AATCC Review

29 January 2023: Received
02 June 2023: Revised
07 August 2023: Accepted
10 October 2023: Available Online

www.aatcc.peerjournals.net

Research Article Open Access

Wide Hybridization of Linseed species to enhance resistance to Budfly and Alternaria blight using morphological characters and SSR markers

Renjini J. S[1]*, Beena Nair[2], J.G. Manjaya[3], Rani Jadhav[2], S.K. Gupta[3], Vandana Madke[1]

[1]Agricultural Botany Section, College of Agriculture, Nagpur, India.
[2]AICRP on Linseed and Mustard, College of Agriculture, Nagpur, India.
[3]Baba Atomic Research Centre, Trombay, Mumbai, India, (Dr. Panjabrao Deshmukh Krushi Vidyapeeth, Akola, MH-440001, India)

ABSTRACT

In the present study the parents, F_1 crosses, and F_2 individuals were screened on the field condition to evaluate resistance to budfly and alternaria blight in linseed. The parental lines included wild accessions exhibiting resistance and susceptibility to budfly and alternaria blight. EC-993391 and IC-633096, displayed minimal infestation (0), while others, like EC-993389, were more susceptible, with infestation levels reaching as high as 27.8 for bud fly and 21.88 for alternaria blight. Notably, the F_1 hybrids T-397 × IC-633096 and T-397 × IC-633096 (treated with 0.15 mg/L in the shoot tip) displayed resistance to budfly and alternaria blight. Although, the overall F_2 population was moderately susceptible to bud fly and alternaria blight, specific individual plants within this generation exhibited lower infestations, suggesting their potential as valuable pre-breeding materials. Molecular studies using identified specificSSR markers including "Lu 2853, Lu 2850, Lu 2840, Lu 2332," which distinguished F_1 hybrids derived from the cross between T-397 × EC-993389 from the parental lines. Besides molecular markers, morphological characteristics such as plant height, number of branches plant^{-1}, capsule size, seed size, and 1000 seed weight were also assessed. Breeding strategies included the possibility of intermating selected F_2 plants to preserve resistance to both budfly and alternaria blight while potentially disrupting undesirable linkages between different traits.

Keywords: *Interspecific, Linseed, Bud fly infestation, Alternaria blight infestation, SSR markers*

I. INTRODUCTION

Linseed, or flax, is an annual, self-pollinating, autogamous diploid plant with a chromosomal count of 2n=2x=30. It belongs to the *Linaceae* family, encompassing 14 genera and over 200 species [5]. In India, the cultivation of linseed is challenged by several diseases and insect pests, resulting in significant yield losses. Notably, bud fly (*Dasyneuralini*) infestations can cause losses ranging from 20% to as high as 97%, while Alternaria blight *(Alternaria lini*) and powdery mildew (*Odium lini*) contribute to losses of up to 60% [11]. Leaf infections are responsible for a range of 27% to 60% of these losses, with bud infections leading to staggering losses of up to 90%. Reports from Kanpur suggest that Alternaria blight can result in losses of 28% to 60% [3].

In response to these challenges, the development of linseed varieties with robust resistance to these pests and diseases has become a necessity. In this context, the untapped potential of the wild gene pool, specifically *L. grandiflorum* (for countering budfly and Alternaria blight) and *L. bienne* (for enhancing oil and fiber quality and bolstering resistance against biotic and abiotic stressors), needs to be explored through well-structured breeding programs.

*Corresponding Author: ***Renjini J. S***
*Email Address: ***renjinijs1997@gmail.com***

DOI: https://doi.org/10.58321/AATCCReview.2023.11.04.162

The scarcity of sources offering resistance against Alternaria blight and bud fly, coupled with limited knowledge regarding the genetic mechanisms underpinning resistance and tolerance, underscores the importance of securing the long-term sustainability of linseed production. The present study was initiated to address these gaps in linseed production research.

II. MATERIALS AND METHODS

Two genotypes of *L. bienne* (EC-993391, EC-993389) and seven genotypes of *L. grandiflorum* [IC-633096, IC-633096 – yellow anther, IC-633096 (0.10 mg/L colchicine treated bud - black anther- 72 hrs), IC-633096 (0.15 mg/L colchicine treated bud - black anther - 72 hrs), IC-633096 (0.20 mg/L colchicine treated bud - black anther - 72 hrs, IC-633096 (0.05 mg/L colchicine treated bud - black anther - 72 hrs,IC-633096 (0.15 mg/L colchicine treated shoot tip - black anther - 24 hrs)] were crossed with T-397 *(L.usitatissimum)*during *rabi* 2022. The F_1 crosses were grown along with the parents in a non-replicated trial with the spacing 30 × 10 cm. A recommended package of practices was followed to raise a good crop. The data were recorded on 5 competitive plants in each parent and F_1 (Table 1)and all F_2 plants (Table 2) for days to maturity, plant height, capsule size, seed size, 1000 seed weight, % bud fly infestation, % alternaria blight infestation[4] and [13]. Statistical analysis was done for mean, variance, and coefficient of variation(%) as per standard formulae suggested by [10].

Field screening

The intensity of disease in the field was estimated from five randomly selected plants which were tagged with labels, at the flowering stage of the crop. Individual plant was scored for budfly infestation. In each plant buds infected by bud fly

(*Dasyneura lini*) were counted and the percentage was taken from the total number of buds as follows.

$$\text{Bud fly infestation (\%)} = \frac{\text{Number of infected buds} \times 100}{\text{Total no. of buds in the plant}}$$

Categorization of bud fly infestation[1].

Score	Category	Symbol	Bud fly infestation (%)
1	Resistant	R	Upto 10.00
2	Moderately resistant	MR	10.01 to 25.00
3	Moderately susceptible	MS	25.01 to 50.00
4	Susceptible	S	50.01 to 75.00
5	Highly susceptible	HS	>75.00

Infected buds by Alternaria lini were counted in each plant and percentage was taken from the total number of buds.

$$\text{Alternaria blight infestation (\%)} = \frac{\text{Number of infected buds} \times 100}{\text{Total no. of buds in the plant}}$$

III. RESULTS AND DISCUSSION

Data on the mean performance of parents and F_1's for various morphological characters are presented in Table 1.

The morphological characters of F_1 crosses revealed that some of the combinations performed better over their respective parents for days to maturity, % bud fly infestation, % alternaria blight infestation, number of branches plant^{-1}, capsule size, and seed size. The mean value of the F_1 for days to maturity showed that all F_1 attained earlier maturity than their parents. 1000 seed weights of crosses were intermediate between the parents involved in the crosses. [8] reported a wide range of variation among the crosses for character 1000 seed weight. [9] reported the same conclusion that there was variation in 1000 seed weight amongst the species and 1000 seed weight of all hybrids was intermediate between the parents. The number of branches plant^{-1} were lower for *Linum grandiflorum* species (control). The F_1 hybrid obtained from the cross between T-397 and IC-633096 showed the lowest value among the crosses, while the highest number of branches plant^{-1} were found in T-397 × EC-993391. Thus, a wide range of variation was observed among crosses.T-397 × IC-633096 (Y) was tallest among F_1 crosses. [8] also put forward the same conclusion in Mustard interspecific crosses that a wide range of variation was observed among crosses for the character number of branches plant^{-1}. The F_1 cross between T-397 and EC-993389 showed higher vigor than their parents.

Analysis conducted in F_2 generation (Table 2) showed that the progeny of T-397 × EC-993389 showed higher variation for characters like % bud fly infestation, % Alternaria blight infestation, number of branches plant^{-1}, capsule size and 1000 seed weight. Maximum variation was found for the character 1000 seed weight followed by % Alternaria blight infestation. Those F_2 plants showing more seed size also showed more test weight (1000 seed weight). The plant height of F_2 progenies was also higher than the F_1 crosses and their wild parents. Height was one of the key traits for assessing the length of fiber in flax. The study revealed that the height of T-397 × EC-993389 was better than all the hybrids. It can be further utilized in fiber breeding programmes.

Table 1. Mean performance of parents and F1's for various morphological characters

Sr. No	Parents and F_1	Days to maturity	Plant height (cm)	Number of branches plant^{-1}	Bud flyinfestation (%)	Alternaria blight infestation(%)	Capsule size(mm)	Seed size (mm)	1000 seed weight (g)
1	T-397	91.00	32.12	5.26	21.2	20.2 (28.28%)	7.60	4.70	7.50
2	EC-993391	91.00	27.10	4.45	0.0	21.1 (29.83%)	4.80	2.70	4.20
3	EC-993389	91.00	24.00	5.20	27.8	18.5 (26.16%)	4.20	2.50	4.00
4	IC-633096	93.00	34.98	4.60	0.0	8.4 (11.87%)	8.70	4.90	6.70
5	IC-633096 (0.10 mg/L)	94.00	32.08	3.80	18.5	17.3 (24.46%)	8.30	4.50	6.40
6	IC-633096 (0.15 mg/L)	95.00	29.70	4.40	20.00	21.7 (30.68%)	6.20	4.60	5.50
7	IC-633096 (0.20 mg/L)	94.00	29.58	5.40	14.10	21.3 (30.1%)	7.90	4.70	5.90
8	IC-633096 (0.05mg/L)	95.00	28.80	5.00	15.50	14.7 (20.7%)	7.00	4.20	6.00
9	IC-633096 (0.15 mg/L - shoot tip)	95.00	29.40	4.80	24.30	19.5 (27.57%)	6.40	4.50	6.20
10	IC-633096 (Y)	94.00	32.96	5.20	19.30	13.4 (18.95%)	7.60	4.50	5.60
11	T-397 × EC-993391	89.00	23.84	5.40	13.20	16.4 (23.19%)	6.60	4.18	5.50
12	T-397 × EC-993389	89.00	30.78	5.00	24.00	16.8 (23.75%)	6.74	3.76	5.41
13	T-397 × IC-633096	90.00	30.00	4.50	9.60	10.9 (15.41%)	8.22	5.02	6.17
14	T-397 × IC-633096 (0.10 mg/L)	91.00	29.30	4.80	18.60	20.9 (29.55%)	8.16	5.14	6.31
15	T-397 × IC-633096 (0.15 mg/L)	93.00	31.20	4.40	26.00	12.2 (17.25%)	7.80	4.74	5.76
16	T-397 × IC-633096 (0.20 mg/L)	91.00	32.52	5.20	33.40	13.9 (19.65%)	7.40	4.80	5.90
17	T-397 × IC-633096 (0.05 mg/L)	92.00	31.00	4.20	21.00	10.6 (14.99%)	6.20	4.26	6.36
18	T-397 × IC-633096 (0.15 mg/L - shoot tip)	91.00	31.32	5.30	19.80	9.5 (13.43%)	6.40	4.10	6.12
19	T-397 × IC-633096 (Y)	90.00	36.42	4.60	13.6	12.0 (16.97%)	7.60	4.58	5.81

Table 2. Mean, Variance and Coefficient of variation for various characters of parents and F_2

Sr. No	Character	Parameter	Parents					F_2 population			
			T-397	EC-993391	EC-993389	IC-633096	*L. grandiflorum*	T-397 × EC-993391	T-397 × EC-993389	T-397 × IC-633096	T-397 ×*L. grandiflorum*
1	Days to maturity	Mean	91.40	90.60	91.20	94.20	94.60	92.40	91.60	91.00	94.80
		Variance	16.70	4.50	21.20	6.30	8.80	58.30	52.80	34.50	34.70
		CV	4.50	2.41	4.85	2.71	3.24	8.26	7.93	6.45	6.21
2	Plant height (cm)	Mean	33.88	30.03	26.39	33.99	35.57	29.20	31.00	34.20	31.40
		Variance	1.58	0.25	0.19	0.27	1.19	12.70	25.50	17.20	32.80
		CV	3.71	1.66	1.63	1.66	3.06	12.20	16.28	12.12	18.23
3	Number of branches plant^{-1}	Mean	5.66	4.45	5.20	4.60	3.80	5.20	5.30	4.60	4.50
		Variance	0.46	0.26	0.41	0.36	0.42	5.80	5.20	4.00	4.32
		CV	11.98	11.45	12.31	13.04	17.05	46.31	43.02	49.26	46.18
4	Bud fly infestation (%)	Mean	18.24	0	25.25	0	18.18	21.38	14.88	21.39	21.33
		Variance	9.70	0	11.88	0	9.04	37.03	38.96	16.48	38.81
		CV	17.07	0	13.65	0	16.53	28.46	41.94	18.97	29.20
5	Alternaria blight infestation (%)	Mean	20.20	21.88	20.59	7.89	9.77	18.07	17.90	17.11	17.80
		Variance	6.68	4.71	6.58	5.83	4.46	68.74	82.54	80.51	121.73
		CV	12.79	9.91	12.45	13.37	21.61	45.87	50.75	52.44	61.98
6	Capsule size (mm)	Mean	6.40	4.18	4.20	8.14	8.39	5.12	5.26	6.30	5.19
		Variance	0.03	0.02	0.03	0.07	0.13	0.72	2.23	1.78	1.69
		CV	2.70	3.38	4.12	3.25	4.29	16.66	28.44	21.17	25.04
7	Seed size (mm)	Mean	4.9	2.7	2.5	5.0	5.1	3.78	3.46	4.69	4.84
		Variance	0.04	0.03	0.01	0.02	0.05	0.05	0.10	0.09	0.11
		CV	4.08	6.41	4.00	2.82	4.38	5.91	9.13	6.39	2.70
8	1000 seed weight (g)	Mean	7.50	4.25	4.06	6.73	6.49	4.45	4.29	5.75	6.05
		Variance	0.22	0.17	0.07	0.21	0.27	8.31	12.62	11.46	13.94
		CV	6.25	9.70	6.51	6.80	8.00	64.77	82.80	58.87	61.71

Table 3. Selection of superior F2 for % budfly infestation and % alternaria blight infestation

Crosses	Plant No.	Bud fly infestation (%)	Plant No.	Alternaria blight infestation (%)
T-397 × EC-993391	3 5	6.56% 5.23%	3	8.34%
T-397 × EC-993389	4	6.85%	5 9	5.91% 9.23%
T-397 × IC-633096	3 8	7.21% 3.55%	2 8	7.42% 8.69%
T-397 ×*L. grandiflorum*	4 9	5.55% 7.22%	5	2.64%

In the F_2 population, certain individual plants displayed lower infestation levels for both bud fly
(% bud fly) and Alternaria blight (% alternaria blight). These exceptional plants have been identified for further use as pre-breeding material.
For bud fly infestation, two plants (plant no: 3 and 5) in the F_2 progeny of the T-397 × EC-993391 cross showed remarkable resistance, with infestation rates of 6.56% and 5.23%, respectively. These plants demonstrated a strong resistance to bud fly infestation compared to the other plants, which showed various levels of susceptibility.
In the case of Alternaria blight, one plant (plant no: 3) from the F2 progeny of the T-397 × EC-993391 cross displayed a resistance level of 8.34%, making it the only plant resistant to Alternaria blight, while the rest showed different degrees of susceptibility. Moreover, Plant 2 in the same cross exhibited resistance to both bud fly and Alternaria blight infestations.
From the F_2 progeny of the T-397 × EC-993389 cross, one plant (plant no: 4) exhibited resistance to bud fly infestation, with a rate of 6.85%. In the case of Alternaria blight infestation, two plants (plant no: 5 and 9) from the same cross displayed resistance, with infestation rates of 5.91% and 9.23%, respectively. These plants showcased resistance, while others in the population exhibited varying degrees of susceptibility.
Similarly, from the F_2 progeny of the T-397 × IC-633096 cross, two plants (plant no: 3 and 8) displayed resistance to bud fly infestation, with rates of 7.21% and 3.55%, respectively. For Alternaria blight infestation, two plants (plant no: 2 and 3) from the same cross exhibited resistance, with infestation rates of 7.42% and 8.69%, respectively. Among the selected plants, Plant no: 3 showed improved resistance to both bud fly and Alternaria blight infestations.
A total of 13 F_2 plants, comprising seven resistant to bud fly and six to Alternaria blight, will be advanced to the F_3 generation. This will allow for the attainment of homozygosity over one or more generations. These selected plants will serve as valuable sources of resistance to bud fly and Alternaria blight infestations.
Among the 7 plants chosen for bud fly resistance and the 6 for Alternaria blight, two plants exhibited resistance to both bud fly and Alternaria blight. These plants, specifically Plant 3 from the T-397 × EC-993391 cross and Plant 8 from the T-397 × IC-633096 cross, can be utilized as valuable breeding materials for developing resistance to both bud fly and Alternaria blight. Additionally, intermating between these selected F_2 plants offers the potential to preserve resistance to both budfly and alternaria blight and break undesirable trait linkages.

MOLECULAR STUDIES

To verify the genetic purity of hybrids, a study was conducted using SSR markers involving 10 parental lines and 9 F1 crosses. Genomic DNA was extracted using the mini kit method from 100 mg of plant tissue ground with liquid nitrogen. After adding lysis buffer and RNase A, the mixture was incubated and then treated with a precipitation solution. The resulting lysate was purified using a QIA shredder spin column. Further purification was achieved through a series of wash steps with Buffer AW1 and Aw2.

For PCR amplification, 19 SSR primers were utilized in 20 µl reaction mixtures, including template DNA, PCR buffer, MgCl2, dNTPs, primer mix, and Taq polymerase. The thermal cycler was programmed for 40

This study employed SSR marker techniques to identify 9 linseed hybrids and their respective cycles, and the resulting PCR products were analyzed on a 3% agarose gel stained with ethidium bromide, with UV light used for visualization.parental lines (Table 1). SSR markers, known for their high polymorphism and co-dominant nature, were used. Among the 9 crosses, the hybrid resulting from the T-397 × EC-993389 cross could be uniquely identified using specific SSR markers. Four SSR markers, namely "Lu 2853, Lu 2850, Lu 2840, Lu 2332," were found to offer distinctive banding patterns for this F_1 hybrid, distinguishing it from its male and female parents. These SSR markers played a crucial role in discerning the F_1 hybrid from its parental lines by showcasing their unique banding patterns.
In hybrid plants, the SSR marker Lu 2850 amplified the allele of size 270bp in the female parent (T-397). The pollen parent (EC-993389) had an amplicon at 230bp. However, the F_1 hybrid exhibited both the alleles of the parents confirming the heterozygosity of the hybrid by having two bands at 270 and 230bp. The hybrid could also be distinguished by SSR marker Lu 2840. The marker had an amplicon of size 250bp in its female parent (T-397). The same marker had another amplicon of size 280bp in the pollen parent (EC-993389). The banding pattern of this hybrid showed both the amplicon at 250 and 280bp.
The Lu 2332 marker amplified the allele of size 250pb in the female parent (T-397). The pollen parent (EC-993389) had an amplicon at 300bp. However, the F_1 hybrid exhibited both the alleles of parents confirming the heterozygosity of the hybrid by having two bands at 250 and 320bp. The hybrid can also be distinguished by the marker Lu 2853. The marker had an amplicon of size 300bp in its female parent (T-397). The same marker had another amplicon of size 270bp in the pollen parent (EC-993389). The banding pattern of this hybrid showed both the amplicon at 300 and 270bp which was important to distinguish the F_1 from their male and female parents.
Simple Sequence Repeat (SSR) markers are of great importance for rapid assessment of hybrid and parental lines seed purity [15] and [12]. The use of SSR markers for genetic purity testing has also been demonstrated in maize [14], in rice [6], in cotton [2] and in sunflower [7].

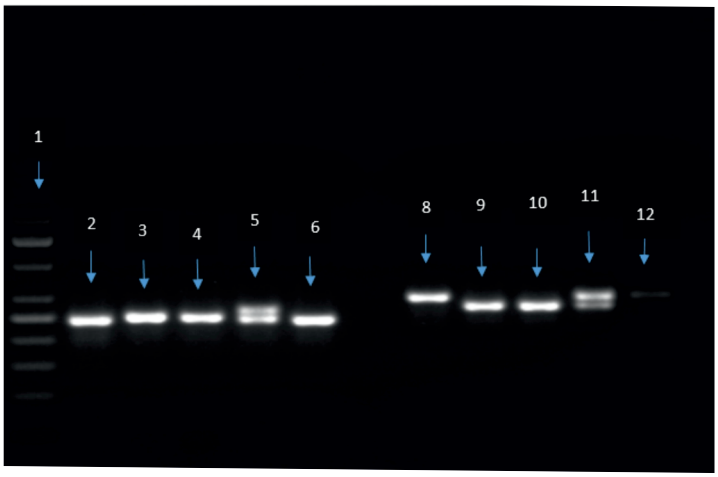


Plate 1. SSR banding profile of parents and hybrids amplified with primers Lu 2840 and Lu 2332. Lane 1 - Ladder, lane 2,8 - EC-993391, lane 3,9- T-397 × EC-993391, lane 4,10 -T-397, lane 5,11 - T-397 × EC-993389, lane 6,12 - EC-993389

IV. CONCLUSION

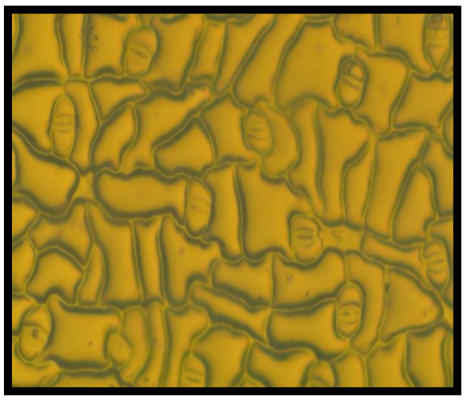
Furthermore, an analysis conducted in the F2 generation also revealed greater diversity in traits such as the number of

branches plant-1, capsule size, 1000 seed weight, as well as the percentages of bud fly infestation and Alternaria blight infestation among the offspring resulting from the cross between T-397 and EC-993389. This heightened diversity in these traits indicated the separation of characteristics within the F2 generation. Consequently, based on both molecular and morphological investigations, it can be inferred that T-397 × EC-993389 represents a genuine hybrid. Despite the fact that the overall population displayed higher than anticipated levels of bud fly and Alternaria blight infestations, certain plants within the population exhibited resistance to these diseases. This suggests that individual plant selection can be employed for further breeding and research in subsequent generations.

T-397

EC-993389

T-397×EC-993389



Stomata size of T-397 × EC-993389

Plate 2. T-397 × EC-993389 (F_1) and their parents along with stomata size of T-397 × EC-993389

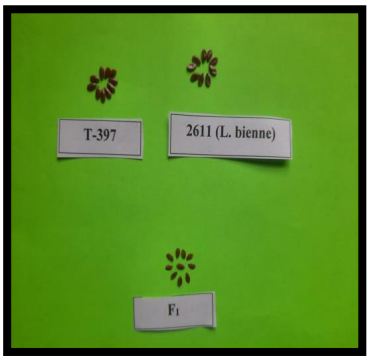
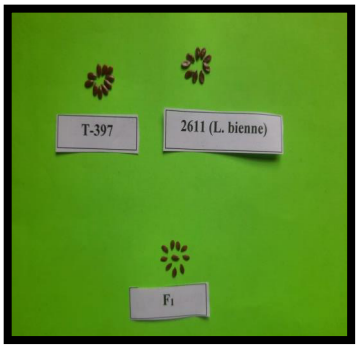


Plate3. Capsule size and seed size of T-397 × EC-993389 and T-397 × L. grandiflorum



V. ACKNOWLEDGEMENT

We are thankful to AICRP on Linseed and Mustard, College of Agriculture, Nagpur, for providing facilities and support during the course of our studies.

VI. REFERENCES

1. Anonymous. 2018. Annual report of linseed. pp.208-220.
2. Ashok, D. and P. Vilas, 2005. Identification of Cotton hybrid through Combination of PCR Based RFLP, ISSR and Microsatellite Markers. J. Plant Biochem. Biotechnol. 14: 53-55.
3. Chauhan, L. S and Srivastava, K. N. 1975. Estimation of loss of yield caused by blight disease of linseed. Indian J. Farm Sci. 3: 107-109.
4. Ghige, A. B., Nair, B., Manjaya, J. G., Jadhav, R.A., Katore, J. R, 2022. Genetic Diversity and selection of parents of Linseed (*Linum usitatissimum* L.) Germplasm using Morphological and Molecular Characterization. J. Ecol. Environ. 40(3): 1214-1221.
5. Kumar, N., S. Paul, H. K. Chaudary, N. S. Jamwal and A. D. Singh, 2016. Wide hybridization and characterization of hybrids of *Linum usitatissimum* L. for crossability, agro-morphological traits and rust resistance. SABRAO J. Breed Genet. 48: 136-144.
6. Nandakumar, N., A. K. Singh, R. K. Sharma, T. Mohapatra, K. V. Prabhu and F. U. Zaman, 2004. Molecular fingerprinting of hybrids and assessment of genetic purity of hybrid seeds in rice using microsatellite markers. Euphytica. 136: 257-264.
7. Pallavi, H. M., R. Gowda, Y. G. Shadakshari, K. Bhanuprakash and K. Vishwanath, 2011. Identification of SSR Markers for Hybridity and Seed Genetic Purity Testing in Sunflower. Helia. 34(54): 59-66.
8. Patil, S., V. Khambalker, P. P. Gawande and S. U. Charjan, 2003 Studies on interspecific hybridization in Brassica Species. J. Soil. Crop. 13(2): 343-347.
9. Seetharam, 1972. Euphytica, 21(3): 489-495.
10. Singh, F.R.K. and Chaudhary, D. 1977. Biometrical Methods in Quantitative Genetic Analysis.

11. Srivastava, R.L., Singh, J., Husain, K., Malik, Y.P., Dubey, S.D., Rai, J. and Bajpai, M. (1997). Linseed: In-Efficient Management of Dryland crops in India. Oilseed, pp. 228-256.

12. Sundaram, R. M., B. Naveenkumar, S. K. Biradar, S. M. Balachandran, B. Mishra, M. Ilyasahmed, B. C. Vikraktamanth, M. S. Ramesh and N. P. Sarma, 2008. Identification of informative SSR markers capable of distinguishing hybrid rice parental lines and their utilization in seed purity assessment. Euphytica. 163: 215-224.

13. Thikare, D. S, Nair, B., Jadhav, R. 2023. Morphological characterization of linseed (*Linum usitatissimum* L.) germplasm using DUS descriptors. J. Ecol. Environ. 41(1A): 273-283.

14. Wang, J., G. Y. Zhong, E. C. L. Chin, J. C. Register, R. D. Riley, W. S. Nieber and J. S. C. Smith, 2002. Identification of parents of F_1 hybrids through SSR profiling of maternal and hybrid tissue. Euphytica. 124: 29-34.

15. Yashitola, J., T. Thirumurugan, R. M. Sundaram, M. K. Naseerullah, M. S. Ramesha, N. P. Sarma and R. V. Sonti, 2002. Assessment of purity of rice hybrids using microsatellite and STS markers. Crop Sci. 42: 1369-1373.

1.

2.

3.

4.

5.

6.

7.

8.

9.

10.