world (Robbelen et al., 1989; Bhatty, 1995; Baydar & Turgut, 1999; Van Ruth et al., 2001; Bean & Leeson, 2002; Krist et al., 2006).

Table 1. Oil percentage and fatty acid composition of flax varieties

S.No	Varieties	TW	OC (%)	PA (%)	SA (%)	TS (%)	OA (%)	LA (%)	LLA (%)	TUS (%)
1	Kiran	5.03	36.60	6.16	6.12	12.28	34.60	19.98	33.14	87.72
2	Deepika	6.23	42.27	5.51	5.97	11.48	25.60	16.33	46.63	88.51
3	Kartika	5.43	41.11	6.77	5.09	11.86	27.30	17.42	43.39	88.14
4	Indira Alsi-32	5.80	42.12	7.57	6.91	14.48	28.20	18.55	38.74	85.52
5	Shekhar	8.53	39.76	6.12	7.38	13.5	26.30	14.08	46.13	86.50
6	Neela	6.33	33.97	6.42	6.76	13.18	30.50	19.49	36.85	86.82
7	Rashmi	5.10	37.82	5.69	6.06	11.75	29.50	20.41	38.36	88.25
8	Sharda	8.07	41.12	6.50	6.24	12.74	31.80	21.04	34.41	87.26
9	Meera	7.03	39.65	6.32	9.90	16.22	32.60	13.19	38.00	83.78
10	PKDL-43	5.53	39.65	6.07	5.07	11.14	27.60	19.25	42.04	88.85

11	PKDL-58	4.43	38.48	6.30	6.71	13.01	30.80	12.52	43.66	86.99
12	PKDL-62	4.83	38.04	5.94	4.62	10.56	30.20	17.55	41.69	89.44
13	JRF-5	5.03	38.22	6.21	7.41	13.62	26.00	13.81	46.53	86.38
14	JLS-9	7.40	40.50	5.91	7.11	13.02	33.70	14.02	39.31	86.98
15	KL-1	4.27	39.48	6.07	6.22	12.29	29.60	13.79	44.20	87.55
16	KL-168	4.80	40.28	6.78	6.61	13.39	32.80	14.08	39.75	86.61
17	GS-27	4.90	36.74	6.43	6.94	13.37	27.80	15.25	43.57	86.63
18	GS-61	7.77	39.34	6.30	7.51	13.81	26.10	13.65	46.45	86.19
19	GS-64	6.73	39.12	6.01	5.77	11.78	19.80	17.94	50.49	88.22
20	GS-129	6.23	39.18	5.99	6.29	12.28	25.50	15.59	46.64	87.71
21	RLC-92	6.57	40.23	5.51	4.78	10.29	19.00	15.91	54.82	89.72
22	RLC-94	5.50	36.74	5.51	4.87	10.38	28.10	11.84	49.71	89.62
23	RLC-122	6.43	38.55	6.62	7.21	13.83	23.10	12.00	51.08	86.16
24	RLC-123	7.03	39.82	5.83	7.39	13.22	26.40	17.20	43.21	86.78
25	RLC-128	5.10	37.08	5.91	4.87	10.78	29.90	18.88	40.48	89.22
26	RLC-129	5.57	36.93	5.94	5.69	11.63	25.90	15.90	46.59	88.38
27	RLC-132	8.63	38.31	6.15	7.79	13.94	22.90	14.05	49.09	86.06
28	RLC-133	7.13	40.38	5.26	5.21	10.47	22.40	17.56	49.60	89.52
29	RLC-134	7.20	37.34	5.51	5.88	11.39	21.50	14.11	53.01	88.61
30	RLC-135	6.8	36.80	5.81	6.27	12.08	29.40	20.43	38.14	87.92
31	RLC-137	6.00	40.11	5.74	5.92	11.66	22.90	15.52	49.97	88.34
32	R-552	5.00	36.52	5.97	6.08	12.05	23.10	12.46	52.41	87.94
33	CI-229	5.63	37.96	6.16	6.52	12.68	26.50	18.06	42.79	87.33
34	NL-97	6.4	36.15	5.40	5.69	11.09	36.50	14.67	37.76	88.91
35	Polf-22	5.27	37.76	5.93	4.56	10.49	28.20	23.36	37.94	89.50
36	T-397	5.00	37.01	7.17	7.29	14.46	28.10	16.64	40.85	85.55
37	LC-54	5.9	35.81	6.33	7.27	13.60	35.50	13.17	37.77	86.40
38	LCK-88068	6.00	37.59	6.41	5.90	12.31	29.90	16.76	41.04	87.68
39	FRW-12	6.13	37.30	6.02	6.27	12.29	31.20	12.13	44.36	87.71
40	Gcwargi 1-2	5.70	37.66	6.31	7.03	13.34	29.40	17.79	39.50	86.67
41	R-2678	6.10	36.94	5.58	4.44	10.02	25.60	18.26	46.07	89.97
42	R-4129	6.13	39.91	5.02	6.26	11.28	31.20	12.13	45.36	88.72
43	R-4140	8.37	38.30	5.56	6.95	12.51	24.00	12.36	51.14	87.50
44	R-4141	7.43	36.72	6.02	5.80	11.82	29.80	14.03	44.33	88.18
45	R-4152	7.00	36.85	5.41	5.33	10.74	27.20	14.09	47.98	89.26
46	R-4154	7.87	38.98	6.20	5.86	12.06	25.50	12.76	49.65	87.94
47	R-4158	7.63	38.76	7.62	7.25	14.87	30.80	15.84	38.47	85.13
48	R-4168	5.77	38.04	5.61	5.75	11.36	22.70	16.11	49.79	88.63

TW: 1000 seed weight; **OC**: Oil content; **PA**: Palmatic acid (C 16:0); **SA**: Stearic acid (C 18:0); **TS**: Total saturated fatty acids; **OA**: Oleic acid (C 18:1); **LA**: Linoleic acid (C 18:2); **LLA**: Linolenic acid (C 18:3); **TUS**: Total unsaturated fatty acids.

*** Note -** All observations of oil content and fatty acid compositions are the mean value of three replicated trials.

3.3 Genetic Parameters & Association Analysis

Both genotypic (GCV) and phenotypic coefficients of variation (PCV) were low for oil content and fatty acid components (Table 2). Linoleic acid exhibited highest GCV (17.52%) and PCV (17.63%), respectively. The genotypic coefficient of variation (GCV) ranged from oil content (4.42%) to linoleic acid (17.52%). The phenotypic coefficient of variation and genotypic coefficient of variation for oleic acid is equal whereas,

linolenic acid, linoleic acid, stearic acid, oil content showed very less difference indicating the greater role of genetic factors in expression of these traits. Differences between genotypic coefficient of variation and phenotypic coefficient of variation were observed for 1000 seed weight indicating higher environmental influences. High heritability with moderate genetic advance was observed for oil content and all fatty acid components. The values of genotypic coefficient of variation for all traits were almost equal in comparison to phenotypic coefficient of variation which clearly indicated high variability and supplementary nature of oil content and fatty acid components. Further, high heritability coupled with moderate genetic advance for these traits suggest that selection *per se* will be rewarding for these traits.

Table 2. Mean, range, coefficient of variation for oil content and fatty acid components in flax

Characters	Mean	Range	PCV (%)	GCV (%)	Heritability bs (%)	Genetic advance (GA)	GA as% of mean
Seed weight (g)	6.50	9.1 – 3.6	14.00	12.30	77.14	1.47	22.67
Oil content (%)	38.41	42.27 – 33.97	4.56	4.42	94.14	16.40	42.69
Palmitic acid (%)	6.07	7.74 – 5	8.99	8.57	90.93	1.02	16.85
Stearic acid (%)	6.26	10 – 4.34	16.66	16.36	96.33	2.07	33.08
Oleic acid (%)	27.76	36.62 – 18.91	14.27	14.27	99.87	8.15	29.37
Linoleic acid (%)	15.88	23.74 – 11.72	17.63	17.52	98.69	5.69	35.86
Linolenic acid (%)	44.01	54.82 – 33.14	11.99	11.94	99.12	22.77	51.73

Table 3. Correlation coefficients between oil content and fatty acid components in flax

Characters	Seed weight (g)	Oil content (%)	Palmitic acid (%)	Steraic acid (%)	Oleic acid (%)	Linoleic acid (%)
Oil content (%)	0.200					
Palmitic acid (%)	-0.097	0.117				
Stearic acid (%)	0.293*	0.099	0.432**			
Oleic acid (%)	-0.220	-0.210	0.239	0.204		
Linoleic acid (%)	-0.172	-0.000	0.107	-0.358**	0.065	
Linolenic acid (%)	0.212	0.126	-0.423**	-0.204	-0.851**	-0.521**

*, ** Significant at 5% and 1% level, respectively.

The correlation values between 1000-seed weight, oil content and fatty acid components are presented in Table 3 and the relationship between seed weight, oil content and fatty acid composition are depicted in Figures 2 to 6. The results revealed weak positive association of 1000 seed weight with oil content. Lower values of seed weight along with oil content were observed in fiber flax varieties 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. Among all the fatty acids, palmatic acid showed significant positive association while linoleic acid showed significant negative association. Palmatic and stearic acid had weak non-significant positive association with oil content. However, Bayark et al. (2010) observed positive association between palmatic acid and oil content. Significant positive association was observed between stearic and palmatic acid. Linolenic acid observed significant negative association with palmatic, oleic and linoleic acid.

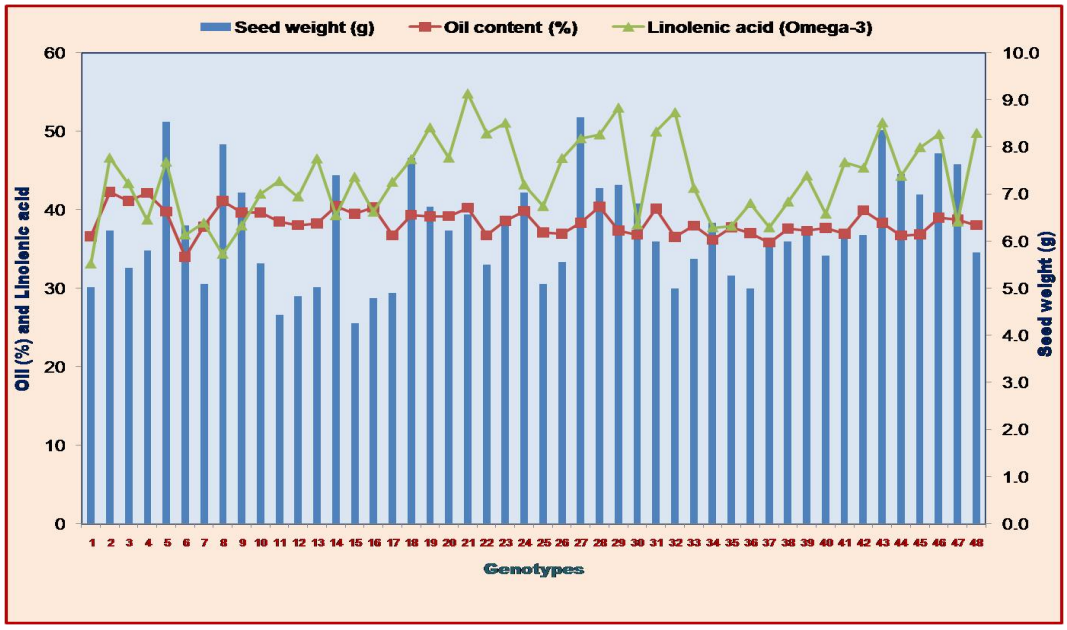

Figure 2. Relationship between seed weight, oil content and linolenic acid (Omega-3)

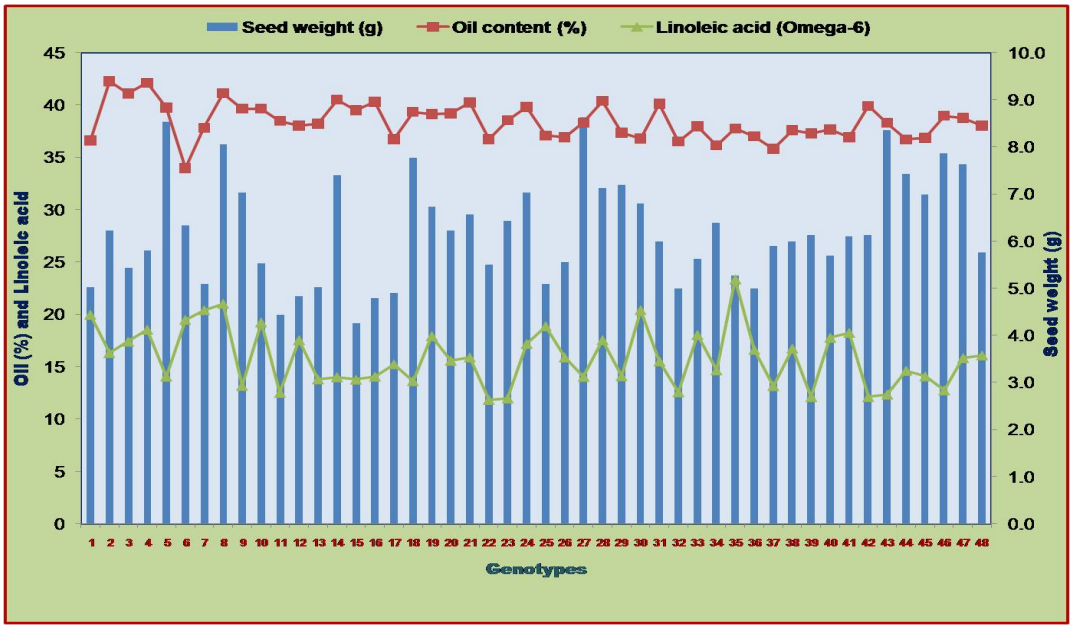

Figure 3. Relationship between seed weight, oil content and linoleic acid (Omega-6)

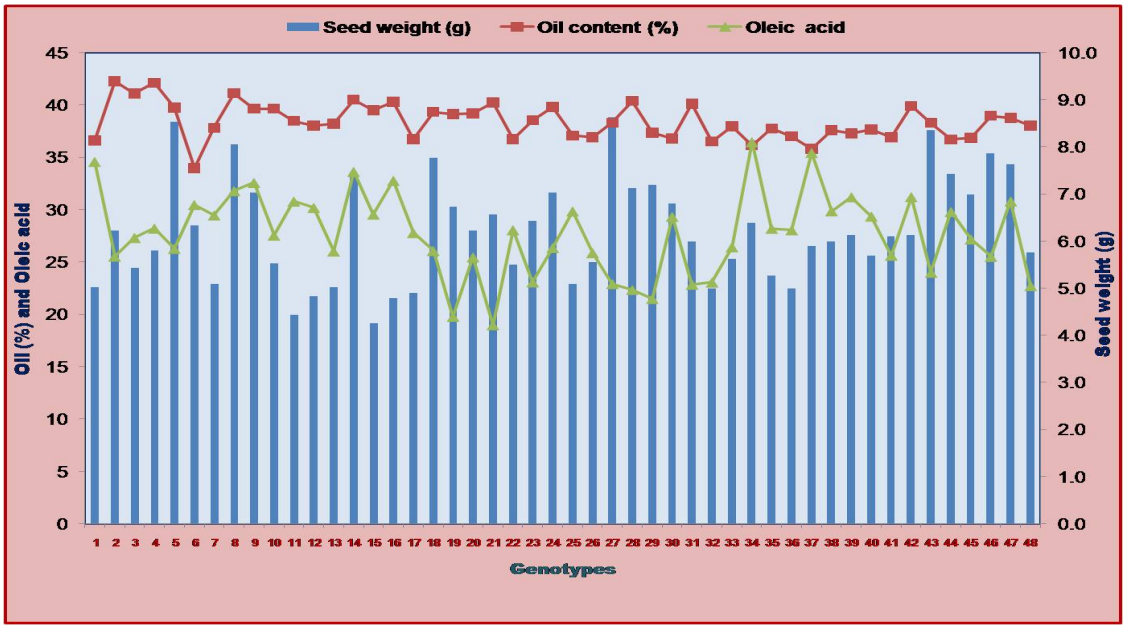

Figure 4. Relationship between seed weight, oil content and oleic acid

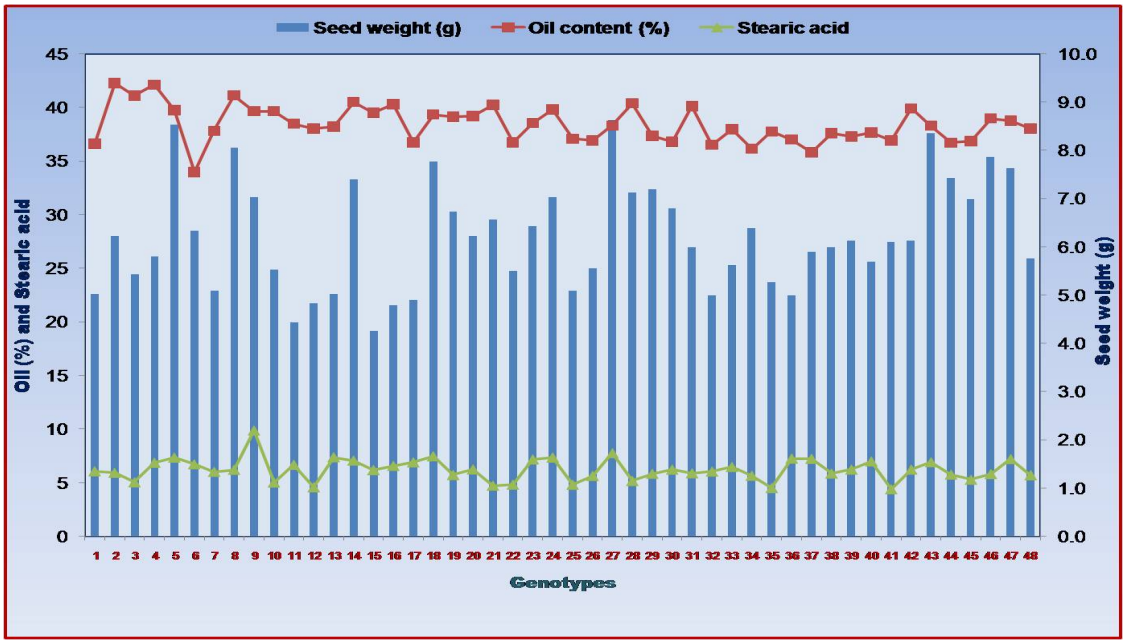

Figure 5. Relationship between seed weight, oil content and stearic acid

Figure 6. Relationship between seed weight, oil content and palmatic acid

These results explained that high oil content will result in low palmatic acid while increase in linolenic acid will result in decrease in palmatic, oleic and linoleic acids. So, the present study clearly indicated that increase in oil content of varieties will definately enhances the linolenic acid. Bayrak et al. (2010) and Bhatty (1995) also observed negative association of linoleic, oleic and stearic 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 palmatic acid.

4. Conclusion

The results of present study demonstrated that the oil content and fatty acid composition of flax varieties grown in Chhattisgarh state of India were quite similar to studies conducted in other regions. Large variation was observed for oil content and fatty acid composition in flax varieties. The variety RLC-92 (54.82%) exhibited the highest linolenic acid followed by RLC-134 (53.01%), R-552 (52.41%), R-4140 (51.14%), RLC-122 (51.08%) and GS-64 (50.49%). All oil and fatty acid composition expressed high heritability with moderate genetic advance implies that selection *per se* will be rewarding for these traits. It can be suggested from the present study that increase in oil content will enhances the linolenic acid of flax varieties.

Acknowledgements

We gratefully acknowledge the help and support provided by Directorate of Oilseed Research, Hyderabad**.**

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