Environment and Ecology 40 (3) : 1214—1221, July—September 2022
ISSN 0970-0420

Genetic Diversity and Selection of Parents of Linseed (*Linum usitatissimum* L.) Germplasm using Morphological and Molecular Characterization

A. B. Ghige, Beena Nair, J. G. Manjaya,
R. A. Jadhav, J. R. Katore

Received 31 March 2022, Accepted 5 May 2022, Published on 15 July 2022

ABSTRACT

Linseed is the main oilseed crop of India. The present investigation was carried out at the experimental farm of AICRP on Linseed and Mustard, College of Agriculture, Nagpur and the laboratory facilities were utilized from BARC, Trombay, Mumbai. For enabling better exploitation of genetic resources, it is desirable to know the genetic diversity at morphological as well as molecular levels. In present study, nine parents namely, NL 451, NL 442, NL 441, NL 431, NL 456, NL 457, NL 371, NL 458 and NL 435 possessed positive significant GCA effects for seed yield per plant and were distributed in the different clusters than that of the checks indicating molecular diversity than the checks. Hence, these parents can be used in hybridization program to get better transgressive segregants. The parents NL 351 and NL 450 possessed higher mean performance for seed yield per plant and were placed in different clusters than the checks, hence can be utilized in varietal development program. The results of SSR analysis generated a dendrogram which divided 37 parents into five main clusters in which the cultivated check genotypes were grouped into separate cluster.

Keywords SSR, Genetic diversity, Marker, Cluster**.**

A. B. Ghige, Beena Nair, R. A. Jadhav* J. R. Katore
All India Coordinated Research Project on Linseed and Mustard, College of Agriculture, Nagpur-440012, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola, Maharashtra.

J. G. Manjaya
Bhabha Atomic Research Centre (BARC), Trombay, Mumbai, Maharashtra.
Email: ranijadhav74@gmail.com
*Corresponding author

INTRODUCTION

Linseed (*Linum usitatissimum* L.) belongs to genus Linum of the family Linaceae and consists of 22 genera and 300 species (Hickey 1988). It is commonly known as 'Alsi' or 'Tisi'. In India, it is grown mostly for seeds, used for extracting oil. The oil content of the seed varies from 35 to 45% (Gill 1987). It has high content of omega-3 (57%) and omega-6 fatty acids (8%). India ranks third in production. In India, it occupies about 3.0 lakh hectares with production of 1.84 lakh tones and average productivity of 613 kg/ha (Anonymous 2018). It has been proposed that the cultivated flax is came from a solitary domestication event from *L. bienne* for its oil. Molecular studies have also supports this event to oil and fiber flax (Allaby *et al.* 2005).

In the field of plant breeding, the most important breakthrough has been considered as heterosis and molecular marker is useful for varietal identification, evaluation of DNA and provides a powerful tool for the analysis of plant genome structure and function (Liu *et al.* 2000). Molecular marker provides a powerful tool for the analysis of plant genome structure and function. Once the molecular markers associated with any desirable agro-morphological traits 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 (Jiang 2013).

As per the present available literature pertaining to DNA markers in linseed, the SSR markers reported, newly developed genomic SSRs or EST-SSRS and that too mostly in exotic linseed genotypes (Kumari *et al.* 2017, Cerda *et al.* 2014). Therefore there is great need and scope of using such novel DNA markers towards screening, identifying and validating the specific positive markers linked to yield, its major components and oil content of linseed genotypes developed in India. Hence, it was proposed to undertake the present study with a view to study the combining ability, heterosis of linseed genotypes and their crosses to identify good combiner for best cross combinations and also to characterize selected linseed genotypes and identify the DNA markers in linseed.

The choice of parents for hybridization program influences the success in any crop improvement programme. It is a common experience that certain crosses nick well and produced superior transgress and some crosses between promising parents producing disappointed progenies. Thus the selection of parents based on the *per se* performance is not always a good indicator of superior combining progenies (Allard 1960). Therefore, identification of parents on the basis of general combining ability, high mean performance and molecular diversity is lightly to give high proportion of superior segregants. Hence, it was proposed to undertake the present study with a view to select the parents for hybridization and characterize selected linseed genotypes by using SSR markers.

MATERIALS AND METHODS

The present research work was conducted at the farm of AICRP on Linseed and Mustard, College of Agriculture, Nagpur. The 34 parental lines were crossed with 3 testers in line x tester mating design to produce 102 crosses during first year (*rabi* 2018-19) and evaluation of combining ability was done. In *rabi* 2019-20, a total of 139 entries (37 parents (34 lines + 3 testers) + 102 F_1) were evaluated in Randomized Block Design in two replications with the spacing of 30 cm × 5 cm. The recommended practices were followed to raise good crop. The list of genotypes is given in Table 1. The diversity of 37 parents was done by using 27 SSR primers. The laboratory facilities to carry out this work were utilized from BARC, Trombay, Mumbai, India. The details of markers used during course of investigation is given in Table 2.

Table 1. List of the parents.

Sl. No.	Name of parents (lines)	Sl. No.	Name of parents (lines)
1	NL 350	20	NL 446
2	NL 351	21	NL 447
3	NL 356	22	NL 448
4	NL 371	23	NL 449
5	NL 430	24	NL 450
6	NL 431	25	NL 451
7	NL 432	26	NL 452
8	NL 433	27	NL 453
9	NL 434	28	NL 454
10	NL 435	29	NL 455
11	NL 436	30	NL 456
12	NL 438	31	NL 457
13	NL 439	32	NL 458
14	NL 440	33	NL 459
15	NL 441	34	NL 460
16	NL 442	Sl. No.	Name of parents (testers)
17	NL 443	35	LSL 93
18	NL 444	36	PKV NL 260
19	NL 445	37	T 397

Table 2. The score of powdery mildew infestation.

Score	Bud infection	Disease reaction	
0	0%	Immune	Free
1	0-10%	Resistant	R
2	10.1-25%	Medium resistant	MR
3	25.1-50%	Medium susceptible	MS
4	50.1-75%	Susceptible	S
5	Above 75%	Highly susceptible	HS

Observations recorded

Observations were recorded on five randomly selected plants (except days to 50% flowering and days to maturity for which plot wise observations were recorded) from each genotype in each replication on following characters. The procedure followed for recording observations on each of the character is described below:

1. Days to 50% flowering (on plot basis): The number of days taken by 50% of plants to initiate flowering in each genotype from the date of sowing were counted and recorded as days to 50% flowering.

2. Days to maturity (on plot basis): The number of days taken by all the plants in each genotype to attain physiological maturity from the date of sowing was counted and presented as days of maturity.

3. Plant height (cm): The height of plant from the base, to the tip of the main stem was recorded in centimeters, plant height divided into 3 classes namely, long (>70 cm), medium (51-70 cm), short (<51 cm) [DUS UPOV, 2011 and www.UPOV.int.].

4. Number of branches/plant: The number of primary branches emerging from the base of the plant was recorded on the five randomly selected plants of each genotype at the time of maturity and the average number of primary branches was noted.

5. Number of capsules /plant: The number of seeds bearing capsules on each of the five randomly selected plants of each genotype was counted at maturity and the average was worked out and presented as number of capsules /plant.

6. 1000 seed weight (g): Weight of 1000 well-developed grains collected from the bulk of plants selected was recorded and expressed in grams. According to weight, it is grouped in 3 classes viz., high (>8 g), medium (6-8 g), low (<6 g) [www.UPOV.int.].

7. Seed yield /plant (g): The seeds obtained from each of the five randomly selected plants were weighed in gram separately on precision electronic balance and mean yield plant^{-1} were calculated and recorded in gram.

8. Budfly infestation (%): Individual plant was scored for bud fly infection. In each plant buds infected by bud fly (*Dasyneure lini*) were counted and percentage was taken from the total number of buds as follows (Anonymous 2018). Percentage data do fall within the range of 0-30% hence, no transformation was done

$$\text{Bud fly \%} = \frac{\text{Infected buds}}{\text{Total no. of buds}} \times 100$$

9. Alternaria blight infestation (%): Infected buds by *Alternaria lini* were counted in each plant and percentage was taken from the total number of buds (Anonymous 2018).

$$\text{Alternaria blight \%} = \frac{\text{Infected buds}}{\text{Total no. of buds}} \times 100$$

10. Powdery mildew infestation (score): Each plant was scored visually in the field and plants were rated in 0 to 5 scale as shown in the table. Based on the visual score of disease incidence the mean score was determined (Anonymous 2018).

Statistical analysis:

The combining ability analysis was carried out following the methodology of Kempthorne (1957) with fixed effect model (model-1).

SSR marker for polymorphism

The present study related to SSR marker polymorphism was conducted at Nuclear Agricultural Biotechnology Division at BARC, Mumbai. The details of material used and methods adopted during course of investigation were described as under.

Experimental material

Total genomic DNA was extracted from young seedling using the method described by Dellaporta (1983). The quality of DNA was checked by nanodrop spectrophotometer (Thermo Scientific NanoDrop TM 1000 Spectrophotometer).

Molecular marker evaluation

All SSR fragments were scored manually and converted into binary data, i.e., 1 for presence of band and 0 for absence of band. Polymorphism information content (PIC) was calculated by using the formula given by Roldan *et al.* (2000).

Data analysis and detection of genetic diversity for SSR markers

All SSR fragments were scored manually and converted into binary data, i.e., 1 for presence of band and 0 for absence of band. Polymorphism information content (PIC) was calculated using formula given by Roldán *et al*. (2000). PICi = 2fi (1 − fi), Where, PICi was the polymorphic information content of marker i, *fi* was the frequency of the marker bands present and (1−*fi)* was frequency of marker bands absent.

The marker index can be calculated by the multi-

Table 3. Pooled mean performance of parents for nine quantitative characters.

Sl. No.	Genotypes	Days to 50% maturity flowering	Days to maturity	Plant height (cm)	Number of branches plant^{-1}	Number of capsules plant^{-1}	1000 seed weight (g)	Seed yield plant^{-1} (g)	Bud fly infestation (%)	Alternaria blight infestation (%)
	Lines									
1	NL 350	63.75	125.00	56.35	3.2	43.20	8.1	2.94	7.50	3.10
2	NL 351	64.00	125.75	59.30	3.8	46.60	8.4	2.86	3.20	2.50
3	NL 356	62.25	120.25	68.60	3.2	40.20	8.8	2.21	7.00	3.85
4	NL 371	62.75	127.00	66.10	2.9	28.35	7.7	1.59	2.40	2.30
5	NL 430	65.75	133.25	79.75	3.3	39.25	8.9	2.13	3.65	1.60
6	NL 431	66.50	130.50	80.70	3.2	43.00	9.2	2.70	3.40	2.75
7	NL 432	64.25	131.00	65.30	3.3	36.00	8.9	2.03	3.15	2.05
8	NL 433	62.50	130.25	69.65	3.4	37.25	10.3	2.68	4.05	2.75
9	NL 434	65.50	130.50	67.50	3.4	37.60	9.5	2.67	3.60	3.80
10	NL 435	60.25	128.25	63.90	3.0	38.20	8.3	2.57	5.05	3.95
11	NL 436	58.50	117.00	72.80	3.0	44.15	8.4	2.42	5.00	4.95
12	NL 438	62.75	122.75	71.00	3.5	45.05	8.0	1.49	2.55	2.35
13	NL 439	61.25	127.00	61.50	2.7	39.55	8.8	2.16	3.20	1.80
14	NL 440	62.25	126.00	64.85	3.7	42.40	8.3	2.71	3.65	2.65
15	NL 441	57.75	124.25	62.65	3.3	41.45	7.6	2.30	2.20	2.15
16	NL 442	64.25	121.75	67.80	4.2	45.05	10.00	2.84	3.80	3.60
17	NL 443	62.00	129.00	64.90	3.5	40.10	9.3	2.28	2.95	2.85
18	NL 444	59.25	131.00	61.35	4.3	39.75	9.4	2.69	4.10	3.60
19	NL 445	57.75	123.50	66.45	3.6	43.40	7.3	2.07	2.35	1.75
20	NL 446	60.00	123.25	65.95	2.9	33.65	8.3	2.14	2.15	1.80
21	NL 447	64.00	126.00	67.80	3.0	34.65	8.9	1.95	2.25	1.95
22	NL 448	64.50	120.00	71.95	3.5	43.85	10.2	2.23	3.50	1.70
23	NL 449	67.00	127.00	58.95	4.3	36.00	10.1	1.78	2.60	1.75
24	NL 450	63.75	129.25	62.25	3.8	47.55	7.7	3.25	3.50	2.25
25	NL 451	64.00	125.00	60.40	3.0	43.20	8.5	2.91	2.20	1.95
26	NL 452	61.50	157.00	67.25	3.3	35.50	8.4	2.50	2.20	2.90
27	NL 453	59.75	125.00	60.35	3.1	40.35	9.8	1.78	2.90	3.20
28	NL 454	64.00	130.25	68.35	3.0	43.70	8.5	1.86	2.65	0.95
29	NL 455	66.00	132.50	58.10	3.2	30.65	10.2	2.69	2.40	2.05
30	NL 456	63.00	130.50	55.35	3.2	37.00	10.8	2.54	1.90	2.35
31	NL 457	61.75	135.00	64.35	3.2	34.95	11.4	2.61	1.50	1.40
32	NL 458	54.50	129.75	60.25	4.0	34.75	11.6	1.71	3.10	3.00
33	NL 459	48.25	124.00	48.80	4.5	35.90	8.8	2.15	2.70	3.10
34	NL 460	59.50	124.50	60.55	4.6	54.25	9.7	2.97	4.55	2.25
	Testers									
35	LSL 93	66.75	134.00	71.15	2.8	30.25	11.2	3.32	3.00	2.90
36	PKV NL 260	67.00	132.50	80.55	3.5	39.20	7.7	2.69	3.05	2.15
37	T 397	64.75	130.25	66.05	3.4	36.30	10.1	2.11	3.15	2.60
	Mean	62.25	128.10	65.38	3.44	39.52	9.11	2.39	3.30	2.56
	CD 5%	4.88	1.67	8.28	0.96	8.20	1.43	1.32	2.22	1.42
	CV %	4.12	2.07	6.57	15.03	6.49	12.29	11.91	31.87	30.94

plication of the PIC value of each primer combination with the EMR (effective multiplex ratio) value as given by Varshney *et al.* (2007). MI = PIC × EMR, Where, EMR was the effective multiplex ratio, defined as the product of the total number of loci fragments per primer (n) and the fraction of polymorphic loci/fragments (β) i.e.: EMR = n. β.

Distance-based cluster analysis was performed and dendrogram based on the unweighted pair group method of arithmetic mean (UPGMA) was constructed using Jaccard's similarity coefficient with the help of DARwin (Perrier & Jacquemoud 2015). The robustness of each dendrogram was evaluated by bootstrap analysis.

Table 4. General combining ability effects of the parents for different characters. Note : * Significant at 5% level, ** Significant at 1% level .

Sl. No.	Genotypes	Days to 50% flowering	Days to maturity	Plant height (cm)	Number of branches per plant^{-}	Number of capsules per plant	1000 seed weight (g)	Seed yield plant^{-1} (g)	Bud fly infestation (%)	Alternaria blight infestation (%)
	Lines									
1	NL 350	-0.245	-1	-4.088*	0.306	-11.875**	-0.043	-0.713	-1.301**	0.227
2	NL 351	-0.245	-0.667	-0.465	-0.172	-3.856**	-0.576**	-1.839**	-0.035	0.244
3	NL 356	3.422	2	-4.546*	-0.294	14.941**	-0.143	0.274	-2.051**	-1.262**
4	NL 371	0.088	2.333*	0.054	-0.011	9.4**	0.190	1.404**	0.849**	0.713
5	NL 430	6.755**	4.667**	-4.438**	-0.077	-5.809**	-0.843	-0.803**	-0.51**	0.622**
6	NL 431	-1.912	-1	0.757	0.439	-1.167	-0.143	1.437**	-0.154	1.055*
7	NL 432	-0.245	3	8.521**	-0.027	6.875	0.357	0.874	-0.251	-0.478
8	NL 433	-2.245	-	-5.513**	-0.111	-3.725	0.324	-1.106**	0.599**	0.538
9	NL 434	0.422	-0.333	-6.457*	-0.027	-10.409**	-0.310	-1.609**	1.382**	0.222
10	NL 435	-0.912	-0.667	-4.043**	0.056	-0.962	-0.976**	2.937**	0.035	0.083
11	NL 436	-1.578	0.167	-4.946	0.006	-14.625**	0.190	-0.159	1.324**	1.005**
12	NL 438	-2.578	-	-5.418**	0.178	-9.692**	0.357	0.197	0.849	1.372**
13	NL 439	-2.578	-	6.787**	-0.136	2.325	0.124	0.287	-0.36	0.097
14	NL 440	1.088	1.667	4.287	-0.327	14.475**	0.957**	0.567	0.149	0.805**
15	NL 441	-2.912	-1.333	-2.813**	-0.561**	-1.142	0.457	2.811**	-0.218	0.688**
16	NL 442	-2.912	-0.667	0.187	-0.077	2.625	0.124	0.954*	-0.335	0.438
17	NL 443	-1.578	-0.333	-1.068	0.328	-1.487	0.057	1.047	-1.812	-0.612
18	NL 444	-1.245	-1.333	5.048**	0.259	-3.917	-0.460	-1.206**	-1.482**	-0.395**
19	NL 445	2.422	2	5.412	-0.194	-10.317**	0.440	-1.203*	1.015*	0.072
20	NL 446	3.088	-1	2.937	-0.172	-5.917	-1.010**	-2.373**	0.29	0.063
21	NL 447	-1.245	-0.333	-0.577	-0.066	-2.942	0.524	-0.549	0.593	-0.406
22	NL 448	-1.245	-0.333	-8.69**	0.123	3.219	0.407	-1.463**	0.226	-0.806*
23	NL 449	1.755	-0.667	-1.213	-0.027	1.241	0.857**	0.111	1.215**	0.738
24	NL 450	0.088	1.333	4.054	0.056	-0.037	0.274	-0.059	-0.296**	-0.82**
25	NL 451	1.088	0	3.118	0.084	1.277	-0.210	1.777**	-0.376	0.008
26	NL 452	-1.245	0.333	-2.09	0.523**	-10.903**	0.157	0.344	-0.196	-1.423**
27	NL 453	2.088*	-0.333	1.771	0.473	10.125	-0.876**	-1.939**	-0.418	-1.062
28	NL 454	1.588	1.667	-0.454	-0.486*	-10.684**	-0.143	-2.306**	1.374**	1.097**
29	NL 455	3.255**	-0.5	3.076**	-0.144	-6.048*	-0.643**	-0.753**	0.938**	-0.395
30	NL 456	0.755	-0.667	4.571**	0.414**	8.583**	-0.476**	1.007**	1.124	0.497
31	NL 457	1.422	0.333	1.504*	0.273	23.058**	0.557**	1.934**	0.099	-0.595
32	NL 458	-0.578	-2.333	2.621	-0.361	8.341**	0.090	3.677**	-1.118	-0.078
33	NL 459	-1.912	-4	3.254	-0.111	11.975**	0.424**	-1.116**	-0.418	-1.178**
34	NL 460	-1.912*	-2	-1.14**	-0.138	-2.942	-0.010	-2.446**	-0.729	-1.078**
	Standard error (g)	0.208	0.236	0.422	0.053	0.415	0.079	0.069	0.122	0.076
	Testers									
35	LSL 93	0.529	- 0.113	0.93	-0.007	0.669	-0.153	-0.082	0.287	-0.147
36	PKV NL 260	-0.147	-1.539	-1.749	-0.003	-0.24	0.279**	0.122	-0.449	-0.033
37	T 397	-0.382	1.652	0.819	0.01	-0.429	-0.125	-0.04	0.162	0.18
Standard eSSSrror (g)		0.847	0.958	1.714	0.213	1.686	0.321	0.281	0.495	0.307

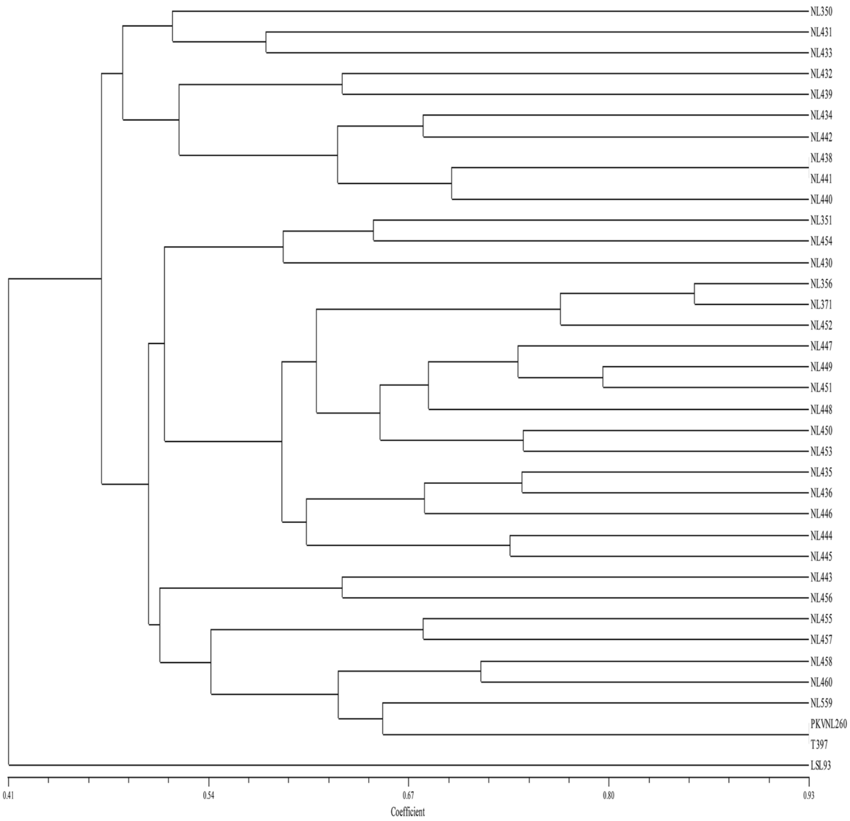

Fig. 1. Dendrogram derived from banding pattern of SSR marker analysis of 37 parents.

RESULTS AND DISCUSSION

The analysis of variance to test the significant differences in the mean values of pooled data of parents revealed that highly significant differences existed among genotypes for all nine quantitative characters. Data of high pooled mean performances for seed yield /plant, number of capsules /plant, 1000 seed weight were exhibited by the tester LSL 93 is (3.32 g), (30.25) and (11.2 g) respectively. The lines NL 450 exhibiting high mean performance for seed yield plant^{-1} was (3.2 g), number of capsules /plant (47) and 1000 seed weight (7.7 g) which was followed by NL 460 exhibiting seed yield /plant of (2.97 g), number of capsules /plant (54.3) and (9.7 g) as 1000 seed weight (Table 3). The data of pooled mean indicated presence of sufficient variability in the material used for this study which allows exploitation of the material for further analysis. Similar results were also reported by Reddy *et al.* (2013) and Pali and Mehta (2014), Kumar *et al.* (2015), Terfa and Gurmu (2020) where they also reported significant mean squares for genotypes in linseed.

General combining ability effect

The GCA effects of lines and testers were estimated for nine characters and are presented in Table 4. In linseed, positive GCA effects are desirable for all the traits studied except for days to 50 % flowering, days to maturity, plant height, budfly infestation and alternaria blight infestation for which negative GCA effects are desirable.

SSR marker studies

In any crop improvement program, genetic diversity

Table 5. Details of informative markers based on PIC, EMR and MI.

Sl. No.	Marker	Total	Number of fragments Monomo-rphic	Polymor-phic	Polymor-phism %	PIC	EMR	MI
1	Lu 2235	4	0	4	100	0.614	4	2.46
2	Lu 2332	8	2	6	150	0.780	6	4.68
3	Lu 2360	3	0	3	100	0.105	3	0.31
4	Lu 2764	4	0	4	100	0.747	4	2.99
5	Lu 3201	6	3	3	100	0.437	3	1.31
6	Lu2536	2	0	2	100	0.105	2	0.21
7	Lu 2739	3	0	3	100	0.614	3	1.84
8	Lu 1148	2	0	2	100	0.277	2	0.55
9	Lu 1165	4	1	3	75	0.475	3	1.43
10	Lu 2420	2	0	2	100	0.397	2	0.79
11	Lu 2850	3	0	3	100	0.530	3	1.59
12	Lu 2486	4	0	4	100	0.634	4	2.54
13	Lu 2509	4	0	4	100	0.284	4	1.14
14	Lu 2853	3	0	3	100	0.409	3	1.23
15	Lu 2921	4	0	4	100	0.561	4	2.24
16	Lu 2968	3	0	3	100	0.613	3	1.84
17	Lu 3180	2	0	2	100	0.315	2	0.63
18	Lu 3216	3	0	3	100	0.155	3	0.47

Note : PIC: polymorphism information content, EMR: effective multiplex ratio, MI: marker index

plays an important role. In fact, it is an essential prerequisite while initiating a breeding program. For enabling better exploitation of genetic resources, it is desirable to know the genetic diversity at morphological as well as molecular levels (Kumar *et al.* 2011). Distance-based cluster analysis was performed and dendrogram based on the unweighted pair group method of arithmetic mean (UPGMA) was constructed using Jaccard coefficient (Fig. 1).

27 SSR primers were used to evaluate 37 parents of linseed. The PCR amplified products of each primer were resolved on 3% agarose gel electrophoresis. Out of 27 SSR primers screened during present study, four primers viz., Lu 3289, Lu a58, Lu 2752, Lu 956 were found monomorphic and eighteen primers viz., Lu 2235, Lu 2332, Lu 2360, Lu 2764, Lu 3201, Lu 2536, Lu 2739, Lu 1148, Lu 1165, Lu 2420, Lu 2850, Lu 2486, Lu 2509, Lu 2853, Lu 2921, Lu 2968, Lu 3180, Lu 3216 were found polymorphic for the set of parents. The polymorphic information content (PIC) value of 18 SSR loci were calculated across 37 parents and are presented in Table 5. 18 markers showed polymorphism. The PIC values calculated for these 18 polymorphic primers were in the range of 0.105 (Lu 2360 and Lu 2536) to 0.78 (Lu 2332) seems to be highly informative and could be further utilized in evaluating other genotypes. Similar work was also conducted by Fayyaz *et al.* (2014), where 12 SSR primer combinations generated a total of 33 alleles, of that 32 were polymorphic loci, whereas only one was monomorphic locus. The primer Lu 2360 and Lu 2536 observed minimum polymorphism with PIC Value of 0.105. While Deng *et al.* (2010) stated higher average PIC value (0.56) than that found in the present experiment. Among the other studies with Indian linseed, Rajwade *et al.* (2010) studied the genetic diversity and said that lower genetic diversity (0.15) and PIC (0.18) value has been compared with our study. Among the primers used in the present study, Lu 2332 was highly informative since it recorded high polymorphic information content (PIC), effective multiplex ratio (EMR) and marker index (MI) value of 0.78, 6 and 4.68 respectively. High PIC value indicates high degree of polymorphism among the parents which helps to estimate genetic distance with more and more precision. Raza *et al.* (2018) also obtained similar PIC values ranging from 0.37 to 0.71 in which ten advanced mutant genotypes were diverged into three super clusters.

REFERENCES

Allaby RG, Peterson GW, Merriwether DA, Fu YB (2005) Evidence of the domestication history of flax (*Linum usitatissimum* L.) from genetic diversity of the sad 2 locus. *Theor Appl Genet* 112:58–65.

Anonymous (2018) Annual Progress Report of Linseed: AICRP (Linseed). ICAR pp 126-127.

Allard RW (1960) Principle of Plant Breeding. John Willey & Son's, Inc. New York.

Cerda S, Duguid J, Booker H, Rowland G, Diederichsen A, Cloutier S (2014) Association mapping of seed quality traits using the Canadian flax (*Linum usitatissimum* L.) core collection. *Theor Appl Genet* 127: 881–896.

Dellaporta (1983) Plant Molecular Biology. *Reporter* 4: 19-21.

Deng X, Long SH, He DF, Li X, Wang YF, Liu J, Chen XB (2010) Development and characterization of polymorphic microsatellite markers in *Linum usitatissimum*. *J Pl Res*.123:119–123.

Fayyaz F, Rabbani, Iqbal S, Mehwishand I, Nawaz. (2014) Genetic diversity analysis of *Brassica napus* / *Brassica campestris. Pak J Bot* 46 (3): 779-787.

Gill S (1987) Linseed. ICAR publication, New Delhi, India, pp 342–355.

Hickey M (1988) 100 families of flowering plants, 2nd edn. University Press, Cambridge.

Jiang GL (2013) Molecular Markers and Marker-Assisted Breeding in Plants. Plant Breeding from Laboratories to Fields. doi:10.5772/52583.

Kempthorne O (1957) An Introduction to general statistics. John Wiley and Sons. Inc. New York. Chapman and Hall Ltd. London, pp 468-470.

Kumar H, Anubha M, Vishwakarma K, Lal P (2011) Morphological and molecular characterization of *Brassica rapa* ssp. yellow sarson mutants. *J Oilseed Brassica* 2 (1) : 1-6.

Kumari A, Paul S, Sharma V (2017) Genetic diversity analysis using RAPD and ISSR markers revealed discrete genetic makeup in relation to fiber and oil content in (*Linum usitatissimum* L.) genotypes. *Nucleus*. 12: 56-59.

Kumar N, Satish P, Ranjana P (2015) Assessment of genetic variability, heritability and genetic advance for seed yield and it's attributes in linseed (*Linum usitatissimum* L.). Plant Archives 15(2): 863-867.

Liu F, Zhuang BC, Zhang JS, Chen SY (2000) Construction and analysis of soybean genetic map. Yi Chuan Xue Bao 27(11):1018-26.

Pali V, Mehta N (2014) Combining ability and heterosis for seed yield and its attributes in linseed (*Linum usitatissimum* L.). *The Bioscan* 9(2): 701-706.

Perrier X, Jacquemoud JP (2015) DARwin software 2006.

Rajwade AV, Arora RS, Kadoo NY, Harsulkar AM, Ghorpade PB, Gupta VS (2010) Relatedness of Indian flax genotypes (*Linum usitatissimum* L.): An inter-simple sequence repeat (ISSR) primer assay. *Mol Biotechnol* 45:161–170.

Raza A, Mehmood S, Ashraf F, Khan AR (2018) Genetic diversity analysis of *Brassica* species using PCR-based SSR markers. Springer Nature.

Reddy MP, Arsul BT, Shaik NR, Maheshwari JJ (2013) Estimation of heterosis for some traits in linseed (*Linum usitatissimum* L.). *J Agric Vet Sci* 2(5): 11-17.

Roldan R, Dendauw J, Bockstaele E, Depikar A (2000) AFLP markers reveal high polymorphic rates in ryegrasses (*Lolium* spp.). *Mol Breed* 6 (2): 125-134.

Sing VK, Sharma V, Chaudhary M, Paswan SK, Ahmad A, Verma M, Chauhan MP (2016) Combining ability analysis in linseed (*Linum usitatissimum* L.) for improvement of seed yield and its component traits. *J Appl Natural Sci* 8(1): 1-4.

Terfa GN, Gurmu GM (2020) Genetic variability, heritability and genetic advance in linseed (*Linum usitatissimum* L.) genotypes for seed yield and other agronomic traits. *Oil Crop Sci* 5: 156-160.

Varshney K, Marcel T, Russel R, Roder M, Stein N, Waugh R, Langridge P, Niks R, Graner A (2007) A high density barley microsatellite consensus map with 775 SSR loci. *Theor Appl Genet* 114(6): 1091-1103.

Vinu V, Singh N, Vasudev S, Yadava DK, Kumar S, Naresh S, Bhat SR, Prabhu KV (2013) Assessment of genetic diversity in *Brassica juncea* (Brassicaceae) genotypes using phenotypic differences and SSR markers. *Int JTrop Biol* 61(4): 1919-1934. www.UPOV.int.