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Evaluation of linseed (*Linum usitatissimum* L.) germplasm lines for drought tolerance using morphological and molecular markers

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Abstract
Linseed (*Linum usitatissimum* L.) is a vital oilseed and fiber crop grown for its seed still as fiber which is employed for the manufacture of linen. The oil is edible and even has industrial use like preparation of paints, varnishes, printing ink, soap, oil, cloth, leather, and waterproof fabrics. In the present study the investigation done on linseed morphology and molecular screening for drought tolerance. Under normal conditions among all genotypes earliest flowering was observed in DEEPIKA (41 days) and under stress condition KARTIKA (41 days) and DEEPIKA (41 Days). Under normal conditions among all lines, the tallest plant was observed in normal condition is RLC-172(55.80 cm) and under stress condition is RLC-172 (56.10 cm). Under normal conditions the maximum number of fertile capsules observed in RLC-161(24) and under stress condition in RLC-143 and RLC- 92(15). The maximum 1000 seed weight observed under normal conditions in RLC- 167(6.80 gm) and under stressed conditions in RLC-172 (6.15 gm). The stressed linseed genotypes showed rise in proline accumulation over unstressed control. Under normal conditions the highest proline content observed in KIRAN (6.62 µmol/g) and under stress condition in KIRAN (18.89 µmol/g). The maximum root length observed in normal condition is KARTIKA (8.6 cm) and under stressed condition observed in KARTIKA (8.96 cm).
The present ISSR primers investigation revealed that the mean polymorphic information content (PIC) score for each primer ranged from 0.34 to 0.71 with a mean of 0.55. The least relevant primer was noticed to be ISSR primer UBC-807 of PIC value of 0.34, while the maximum informative marker UBC-825 had a PIC value of 0.71. The similarity matrix of 18 linseed lines varies from 0.20(IA-32-DEEPIKA) to 0.92(RLC-167-RLC-148). Thus, most similar genotypes observed were RLC-167 and RLC-148. The most dissimilar genotypes observed were IA-32 and DEEPIKA were noticed. The dissimilarity matrix of 18 linseed lines varies from 0.08(RLC-167- RLC-148) to 0.77 (IA-32-DEEPIKA). Thus, most dissimilar genotypes observed were IA-32-DEEPIKA. The most dissimilar genotypes observed were RLC-167-RLC-148 were noticed. The present SSR primers investigation revealed that the mean polymorphic information content (PIC) score for each primer ranged from 0.15 to 0.84. The least relevant primer was noticed to be SSR primer LU-9 of PIC value of 0.15, while the maximum informative marker LU-3 had a PIC value of 0.84. Thus, most similar genotypes observed were RLC-92 and RLC-153 and RLC-133. The most dissimilar genotypes observed were IA-32 and KIRAN were noticed. The dissimilarity matrix of 18 linseed lines varies from 0.00(RLC-92-RLC-153) to 0.77 (IA-32-KIRAN). Thus, most dissimilar genotypes observed were IA-32-KIRAN. The most dissimilar genotypes observed were RLC-92 and RLC-153 were noticed.

Keywords: Linseed, drought, ISSR, SSR, PIC, proline

Introduction
Linseed (*Linum usitatissimum* L.) (2n = 30) belong to the family "Linaceae" and genus "Linum" is widely cultivated India during Rabi season. Linseed is a vital oilseed and fiber crop grown for its seed still as fiber which is employed for the manufacture of linen. The oil is edible and even has industrial use like preparation of paints, varnishes, printing ink, soap, oil, cloth, leather, and waterproof fabrics. About 20% of the full oil produced is employed in domestic purpose and about 80% of the flaxseed oil is employed for industrial purposes [24]. Flaxseed oil, rich in omega–3 and omega-6 fatty acids which improve human system [20].

The rich in dietary fiber may help to scale back the chance of heart condition, diabetes, cancer, obesity, and inflammation [32]. India ranks third in linseed production area after Canada and Kazakhstan. After mustard, linseed is second most vital Rabi crop grown for oil purpose in respect of area further as production. Despite, the economic importance of this crop, it's mostly grown in arid and semi-arid regions under abiotic stress mainly because of drought that affects its yield and stability.

Drought is a worldwide phenomenon that reduces the productivity of all field crops. In comparison, flax is susceptible to drought stress and the resulting yield loss. Drought research has received significant attention in model and field crops due to the vagaries of climate change, erratic monsoon, and global warming. While priority crops such as rice, wheat, corn, and canola have seen significant advances in drought research [1, 17], the industrially important crop flax/linseed has seen little progress (*Linum usitatissimu*) [16]. Transcriptome data for legumes such as pigeonpea (*Cajanus cajan*) and its wild relative C. scaraboides have recently become available for translational research [35]. Drought is one among the prevailing environmental conditions that induce adverse effects on plant growth. The role of drought stress is more for limitation the expansion and productivity of the crop than other stresses, especially that the recent climate changes increase the chance of this situation. Linseed is drought tolerant [33]. However, genotype environment interactions are shown to be high for linseed [10], and yields vary considerably between seasons, reckoning on location and weather. The purpose of this study was to analyze the consequences of water stress on the growth of linseed seedlings, including effects on accumulations of proline, relative water content. Understanding how linseed manages water stress is very important for the reclamation of drought prone soils and crop production, and possibly also to find water stress tolerant genes and hence to develop drought tolerant crop. Based on above observations and considering in importance studies on "Evaluation of Linseed (*Linum usitatissimum* L.) germplasm lines for Drought Tolerance" is being proposed with objectives to evaluate linseed germplasm lines for drought tolerance on the basis of morphological and molecular chrecterization.

Materials and Methods

Linseed genotypes were obtained for the current study through MTA from the germplasm kept at the Indira Gandhi Agricultural University in Raipur (C. G.). During the current study, selected 18 germplasm lines were used to evaluate for drought tolerance.

List of linseed germ plasms used in the present investigation

Sr. No.	Entries	Sr. No.	Entries	Sr. No.	Entries	Sr. No.	Entries
1	R-7	6	Deepika	11	RLC-148	16	RLC-167
2	R-17	7	IA-32	12	RLC-153	17	RLC-171
3	KIRAN	8	RLC-92	13	RLC-161	18	RLC-172
4	R-552	9	RLC-133	14	RLC-164		
5	Kartika	10	RLC-143	15	RLC-165		

Fig 1: Seed Samples of 18 Linseed Germplasms

Methodology

Selected germplasm lines grown in pots with two replications and two different environmental conditions *viz*., 1. Water stress (WS) and 2. Preventive irrigated conditions (PI). All normal packages of practices were followed to raise a good crop. The drought condition was imposed by stopping irrigation after 50% flowering stage. Morphological characters were score by using DUS guidelines by laid out in a Randomized Block Design (RBD) in two replications. For pot experiment genotypes were grown in pots by hand dibbling. Agronomical package of practices was followed to raise the good crop. Observations were recorded on five randomly selected plants in each entry from each replication.

Fig 2: Pot Experiment for Drought Tolerance Studies

Observations were recorded on five randomly selected plants in each germplasm line from each replication on standard agro-morphological characters. Leaf rolling score were taken by following method given by Pandey and Shukla [37]. Relative water content was calculated by follow stander procedure. Proline content (PC) was assessed using Bates *et al*. 1973's acid-ninhydrin technique. Stander procedure was followed for statistical analysis to calculate analysis of variance. Genomic DNA was extracted from the leaves of linseed genotypes following the Cetyltrimethyl Ammonium Bromide (CTAB) method with some modifications as described by Doyle and Doyle [11].

Screening with primers linked to genetic diversity in linseed lines

Linseed specific 20 Primers were preselected for the present study. In which 10 SSR and 10 random ISSR were obtained from the available literature. These primers were custom oligo-synthesized and dissolved in a suitable volume of sterile distilled water to get desired concentration of 10picomole/µl of primer. It was thoroughly mixed by repeated vertexing and incubation at room temperature for 1hr. The details of 20primers and their sequences are presented in (Table No. 1 and Table No. 2). The amplification reaction mixture was prepared in sterile 0.2ml thin- walled flat-capped PCR tubes, containing the following

components (Table No. 3). The total volume of each reaction mixture was 20-25 µl.

Table 1: ISSR primers pairs used for screening of the linseed lines

Sr. No.	Primers	Sequences	No. of Bases
1	UBC-807	AGAGAGAGAGAGAGAGT	17
2	UBC-810	GAGAGAGAGAGAGAGAT	17
3	UBC-815	CTCTCTCTCTCTCTCTG	17
4	UBC-818	CACACACACACACACAG	17
5	UBC-819	GTGTGTGTGTGTGTGTA	17
6	UBC-825	ACACACACACACACACT	17
7	UBC-840	GAGAGAGAGAGAGAGAYT	18
8	UBC-841	GAGAGAGAGAGAGAGACC	18
9	UBC-850	GTGTGTGTGTGTGTGTYC	18
10	UBC-855	ACACACACACACACACYT	18

Table 2: SSR primer used for screening of the linseed genotypes

Sr. No	Primer's	Sequences	No. of bases
1	LU-1	F: TCATTCATCTCCTTCCACTAAAA	23
		R: TTGAAAGCCCTAGTAGACACCA	22
2	LU-2	F: TCCGGACCCTTTCAATATCA	20
		R: AACTACCGCCGGTGATGA	18
3	LU-3	F: GCTCGTGATCTCCTTCATCC	20
		R: AAAACCACGTCCAGATGCTC	20
4	LU-4	F: TTATTTCCGGACCCTTTCAA	20
		R: AAACTACCGCCGGTGATGAT	20
5	LU-5	F: GTCACTGGGTGTGTGTTTGC	20
		R: AGCAGAAGAAGATGGCGAAA	20
6	LU-6	F: CCCCATTTCTACCATCTCCTT	21
		R: CAACAGCGGAACTGATGAAA	20
7	LU-7	F: CATCCAACAAAGGGTGGTG	19
		R:GGAACAAAGGGTAGCCATGA	20
8	LU-8	F: TCCCGTAATATTCTATGTTCTTCC	24
		R: TGAGTTGGACCTTACAAGACTCA	23
9	LU-9	F: TTGCGTGATTATCTGCTTCG	20
		R: ATGGCAGGTTCTGCTGTTTC	20
10	LU-10	F: GCCTAAAGCTGATGCGTTTC	20
		R: TGTCAGGCTCCTTCTTTTGC	20

PCR protocol for ISSR and SSR analysis

PCR amplification reactions were set in a final 25µl reaction volume. A master mix in sterile double distilled water having all the compounds in required quantities was prepared.

PCR amplification program

Polymerase chain reactions of ISSR and SSR were performed in Eppendorf® (AG- 22331, Hamburg-5345) or Biometra© gradient thermal cycler using the following cyclic parameters and Bands were scored as present (+/1) or absent (-/0).

Table 3: PCR cyclic parameters for ISSR SSR primer amplification

Step	Temp. (^{0}C)	Duration	Cycles	Function
1	94	5 min	1	Initial denaturation
2	94	1 min	up to 40	Denaturation
3	53 (For ISSR)	1 min		Annealing
	58-60 (For SSR)	1 min		Annealing
4	72	2 min		Extension
5	72	10 min	1	Final Extension of target Molecules
6	04	Hold	1	Storage

Results and Discussions

Mean performance of agro-morphological traits

Days to first flowering under normal conditions ranges between 41 to 46 days with a mean of 43.83. The earliest flowering line is DEEPIKA (41 days) and the late flowering observed in RLC-143, RLC-148 and RLC-172 (46 days). Under stressed condition, ranges between 41 to 47 days with an average of 43.61. The earliest flowering variety is KARTIKA and DEEPIKA (41 days) and the late flowering observed in RLC-172(47 days). Plant height measured with scale in centimeter at the stage of flowering completion. Under normal conditions plant height ranges between 32.50 cm to 55.80 cm with a mean of 43.85 cm. Among all lines, the tallest plant was observed in normal condition is RLC-172 (55.80 cm) and smallest plant height was observed R-7 (32.50 cm) Under stress conditions plant height ranges between 32.30 cm to 56.10 cm with a mean of 44.39 cm. The tallest plant was observed is RLC-172 (56.10 cm) and the smallest plant were observed is R- 17 (32.30 cm). Number of capsules per plant under normal conditions from 7 to 34 capsules was observed with overall mean of 18.22. The maximum number of capsules observed in DEEPIKA (34) and minimum number of capsules observed in R-552(7). Under stressed conditions range of variation 4 to 27 number capsules were observed with overall average of 11.05. The maximum number of capsules noticed in DEEPIKA (27) followed by IA-32 (18). DEEPIKA genotype observed maximum capsules in both normal and stressed condition, but in stressed condition 20.59% reduction observed as compared to the normal condition. The highest reduction observed in stressed RLC-161 i.e., 46% as compared to normal condition. DEEPIKA shows best performance under stress condition. Number of Fertile capsules per plant under normal conditions from 4 to 24 was observed for this character with overall mean of 16. The maximum number of fertile capsules observed in RLC-161(24) and minimum number of fertile capsules observed in R-552 (4). Under stressed conditions wide range of variation 3 to 15 was observed with an average of 10.16. The maximum number of fertile capsules observed in RLC-143 minimum number of fertile capsules observed in R-7 and R-552 (3). Grain Yield per Plant under normal conditions from 2.95 to 5.55 gm was observed with an average of 4.03 gm. The maximum grain yields observed in R-552 and RLC-148(5.55 gm) and the minimum grain yields observed in KARTIKA (2.95 gm). Under stressed condition 2.1 to 3.7 gm grain yield per plant observed with an average of 2.97 gm. Maximum grain yield observed in RLC-161 (3.7 gm) and the minimum grain yield observed in KIRAN (2.1 gm). Grain yield in stressed genotypes is low as compared to normal condition grain yield. Drought affects the grain yield of linseed. KARTIKA genotype showing only 11% reductions in grain yield. KIRAN genotype in stressed condition showed 51.09% reduction as compared to normal yield. 1000 seed weight under normal conditions ranges from 4.25 gm to 6.80 gm was observed with an overall mean of 5.42 gm. The maximum 1000 seed weight observed in RLC-167(6.80 gm) and the minimum 1000 seed weight observed in R552(4.25 gm). Under stressed conditions it ranges from 3.52 gm to 6.15 gm with an average of 4.72 gm. The maximum 1000 seed weight observed in RLC- 172(6.15 gm) and the minimum observed in R-17(3.52 gm). 1000 seed weight of drought stressed genotypes is less as compared to the normal genotypes. Seed Length under normal conditions ranges from 0.28 cm to 0.39 cm with an average of 0.31 cm. The highest seed length observed KIRAN (0.39 cm) and the minimum in RLC-164, RLC- 167, RLC-165, R-7(0.28 cm). Under stressed conditions it ranges from 0.25 cm to 0.39 cm with an average of 0.30 cm. The highest length of seed observed in KIRAN (0.39 cm) and the minimum length of seed observed in RLC- 164 and R-7 (0.25 cm). Seed length of normal condition genotypes is more than the stressed genotype seed length. Seed Breadth under the normal conditions varies

from 0.10 mm to 0.16 cm with an average of 0.124 cm. The maximum seed breadth observed in RLC- 148(0.16 cm) and the minimum were observed in R-552, RLC- 92, RLC-133(0.10 cm). Under stressed condition it varies from 0.08 cm to 0.15 cm with an average of 0.107 cm. The maximum seed breadth observed in RLC-148(0.15 cm) and the minimum seed breadth observed in DEEPIKA and RLC-165(0.08 cm). Seed breadth of normal condition genotypes is more than the stressed condition genotype. Seed Length-Breadth Ratio under normal conditions ratio varies from 1.86 to 3.70 with an average of 2.58. The maximum seed length-breadth ratio observed in RLC-92 is 3.70and the minimum were observed in RLC-167 i.e., 1.86. The maximum seed length-breadth ratio observed in DEEPIKA (4) followed and the minimum seed length-breadth ratio observed in drought condition was RLC-148(2.13). Capsule Fertility Percentage under normal conditions ranges from 57.14% to 100% with an average of 86.50%. The maximum fertility was observed in RLC-143, LC-148, RLC-167 and RLC-171(100%) and the minimum capsule fertility observed in R-552(57.14%). Under stress condition the capsule fertility varies from 48.14% to 100% with an average of 72.99%. The maximum capsule fertility observed in RLC-92, RLC-133 and RLC- 165(100%), and the minimum capsule fertility observed in DEEPIKA (48.14%).

Capsule Diameter under normal conditions varies from 0.5 cm to0.95 cm with an average of 0.71 cm. The maximum capsule diameter observed in RLC- 172(0.95 cm) and the minimum capsule diameter observed in KARTIKA (0.50 cm). Under stressed condition the diameter ranges from 0.55 cm to 0.95 cm with an average of 0.69 cm. The maximum capsule diameter observed in RLC-164(0.95 cm) and the minimum capsule diameter observed in KARTIKA (0.55 cm).

Leaf Rolling Score

Leaves curling were recorded for three days continuously under water stress. The level of stress was good enough to discriminate the line seed lines for their reaction to leaf rolling. The following scoring Standard Evaluation System (SES) was used for recording observations on leaf curling. The Scale on all three continuous days in normal condition is zero except on 3rd day of genotypes KARTIKA and RLC-167 showing slight fold to lower leaves. Under stress condition the continuous three days observations were taken as on first day KIRAN, RLC-143, RLC-167, these three genotypes shown shallow fold of lower leaves and other genotypes show healthy leaves no folding occur on first day. On second day RLC-143 and RLC-167 genotypes shown V-shaped folding and KIRAN, IA-32, R-17, RLC-92, RLC-133, RLC-148, RLC-153, RLC-161, RLC-164, RLC-165, RLC-171 and RLC-172 shown shallow V-shaped folding. On day 3 RLC- 167 genotype shown U-shaped folding means the leaves about to die hence genotype RLC-167 couldn't tolerate water stress. Some genotypes shown shallow V-shaped, and some genotypes shown V-shaped folding. R-7, KARTIKA, and DEEPIKA genotypes shown less folding than other hence these three genotypes could tolerate water stress.

Relative Water Content (%)

Fig 3: Relative Water Content of Leaf under Stressed and Unstressed Plants

Twelve days after the start of the stress, sampling was carried out. Sampling was done at morning and turgid weight and dry weight were taken as given method in protocol. Under normal condition genotypes leaf water content ranges from 56.1% to 75.55% with an average of 67.44%. The maximum Leaf Water Content is observed in RLC-164 genotype i.e., 75.55%, and the minimum is observed in RLC-148 i.e., 56.1%, Under stressed condition genotypes leaf water content varies from 37.85% to 55% with an average of 45.06%. The maximum leaf water content in stress condition observed in DEEPIKA i.e., 55%, and the minimum leaf water content observed in RLC- 165(37.85%) [30]. showed that water stress significantly decreased the seed production and pod number in rapeseed, which lends support to the conclusion. Leaf water potential, relative water content, and leaf area all dramatically decreased as stress severity increased [14]. investigated how metabolic parameters related to yield potential were affected by soil moisture stress. The seed production, number of pods, and seed oil content were all significantly reduced by water stress. The interaction of the severity, length of the drought event, and species had an influence on the relative moisture content [55]. According to [52], *P. vulgaris* foliage had a 10% fall in RLWC on the fourteenth day of treatment, indicating a rapid decline in the species' water potential. Also reported decreases in RLWC in chickpea cultivars [9] and in groundnut cultivars, while the fall in RWC was least in cv. K-134 [29]. The maximum RLWC was found in Pusa-362 by 86.33 percent at blooming stage and in Pusa-1103 by 81.07% at pod formation stage when plants were under moisture stress [43]. In comparison to unstressed plants, all genotypes of linseed had significantly lower relative water content. In all the linseed genotypes, the relative water content lowered as crops matured (Fig. No. 3).

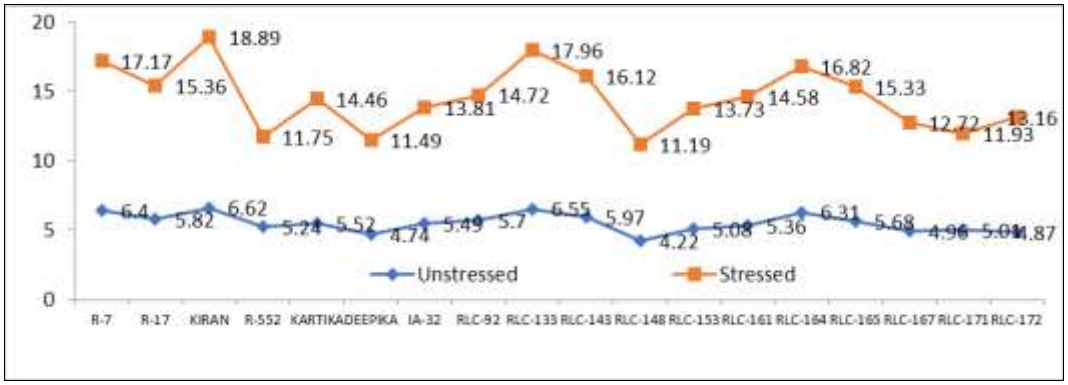

Fig 4: Proline Accumulation under Stressed and Unstressed Plants

Proline Content (µmol/g)

The stressed linseed genotypes showed rise in proline accumulation over unstressed control (Fi. No. 4). Under normal conditions, the proline content ranges from 4.22 to 6.62 µmol/g with an average of 5.53 µmol/g. The highest proline content under normal condition observed in KIRAN (6.62 µmol/g) followed by RLC-133 (6.55 µmol/g), and the lowest proline content observed in RLC-148 (4.22 µmol/g), followed by DEEPIKA (4.74 µmol/g). Under stress condition the proline content varies from 11.19 µmol/g to 18.89 µmol/g with an average of 14.52 µmol/g. The results of this study showed that under drought stress conditions, proline levels in the leaf linseed genotypes rise. It is suggested that proline may be a suitable indication for determining drought tolerance in linseed given the higher tolerance of KIRAN and RLC-133 to water stress with higher level of proline [15]. Revealed a 12-fold gain in free proline in B. *juncea* when water stress was imposed during germination compared to a 7-fold increase in B. *campestris.* Increased proline levels were found in the roots and leaves of the less drought-tolerant *Brassica juncea* variety [38]. As seen in the current study, several researchers have noted a significant positive link between the amount of free proline in leaves and water stress in diverse crops [53]. Higher proline buildup was caused by PEG-induced water stress in *P. vulgaris* [52]. Proline buildup in leaves at the post anthesis stage increased significantly in chickpea cultivars because of progressive water shortage, more than two times as much in severely stressed plants as compared to well-watered plants [31]. In shoot tissues of melon cultivars under PEG-induced water stress, [23] noticed comparable substantial increase patterns in proline accumulation in proportion to the severity of drought stress. Both black gramme and green gramme cultivars' leaf proline concentration rises in during stressing period and was discovered to decrease during the healing period [3]. A more typical argument for the buildup of proline is that it benefits by safeguarding proteins and membranes when RWC lowers [41].

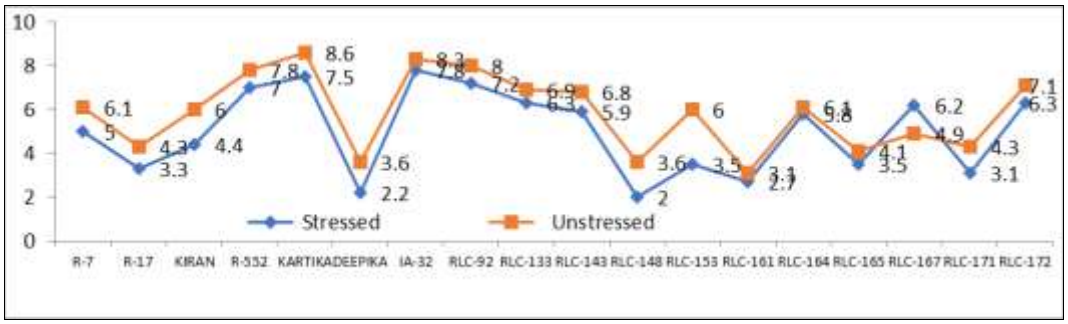

Fig 5: Total Root Length under Stressed and Unstressed Plants

Total Root Length

Under normal condition the root length varies from 3.1 cm to 8.6 cm with an average of 6.12 cm. The maximum root length observed in normal condition is KARTIKA (8.6 cm) and the minimum root length observed in RLC-161(3.1 cm). Under stressed condition the root length varies from 3.75 cm to 8.96 cm with an average of 6.37 cm. and the maximum root length in stressed condition observed in KARTIKA (8.96 cm) and minimum root length observed in RLC-161 (3.5 cm). Absorption by plants from the soil is closely correlated with root growth and extension when there is a lack of water [19, 42]. While root features do change with biotic and abiotic and climatic conditions [48], several species have shown connections between specific root metrics and drought tolerance. The root system of the sunflower is deep and wide, and it can draw water up to 270 cm [13, 7, 40]. [36], seedlings under drought stress displayed a slight improvement in root length but a decreased diameter. However, [39] demonstrated that insufficient soil moisture decreased root elongation. In the current investigation, most genotypes have longer rooted under stress than under control. Increased root elongation is the cause of the rise in root length under stress. Despite stress, several genotypes including R-552, KARTIKA, IA-32, RLC-92, RLC-153 maintain greater root length. Longer roots may aid in locating water and nutrients further down in the soil profile. When plants experience water shortage stress, their roots typically continue to grow and encroach on deeper soil layers [18, 49, 8, 54]. One of the key elements of drought tolerance, according to [25], was "deep, wide-spreading, much-branched root system" fifty years ago. The plant adjusts to increased rooting depth and root biomass to access the increased amount of accessible soil moisture [6, 12]. Numerous crops, including rice [34, 22, 28, 50, 5], & chickpea [44]. According to [26], our findings were supported by their assertion that the root length, root width, root entry ability, and elongation rate of roots in water stress resistant cultivars were much higher than those in susceptible cultivars. Under situations of water deficiency, (2005) [51] found that the

ratio of root to shoot dry weight was positively and significant correlated with total root length. When considering drought tolerance breeding, a plant's root system is crucial since certain root traits, such as root length, root biomass, density, will affect how effectively water is drawn from the soil. The plant is anticipated to perform much better under water deficit when its growth is dependent on water stored deeper in the soil because a deepened root system would facilitate water extraction from lower soil profiles (Fig. No. 5 & 3).

Fig 6: Root Length observation in normal and stressed condition

Analysis of variance

ANOVA demonstrated that the difference between treatments, i.e., genotypes, was significant at the 5% and 1% levels of significance for all the traits tested, demonstrating the presence of adequate variability among these variables.

Table 4: Analysis of variance for different traits in linseed in normal and stress condition

Source of variation	Days of Flowering	Plant height (cm)	No. of capsule/ plant	No. of Fertile capsule/ plant	Capsule fertility%	Grain Yield per plant(gm)	1000 seeds weight (gm)	Seed Length (cm)	Seed Breadth (cm)	Total Root Length (cm)
Replication	0.44	2.6	306.25	441	1641	10.08	4.45	0.02	0.01	2.36
Treatments	4.78	91.53	67.24	43	322	0.44	0.99	0.02	0.01	5.80
Errors	0.21	1.10	4.49	7.71	110.09	0.17	0.03	0.25	0.15	0.10
G. Mean	43.72	44.12	15.30	12.5	86.50	3.50	5.07	0.30	0.12	6.12
SEM	0.32	0.74	1.50	1.96	7.42	0.29	0.12	0.01	0.01	0.22
CD-1%	0.96	2.21	4.47	5.86	22.13	0.86	0.36	0.02	0.02	0.66
CD -5%	0.98	2.24	5	5.89	23	0.89	0.39	0.03	0.05	0.69
CV	1.05	2.37	13.84	22.21	13.16	11.66	3.41	2.48	6.9	5.12

Similarity matrix of 18 linseed genotypes by morphological analysis

For a collection of 18 linseed genotypes, Jaccard's estimations of similarity matrix were evaluated and given in Table 4.5 in accordance with the morphological trait scoring. The similarity ranged from 0.10 to 1.0 with an average of 0.45 among these 18 promising genotypes of linseed under normal conditions. The similarity matrix of 18 linseed genotypes under normal conditions varies from 0.10 to 1.00 from morphological analysis, thus most similar lines are whose similarity is 1 i.e., RLC-92-RLC-153. And most dissimilar genotypes are KARTIKA- RLC-165 and RLC-172-R552 under normal condition. Under stress condition the similarity ranges from 0.00 to 0.83 with an average of 0.41. Thus, most similar lines under stress condition are RLC-161-RLC- 148 whose similarity is 0.83 and most dissimilar genotypes are KARTIKA-RLC-165 whose similarity is 0.00.

Molecular characterization using molecular markers

A set of 10 ISSR primers were used in PCR to amplify 18 genomic DNAs. The genomic DNAs were amplified by six of the ten ISSR primers used in this study. In the current experiment, 210 amplicons were generated using six ISSR primers. Amplicons were found to be polymorphic, with a mean polymorphism of 65.10 percent. On average, 35 amplicons were produced by each primer. Sizes for amplification products ranged from 400 to 1000 bp. The average polymorphic information content (PIC) score for each primer was 0.55, with a range of 0.34 to 0.71. The ISSR primer UBC-807 with a PIC value of 0.34 was found to be the least pertinent, whereas the most informative marker was UBC-825 with a PIC value of 0.71. Similar findings were found for M. *caesalpiniifolia* (0.397), which is found in the semi-arid region of Brazil [2].

Similarity matrix linseed genotypes by ISSR analysis

Based on the ISSR results, Jaccard's estimates of the similarity matrix for a group of 18 linseed genotypes were assessed. These 18 promising linseed genotypes shared an average similarity of 0.56, with a range of 0.20 to 0.92. The 18 linseed lines'

similarity matrices range from 0.20 (IA-32- DEEPIKA) to 0.92. (RLC-167-RLC-148). Thus, the genotypes RLC-167 and RLC-148 were the most comparable ones found. IA-32 and DEEPIKA were found to have the most different genotype.

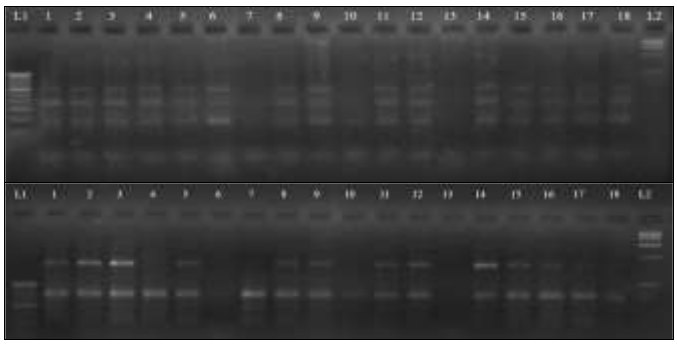

Fig 7: Molecular characterization using ISSR markers

1	R-7	5	KARTIKA	9	RLC-133	13	RLC-161	17	RLC-171
2	R-17	6	DEEPIKA	10	RLC-143	14	RLC-164	18	RLC-172
3	KIRAN	7	IA-32	11	RLC148	15	RLC-165		
4	R-552	8	RLC-92	12	RLC-153	16	RLC-167		
L1 = 100bp DNA Ladder, L2 = 1kb DNA Ladder									

Genetic distance values between germplasm accessions by ISSR analysis

Based on the ISSR scoring, Jaccard's estimates of the dissimilarity matrix for a group of 18 linseed genotypes were assessed and provided in Table 5. These genotypes have a mean dissimilarity of 0.46 with a range of 0.08 to 0.80. Dissimilarity matrices range from 0.08 (RLC-167-RLC-148) to 0.77. (IA-32 and DEEPIKA). The genotypes with the greatest genetic variance were IA- 32 and DEEPIKA. The genotypes RLC-167 and RLC-148 were the ones that were found to be the most different. Likewise, using Jaccard's similarity score, the average genetic distances between every genotype and the other genotypes in all linseed cultivars were independently calculated. According to ISSR analysis, the genetic distance between promising linseed germplasms ranges from 0.34 (KARTIKA) to 0.60 (IA-32). As a result, it was determined that IA-32 (0.60), RLC-161 (0.59), and RLC-143, DEEPIKA (0.59), had the most prevalent and diverse genotypes.

Table 5: Average genetic distance for ISSR

Sr. No	Genotypes	AGD	Sr. No	Genotypes	AGD	Sr. No	Genotypes	AGD
1	R-7	0.48	7	IA-32	0.60	13	RLC-161	0.59
2	R-17	0.40	8	RLC-92	0.41	14	RLC-164	0.42
3	KIRAN	0.52	9	RLC-133	0.45	15	RLC-165	0.43
4	R-552	0.41	10	RLC-143	0.57	16	RLC-167	0.41
5	KARTIKA	0.34	11	RLC-148	0.36	17	RLC-171	0.43
6	DEEPIKA	0.57	12	RLC-153	0.48	18	RLC-172	0.54

Principal Coordinate Analysis (PCoA) based on ISSR analysis

The relationship amongst 18 linseed genotypes were further examined by the PCoA based on collective derived values of Jaccard's similarity coefficient generated via ISSR analysis. The two-dimensional (Fig.No.5) and three-dimensional (Fig.No.6)) plots were prepared by using the first two and first three principal coordinates (PCs), respectively. The combined data showed that 57% of the total variation could be assessed by the three principal components based on the first, second and third most revealing Eigen vectors, which accounted for 22.78%, 20.86% and 13.36% variations, respectively. The groupings of the 18 genotypes are shown in the 2-dimensional and 3-dimensional scaling plots from this analysis, it was also apparent that majority of groupings followed the equivalent pattern as revealed in the dendrogram with minor differences. In 2-dimensional plot, nearly all linseed genotypes were categorized in the pattern like the clustering in dendrogram. Further, the 3-dimensional scaling plot also revealed the clustering conforming to the dendrogram constructed, where in the 3-dimensional plot differentiated all 18 genotypes into four major clusters.

Consequently, like UP GMA analysis, PCoA analysis confirmed the clear separation of the genotypes studied into equivalent clusters. Therefore, it can be suggested that both UP GMA and PCoA should be performed for more precise elucidation of genetic diversity analysis under study.

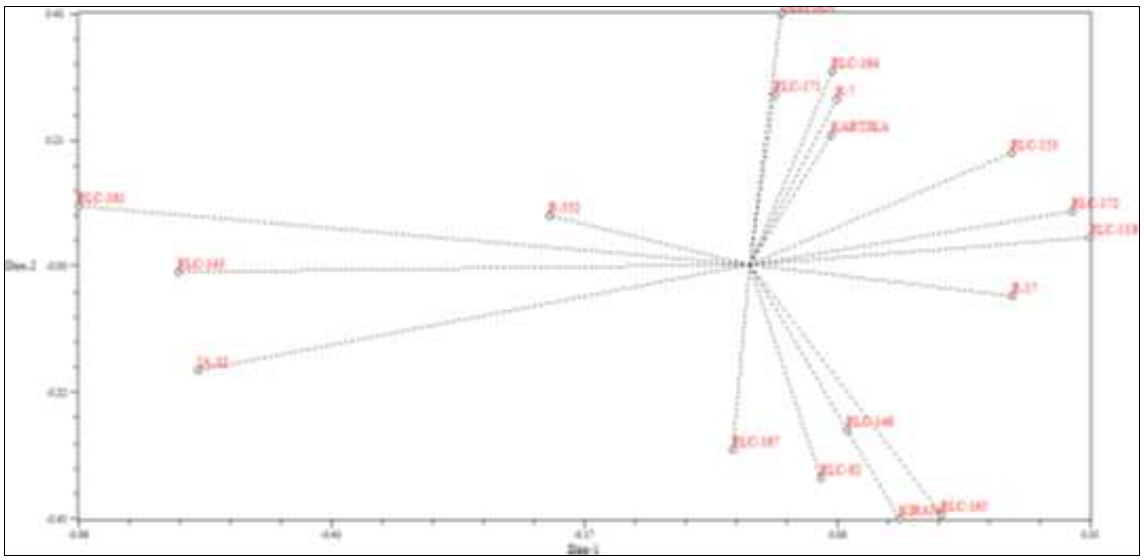

Fig 8: 2D Scaling plot based on ISSR primers analysis by using PCoA

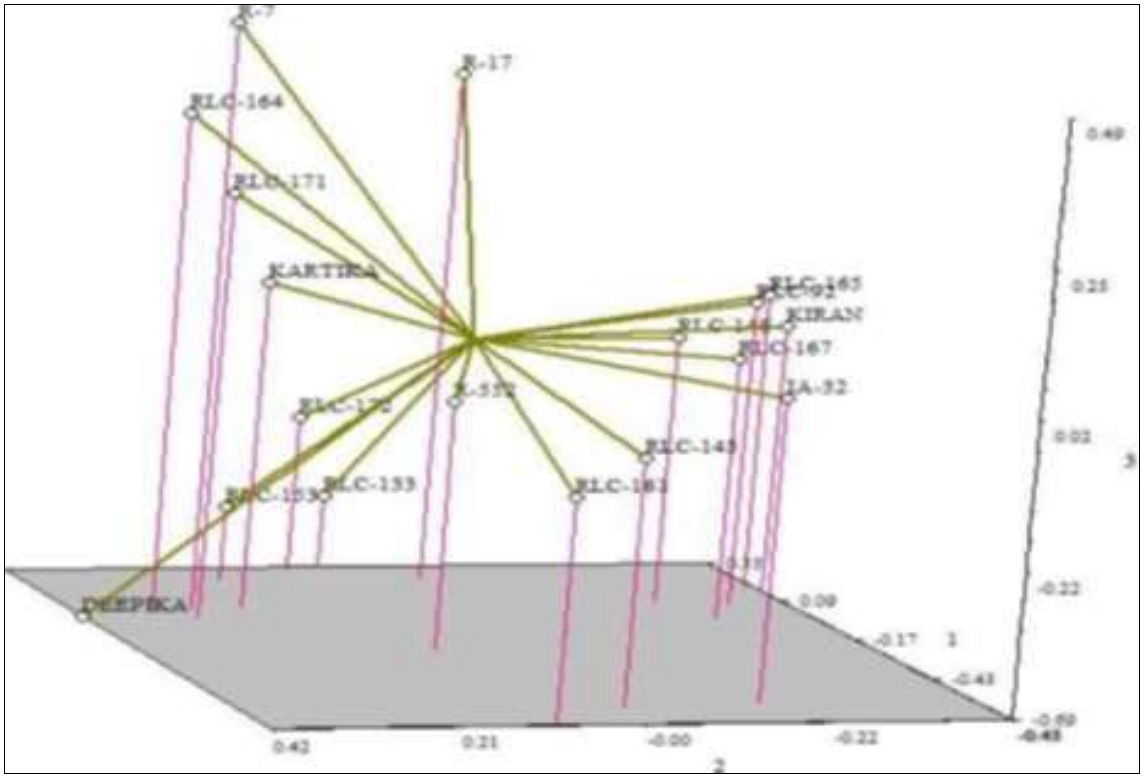

Fig 9: 3D Scaling plot based on ISSR primers analysis by using PCoA

Clustering of accessions based on ISSR markers analysis

The examination of clusters based on ISSR markers across multiple linkage groups reveals that indigenous linseed lines have a reasonably diverse genetic origin. Two different and important clusters (I and II) representing distinct groupings of 18 linseed germplasm were detected. The number of genotypes in each cluster ranged from one to five (Fig.7). At the 40% cut-off on the scale, two minor clusters, IA and one out group, were found to be like each other in major cluster I. DEEPIKA was discovered to be separated from IA. The IA minor cluster has been separated into two sub clusters, IAa and IAb, both of which have a cut-off of roughly 57% on the scale and comprise 14 linseed lines. Sub cluster IAa is further subdivided into IAa1 and IAb2, which have comparable cut-off values of roughly 62% on the scale. Germplasm R-7 and R-17 were discovered to be dissimilar to IAa1. KARTIKA, RLC-164, and RLC-171 from cluster IAa1 show 87% commonality on the scale. Six germplasm are found in sub cluster IAa2. R-552 was shown to be out group IAa2. Sub cluster IAb had two germplasm RLC-133 and RLC-153 that were identical at roughly the 83% cut-off on the scale, whereas RLC-172 was shown to be out-grouped from IAb. Minor cluster IIA has two germplasm samples that are comparable to one another at a cut-off of about 33% on the scale, and IA-32 was shown to be out grouped from cluster IIA. [47] obtained a comparable outcome using UP GMA, cluster analysis based on Jaccard's similarity coefficient separated the 48 flax genotypes into two groups, with similarity indices ranging from 0.17 to 0.97 and an average of 0.46.

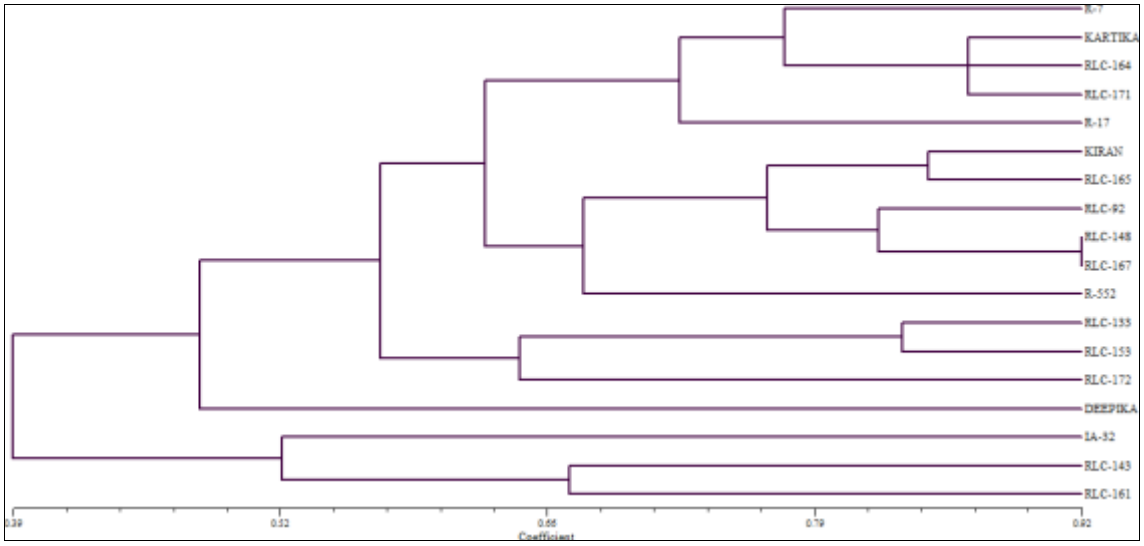

Fig 10: Dendrogram generated by UP GMA analysis based on ISSR markers

Molecular characterization by using SSR primers

PCR was used to amplify 18 genomic DNAs using a set of 10 SSR primers. Five of the 10 SSR primers used in this study successfully amplified the genomic DNAs. In the current study,

five SSR primers were employed to generate a total of 120 amplicons. 43 amplicons were found to be polymorphic, with a mean polymorphism of 35.83%. On average, each primer produced 24 amplicons. Amplification products ranged in size from 300 to 100bp. For each primer, the average polymorphic information content (PIC) score ranged from 0.15 to 0.84. SSR primer LU- 9 with a PIC value of 0.15 was found to be the least pertinent, whereas LU-3 with a PIC value of 0.84 was the most informative primer. This result is consistent with the findings of Sajib *et al.* 2012 [45], who discovered that PIC values for all examined SSR loci differed significantly (from 0.14 to 0.71 with a median of 0.48). Better average PIC values of 0.57 and (0.71) were discovered, according to Zeng *et al.* 2004 [56]. According to Botstein *et al.*, (1980) there were eight extremely informative markers (PIC>0.50), four informative markers (50>PIC>0.25), and the least informative markers (PIC<0.25).

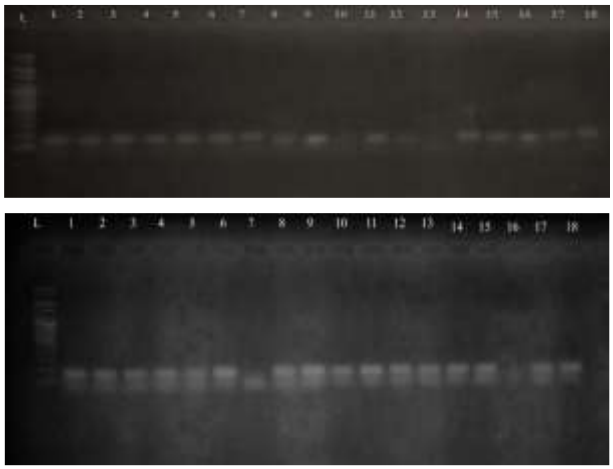

Fig 11: SSR profiling of 18 linseed lines obtained with primer LU-3 and LU-9

Similarity matrix of 18 linseed genotypes by SSR analysis

Based on the SSR scoring, Jaccard's estimates of the similarity matrix for a group of 18 linseed genotypes were assessed and provided in Table 4.13. These 18 promising linseed genotypes showed an average similarity of 0.58, with a range of 0.22 to 1.0. 18 linseed lines have similarity matrices that range from 0.22 (IA-32-KIRAN) to 1. (RLC-153-RLC-92, RLC-153-RLC-133). Therefore, the genotypes RLC-92, RLC-153, and RLC-133 were shown to be the most comparable. IA-32 and KIRAN were found to have the most different genotypes [46]. Reported similar results. The SSR markers' binary data were used to compute similarity parameters. From 0.30 to 0.90, the similarity coefficient was found [27] and 2022 [21] reported similar results.

Genetic distance values between germplasm accessions by SSR analysis

According to the SSR scoring, Jaccard's estimates of the dissimilarity matrix for a set of 18 linseed genotypes were assessed and shown in Table No. 6. These 18 potential linseed genotypes have a mean dissimilarity of 0.46 with a range of 0.00 to 0.77. Linseed lines' dissimilarity matrices range from 0.00 (RLC-92-RLC-153) to 0.77. (IA-32-KIRAN). IA-32- KIRAN was the genotype with the greatest degree of dissimilarity. The genotypes RLC-92 and RLC-153 were found to be the most different. Additionally, using Jaccard's similarity score, the average genetic distances between each genotype and the other genotypes in all linseed cultivar lines were independently calculated. Table No.6 and Figure 10 and 11 revealed that the genetic separation across 18 potential linseed accessions varies from 0.24 (R-552) to 0.52 based on SSR analysis (RLC-167). Thus, it was determined that RLC-167 (0.52), RLC-171 (0.44), and RLC-143 (0.44) had the most prevalent variant genotypes.

Table 6: Average Genetic Distance for SSR

Sr. No	Genotypes	AGD	Sr. No	Genotypes	AGD	Sr. No	Genotypes	AGD
1	R-7	0.34	7	IA-32	0.45	13	RLC-161	0.28
2	R-17	0.31	8	RLC-92	0.25	14	RLC-164	0.32
3	KIRAN	0.38	9	RLC-133	0.36	15	RLC-165	0.37
4	R-552	0.24	10	RLC-143	0.44	16	RLC-167	0.52
5	KARTIKA	0.38	11	RLC-148	0.43	17	RLC-171	0.44
6	DEEPIKA	0.35	12	RLC-153	0.27	18	RLC-172	0.43

Principal coordinate analysis (PCoA) based on SSR analysis

Moreover, the relationship amongst 18 linseed accessions maintained at V.D. College of Agriculture Biotechnology, Latur were further examined by the PCoA based on collective derived values of Jaccard's similarity coefficient generated via SSR analysis. The two-dimensional (Fig.9) and three-dimensional (Fig 10) plots were prepared by using the first two and first three principal coordinates (PCs), respectively. The combined data showed that 46.98% of the total variation could be assessed by the three principal components based on the first, second and third most revealing Eigen vectors, which accounted for 19.26%, 14.99% and 12.73% variations, respectively. The groupings of the 18 genotypes are shown in the 2-dimensional and 3-dimensional scaling plots from this analysis, it was also apparent that majority of groupings followed the equivalent pattern as revealed in the dendrogram with minor differences. In 2-dimensional plot, nearly all accessions were categorized in the pattern like the clustering in dendrogram. Further, the 3-dimensional scaling plot also revealed the clustering conforming to the dendrogram constructed, wherein the 3-dimensional plot differentiated all 18 genotypes into two major clusters. Consequently, like UP GMA analysis, PCoA analysis confirmed the clear separation of the genotypes studied into equivalent clusters. Therefore, it can be suggested that both UP GMA and PCoA should be performed for more precise elucidation of genetic diversity analysis under study. Principal coordinates analysis helps to identify the most relevant characters by explaining the total variation in the original set of variables with few of the components as possible, and it reduces the complexity of the problem. The traits with the largest impact on the components showed the highest rate of variation and hence can be used for grouping genotypes effectively.

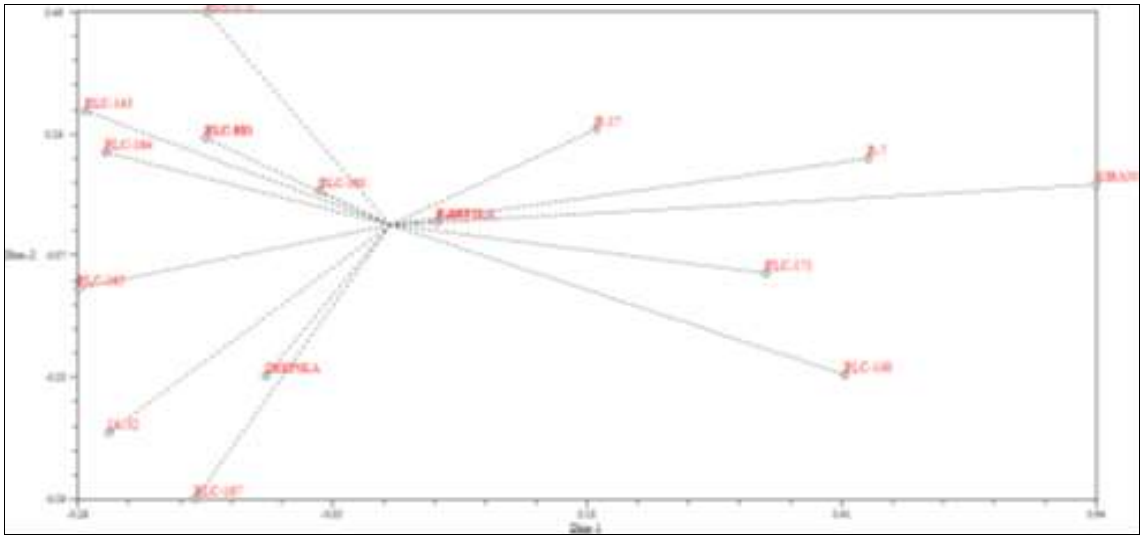

Fig 9: 2D Scaling plot of linseed genotypes based on SSR primers analysis by using PCoA

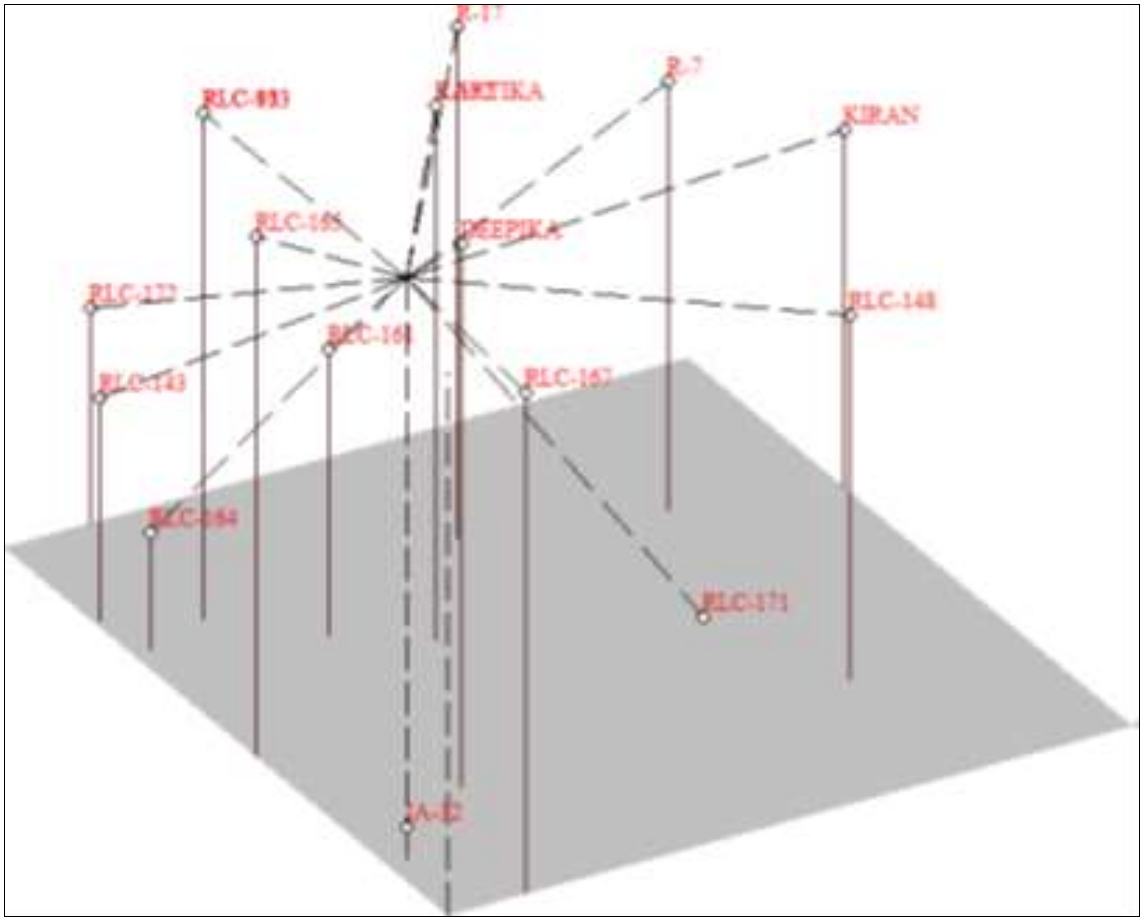

Fig 10: 3D Scaling plot of linseed genotypes based on SSR primers analysis by using PCoA

Clustering of accessions based on SSR markers analysis

Using UP GMA-based cluster analysis, the genetic similarity matrix was used to create a dendrogram (Figure 11), which revealed two significant clusters (I and II). Only two genotypes, IA-32 and RLC-167, are found in the II main cluster, while I have 16 germplasm. At a size cutoff of about 57%, major cluster I was further separated into minor clusters IA and IB that were similar. Only two germplasm samples, RLC-148 and RLC-171, were found in the IB minor cluster and were like one another by 63%. Minor cluster IA was broken into IAa and IAB subclusters. IAa subcluster was split into IAa1 and IAa2 at a comparable 57%. At the 86% cut-off on the scale, IAa1 had two germplasms that were identical to one another: R-7 and R-17. It was discovered that KIRAN outperformed IAa1. IAa2i and IAa2ii, two subclusters of subcluster IAa2, exhibit commonality of about 66%. RLC-161 and RLC-164 were found to be out grouped from IAa2i in their germplasm. Two germplasm samples, RLC-143 and RLC-172, are part of sub-cluster IAa2ii and are like one another by a cut-off of 87% on the scale.

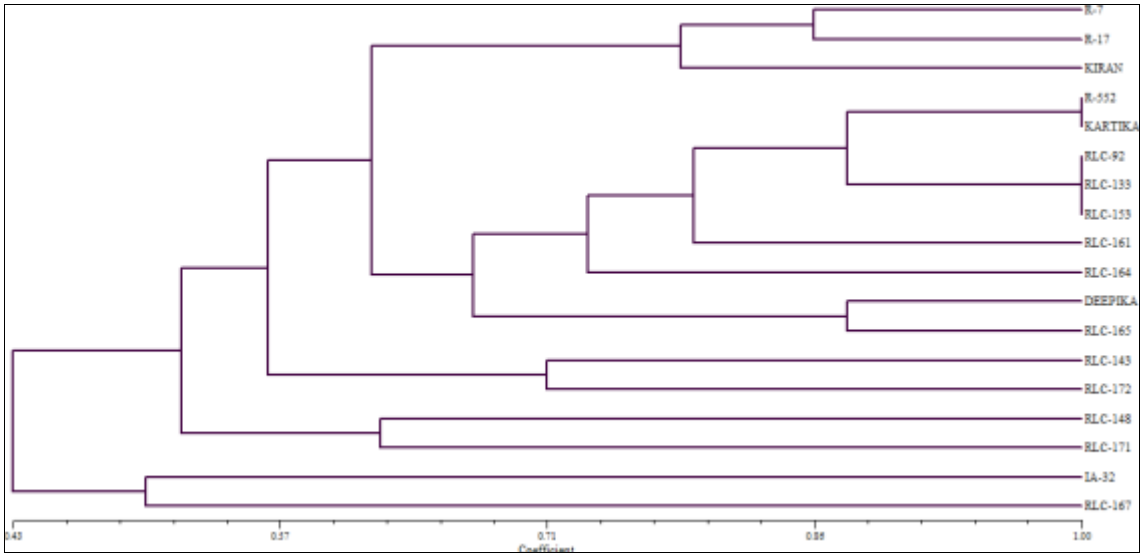

Fig 11: Dendrogram generated by UP GMA analysis based on SSR markers

Conclusion

In the present study the investigation was done on linseeds germplasm lines for drought tolerance using morphological and molecular markers. It was observed that for earlier flowering in Deepika and late first flower was observed in RLC-143, RLC-148, RLC-172. Under stressed condition the earliest flowering genotype was KARTIKA and late flowering observed in RLC-172. For plant height under both conditions among all lines, the tallest plant was observed RLC-172 and smallest plant height was observed is R-7. Number of capsules per plant under normal and stressed conditions the maximum number of capsules observed in DEEPIKA was 34 and 27 respectively. The minimum number of capsules observed under normal and stressed conditions in R-552 was 7 and 4 respectively. For Grain Yield per Plant under normal conditions maximum grain yields observed in R-552 and RLC-148 and minimum in KARTIKA. Under stress condition maximum grain yield observed in RLC-161 and the minimum in KIRAN. For 1000 seed weight under normal conditions the maximum weight observed in RLC- 167 and the minimum in R552 and under stressed conditions the maximum seed weight observed in RLC-172. For Capsule Fertility percentage under normal conditions RLC- 143, RLC-148, RLC-167, RLC-171showed 100% fertile capsule and under stress condition RLC-92, RLC-133 and RLC-165 showed 100% fertile capsule. For Leaf Rolling Score the genotypes KIRAN, RLC-143, RLC-167 show susceptible under stress condition and couldn't tolerate water stress. R-7, KARTIKA, and DEEPIKA genotypes shown less folding could tolerate water stress. For Leaf Relative Water Content under normal condition the maximum leaf water content was observed in RLC-164 genotype and the minimum in RLC-148. Under stressed condition maximum leaf water content observed in DEEPIKA and the minimum in RLC-165. For Proline Content under stressed showed rise in proline accumulation over unstressed control. Under stress conditions the highest proline content observed in KIRAN and the lowest in RLC-148. KARTIKA showed maximum root length in normal condition and minimum root length observed in RLC-161. Despite stress, several genotypes including R-552, KARTIKA, IA-32, RLC-92, RLC-153 maintain greater root length. Longer roots may aid in locating water and nutrients further down in the soil profile.

The similarity matrix of 18 linseed genotypes under normal conditions, most similar genotypes are RLC-92 and RLC-153 and most dissimilar genotypes are KARTIKA-RLC-165 and RLC-172-R552. Under stress condition the most similar lines are RLC-161-RLC-148 and most dissimilar genotypes are KARTIKA and RLC-165. Five SSR primers were used in the current investigation polymorphism. It was discovered that a mean polymorphism of 35.83%. On the basis of mean polymorphic information content (PIC) the least relevant primer was noticed to be SSR primer LU-9 of PIC value of 0.15, while the maximum informative marker LU-3 had a PIC value of 0.84. The most similar genotypes observed were RLC-92, RLC-153 and RLC-133 and most dissimilar genotypes were IA-32 and KIRAN. According to ISSR analysis, the genetic distance between linseed accessions ranges from 0.34 (KARTIKA) to 0.60(IA-32). Thus, IA-32 (0.60), RLC-161 (0.59), and RLC-143, DEEPIKA (0.59) was shown to have the most common varied genotypes.

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