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FULL-LENGTH RESEARCH ARTICLE

Genetic Diversity, Combining Ability and Stability Analysis of Linseed (*Linum Usitatissimum*) Under Acidic Soil Conditions in the North-Eastern Region of India

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Abstract

Linseed is a potential oilseed crop with various industrial and nutraceutical applications. The climatic conditions of the North-Eastern Region of India favour linseed cultivation, but the crop occupies a limited area and yields remain low. This is primarily due to soil acidity, which leads to phosphorus deficiency, as well as iron and aluminium toxicity in the soil. A set of 35 linseed genotypes was grown in the ICAR research complex for the NEH region, and the genotypes were evaluated for eight yield contributing traits. Analysis of variance revealed a significant difference among the genotypes for all the traits, indicating a considerable variability among the genotypes. Pearson correlation studies revealed a positive and significant correlation of yield per plant with number of capsules per plant and number of secondary branches per plant. Further, the hierarchical clustering method grouped 35 genotypes into four different clusters. Among that, cluster I consists of 23 genotypes, and clusters II, III and IV consist of six, five and one (NL294) genotypes, respectively. Additionally, AMMI and GGE biplot analysis revealed stable and high-yielding genotypes NL356, BAU-15-06 and T397 across environments. The genotypes SLS115 and PKDL166 were identified as high-yielding and moderately stable genotypes. Based on yield performance, five parental lines, namely RLC163, SLS115, RLC164, LMS-2015-14 and T397, were selected, and a total of 20 crosses were made in diallel fashion, including the reciprocals. GCA effects were non-significant for all traits, indicating the presence of non-additive gene action. Specific combining ability variances showed a significant variation for all traits except yield per plant. Among 20 crosses, C8 (SLS115 XT397) exhibited a high SCA effect for plant height, primary branches per plant, secondary branches per plant and performed better under acidic soil conditions. Thus, the population was advanced up to the F_5 generation, and the mean value comparison for selected plants of F_3, F_4 and F_5 generations revealed that the F_5 generation exhibited a higher mean value than the F_3 and F_4 generations, suggesting that the population performance is moving in a positive direction for yield-contributing traits. The presence of non-additive gene action suggests the use of heterosis breeding for the genetic improvement of the traits. The present study identified that the genotypes NL356, BAU-15-06, T397, SLS115 and PKDL166 can be used as parents in the future breeding programme and that the genotypes SLS115 and RLC163 were identified as the best combiners to increase yield per plant.

Keywords Acidic soil · Combining ability · Diallel analysis · Genetic diversity · Linseed

Introduction

Linseed (*Linum usitatissimum* L.) is an annual, self-pollinated, diploid plant with a chromosome number of 2n = 30 and belongs to the family Linaceae. It is also known as flax, which is well-suited to temperate climate conditions and used as a source of cellulose-rich bast fibres and high-quality oil. Linseed oil contains 40–50% α-linolenic (omega-3 fatty acid) acid, which is rich in phytosterols and tocopherols. These bioactive components can enhance the human immune system, prevent inflammation, reduce the absorption of bad cholesterol and contribute to the prevention of cardiovascular diseases [31].

Linseed occupies an area of 3.22 mha, yielding 3.01 mtonnes with an average productivity of 952 kg/ha. India is the fifth largest producer with an area of 1.7 lakh ha, with

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Extended author information available on the last page of the article about 1 lakh tonnes production and 574 kg/ha productivity [2]. The North-East Hill Region (NEHR), where the climate is highly favourable for linseed production but still has low area, production and productivity due to extreme forms of soil acidity with pH below 5.5, which causes Al^{3+} toxicity and phosphorus deficiency [11, 24, 41]. After the rainy season, the main rice crop land remains fallow in most of the NEHR.

The commercial cultivation of linseed in India is primarily for its seed containing oil, and it possesses several industrial applications, such as the manufacturing of paints, varnishes, soaps, printing inks and linoleum [8, 40]. After synthetic paints became a cheaper option, there has been increased interest in linseed cultivation due to its wide range of industrial properties. In addition to that, linseed also possesses several nutraceutical applications, high omega-3 fatty acids and high lignans known for anti-cancer properties [5, 17].

Considering the potential industrial and medicinal values, it is essential to assess the genetic diversity and identify the suitable parents for linseed improvement in the NEH region. Since the climatic conditions are favourable for linseed cultivation and to increase the cropping intensity of the NEHR region, the present study was carried out to identify stable linseed genotypes that can be grown under adverse soil conditions. Further, the identified lines can be used as parents in the hybridization programme to develop elite progenies.

Materials and Methods

Experimental Design and Plant Material

A set of 35 linseed genotypes obtained as test entries for rainfed ecosystems under AICRP (All India Coordinated Research Project) on linseed were used in the present study (Online Resource 1). The materials were evaluated at the Indian Council of Agricultural Research Complex for North-East Hill Region (ICAR-RC NEH) plant breeding farm at Umiam, Meghalaya, in three winter seasons, i.e. *rabi* 2018 (E1), *rabi* 2019 (E2) and *rabi* 2021(E3). The metrological data, including maximum temperature (°C), minimum temperature (°C), rainfall (mm) and radiation (W/m^2) for the cropping seasons, are presented in Online Resource 2. The experiment was laid out in a randomized block design with three replications. The genotypes were grown in rows by maintaining row length of 1.5 m, row-to-row spacing of 25 cm and plant-to-plant spacing of 5 cm, and each genotype was grown in three rows.

Yield and yield-contributing traits like plant height (PH), days to 50% flowering (DF), number of primary branches per plant (PB), number of secondary branches per plant (SB), number of capsules per plant (CPP), number of seeds per capsule (NSPC), biological yield (BY) and yield per plant (YPP) were recorded on ten random plants from each genotype.

Development of Diallel Crosses and Evaluation of Crosses

Based on the seed yield performance under field conditions (*rabi* 2017) (unpublished data), five genotypes, namely RLC163 (P1), SLS115 (P2), RLC164 (P3), LMS-2015-14 (P4) and T397 (P5), were selected as parents, and diallel crosses, including reciprocals, were made in *rabi* 2018 (Online Resource 3). The F_1 plants were confirmed based on field performance, and the seeds of 20 crosses and five parents were sown in a randomized block design (RBD) with three replications in *rabi* 2019. Standard agronomic practices were carried out to raise a healthy plant [8]. Ten random plants from each cross as well as parents were used to record data for the morphological traits like plant height (PH), days to 50% flowering (DF), number of primary branches per plant (PB), number of secondary branches per plant (SB), inflorescence length (IL), root length (RL), number of capsules per plant (CPP), number of seeds per capsule (NSPC), plant weight (PW), test weight (TW), harvest index (HI) and grain yield per plant (YPP). The mean value of the ten plant data was subjected to analysis of variance and combining ability analysis. The crosses were advanced by single-seed descent (SSD) method [9, 16], and the phenotypically best-performing cross C8 (SLS115 × T397) was further evaluated for seed yield and related traits at F_3 (*rabi* 2020), F_4 (*rabi* 2021) and F_5 (*rabi* 2022) generations.

Statistical Analysis

The mean data of 35 linseed genotypes recorded over three seasons were used for the analysis of variance using the '*agricolae*' package in RStudio [25]. Variability parameters like GCV (genotypic coefficient of variation) and PCV (phenotypic coefficient of variation), and heritability were estimated using Microsoft Excel. The mean data were subjected to Pearson correlation analysis, and a correlogram was constructed using the '*metan*' package in RStudio [28]. Further, based on morphological data, the genotypes were grouped into various clusters (unrooted clustering) by employing hierarchical clustering using the SR plot (Science and Research Plot) [38]. The stable and best genotypes over three seasons were identified using stability analysis, viz. 1. AMMI model [12] and 2. GGE biplot analysis [42] in the RStudio package '*metan*' [28]. AMMI model uses analysis of variance (ANOVA) and principal component analysis (PCA) for assessing the main effects and residual effects, respectively [13]. The GGE biplot displays both genotype (G) and genotype x environment (GE) variation for traits using the study [20]. The multi-trait stability index (MTSI) was performed using the RStudio package '*metan*' [28].

Based on grain yield performance, five parents were selected for hybridization, including normal and reciprocals, which were subjected to model 1 (parents, direct cross and reciprocals), method 1 (fixed effects) of full diallel analysis [36]. The diallel analysis was carried out using the '*Diallel Analysis R*' package in RStudio [43].

Results

Descriptive Statistics and Association Studies for Yield and Yield-Related Traits in Linseed

Thirty-five linseed genotypes were evaluated for eight yield-related traits, and the mean data are presented in Online Resource 4. Analysis of variance results showed a significant difference among the genotypes for all traits. It indicates a considerable variability among the genotypes used in the present study. Season or environment was found significant for days to 50% flowering and days to maturity, indicating their seasonal variability. Season and genotype interaction effect (SXG) was found significant for all the traits (Table 1). The genetic components of variation and heritability were calculated for all the traits, and the result showed the highest GCV for biological yield (45.75%), followed by yield per plant (25.64%) and number of capsules per plant (20.13%). For all the traits, PCV is greater than the GCV, indicating the influence of the environment on the traits studied. Heritability was observed to be highest in plant height (80.56%) and biological yield (80.49%). Medium heritability was observed for yield per plant (60.02%), number of seeds per capsule (56.79%), number of capsules per plant (41.94%), number of primary branches per plant (41.75%) and days to 50% flowering (40.84%) (Online Resource 5).

Correlation studies (Pearson correlation) were carried out between yield per plant and other yield-contributing traits (Fig. 1a). The results showed that yield per plant exhibited a positive significant correlation with number of capsules per plant ($r = 0.64$), followed by number of secondary branches per plant ($r = 0.43$). Biological yield was significantly positively correlated with plant height ($r = 0.48$) and negatively correlated with days to 50% flowering ($r = 0.46$) (Online Resource 6).

Cluster Analysis

The three-season mean data were subjected to hierarchical clustering. Based on the analysis, 35 linseed genotypes were grouped into four distinct clusters (Fig. 1b). Cluster I was the largest, containing 23 genotypes, followed by Cluster II with six genotypes, Cluster III with five genotypes and Cluster IV with only one genotype, NL294. This indicates that NL294 was the most genetically diverse compared to the other genotypes. Additionally, DLV54, RL15559, LCK1727, LMS2015-11 and NL356 formed a separate cluster, while RMLS11, RL15584, LMS2015-14, LCK1627, PKDL166 and SLS109 formed another cluster. The remaining genotypes were grouped into two major subgroups within the same cluster.

Stability Analysis of Genotypes Across Environments

AMMI Biplot Analysis

The additive main effects and multiplicative interaction (AMMI) model was used to describe the effect of yield on 35 genotypes under three environments (Fig. 2a and b). The environment E2 has a shorter vector with an IPC1 score closer to zero compared to other environments,

Table 1 Analysis of variance (ANOVA) of linseed genotypes for seed yield and other related traits

Source of variation	df	PH	PB	SB	CPP	NSPC	BY	YPP	DF
Replication	2	140.03	1.41.93.7	93.7	895.0	0.4901	45.44	3.313	29.7
Genotype	34	299.45***	6.52***	69.8***	2844.5***	2.675***	263.08***	20.69***	134.7***
Season	2	364.41*	22.27**	4811.5***	23,118.4*	16.66**	2956.93**	229.701**	7846.2***
Genotype x season	68	76.62***	3.986***	82.4**	2485.4***	1.28***	256.88***	16.963***	107.5***
Error a	4	40.22	0.968	18.7	1589.4	0.615	17.27	4.243	50.8
Error b	204	24.69	1.48	32.9	911.8	0.571	20.45	4.188	37.7

*** indicates significance at 0.1%, ** indicates significance at 1% and * indicates significance at 5%. df—degrees of freedom; PH—plant height; PB—number of primary branches per plant; SB—number of secondary branches per plant; CPP—number of capsules per plant; NSPC—number of seeds per capsule; BY—biological yield; YPP—seed yield per plant; and DF—days to 50% flowering

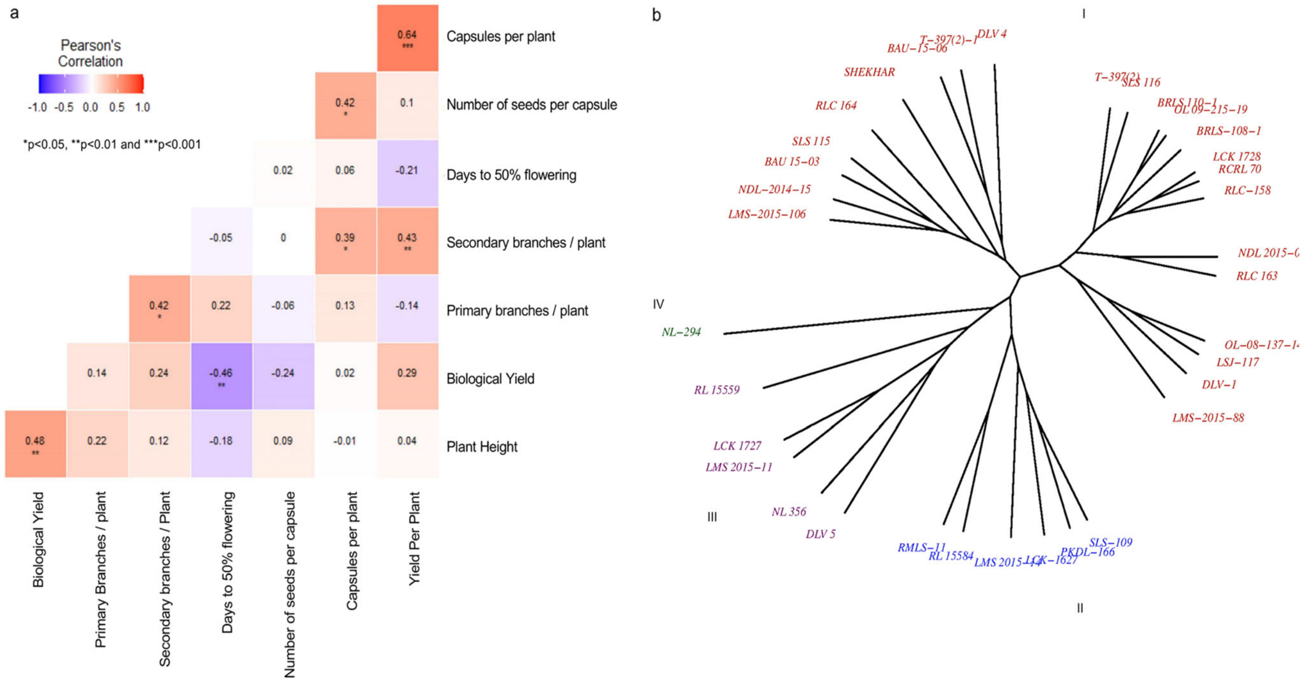

Fig. 1 **a** Correlation analysis between yield-contributing traits in linseed genotypes. High intensity of red colour indicates a positive correlation, and blue colour indicates a negative correlation. **b** Unrooted cluster analysis of linseed genotypes for yield traits. I, II, III and IV indicate the first, second, third and fourth clusters, respectively

indicating that E2 had a less-pronounced interaction effect, ensuring that all genotypes performed better. Genotypes NL356, BAU-15-06 and T397, having IPC1 scores near to zero line, indicated that they have less interactions with the environment and can be considered stable. Genotypes LMS2015-14, PKDL166 and RLC164 showed higher yield per plant. SLS115 is considered both high yielding and moderately stable.

GGE Biplot Analysis

The phenotypic performance and stability of genotypes under different seasons were graphically visualized using GGE biplots (Fig. 2c). The GGE biplot under the study was constructed with scaling = 0, centring = 2 and SVP = 2. A total of 75.92% of variation was explained by GGE biplot analysis, with PC1 and PC2 contributing around 41.85% and 34.07% of variation, respectively. The biplot is divided into eight sectors representing mega-environments, where environments E1, E2 and E3 fall into separate mega-environments. Genotype RL15584 performs better under E3, whereas SLS-109 performs better under E2. In the E1 environment, genotypes LCK-1627 and OL-08-137-14 were exhibiting better performance. Genotypes LMS2015-106, OL-09-215-19, NL294, T-397(2) and RL15559 landed in the segments with no environment, indicating these lines performed poorly in all the environments.

Mean vs stability analysis of the genotypes was constructed with SVP = 1 (Fig. 2d). The average environment coordinate (AEC) line represented by a single arrowed line directed towards the genotype with increasing order of average yield across environments, while the perpendicular position of the genotypes from the AEC line represents the stability. Genotypes SLS109, LMS2015-14 show higher yield, whereas SLS115 and PKDL166 show both higher mean yield and stability across all the environments. Genotypes T-397(2), NL294 and RLC158 were observed as stable genotypes with lower yields. Here, it is worth mentioning that T-397 is used as a national check over decades due to its very high stability.

The which-won-where biplot for yield per plant explains the best-suited genotypes for the respective traits and specific environments (Fig. 3). For yield per plant, the genotypes RL15584, SLS-109, OL-08-137-14, LCK-1627, OL 09-215-19, NL-294 and RL15569 were identified as best performers.

Multi-Trait Stability Index (MTSI)

Selection of genotypes for multiple traits across environments is carried out by computing the WAASBY score, which is a weighted average of the absolute score index. The genotypes were assigned MTSI ranks based on the WAASBY score, and 15% of selection intensity was applied. Genotypes with the highest MTSI values were

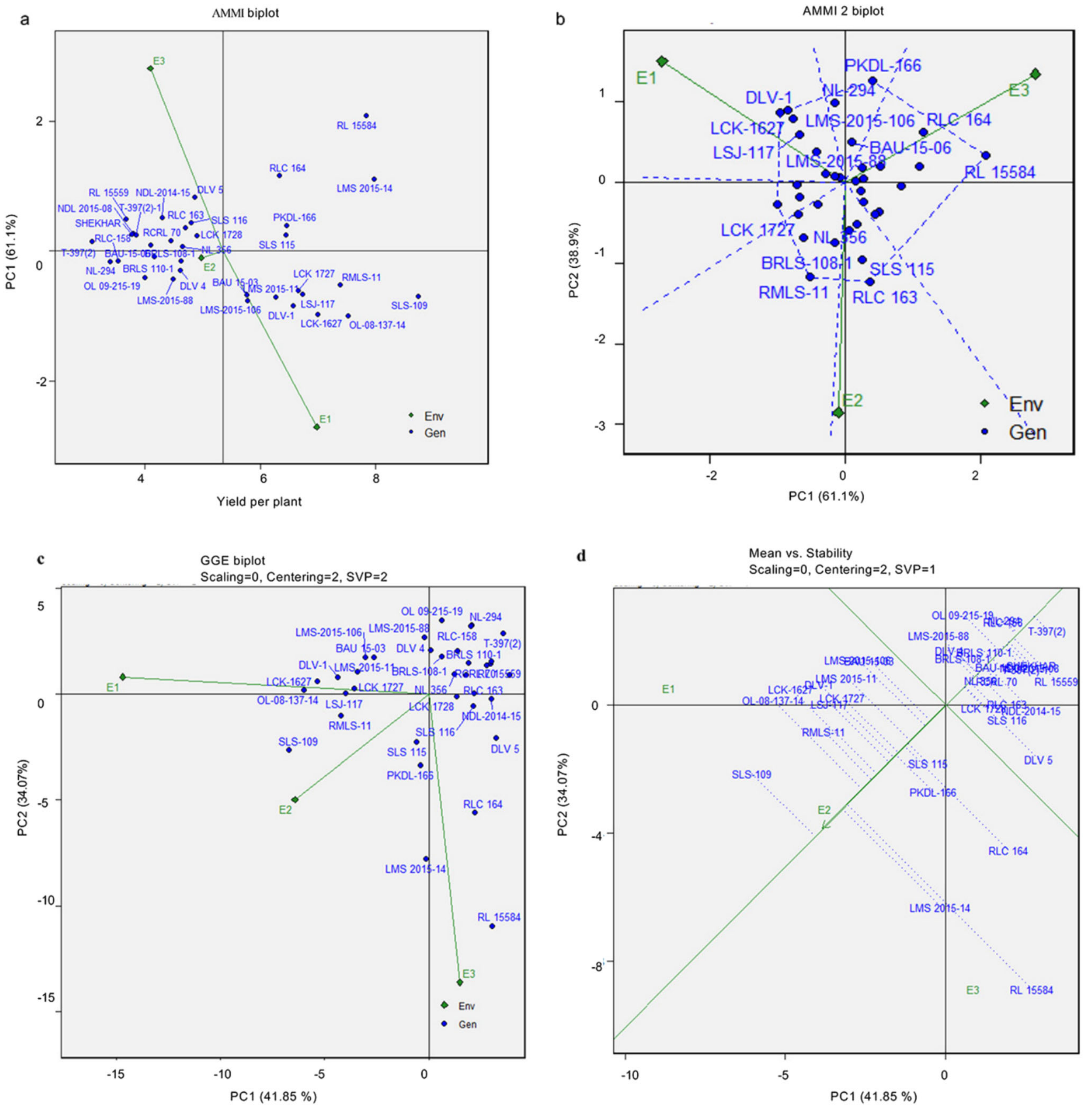

Fig. 2 AMMI and GGE biplot view of linseed genotypes for seed yield trait in three different environmental conditions. E1- rabi 2018; E2- rabi 2019; and E3- rabi 2021

placed at the centre and the lowest at the periphery. Genotypes LSJ-117, Shekhar, RCRL70, and NDL2015-08, denoted as red dots with lower MTSI values, are considered as ideal genotypes showing better performance and stability. The genotypes NL358, RL15584, RL15559, RLC164 and NL294 recorded high MTSI values, and hence were considered as poor performers with low stability (Fig. 4).

Diallel Analysis

Based on the yield performance, five promising parental lines (RLC163, SLS115, RLC164, LMS-2015-14 and T397) were identified for hybridization, and a total of twenty crosses were derived. The parents and 20 crosses, were subjected to diallel analysis, resulted in a significant variance for all the characters (Table 2). Variance due to general combing ability (GCA) was significant for plant

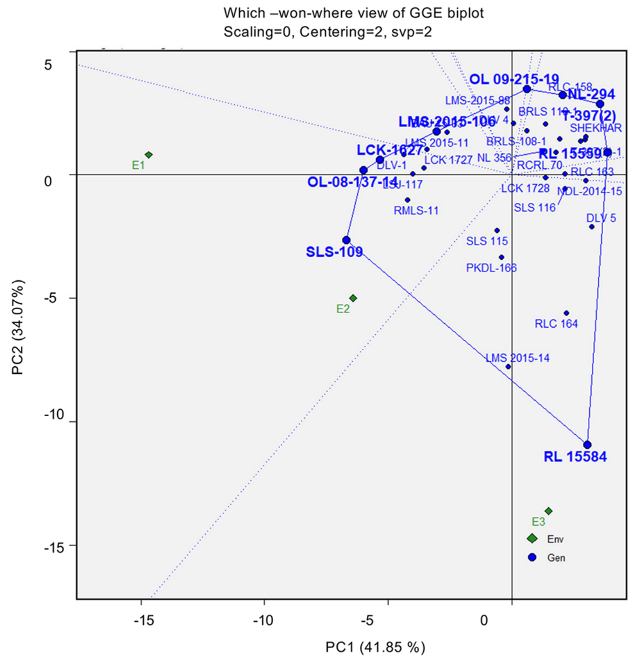

Fig. 3 'Which-won-where' polygon view of the GGE biplots for seed yield trait in three environments. The green colour diamond shape indicates the environments, and blue circle indicates linseed genotypes. E1- rabi 2018; E2- rabi 2019; and E3- rabi 2021

height (41.78), primary branches per plant (2.47), secondary branches per plant (5.29), inflorescence length (12.87), number of capsules per plant (1748), number of seeds per capsule (3.87), test weight (7.37), days to 50% flowering (4.38) and days to maturity (20.64) (Table 2). Specific combining ability (SCA) showed a significant variance for all the traits, except primary branches per plant and secondary branches per plant. Significant variances were observed for reciprocals for number of capsules per plant (43.75), biological yield (20.95), inflorescence length (6.24), days to 50% flowering (3.92) and number of primary branches per plant (1.65).

Components of Variance and Combining Ability Analysis

The combining ability analysis showed that the GCA effect was non-significant for all the traits (Online Resource 7). Variance of specific combining ability (Vsca) was higher than the variance of general combining ability (Vgca) for the majority of the characters, indicating that all the studied traits were governed by non-additive gene action (Table 3). For plant height, a high SCA effect was observed in C8 (3.34) followed by C16 (3.01) and C9 (2.01). Similarly, for

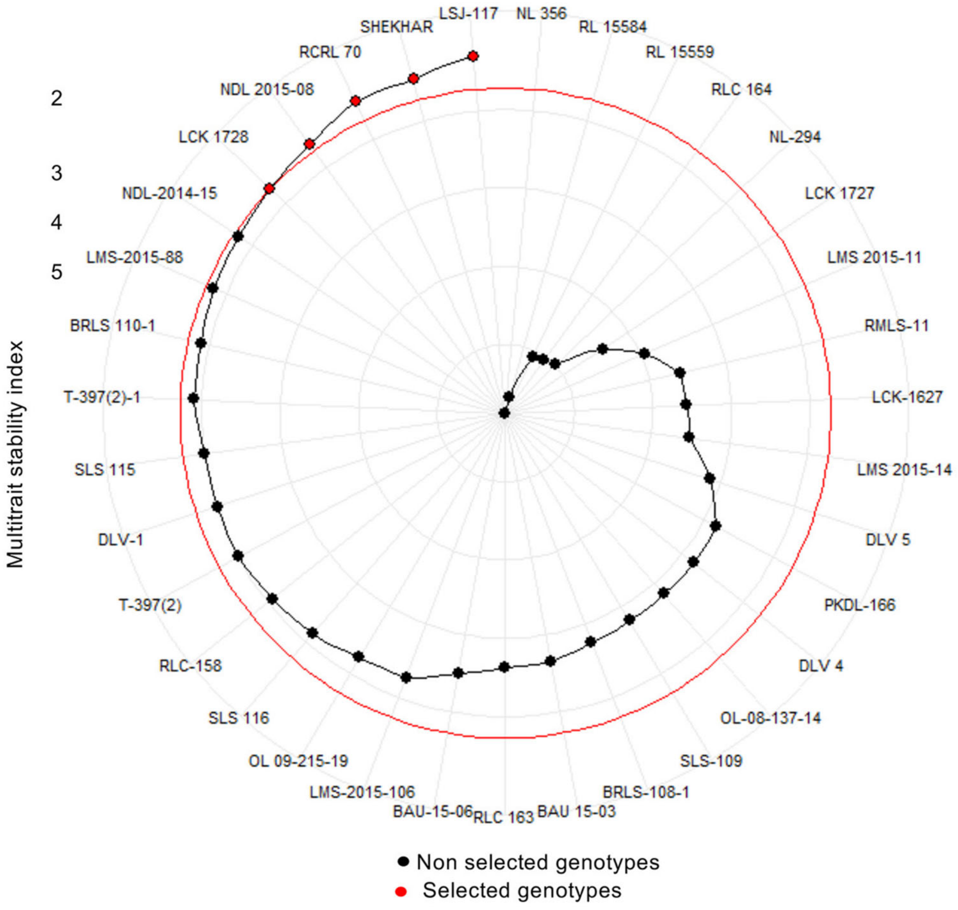

Fig. 4 Ranking of linseed genotypes based on multi-trait stability index (MTSI) considering a selection intensity of 15%. The genotypes with highly stable and maximum mean performance are represented in red colour, while the poor performing and less stable genotypes are shown with black dots

Table 2 Diallel analysis of variance (ANOVA) for 20 crosses and five parents for seed yield and related traits

Source of variation	df	PH	PB	SB	IL	RL	CPP	NSPC	BY	TW	YPP	DF	DM
Genotypes	24	57.13**	4.18**	47.63*	32.35**	3.93**	10,247.1**	5.11**	44.05**	6.97**	19.63**	1174.51**	0
Replication	2	124.68**	22.23**	438.41**	155.75**	1.98	1943.1	0.014	10.75	0.25	4.1	6.55	50**
Residual	48	13.4	2.17	30.6	7.10	1.42	2216.5	0.51	11.62	0.43	6.35	241.57	0
GCA	4	41.78**	2.47*	5.29*	12.87*	0.89	1748**	3.87**	0.82	7.37**	1.66	4.38*	20.64**
SCA	10	24.69**	0.70	16.72	14.78**	1.45*	3123.4**	2.31**	13.96**	2.21**	3.78*	19.72**	90.94**
Reciprocal	10	4.29	1.65*	19.26	6.24*	1.34	43.75**	0.22	20.95**	0.41	3.92*	17.67**	3.45**

** indicates significance at 1% and * indicates significance at 5%

df—degrees of freedom; PH—plant height; PB—number of primary branches per plant; SB—number of secondary branches per plant; IL—inflorescence length; RL—root length; CPP—number of capsules per plant; NSPC—number of seeds per capsule; BY—biological yield; TW—test weight; YPP—seed yield per plant; DF—days to 50% flowering; DM—days to maturity; GCA—general combining ability; and SCA—specific combining ability

the number of primary branches per plant, C4 (0.73) recorded the highest SCA effect, followed by C8 (0.58) and C20 (0.53); for the number of secondary branches per plant, the highest SCA effect was recorded on C8 (4.42) followed by C20 (3.33) and C18 (2.33). Number of capsules per plant showed a high SCA effect in C16 (61.13), C9 (56.67) and C20 (29.37); for number of seeds per capsule, high SCA effect was recorded on C11 (1.21) followed by C4 (0.84) and C9 (0.53). The crosses C9 (5.05), C11 (2.63) and C5 (2.72) recorded the highest SCA effect for biological yield. Yield per plant exhibited a high SCA effect C5 (2.6) followed by C1 (1.06) and C20 (0.83); for days to 50% flowering, C2 (4.37), C20 (4.33) and C17 (4) exhibited the highest SCA effects. Overall, among the crosses, C20 (T397 x LMS2015-14) had high SCA effect for primary branches, secondary branches, capsules per plant, yield per plant and days to 50% flowering. Cross 8 (SLS115 X T397) had a high SCA effect for plant height, primary branches and secondary branches. Similarly, C9 (RLC164 X RLC163) exhibited a high SCA effect for plant height, capsules per plant, number of seeds per capsule and biological yield. Thus, these crosses were advanced for further evaluation.

Performance of Advanced Lines (F3, F4 and F5) for Yield and Other Related Traits

Based on the phenotypic performance of three crosses (C8, C9 and C20), the cross C8 performed better under acidic soil conditions. Thus, the cross was forwarded to F_3, F_4 and F_5 generations by using single-seeded descent method. Selected advanced lines from F_3, F_4 and F_5 generation of C8 (SLS115 × T397) population were compared with parents for various yield-related traits (Fig. 5). The results revealed a significant improvement for yield per plant, primary branches per plant, secondary branches per plant, capsules per plant and number of seeds per capsule in advanced generations compared to parents. The higher mean values of F_5 generation than F_3 and F_4 generations revealed that the population performance is moving forward in the positive direction for these traits.

Discussion

Genetic variation or diversity is the primary factor that determines the efficacy of the selection process. Hybridization and selection are the basic breeding approaches followed to develop new varieties. The experiment demonstrated a significant difference among the genotypes for yield and other yield-contributing traits, indicating the diverse nature of germplasms for these traits. Gidey et al. [15] reported significant differences among

Table 3 Specific combining ability (SCA) effect of crosses for seed yield and related traits in linseed genotypes

Cross name	Parents	PH	PB	SB	IL	RL	CPP	NSPC	PW	TW	YPP	DF	DM
C1	P1XP2	0.57	0.03	– 0.33	– 0.97	1.18	17.2	0.12	0.03	– 0.29	1.06	0.85	– 0.09
C2	P1XP3	1.66	– 0.12	– 0.55	1.57	0.83	6.1	– 0.47	1.17	1.02	– 1.50	4.37	1.02
C3	P1XP4	1.70	0.01	0.72	1.84	– 0.33	– 1.33	– 0.60	– 0.38	0.62	0.32	3.78	1.70
C4	P1XP5	– 0.43	0.73	2.24	– 1.62	0.14	20.01	0.84	2.51	– 0.60	– 0.18	– 2.02	2.80
C5	P2XP1	1.49	0.51	0.46	2.74	0.88	34.2	0.06	2.72	– 0.52	2.6	1.83	– 1.33
C6	P2XP3	1.12	– 0.48	– 1.50	0.58	– 0.76	– 0.58	0.20	0.84	0.23	0.59	– 0.62	2.38
C7	P2XP4	0.61	0.01	0.74	– 1.23	0.64	– 20.80	0.47	– 0.67	– 0.08	– 0.68	– 0.71	2.72
C8	P2XP5	3.34	0.58	4.42	3.95	– 0.38	15.65	0.03	1.60	0.14	0.78	– 0.52	1.74
C9	P3XP1	2.01	0.01	– 0.95	1.64	1.11	56.67	0.53	5.05	0.06	0.66	0	0.62
C10	P3XP2	– 2.53	– 0.2	– 0.97	0.90	– 0.40	7.55	0.01	1.23	– 0.13	0.64	2.6	– 0.69
C11	P3XP4	1.18	0.27	1.86	0.61	– 1.09	37.32	1.21	2.63	0.25	1.29	1.63	4.81
C12	P3XP5	1.84	– 0.45	– 2.62	1.73	0.53	– 2.95	– 1.41	– 0.43	0.79	– 0.89	-1.1	5.32
C13	P4XP1	0.93	0.03	– 1.5	– 0.13	0.11	6.48	– 0.43	0.91	0.26	0.59	– 0.66	– 0.5
C14	P4XP2	0.13	– 0.10	– 1.37	– 0.10	– 1.30	– 16.19	– 0.01	– 1.11	0.05	– 1.16	– 3.16	– 1.83
C15	P4XP3	– 1.36	– 1.63	– 6.03	– 2.76	1.10	– 98.23	0.17	– 5.51	0.01	– 2.07	– 4.16	– 0.49
C16	P4XP5	3.01	0.52	1.78	2.2	0.07	61.13	– 1.76	2.58	1.33	1.98	1.07	5.21
C17	P5XP1	– 2.39	– 2.2	– 5.96	– 2.08	0.03	– 81.08	– 0.70	– 5.86	0.70	– 2.18	4	– 2.24
C18	P5XP2	0.14	– 0.32	2.33	1.52	– 0.48	– 3.14	– 0.2	0.26	0.05	– 0.32	– 3.83	1.5
C19	P5XP3	– 0.20	– 0.22	1.24	– 2.05	– 1.12	– 5.76	0.17	0.27	0.84	– 0.18	0.76	– 1.3
C20	P5XP4	0.92	0.533	3.33	1.24	0.31	29.37	0.23	1.80	0.70	0.83	4.33	– 1.33

C1–C20 indicate the twenty different crosses made using selected five parents in diallel fashion

P1—RLC163; P2—SLS115; P3—RLC164; P4—LMS-2015–14; and P5—T397

PH—plant height; PB—number of primary branches per plant; SB—number of secondary branches per plant; IL—inflorescence length; RL—root length; CPP—number of capsules per plant; NSPC—number of seeds per capsule; BY—biological yield; TW—test weight; YPP—seed yield per plant; DF—days to 50% flowering; and DM—days to maturity

genotypes for traits such as days to 50% flowering, days to maturity and seed size. Significant differences for all the traits were also observed by Paul et al. [30]. Genotype x environment interactions showed significant differences for all 12 traits. Similarly, Adugna and Labuschagne [1] reported a significant variation for genotypes, locations and their interactions. However, Anand and Murty [4] observed significant GXE interactions for days to 50% flowering, plant height, number of branches per plant and number of capsules per plant only. Genotype x environment interactions are used to rank the genotypes across environments and draw meaningful results.

The correlation coefficient was used to study the interrelationship between yield and yield-contributing traits. Character association studies are of great significance in the process of selection by which simultaneous improvement of more than one character is possible. Correlation coefficients at the genotypic level were generally of higher magnitude than the corresponding phenotypic level, indicating the strong association between the characters. A high positive correlation was observed between yield per plant and with number of capsules per plant and the number of secondary branches per plant. Similarly, the number of seeds per capsule is found to be positively correlated with the number of capsules per plant. Akbar et al. [3] and Sandhya et al. [33] observed a similar positive significant correlation for the number of seeds per capsule with the number of capsules per plant.

The genetic divergence among linseed genotypes was identified using hierarchical clustering (unrooted cluster analysis), and the 35 genotypes were grouped into four major clusters. Genotype NL-294 was identified as the most diverse genotype, showing its value to generate further variability in future breeding programmes.

The performance in terms of yield and stability was assessed by evaluating the genotypes across three environments, and the results were used to construct AMMI and GGE biplots. In the AMMI biplot, environment E2 has a shorter vector with IPC1, indicating a less interaction effect. This results to the findings by Chobe and Ararsa [10]. The genotype SLS115 was found to be high-yielding and stable genotype. With the help of GGE *which-won-*

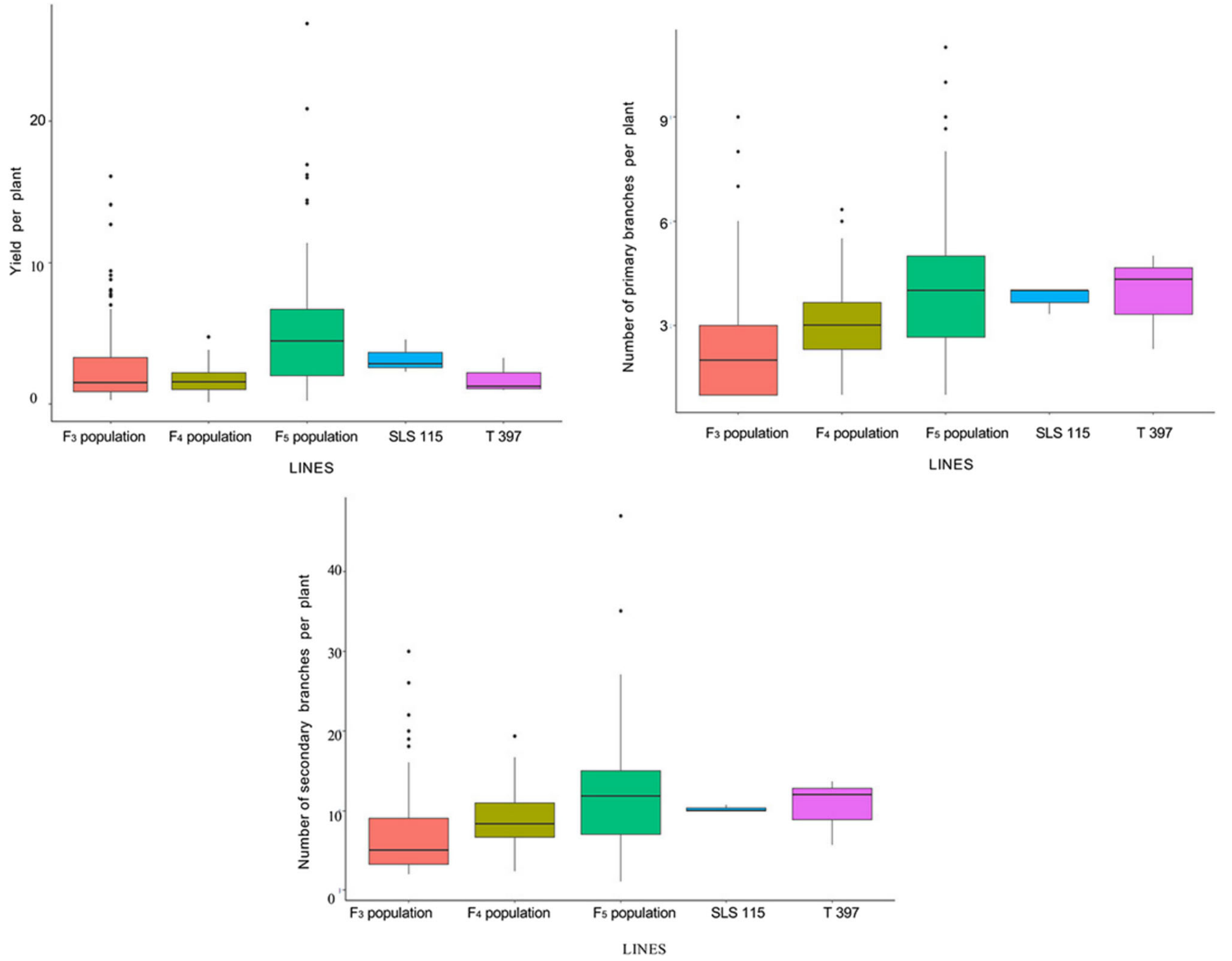

Fig. 5 Boxplots representing the comparison of parents (SLS115 and T397), F_3, F_4 and F_5 populations, respectively, for seed yield and related traits. The boxes correspond to the distribution of yield per plant, number of primary branches and secondary branches per plant values between the 25th and 75th percentiles; the median value is indicated by the horizontal line in the box; the bars indicate the highest and lowest values; and black dots correspond to extreme values

where biplots, all the environments were split into various mega-environments for identifying the suitability of various genotypes for mega-environments [18, 37, 39]. It was observed that genotypes RL15584 and SLS109 perform better under E3 and E2, respectively, whereas in the E1 environment, both LCK1627 and OL-08-137-14 were observed as high-yielding genotypes. The results agree with those of Hashim et al. [19], who analysed the data across two seasons and two locations, as well as Khan et al. [21], who conducted similar research on Bambara groundnut. The high-yielding genotypes across all environments were identified using the GGE *mean vs stability* biplot. Genotypes SLS115 and PKDL166 were identified with both higher mean yield and stability across all the environments. A similar trend of results was observed previously [14, 27, 32]. The multi-trait stability index is a novel technique used to identify genotypes by simultaneously considering all traits across multiple environmental conditions [34, 44]. Genotypes with a low MTSI score are considered as better performers and vice versa [35]. Four genotypes, namely LSJ117, SHEKHAR, RCRL70 and NDL2015-08, with lower MTSI scores, were selected as the best genotypes. The genotypes RL15559 and NL294 were identified as poor-performing based on GGE biplot and MTSI analysis.

Combining ability studies were used to assess the ability of parents to combine during the hybridization. General combining ability (GCA) determines the average performance of lines, and the specific combining ability (SCA) determines the performance of a combination of specific lines. GCA was non-significant for all the traits, and the SCA showed significant variations for all the traits except the number of primary branches per plant and number of secondary branches per plant. Non-significant GCA effects indicate the presence of non-additive gene action. The variance components were estimated to find the nature of gene action controlling the traits. All the traits observed in this study were governed by non-additive gene action since variance due to SCA was higher than GCA variance. Bibi et al. [7] reported that harvest index, test weight, days to 50% flowering and days to maturity were controlled by non-additive gene action. Controversial results were noticed by Patil and Chopde [29] and Kumar et al. [22] that a significant difference of the GCA/SCA ratio for most of the traits indicates that the inheritance of characters was predominantly controlled by the additive gene effect.

Combining ability analysis assists in the identification of the good general combiners and specific combiners for variety development. The GCA effects were found to be non-significant for all the traits, indicating the parents' lack of good general combining ability, and the traits were governed by non-additive gene action. Kurt and Evans [23] detected that none of the parental cultivars selected for a full 8X8 diallel cross possessed general combining ability. However, Bhateria et al. [6] identified good general combiners through line X tester analysis. Mishra and Rai [26] found T397 to be a good combiner for SPP, whereas in the present study, C16 and C20 were found to have high SCA effects for traits like plant height, secondary branches per plant, capsules per plant and days to 50% flowering. Parent T397 (P5) was one of the parents in both crosses. The SCA showed that RL163 X SLS115 (C1) and SLS115 X RLC163 (C5) were the best combiners to increase YPP. The cross C8 (SLS115 XT395) was recorded as the best combiner for primary branches per plant and secondary branches per plant. Further, the cross C8 was advanced to F_3, F_4 and F_5 generations using the SSD method. A comparison of the advanced generation of the C8 population revealed significant improvement for YPP, PB and SB over the parents. It indicates that the performance of the population is moving in a positive direction to increase seed yield in linseed. Although the GCA effects were non-significant across traits, the advanced generation showed an improvement for yield per plant, number of primary branches per plant and number of secondary branches per plant, suggesting the presence of additive x additive gene action. The presence of non-additive gene action suggests the use of heterosis breeding for the genetic improvement of traits.

Conclusions

The present study evaluated the linseed genotypes for seed yield and yield-contributing traits and identified high-yielding, stable genotypes across seasons in acidic soil conditions. Among the 35 linseed genotypes, NL356, BAU-15–06 and T 397 were identified as the stable genotypes across environments, whereas the genotypes SLS115 and PKDL166 were identified as high-yielding and moderately stable genotypes. A set of eight traits was studied among the linseed genotypes, and the number of capsules per plant and secondary branches per plant were found to be strongly associated with yield per plant. The combining ability effects revealed that the parents SLS115 and RLC163 were identified as the best combiners to increase yield per plant under acidic soil conditions. Identified best-performing lines can be used as parents in the breeding pipeline for developing elite varieties that can sustain and produce better yields under acidic soil conditions.

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Author contribution MR conceived and supervised the project. PM, AK and MR interpreted the data. MB and PM did the initial evaluation of material with MK and AB evaluating the material in the third season. PM wrote the original draft. AK, WT and MR edited the manuscript. All authors read and approved of the final manuscript.

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Data availability The data that support the findings of this study are included as the supplementary data.

Declarations

Competing Interest Authors declare that there is no conflict of interest.

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