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Biochemical studies of linseed (*Linum usitatissimum* L.) genotypes

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Abstract
Flaxseed (*Linum usitatissimum* L.) is an oilseed used in industrial and natural health products. It accumulates many biologically active compounds having strong phytochemicals and antioxidant properties. This study aims to provide an entire portfolio of bioactive compounds present in five different genotypes of flaxseed. Biochemical diversity among these five different types of genotypes with respect to total seed protein, total phenol and total zinc were investigated. According to results total four scorable protein bands were recorded among each linseed genotype by using SDS-PAGE. The dendrogram produced from linseed genotypes show two main clusters and joined together at 0.40 genetic distance level. The second cluster was again divided into two sub-clusters which were joined at around 0.46 genetic distance level. The average similarity percentage was 40%. In sub cluster shows 74% similarity and in second sub cluster two genotypes shows 100% similarity. The highest Phenol content of 0.25 mg/ml was recorded in variety R-II 160-119 Kota Barani (ALSI-4) whereas R-II 160-108 LMS 215-88 recorded lowest content of 0.14 mg/ml. with an average of 0.2 mg/ml. Zinc content was assessed by DTZ method. LMS 215-88 and RLC-4 showed high zinc content, PKDL-166 showed medium zinc content, while ALSI-4 and RMLS-11 appears to be low in zinc content.

Keywords: Biochemical, linseed, natural, *Linum usitatissimum* L.

Introduction
The relatively high protein content of flaxseed is of interest for reduction of hypertension and heart diseases, because plant proteins with other components tend to reduce serum cholesterol (Oomah, 2001; Oomah *et al*., 2006) [9]. Beyond the primary importance of flaxseed proteins as a source of nutrients and their status as functional foods, the need has surfaced for the study of biological properties of flaxseed proteins due to their desirable amino acid profile (Oomah, 2001) [9]. The modification of flaxseed proteins for specific applications is needed as a potential source of bioactive peptides. These peptides may already be present in flaxseed proteins as natural components or may be released after enzymatic hydrolysis. Flaxseed has been reported to contain albumin, globulin, glutelin and prolamin, the globulin being the major seed protein fraction (Oomah and Mazza, 1993) [10]. However, there is still limited information on identification, characterization and molecular and biological properties of the various flaxseed protein fractions. The utilization of whole flaxseed or its components as diet for human and animal feed has been established for a long time. Introduction of whole flaxseed or defatted flax meal in foods due to their potential nutritional and health benefits that have been reported; flaxseed consumption for its medical properties has also reported (Oomah, 2001) [9]. Use of flaxseed protein in combination with other components such as dietary fiber, phenolic compounds (isoflavones, lignans), glucosinolates and phytic acid might be useful in prevention and treatment of many diseases (Oomah, 2001) [9].
The total proteins in flaxseed represent about 20-30% of the seed meal which makes it as a good source of proteins (Sammour, 1999) [12]. Flaxseed proteins have similar nitrogen extractability at varying pH and ionic strength with other oilseed sources of proteins (Oomah and Mazza, 1993) [10]. Previous research on flaxseed protein molecular structure has indicated that flaxseed contains mixed or heterogeneous proteins comprising different protein fractions (Sammour *et al*., 1994) [13]. Flaxseed proteins are similar to other oilseed proteins and have been classified based on their solubility in a series of aqueous and non-aqueous solvents into different protein classes based on the Osborne classification of proteins (Osborne, 1924). The flaxseed proteins classes include globulins or linins, albumins or conlinins, glutelins and prolamins (Anonymous, 1962; Sammour *et al*., 1994) [1, 13]. This study focused on the analysis of bioactive features of five different genotypes of flaxseed.

In this concern, we elucidated the biochemical diversity with respect to protein, phenol and zinc.

Material and Methods

The linseed genotypes for present study were obtained from 'Oilseed Research Station, Latur (M. S.) toward fulfillment of diversity study at biochemical level among these cultivars.

Table 1: List of linseed accessions used in the present investigations

Sr. No.	Name of genotype	Source
1	R-II 160-119 Kota Barani (ALSI-4)	Latur
2	R-II 160-11 (PKDL-166)	Latur
3	R-II 160-108 (LMS 215-88)	Latur
4	R-II 160-116 (RMLS_II)	Latur
5	R-II (RLC – 4)	Latur

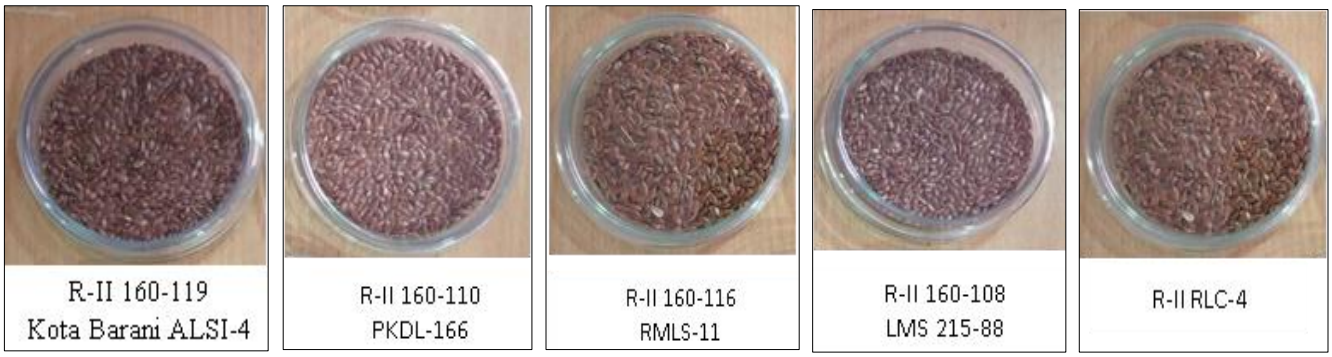

Fig 1: Linseed germplasm variability

Linseed germplasm variability

Methodology

Isolation of total seed protein

Total seed protein content in Linseed grain was estimated by method of Sardar *et al*. 2012 [14] from linseed flour of de-hulled mature grains. 3 mg of flour and 100 µL of extraction buffer (0.055 M Tris-HCl pH 6.8, 2.3% SDS, 5% β-mercaptoethanol, 10% Glycerol and 0.1% Bromo phenol blue) was added and allowed to boil for 10 min. The freshly prepared protein extraction solution was added in each tube. The content of tubes was thoroughly mixed with help of cyclo-mixer. Tubes were incubated at -20ºC for 1-2 hours and after incubation, it was centrifuged at 10,000 rpm for 5 min at 4ºC. The supernatant was collected and kept in water bath at 95^0 C for 5 min by mixing with dye and this denatured protein used for further SDS-protein profiling. Protein profiling of extracted samples were analysed through SDS-PAGE (Laemmli, 1970) [5] in a discontinuous buffer system with a 5% stacking gel and 15% acylamide gel. The extracted proteins (10µL) were loaded along with a protein marker and the gel was subjected to electrophoresis at 30mA using Tris-glycine buffer for 3 h. The banding patterns of protein were considered as "finger print" of the genotype.

Isolation of total phenol content

Total phenolic content in Linseed grain was estimated by method of Malick and Singh (1960) [8]. 1g of grain sample was grinded in 10 times volume of 80% ethanol and centrifuge at 10,000 rpm for 20 min. Supernatant was preserved and extracted with 5 times volume of 80% ethanol, and the supernatant was dried. The residue was dissolved in known volume of distilled water, and 2ml of aliquots were pipette out, and volume was raised to the volume of 3ml and 0.5ml of folin-ciocalteu reagent was added. After 3 minutes 2ml of 20% sodium carbonate was added and placed in boiling water for 1 minute and then cooled. Absorbance was measured at 650nm against a blank. The different concentrations of Catechol was used for standard curve preparation and from that standard curve, the concentration of all samples was calculated.

Isolation of total zinc content

Total zinc content in Linseed grain was estimated by method of Shobhana *et al.*, 2013. The DTZ staining was standardized to assess the concentration of zinc in flour sample by using DTZ and methanol reagent. The whole seed flour samples were obtained by grinding dry seed in mortal and pestle. 1gm of flour sample was placed in borosilicate glass test tube and 5ml of freshly prepared DTZ solution added to test tube and mixed thoroughly by vigorous shaking using vortex apparatus for 2 minutes. The samples were incubated for 15 minutes for maximum color development. The red color produce by DTZ staining was stable at least for 2 hrs. Fading of colour was evident only after 3 hrs. Intensity of color was visually scored on a 1-3 scale, where 1 = less intense color, 2 = medium red colour, 3 = more intense red color which depict high, medium and low concentration of zinc.

Results

Study of total seed protein

Total four scorable protein bands were recorded among each linseed genotype by using SDS-PAGE. Protein band conformed on stacking gel (5ml) to visualized band by using 0.05% Bromophenol Blue in protein assembly.

Table 2: Similarity matrix values among different linseed genotypes

	Kota Barani ALSI-4	PKDL-166	LMS 215-88	RMLS-11	RLC-4
Kota Barani ALSI-4	1.00				
PKDL-166	0.40	1.00			
LMS 215-88	0.40	0.40	1.00		
RMLS-11	0.46	0.74	0.46	1.00	
RLC-4	0.83	074	040	0.46	1.00

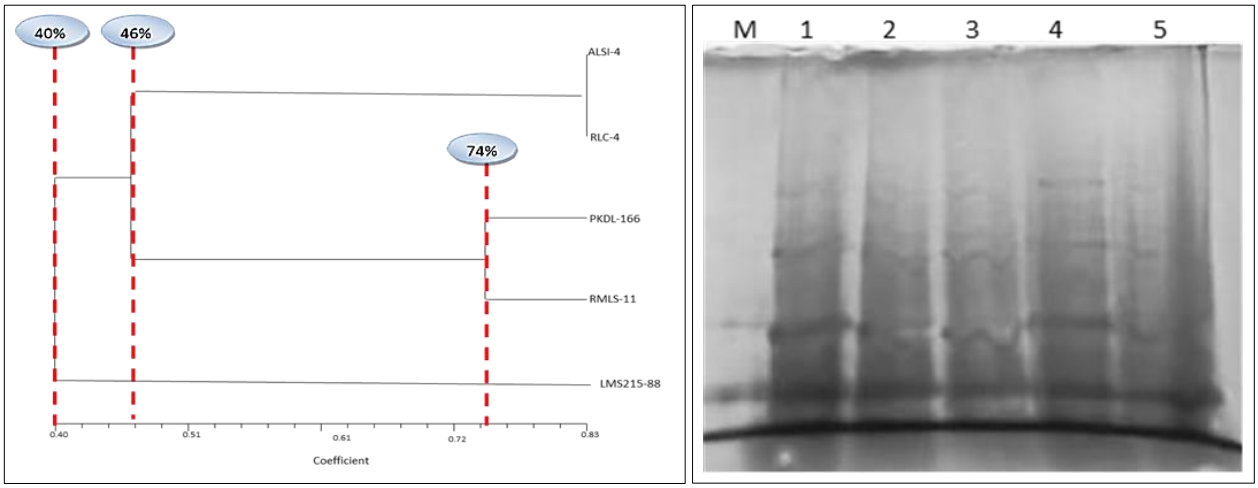

Fig 2: Similarity matrix and protein profiling of linseed genotypes
1) Kota Barani ALSI-4, 2) PKDL-166, 3) LMS 215-88, 4) RMLS-11, 5) RLC-4

The dendrogram produced from linseed genotypes show two main clusters. The first cluster consists of one genotype LMS-215-88. The second cluster consists of four genotypes *viz.* RMLS-11, PKDL-166, RLC-4 and ALSI-4.These two groups were joined together at 0.40 genetic distance level. The second cluster was again divided into two sub-clusters. In each sub clusters there are two genotypes in sub-cluster one (RMLS-11 and PKDL-166) and sub-cluster 2 (RLC-4 and ALSI-4) which were joined at around 0.46 genetic distance level. The average similarity percentage was 40%. In sub cluster one two genotypes *viz.* RMLS-11 and PKDL-166 shows 74% similarity and in second sub cluster *viz.* RLC-4 and ALSI-4 shows 100% similarity.

Study of total phenol and zinc

The linseed of upland seeds germplasm accessions were subjected for Nutrient and biochemical characterization for Phenol, and Zinc content. The results are depicted below:

1. Total phenol

Phenol content ranged from 0.14 to 0.25mg/ml. The highest Phenol content of 0.25 mg/ml was recorded in variety R-II 160-119 Kota Barani (ALSI-4) whereas R-II 160-108 LMS 215-88 recorded lowest content of 0.14 mg/ml. with an average of 0.2 mg/ml.

2. Total zinc

Zinc content was assessed by DTZ method. LMS 215-88 and RLC-4 showed high zinc content, PKDL-166 showed medium zinc content, while ALSI-4 and RMLS-11 appears to be low in zinc content.

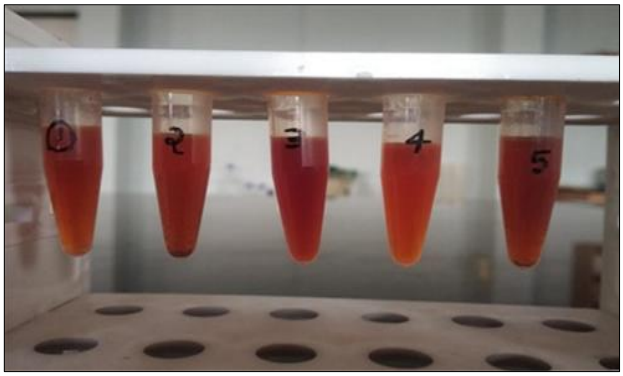

Fig 3: Estimation of zinc content. 1) ALSI-4, 2) PKDL-166, 3 LMS-215-88, 4) RMLS-11, 5) RLC-4

Table 3: Estimation of zinc content

Linseed genotypes	Phenol (mg/ml)	Zinc
R-II 160-119 (ALSI-4)	0.25	Low
R-II 160-110 PKDL-166	0.18	Medium
R-II 160-108 LMS 215-88	0.14	High
R-II 160-116 RMLS-II	0.20	Low
R-II RLC-4	0.24	High

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