



## Variability in linseed (*Linum usitatissimum*) germplasm collections from peninsular India with special reference to seed traits and fatty acid composition

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### ABSTRACT

Eighty-four accessions of linseed (*Linum usitatissimum* L.) collected from Andhra Pradesh and Maharashtra, were analyzed for seed traits, oil content and fatty acid composition. Field observations on floral and other morphological traits were recorded. Variability was observed in all the agro-morphological and biochemical traits studied. DIVA-GIS software was used to assess the diversity of linseed fatty acid composition for the first time in India. The oil content of the linseed germplasm ranged between 29.4% and 42.6%. IC564681 recorded the highest oil content (42.6%), while the accession IC564591 recorded the least (29.4%). With respect to linolenic acid (omega-3 fatty acid), IC 564631 possessed the maximum (57.1%) and IC564687 the minimum (39.5%). Linseed germplasm with high oleic acid content was identified with IC564627 recording the maximum of 32%. Based on DIVA-GIS diversity analysis for omega-3 profile and test weight of seed, it was inferred that Kohir, Jarasangham, Nayalkal, Shankarampet and Zaheerabad which are sub-units (mandals) of Medak district and Jainoor, Koutala and Bhimni mandals of Adilabad district are potential areas for collection of diverse accessions.

**Key words:** Characterization, DIVA-GIS, Fatty Acid, Germplasm, Linseed

Linseed or flax (*Linum usitatissimum* L.) belongs to the family Linaceae under the genus *Linum*, which is reported to have about 100 species distributed globally. It is under cultivation since ancient times for its fibre, seed and oil and occupies a prominent place among the oilseed crops owing to its various uses and special qualities. The seeds are a rich source of drying oil of edible nature and are the most potent source of omega-3 fatty acid, the desirable cholesterol for healthy living. Given the increasing importance of linseed, an exploration was conducted in parts of Andhra Pradesh and Maharashtra during March 2008 and 84 accessions [Andhra Pradesh (80) and Maharashtra (4)] were collected and characterized for their fatty acid composition. DIVA-GIS is a geographic information system designed to assist the plant

genetic resources (PGR) and biodiversity research communities in elucidating the genetic, ecological and geographic patterns of distribution of crops using locality (points) data (Hijmans *et al.*, 2001). The mapping could be extended to other related information on the material of interest, for example with respect to specific traits. The objective of the present study is to apply DIVA-GIS techniques for assessing the variability in fatty acid composition of linseed germplasm for identification of potential areas and germplasm accessions rich in quality omega-3 fatty acids for conservation.

### MATERIALS AND METHODS

The 84 accessions along with two check varieties (JL 23-10 & RLC 6) were grown in the experimental field at National Bureau of Plant Genetic Resources, Regional Station for preliminary characterization on floral and other morphological traits during post rainy season of 2008–09. The plants were raised in Augmented Block design following the standard package of practices during the crop growing season. Agro-morphological classification for selected traits was made following the DUS guidelines (<http://www.plantauthority.gov.in/draftcropguide.htm>). The characterization data on floral traits, viz flower size, colour,

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aestivation, flower venation, stamen and anther colour was recorded at 50% flowering of the germplasm accession. Other morphological traits such as plant height, capsule size, seed colour, size and 1000-seed weight were recorded at maturity. Seed traits such as length, width, thickness were recorded using Mitutoyo digimatic calipers and test weight of linseed accessions were recorded using an electronic balance. The seed materials were subjected to oil analysis and the fatty acid profiles was studied. DIVA-GIS version 5.2 software (Hijmans *et al.*, 2005) was used for mapping the collection sites of linseed germplasm and further analysis were made on point collections using spatial data (seed weight, oil content and omega-3 fatty acid). Geographical coordinates (latitude, longitude and altitude) recorded using the Global Positioning System (GPS) [Garmin 12 GPS] were used for the mapping. Descriptive Statistical analysis and correlation coefficient of seed traits and oil content was carried out using MS-Excel 2003 version. The procedures followed for the extraction of oil and analysis of fatty acid composition is detailed below.

A well mixed linseed kernel (5.0 g) was ground and transferred to an extraction thimble and the top portion was covered with cotton. This packed thimble was placed in the extraction chamber of SER 148 Solvent Extractor (VELP Scientifica, Italy). Around 70 ml of hexane was taken in the extractor and the temperature of the solvent heating block was adjusted to 130°C (recommended set point for hexane). The thimble was soaked in hexane and the solvent was refluxed over a period of 1.0 hr. After 1.0 hr the thimble was lifted from the solvent and the solvent was allowed to pass through the bed of ground seeds for 15 min. This operation ensured washing of the thimble with fresh solvent. Hexane was distilled off to recover linseed oil.

Linseed oil (1.0 g) was taken in a 50 ml round bottom flask and to this 25 ml of 2% sulfuric acid in methanol solution was added and the contents were refluxed over a period of 4.0 hr. At the end of reaction time excess methanol was distilled and the residue was diluted with distilled water (50 ml). The organic phase which was separated from the water was extracted with ethyl acetate (50 ml). The ethyl acetate extract was separated from the water layer and washed with distilled water till free from mineral acid. Ethyl acetate layer was passed through a bed of anhydrous sodium sulphate and concentrated to get the methyl esters (1.0 g).

The fatty acid composition of linseed methyl esters was carried out on a gas chromatograph (Agilent 6890) equipped with a flame ionization detector (FID) on a split injector system. A fused silica capillary column (DB 225, 0.25mm, 30 m × 0.32 mm id) was used for the analysis. Oven temperature was programmed at 160°C for 2 min, increased to 180°C at 6°C/min. held for 2 min. and finally increased to 230°C at 4°C/min. and held for 15 min. The injector and detector temperatures were held at 220 and 250°C respectively. Nitrogen was used as carrier gas at a flow rate of 1 ml/min.

The area percentages were recorded with Agilent chemstation data processing system.

## RESULTS AND DISCUSSION

The germplasm survey in parts of Andhra Pradesh and Maharashtra resulted in the collection of 84 accessions of linseed germplasm from 46 villages spread over four districts (Table 1). No infestation of *Cuscuta* was observed on these 84 accessions. However, Mishra *et al.* (2006) reported relative tolerance of linseed varieties against *Cuscuta campestris*. Agro-morphological characterization and classification of the 84 accessions revealed variation in all the 15 traits studied and in other quantitative biochemical traits, viz oil concentration and fatty acid proportion of palmitic acid (16:0), stearic acid (18:0), oleic acid (18:1), linoleic acid (18:2) and alpha linoleic acid (18:3). Majority of the accessions (50) are medium tall types. Varieties grown for oil production are usually considerably shorter at maturity, while tall types are preferred for fibre. In our study we observed that all the 84 accessions are grown for oil production in the surveyed regions. Observations on seed traits and oil concentration and fatty acid composition diversity of linseed germplasm have been reported earlier (Diederichsen 2001, 2007, Diederichsen and Philip 2008; Madhu Vajpey *et al.* 2008, Singh *et al.* 2009, Awasthi *et al.* 2011). Majority of the linseed germplasm possessed medium sized flowers (15–20 mm) in the present study, which is in contrast to many exotic fibre flax varieties studied earlier by Bhatia *et al.* (2009). As the dietary significance of omega-3 has gained increasing importance in recent years, it is imperative to identify omega-3 rich lines of linseed germplasm and the present investigation led to the identification of three accessions, viz IC564605, IC564582, and IC564631 with 56.4%, 56.8% and 57.1% linolenic acid content respectively.

Three accessions (IC564667, IC564684 and IC564685) were found promising as dwarf types when compared to the best check variety (JL 23-10). Petal colour varied for shades of blue and violet. While oil types usually have bright blue or pale blue or white flowers, flax types favoured for fibre usually have white flowers. Stamen colour was violet in one accession (IC564603) while all others were colourless/white (Table 1).

Descriptive statistical analysis for seed traits including oil content and fatty acids are provided for the germplasm and check varieties in Table 2. The mean values recorded for seed length, width, thickness and test weight are 5.1mm, 2.7mm, 1.1mm and 7.5 g respectively. The oil content and fatty acid profile of all the collected linseed accessions exhibited wide variability. Fatty acid profiles of linseed germplasm exhibited variation in saturated and unsaturated fatty acid composition. The oil content of the linseed germplasm ranged between 29.4 and 42.6%. Thirty-eight accessions of the collected linseed germplasm possessed more than 38% oil content than the best check variety (J 23-10)

Table 1 Flax germplasm collections made in peninsular India and characterized for agro-morphological traits

State	District (acc. collected)	Mandal	Village	Geographical coordinate	
Andhra Pradesh	Adilabad (24)	10	19	19°10' – 19°33' N Lat.	77°56' – 79°03' E Long.
	Medak (53)	8	21	17°35' – 19°13' N Lat.	77°32' – 78°55' E Long.
	Nizamabad (3)	3	3	18°11' – 18°39' N Lat.	77°52' – 78°00' E Long.
Maharashtra	Nanded (4)	2	3	18°53' – 19°37' N Lat.	77°50' – 78°15' E Long.
<i>Phenomorphic trait</i>	<i>Descriptor state – Accessions (no.)</i>			<i>Check-I</i>	<i>Check-II</i>
Plant height	40–50 (cm)- 10; 50–60 (cm)- 50; > 60 (cm)- 24			JL 23-10	RLC 6
Flower shape	Funnel- 82; Disk- 2			Funnel	Funnel
Flower diameter	Small (<15 mm)- 14; Medium (15–20 mm)- 68; Large (>20 mm)- 2			Medium	Medium
Petal colour	Tinge blue- 2; Lilac- 1; Blue- 1; Violet blue- 80			Light violet blue	Light violet blue
Size of corolla	Small- 14; Medium- 69 ; Large- 1			Medium	Medium
Petal aestivation	Twisted-11; Semi twisted-43; Valvate-30			Semi twisted	Semi twisted
Stamen filament colour	Colorless-83; Violet-1			Colourless	Colourless
Anther colour	Cream-1; Grey-1; Blue-82			Blue	Blue
Capsule size	Small (<7.5 mm) – 61; Medium (7.5–8.5 mm) -18; Bold (>8.5 mm)-5			Small	Medium
Capsule tip shape	Pointed – 3; Blunt - 81			Blunt	Blunt
Seed colour	Light brown – 72; Brown -12			Light brown	Light brown

Table 2 Seed and fatty acid characterization of flax germplasm

Seed trait	Minimum	Maximum	Standard deviation	CV%	Check I JL 23-10	Check II RLC 6
Length (mm)	4.6	5.4	0.1	2.0	4.40	4.63
Width (mm)	2.3	8.6	0.7	25.9	2.34	2.56
Thickness (mm)	0.9	1.3	0.1	9.1	1.04	1.12
1000-seed Weight (g)	5.7	8.9	0.6	8.0	5.8	6.3
Oil content (%)	29.4	42.6	2.7	7.1	38.0	37.3
Palmitic acid (%)	5.3	7.6	0.6	9.3	6.6	6.6
Stearic acid (%)	5.8	8.5	0.7	9.6	8.8	8.2
Oleic acid (%)	18.1	32.0	2.7	11.9	29.7	28.1
Linoleic acid (%)	10.9	15.2	0.8	6.6	13.5	12.8
Linolenic acid (%)	39.5	57.1	3.7	7.2	40.8	44.0
<i>Promising lines identified for various traits</i>						
Oil content (>41%)	IC 564636 (41.2%); IC 564687 (41.6%); IC 564645 (41.7%); IC 564681 (42.6%)					
Omega-3 content (> 56%)	IC 564618 (56.1%); IC 564605 (56.4%); IC 564582 (56.8%); IC 564631 (57.1%)					
Oleic acid (>30%)	IC 564624 (30.3%); IC 564627 (32.0%)					
Bold capsule (>8.5mm)	IC 564582 (8.8mm); IC 564583 (8.8mm); IC 564585 (8.8mm); IC 564597 (9.1mm); IC 564612 (9.3mm)					
Test weight (>8.5 g)	IC564669 (8.6g); IC564676 (8.6g); IC564678 (8.7g); IC564642 (8.9g)					

used in the study with the linseed accession IC564681 recorded the highest oil content (42.6%) and accession IC564591 recording the lowest (29.4%) among the germplasm collected. With respect to linolenic acid (omega-3 fatty acid), IC564631 possessed the maximum (57.1%) and IC564687 the minimum (39.5%). Correlation coefficient values for oil content against seed length, width, thickness and 1000 seed

weight were 0.08, –0.07, –0.08 and 0.20 respectively (at  $P = 0.05$ ). This indicates that positive correlation exists between oil content and seed length and test weight. However, negative correlation was observed for seed width and thickness. Test weight has direct influence on oil content in the studied linseed germplasm. Some of the promising lines identified for the traits, viz oil content, omega-3, oleic acid, bold capsule and

test weight are also provided in Table 2. Three accessions, viz IC564605, IC564582, and IC564631 are identified to possess 56.4%, 56.8% and 57.1% linolenic acid (omega-3) content respectively.

Grid maps were generated to study the diversity analysis for oil content, Omega-3 and seed test weight of the collected linseed accessions. The geographical coordinates of the collection sites of linseed germplasm are used for the DIVA-GIS analyses for diversity in oil content, omega-3 and seed weight. High Shannon diversity indices (2.7 – 4.0) for oil content were observed for the collections made from Medak and NE Adilabad regions of South India. However, the high Shannon diversity index (2.97–4.0) for omega-3 content observed from Adilabad region. Further, the diversity indices (1.97–3.0) obtained from the DIVA-GIS analysis revealed that collections made in Medak and Adilabad regions are highly diverse for seed weight also. The DIVA-GIS technique has been successfully adapted earlier by researchers in India for the diversity mapping and analysis of germplasm collections (Sivaraj *et al.* 2010, Babu Abraham *et al.* 2010, Sunil *et al.* 2008, Varaprasad *et al.* 2008, Utpala Parthasarathy *et al.* 2006). Based on DIVA-GIS analysis for oil content, fatty acid profile and test weight, it can be inferred that, Kohir, Jarasangham, Nayalkal, Shankarampet and Zaheerabad sub-units (*mandals*) of Medak district and Jainoor, Koutala and Bhimni *mandals* of Adilabad district are potential areas for collection of diverse accessions. The promising lines identified for various traits can be effectively used in various linseed crop improvement programmes.

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