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Assessment of genetic diversity and physiochemical analysis of linseed (*Linum usitatissimum*) genotypes

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Abstract
The flax (*Linum usitatissimum* L.) is a diploid (2n = 30) autogamous (primarily self-pollinating) and an annual crop plant also called common flax or linseed. Linseed is an important oilseed crop, which belongs to the family Linaceae and order Geraniale having 14 genera and over 200 species. Almost all the species are annual herbs and some are shrubs. *Linumu sitatissimum* L. is the only species of the family Linaceae with non-dehiscent or semi-dehiscent capsules suitable for modern cultivation.
The genetic diversity was investigated in 5 linseed lines which were collected from Oilseed Research Station, Latur. Six RAPD primers were utilized for genetic diversity analysis. In physiochemical characterization seed dimensions and biochemical properties of linseed *viz*. moisture, ash etc were estimated. The dendrogram produced from linseed genotypes show two main clusters. The first cluster consists of NL-356, RLC-156, Padmini, LCK-1625. The second cluster consists of sample LCK-2627.These two groups were joined together at 0.43 genetic distance level. The first cluster was again divided into sub-cluster 1(NL-356, RLC-156, Padmini) and sub-cluster 2 (LCK-1625) which were joined at around 0.92, 0.66 genetic distance level respectively. The average similarity percentage was 63% and the average polymorphic percentage observed with RAPD primers OPG-02, OPG-05, OPI-02 was 82.2%

Keywords: *Linum usitatissimum*, genetic diversity, linseed

Introduction
Flaxseed has been used as a precious nutritional food grain and traditional medicine in human diets for thousands of years and more recently, it has been used as a source of nutraceuticals and identified as a functional food, whose benefits on health are generally attributed to high concentration of linolenic acid (omega- 3) and linoleic (omega-6) fatty acids, which are essential polyunsaturated fatty acids (PUFA) that cannot be synthesized in the organism and must be ingested through food. It also improves cardiac and bone health, when consumed by humans and animals. Due to consumer demand the market for essential fatty acids has grown rapidly in the past few years.
As consumers are becoming more health conscious and are looking for more nutritious foods. Manufacturers, conscious of the needs of the consumer, are seeking ways to improve the nutritional value of their food offerings.
Only by understanding the science supporting the full benefits of all three fatty acids (ALA, EPA and DHA) and the technology required to deliver a naturally stable functional food, can they deliver the best products to consumers. A molecular marker is a fragment of DNA that is associated with a certain location within the genome. They are useful for varietal identification and evaluation of DNA variation. Different molecular markers including random amplified polymorphic DNA (RAPD), restriction fragment length polymorphism (RFLP), amplified fragment length polymorphism (AFLP) and simple sequence repeat (SSR) have been developed to analyze flax genetic diversity.
The use of molecular markers, like simple sequence repeat (SSR), random amplified polymorphic DNA (RAPD), provides a powerful tool for the analysis of plant genome structure and function. Once the molecular markers have been identified in multiple populations over multiple generations and in multiple environments, the plant breeder can use these data to choose such positive markers for development of breeding populations with desirable traits. Hence, current investigation is to be to undertaken to characterize selected linseed genotypes by using RAPD marker and its quality in the linseed (*Linum usitatissimum* L.) germplasm.

Material and Methods

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

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

Accessions	Pedigree	Source
NL-356	-	Latur
RLC-156	LCK-88062 × R- 552	Latur
LCK-2627	Shikha × sheela	Latur
Padmini	-	Latur
LCK-1625	Subhra × Shikha	Latur

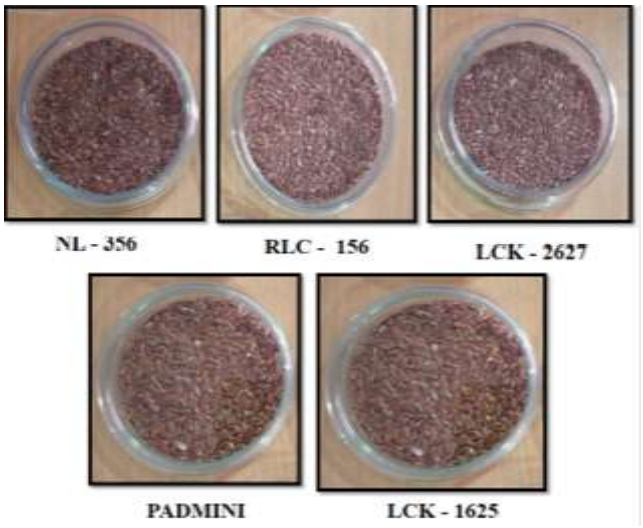


Fig 1: Linseed varieties used in the study

Methodology

Molecular Characterization

Isolation of DNA

High quality genomic DNA was isolated from fresh and young leaves of 5 linseed genotypes. The genomic DNA was extracted from leaf tissue of field grown plants following CTAB (Cetyl Trimethyl Ammonium Bromide) extraction method (Doyle and Doyle, 1987) [8].

Integrity and intactness of DNA samples was checked on 0.8% agarose gel and quantity was measured by recording absorbance ratio A260/280 using Nanophotometer™ (Implen Inc.). Individual samples were then diluted in sterile Milli-Q water to final concentration of 50 ng/µl and stored at 4 °C for further use.

Polymerase Chain Reaction (PCR)

2µl 10× PCR reaction buffer (Jonaki, BRIT), 0.5µl dNTPs, 1µl each RAPD primers, 0.2 µl (5U/µl) Taq DNA polymerase (Jonaki, BRIT), 2µl template DNA (50 ng/µl) and 13.3µl sterile Milli-Q water.

The PCR reaction conditions were, initial denaturation at 94°C for 5 min, 5 cycles of denaturation at 94 °C for 1 min, annealing at 35 °C for 1 min and extension at 72°C for 1 min, followed by 35 cycles of denaturation at 94°C for 1min, annealing at 50°C for 1 min and extension at 72°C for 1 min final extension at 72°C for 5min and reaction was hold at 4 °C. All the PCR products were stored at 4 °C until resolved on 2.0% agarose prepared in 1× TBE buffer containing 0.5µg/ml ethidium bromide (EtBr).

Table 2: List of RAPD primers used in initial screening of linseed genotype

Sr. No	Primer code	Sequence of primer	Number of bases
1.	OPG-02	GGCACTGAGG	10
2.	OPG-05	CTGAGACGGA	10
3.	OPI-02	GGAGGAGAGG	10
4.	OPM-10	TCTGGCGCAC	10
5.	OPM-13	GGTGGTCAAG	10
6.	OPO-03	CTGTTGCTAC	10

Data Analysis

Scoring of bands was done on the basis of presence (scored as '1') and absence (scored as '0') of bands and alleles amplified per primer pair was determined based on the number of clearly visible bands. The binary score data was subjected for statistical analysis to calculate the Jaccard's Similarity coefficient (JS). Dendrogram was constructed based on the Un-weighted Pair-wise Group Method of Arithmetic means (UPGMA) using NTSYS-pc© (Version 2.02i). The polymorphic percentage of the obtained bands was calculated by using following formula

Polymorphism % = (Number of polymorphic bands/Total bands) X 100

Physicochemical characteristics of linseed

Physical characteristics of linseed

1. **Seed dimensions**: Ten seeds were randomly selected from the sample. The three linear dimensions, namely, length, width and thickness of each of the 10seeds were measured with a digital Vernier calliper with least count reading 0.01mm and its average was recorded.
2. **Biochemical Properties of linseed:** Biochemical constituent's *viz.* moisture, ash etc were estimated by following standard procedure. Moisture content in seed sample of linseed varieties/genotypes was determined by following the oven drying method. The 5 g sample was taken in pre-weighed moisture box, dried in oven at 105°C for 5 h and transferred to desiccators for cooling for ½ h. After cooling the sample was weighed. The procedure was repeated until a constant weight was obtained.

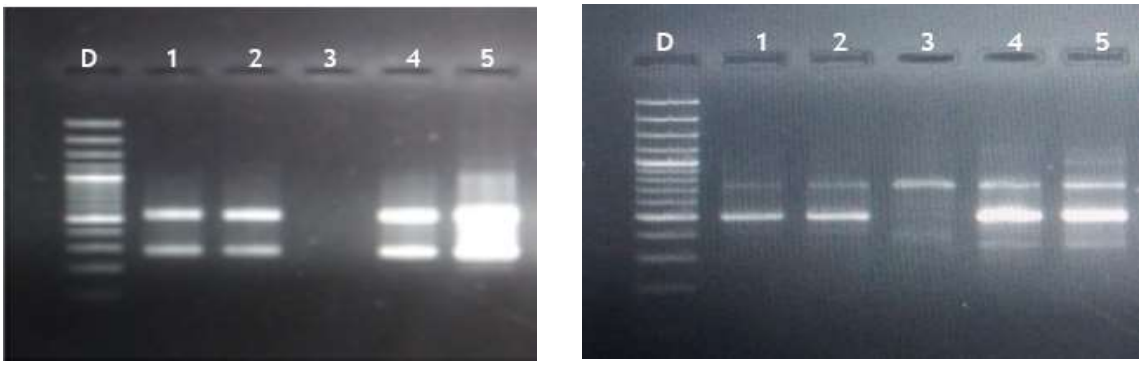
$$\text{Moisture (\%)} = \frac{W1 - W2}{W1 - W} X\ 100$$

Where,
W1= Weight (g) of the dish with the material before drying
W2= Weight (g) of the dish with the material after drying
W = Weight (g) of the empty dish

Result

DNA Polymorphism Study by Molecular Marker

In an attempt to characterize these linseed accessions, a set of six RAPD primers belonging to Operon© series from OPG, OPI, OPM and OPO were used for initial screening to amplify gDNAs from these five linseed genotypes. Out of six decamers, five were found to amplify all genotypes, of which only one *viz*., OPG-02, was revealed to be highly polymorphic and reproducible; while remaining (four) were very less consistent.

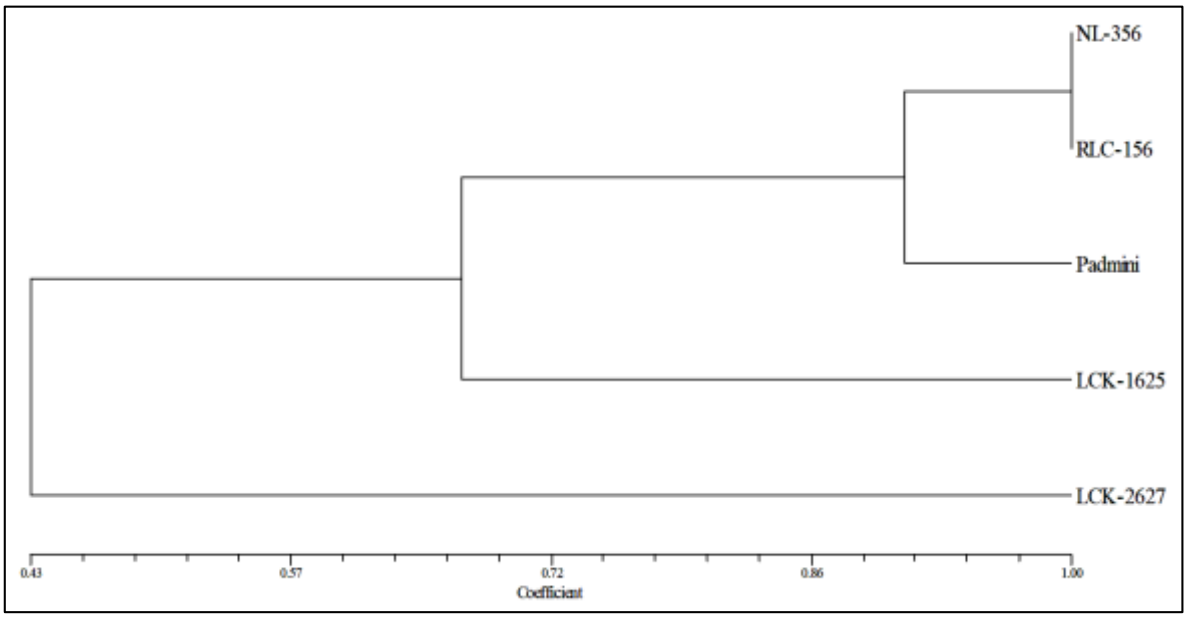


1. NL-356
2. RLC-156
3. LCK-2627
4. Padmini 5. LCK-1625

Fig 2: RAPD profile of linseed genotypes obtained with primer OPG-05 and OPI-02

Table 3: Results for each polymorphic primer

Sr. No	RAPD Primers	No. of polymorphic bands	Total no. of bands	%polymorphism P/T × 100
1.	OPG - 02	4	5	80%
2.	OPG - 05	3	3	100%
3.	OPI - 02	2	3	66.6%
4.	OPM-10	3	3	100%
5.	OPM-13	4	5	80%
6.	OPO-03	4	5	80%



Dendrogram showing genetic diversity in elite linseed genotypes by RAPD analysis

The dendrogram produced from linseed genotypes show two main clusters. The first cluster consist of NL-356, RLC-156, Padmini, LCK-1625.

The second cluster consist of sample LCK-2627.These two groups were joined together at 0.43 genetic distance level. The first cluster was again divided into sub-cluster 1(NL-356, RLC-156, Padmini) and sub-cluster 2 (LCK-1625) which were joined at around 0.92, 0.66 genetic distance level respectively. The average similarity percentage was 63% and the average polymorphic percentage observed with RAPD primers OPG-02, OPG-05, OPI-02 was 82.2%

Table 4: Similarity matrix values among different linseed genotypes

	NL-356	RLC-156	LCK-2627	Padmini	LCK-1625
NL-356	1.00				
RLC-156	1.00	1.00			
LCK-2627	0.54	0.54	1.00		
Padmini	0.90	0.90	0.45	1.00	
LCK-1625	0.63	0.63	0.18	0.72	1.00

Physiochemical characteristics of linseed genotypes

Seed dimensions: The mean value seed dimensions of five linseed genotypes are as follows, length (5.1066 mm), breadth (2.382 mm), thickness (0.914 mm).

Moisture Content: The following table represents the moisture content in five linseed genotypes.

Table 5: Moisture Content in five linseed genotypes

Sr. No	Linseed Genotypes	Moisture Content
1.	NL - 356	6.82
2.	RLC – 156	6.52
3.	LCK – 2627	7.26
4.	Padmini	7.16
5.	LCK – 1625	6.91

Conclusion

The basis of crop improvement is selection operating on genetic variability, which provides adaptability to variables like environment, pest and disease incidences and market. Genetic diversity is, therefore, essential for crop improvement. The enforcement of any plant breeding programme is largely based on the genetics of quantitative characters associated with yield, quality traits, nutritional quality attributes, pest-disease resistance or any economic trait concerned to the breeder. Such quantitative characters require sound understanding of their genetic architecture to make breeding methodology a success.

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