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COMPARISON OF FATTY ACID COMPOSITIONS AND MICRONUTRIENT PROFILE OF ADVANCED BREEDING LINES OF LINSEED (*LINUM USITATISSIMUM* L.)

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ABSTRACT

The current study determined oil content, fatty acid composition and mineral content in ten linseed accessions of elite breeding material procured from linseed genotypes. The fatty acid profiles for the ten genotypes ranged between 5.97% and 7.13% for palmitic acid, 3.9-5.53% for stearic acid, 17.71-29.05% for oleic acid, 8.13-12.7% for linoleic acid, and 47.89-59% for linolenic acid. The overall average values of Na, K, P, Ca, Mg, Fe, Zn, Cu, Mn, and B for the linseed accessions were 882.31, 7641.05, 6043.03, 391.29, 4245.01, 327.25, 63.65, 24.04, 39.18, and 32.88 mg/kg, respectively. Among the macro-minerals, the highest levels of Na, K, P, Ca, and Mg were observed in LCP87, LCP1, LCYP35, LCP3 and LCP87, respectively. Among micro-minerals, Fe and Mn were found in maximum content in LCP3, Cu in LCPY35 and Zn and B content in LDCP1. Identified genotypes with rich nutritional profile can be further utilized towards the development of nutrient-rich varieties in future breeding programs in linseed of India.

Key words : Linseed, Fatty acid, Linolenic acid, Mineral content.

Introduction

Linseed, also known as *Linum usitatissimum* L., is considered to be one of the oldest crops in the world from the *Linum* genus, encompassing more than 300 species all over the world. It is utilized in diverse industries such as food, textiles, pharmaceuticals etc. Specifically, it is grown for oil production, because linseed contains 40% oil, 30% dietary fiber, 20% protein, 4% ash and 6% moisture (Wang *et al*, 2008). Particularly known for high levels of omega3 (ω-3) fatty acids, primarily α-linolenic acid (ALA, C18:3), linseed has been coming under the spotlight as a functional food in recent times (Thomson and Cunnane, 2003; Vaisey-Genser and Morris, 2003).

Linseed has a fat content of 41% on a dry weight basis. Its lipid profile is remarkable; 70% polyunsaturated fatty acids (PUFA), of which more than 50% of the total fatty acids being ALA (Hall *et al*., 2006; Muir, 2006). The ALA serves as a precursor to important ù-3 PUFAs, namely docosahexaenoic acid and eicosapentaenoic acid that are critical for the healthy development of children's brains and for providing resilience to allergies, autoimmune diseases, cardiovascular issues and inflammation. Because mammals, including humans, can never synthesize these fatty acids inside their body, it becomes necessary for them to be part of the diet (Sierra *et al*., 2008). Linoleic acid (C18:2, ω-6) is the second major essential fatty acid, accounting for about 17.00% of the total fatty acid composition (Velek, 2002; Bayrak *et al*., 2010).

Linseed oil is a strong inhibitor of pro-inflammatory mediators, even when used for household cooking. This makes linseed oil a potential candidate for the development

of novel anti-inflammatory drugs for populations without pharmaceutical drugs (Oomah, 2001). Many studies have indicated that linseed presents many positive effects on health: it greatly increases the intake of PUFA in the diet, offering protection against cancer (Rose and Connolly, 1999); reduces the risk of cardiovascular diseases and other numerous health problems (Alexander, 1998). Thus, with its nutritional composition, flaxseed might have a strong role in reducing the risk to health.

The present study compared the fatty acid composition and concentration of macro- and micro-minerals in ten linseed genotypes. Genotypes that are rich in nutritional ingredients will be identified for their probable incorporation into future breeding programs.

Materials and Methods

Ten advanced breeding lines of linseed, namely LDCP25, LCP87, M6, LCPY35, JCB1, LDCP1, LCP1, LCP3, LDCP13 and LC 2063, were either cultivated, harvested, or procured to investigate their fatty acid composition and mineral content. For the extraction of oils and preparation of their methyl esters, one gram of ground linseed seed from each genotype was combined with 75 mL of hexane and then subjected to extraction using the FOSS Soxtec 2055 Apparatus. The extraction process involved boiling, rinsing, recovery and drying, with the procedure repeated twice for optimal oil recovery. The extraction timeline included 15 minutes of boiling, 30 minutes of rinsing, 10 minutes of recovery, and 5 minutes of drying.

To identify fatty acids, they were esterified as methyl esters following the determination of oil yield in the dry matter. Analysis was carried out using an Agilent 6890 N GC equipped with a capillary column DB-23 (60 m long x 0.25 m) and a Flame Ionisation Detector (FID). The carrier gas was helium at 1.2 mL/min; and the injector and detector temperatures were kept constant at 250°C. The temperature of the column was first kept at 165°C for 15 min, then increased to 200°C at a rate of 5°C/min and held at200°C for 15 minutes. Fatty acids were identified by comparing the retention time to standards purchased from Sigma-Aldrich Ltd.

Mineral content estimation involved pre-digestion of the samples overnight and ashing at 450°C in a muffle furnace for three hours. The ash was dissolved in 25 ml acidic buffer prepared by combining 1 L of nitric acid with 23 g of sodium carbonate. The sample was ignited using a 20kV AC-Spark on emission spectrophotometer, simultaneously determining Na, P, K, Ca, Mg, Zn, Cu, Fe, Mn, B, Cr, Cd and Pb by following method given by Eardley and Clark (1965).

The tool used in doing the statistical analysis was representing the results obtained for the composition of fatty acid and mineral content as means ± standard error.

Results and Discussion

Oil content

Seed oil content varied among the entries, with the results ranging from 36.88% to 44.38%,with overall average of 40.2% (Fig. 1). The genotype LDCP-25 recorded the highest content of 44.38%, followed closely by LDCP-13 and LCP3, recording oil contents of 42.73% and 40.7%, respectively. Lowest oil content (36.88%) was recorded in entry JSB 1. According to Nykter *et al*. (2006), the seed oil content of linseed ranged between36.6% and 44.0%. Generally, the oil content in the linseed is around 40% on a dry weight basis according to Choo *et al*. (2007).

Fatty acid profile

The oil content further was further analysed to estimate the fatty acid profile, which is made up of palmitic acid (C16:0), stearic acid (C18:0), oleic acid (C18:1), linoleic acid (C18:2) and linolenic acid, shown in Fig. 2. This figure indicates that the main fatty acid of the entries was linolenic, varying from 47.89% to 59%, with an average of 54.41%. Highest linolenic acid content was observed in entry LCPY35 (59%), followed by M6 (58.53%) and LDCP-1 (56.37%). These findings agree with results obtained by Jhala and Hall (2010) and Hosseinian *et al*. (2004). The alpha-linolenic acid (ALA) content was observed to be around 50%, aligning with findings from Bayrak *et al*. (2010), Diederichsen and Raney (2006), Jhala and Hall (2010).

Oleic acid was the second major fatty acid which ranged from 17.71% to 29.05%, with a mean value of 23.61%. Entry LDCP-25 recorded the highest oleic acid content at 29.05%, followed by LCP-3 and LCP 87 with 27.46% and 27.17%, respectively. The experimental values for oleic acid were comparable to those reported in the earlier studies, specifically (15.81-27.99%) by Nykter *et al*. (2006) and (20.9-24.4%) by El-Beltagi *et al*. (2007).

In the category of polyunsaturated fatty acids, linoleic acid ranged from 8.13% to 12.7%, with an average of 10.23%. Entry LCPY 35 exhibited the highest linoleic acid content at 12.7%, followed by M6 and JSB1 with 12.53% and 11.89%, respectively. Similar linoleic acid content was noted in studies by Jhala and Hall (2010), Bayrak *et al*. (2010), Diederichsen and Raney (2006).

Among saturated fatty acids, palmitic acid ranged from 5.97% to 7.13%, with an average value of 6.65%.

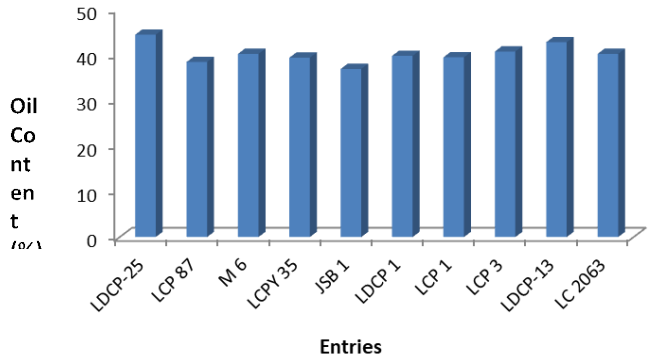

Fig. 1 : Variation in oil content (%) of linseed genotypes.

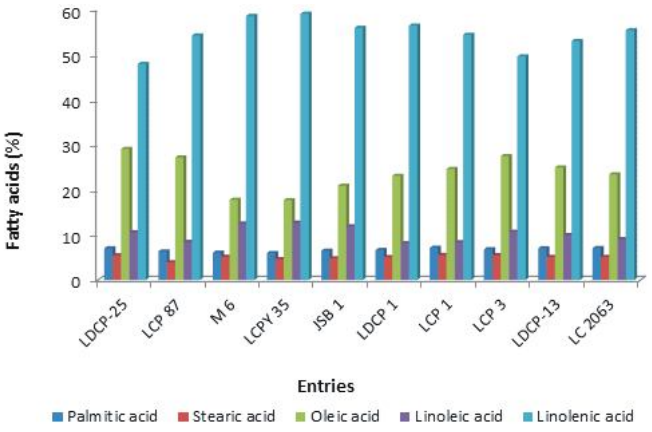

Fig. 2 : Fatty acid profile of different linseed genotypes.

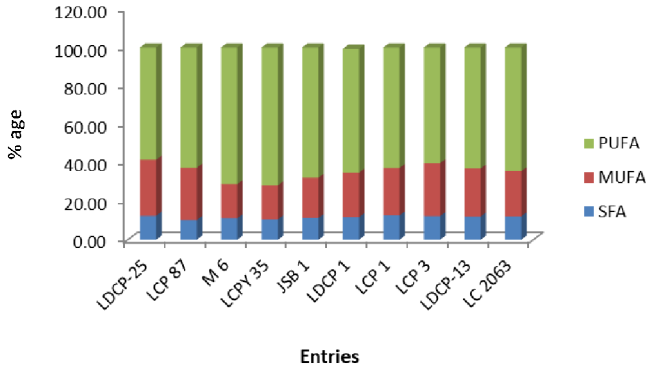

Fig. 3 : SFA, MUFA and PUFA concentration among the linseed genotypes.

All entries demonstrated palmitic acid levels less than or equal to 7%. Entry LCPY 35 had the minimum palmitic acid at 5.97%, followed by M6 with 6.05%. Stearic acid ranged from 3.9% to 5.53%, with an average value of 5.02%. Entry LCP 87 exhibited the minimum stearic acid content at 3.9%, followed by LCPY 35 with 4.62%.

Saturated, Monounsaturated and Polyunsaturated fatty acids

Palmitic and stearic acids are the saturated fat components which ranged from 10.23% to 12.66%, with a mean of 11.67%. Lowest saturated fat was recorded in Entry LCP87 (10.23%), followed by LCPY35 (10.59%). Oleic acid which is categorized as monounsaturated fatty acid (MUFA) was the second major fatty acid, ranged from 17.71% to 29.05%. Entry LDCP-25 recorded the highest MUFA content at 29.05%, trailed by LCP-3 and LCP 87 with 27.46% and 27.17%, respectively. Both linoleic acid and linolenic acid comprised the polyunsaturated fatty acids, ranging from 58.48% to 71.7%, with an average of 64.65%. The saturated fat (SFA), monounsaturated fat (MUFA), and polyunsaturated fat (PUFA) content of different entries has been illustrated in Fig. 3.

The saturated fat content in the genotypes under investigation (10.23% to 12.66%) was higher the recommended range. Similarly, polyunsaturated fat (58.48% to 71.7%) was also higher among the genotypes under study. Monounsaturated fatty acid was present in substantial amounts, ranging from 17.71% to 29.05%. The percentage of total unsaturated fat was notably high, ranging from 87.33% to 89.77%. Our findings regarding fatty acid composition align with prior research (Zhang *et al*., 2016; Yaqoob *et al*., 2016; Zou *et al*., 2017). The studies revealed that oil content and the fatty acid composition were significantly influenced by the cultivar and the geographical location. The genotypes LCPY 35 and M6 having higher linoleic and linolenic acid content are really of huge industrial significance.

Elemental composition of seeds of linseed entries

Macro-minerals Na, K and P ranged from 649.77 to 1128.13, 6845.24 to 8490.29 and 5233.93 to 6788.87 mg/Kg, respectively; values of 882.31, 7641.05 and 6043.03 mg/Kg were recorded as the mean (Fig. 4). The highest Na content was recorded in LCP87 with 1128.13 mg/Kg, followed by LCYP35 with 1096 mg/Kg. The maximum K content was recorded in JSB1 (8490.29 mg/Kg), followed by LCP1 (8224.28 mg/Kg). LCYP35 exhibited highest P content of 6788.87 mg/Kg, followed by LCP1 (6452.43 mg/Kg). Ca and Mg content ranged from 283.45 to 532.67 and 4093.68 to 4379.69 mg/Kg, with average value of 391.29 and 4245.01 mg/Kg, respectively. LCP-3 showcased the highest Ca content at 532.67 mg/Kg, followed by LDCP-13 with 457.53 mg/Kg. Four genotypes, namely LCP87, LDCP-25, LCPY35, and LC2063, exhibited Mg content surpassing 4300 mg/Kg, with values of 4379.69, 4355.41, 4352.38, and 4320.08 mg/Kg. LCP87 exhibited elevated levels of both Ca and Mg content. The genotypes showed high variation for the various elements in linseed genotypes, indicating that linseed can be a good source of Ca and rich source of Mg, which is in concurrence with previous research studies (Gambus *et al*., 2003; Khan *et al*., 2010; Colovic

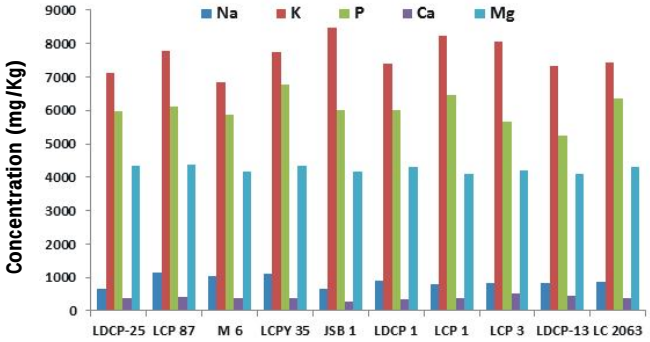

Fig. 4 : Macro-nutrient composition of linseed genotypes.

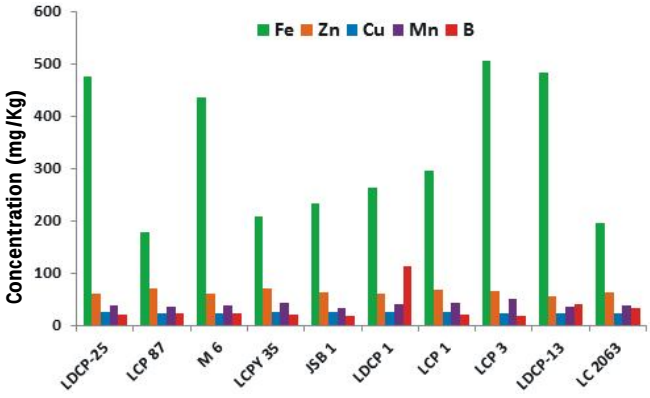

Fig. 5 : Micro-nutrient composition of linseed genotypes.

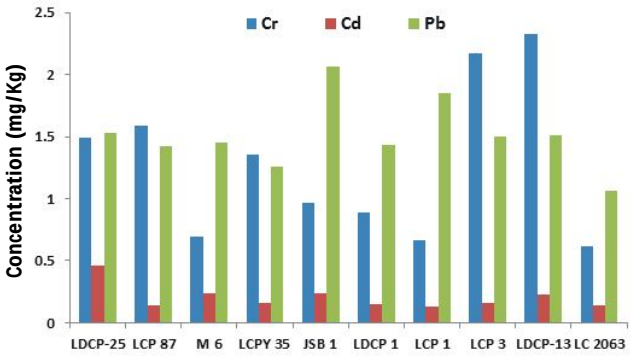

Fig. 6 : Anti-nutritional elements concentration in linseed genotypes.

et al., 2016). Genotype LCP87 proved superior for both Ca and Mg contents and thus can be exploited further in the breeding programmes.

Essential minerals, Fe, Zn, Cu, and Mn, content varied from 176.67 to 505.19, 56.42 to 71.13, 22.03 to 25.6 and 31.68 to 50.42 mg/Kg, respectively; with mean values of 327.25, 63.65, 24.04, and 39.18 mg/Kg (Fig. 5). In LCP-3, the highest contents were found for both Fe and Mn, amounting to 505.19 and 50.42 mg/Kg, while in LCPY35, the highest contents were found for Zn and Cu, 71.13 and 25.6 mg/Kg, with a considerably high amount of Mn (42.33 mg/Kg). Other genotypes with a high content of Fe were LDCP-13 with 484.09, LDCP-25 with 475.12, and M6 with 436.08 mg/K. LCP87 showed the highest content of Zn, with 70.38 mg/Kg, while JSB1 showed the highest Cu content of 25.6 mg/Kg, followed by LCPY35.LCP1 had relatively higher constituents of Zn, Cu, and Mn by 67.42, 25.16 and 41.66 mg/Kg, respectively. B content ranged from 17.33 to 113.08, averaging 32.88 mg/Kg. The best content came from LDCP1 with 113.08 mg/Kg. Essential minerals Fe, Zn, Cu, and Mn had variation from 176.67 to 505.19, 56.42 to 71.13, 22.03 to 25.6, and 31.68 to 50.42 mg/Kg, respectively. Linseed is known to be rich in essential minerals, as reported by Khan *et al*. (2010). There are some good genotypes like LCP-3, LCPY35 and LCP1, which have been found superior for multiple minerals, showing good genetic potential for enhancing linseed's biofortification for essential minerals.

The heavy metals, Cr, Cd, and Pb concentrations ranged from 0.62 to 2.33, 0.13 to 0.46, and 1.06 to 2.07, with mean value of 1.28, 0.205, and 1.51 mg/Kg (Fig. 6). The reported concentrations of heavy metals fell within recommended ranges, indicating that these genotypes are not efficient accumulators of heavy metals. This aligns with observations by Moraghan (1993) and Khan *et al*. (2010).

Author contributions statement

Naresh Kumar & Vijay Kumar: Conceptualization, Data curation, Investigation, Project administration, Resources, Supervision, Visualization, Writing – review & editing

Sanjula Sharma: Formal analysis, Investigation, Methodology, Writing – review & editing

Sandeep Kumar: Data curation, Software, Validation, Writing – original draft

Disclosure of interest

It is hereby declared that there is no conflict of interest between the authors or with any other scientist related to this manuscript.

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