# **RESEARCH ARTICLE**



# Genotype by environment ( $G \times E$ ) interaction analysis for seed yield and other contributing traits in linseed (*Linum usitatissimum* L.) across conventional and zero budget natural farming production systems in north-western Himalayas

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# Abstract

Development of high-yielding and stable linseed (*Linum usitatissimum* L.) genotypes in Himachal Pradesh under zero budget natural farming system (ZBNF) is an absolute need. A study was conducted to evaluate 30 elite genotypes of linseed for yield and stability across sixteen different environments over two years using Additive main effects and a multiplicative interaction model. For seed yield the environment, genotype and genotype x environment interaction (GEI) effects were highly significant (p <0.001), with contributions to total observed variation of 89.74, 0.81 and 8.08%, respectively. Out of the two production systems, ZBNF was observed to show consistently poor mean yields as compared to the conventional system across all locations. However, locations Kangra and Dhaulakaun performed better under the ZBNF system than Palampur and Bajaura. ZBNF was also identified as less discriminating with weak interactive forces. As per Eberhart and Russell model, the most stable and high-yielding genotype was Surbhi whereas, as per AMMI model, the ASV and GSI values indicated Giza-7 (G18), KL-285 (G16), KL-311 (G1) and Surbhi (G28) as stable and high in grain yield. For yield attributing traits such as primary branches per plant genotypes KL-279 (G13), Binwa (G30) and Him Alsi-2 (G19), for trait biological yield per plant Jeewan (G22), for harvest index KL-236 (G6) and Him Alsi-1 (G26) and for 1000 seed weight genotypes Him Alsi-1 (G26) and KL-285 (G16) showed high stability along with high mean performance and therefore could be selected. Genotype KL-284 (G15) showed specific adaptation under ZBNF system of Bajaura and is therefore recommended for production under respective environments after further evaluation.

Keywords:  $G \times E$  interaction, linseed, stability, production systems, AMMI, ZBNF

# Introduction

Although linseed (Linum usitatissimum L.) is a minor crop, it is grown in various locations climates and for various purposes. The major flax-growing countries are Kazakhstan, Russia, Canada, China, India and USA (FAOSTAT 2020). Even though linseed is a plant species with a high adapting capacity to unfavorable environmental conditions, which enables the cultivated land area to expand under various agroecological conditions, the cultivated area of linseed is limited (Ceh et al. 2020). According to the FAO statistics, there has been a decline in linseed production over last decade almost everywhere in the world with current worldwide acreage of 32.23 lakh ha, global production of 30.7 lakh tones and productivity of 0.951t/ha (FAOSTAT 2020). In India, the area under linseed has reduced from 3 lakh ha (2016) to 2 lakh ha (2020), with annual production of 1.21 lakh tonnes and productivity of 0.605 t/ha (FAOSTAT 2020). The area under linseed cultivation in Himachal Pradesh is 0.81 thousand ha and production is 0.30 thousand tonnes with an average Department of Genetics and Plant Breeding, <sup>1</sup>Department of Seed Science and Technology, <sup>2</sup>Department of Organic Agriculture and Natural Farming, CSKHPKV Palampur 176 061, Himachal Pradesh, India.

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productivity of 0.37 t/ha (Statistical Year Book of Himachal Pradesh 2020). However, linseed consumption is expected to increase as a result of the greater attention being paid to its health advantages. It has unique nutrient profile, mainly omega-3 essential fatty acid, which lowers the level of triglycerides in the blood, thereby reducing heart disease (Goyal et al. 2014) and also shows promise in the battle against inflammatory diseases such as rheumatoid arthritis. Linseed oil is also replacing other oils like fish oil as a nutritious supplement because of it's vegetarian origin. Raw consumption of linseed, famously known as flaxseed is also becoming very popular. Due to its rising demands but lower productions it is important to develop high-yielding linseed cultivars for higher productions per unit area. However, in addition to high mean yield, information on a cultivar's stability performance across environments is also important for its recommendation over wide geographical regions. Yield is a complex quantitative character and is greatly influenced by environmental fluctuations. This instability in yield of varieties over time and space and in particular, has negative effects on a farmer's income. Farmers need a consistent amount of output, hence, the need for stable genotypes that perform well in a variety of conditions is becoming more and more crucial (Annicchiarico 2002).

The instability of a genotype across environments/ locations arise due to genotype x environment interaction  $(G \times E)$ . It is the change in the relative performance of a character of two or more genotypes measured in two or more environments (Bowman 1972). Several statistical procedures can be used to measure crop yield stability and predict phenotypic responses to environmental changes. The statistical procedures can be divided into two major groups: univariate and multivariate stability parameters (Mohammadi et al. 2012). The most commonly used methods belong to the univariate group, including the Eberhart and Russell model (1966) used in the present investigation. On the other hand multivariate statistical approaches explore multi-directional aspects of GE interaction and attempt to extract more information from GE interaction components (Karimizadesh et al. 2013). They are based on singular value decomposition (SVD) and biplot concept (Kumar et al. 2016). Among the multivariate methods the additive main effects and multiplicative interaction (AMMI) (Zobel et al. 1988; Gauch 1992) and genotype (G) main effect plus GE interaction (GGE) (Yan et al. 2000) biplot analysis are the most well known and appealing methods for analyzing of GE interaction data (Mohammadi and Amri 2013). The multivariate group AMMI model has been used in the present investigation to measure stability. Proposed by Gauch (1992) AMMI analysis uses ANOVA and PCA in a joint approach that can be used to analyze multiple yield trials and hence is more suitable for characterizing the G×E interaction(Oliveira et al. 2014).

Prakritik Kheti also known as Natural Farming or Zero Budget Natural Farming (ZBNF), which started in Karnataka in India is also being enthusiastically adopted and popularized in Himachal Pradesh. It is a low-input farming system that advocates natural plant growth without adding fertilizers and pesticides. As the name implies, the cost of growing and harvesting the plants is zero. This may ensure the farmer that their dependency on loans will end, their production costs will significantly drop and their debt cycle will be broken. Linseed is also suitable as an oil crop in ZBNF that allows diversification of crop rotation in concern to the state of Himachal Pradesh. This is because in Himachal Pradesh linseed is either sown on poor marginal land viz., under low input production system or is broadcasted in standing paddy crop, 15-20 days before its harvest, popularly known as utera or paira system. The varieties recommended for cultivation on marginal land are evidently poor in yield performance. Most of the improved linseed varieties have been developed and released for use under a high input production system where high doses of fertilizers and plant protective chemicals supplement plant growth. Such varieties lack important traits required under natural and low input production conditions and are a major reason for their poor performance in Himachal Pradesh. Therefore, ZBNF is an excellent opportunity to evaluate linseed genotypes in low-input and natural farming conditions in Himachal Pradesh. Identifying stable and high-yielding genotypes of linseed with wider adaptation across diverse production systems would motivate farmers to take up linseed cultivation on a larger scale in the State even under low-input farming conditions. Keeping in view of this the present study was conducted.

## Materials and methods

## Plant materials and study locations

In this study the experimental material comprised of 30 linseed genotypes (Table 1) which included 13 released varieties, 14 advanced breeding lines and 3 exotic lines, which included KL-241 (Him Palam Alsi-1), KL-263 (Him Palam Alsi-2) and Him Alsi-2 as standard checks. The 30 linseed genotypes were evaluated for stability over 16 environments which included four locations viz., Linseed research farm at CSKHPKV Palampur and ZBNF farm at Holta, Palampur District Kangra (1290 m amsl), Hill Agricultural Research and Extension Centre (HAREC) and KVK, Bajaura District Kullu (1090 m amsl), Shivalik Agricultural Research and Extension Centre (SAREC) and KVK, District Kangra (700 m amsl) and Hill Agricultural Research and Extension Centre (HAREC) and KVK, Dhaulakuan District Sirmaur (468 m amsl), each location comprising of two different production systems viz., conventional and ZBNF production systems repeated across two years viz., rabi 2019-20 and rabi 2020-21. The locations varied in their altitudes as well as climatic conditions (Fig. 1).

## Experimental design and field management

The experiment was laid out in randomized complete block design (RCBD) with three replications with 50cm gap between each replication in each environment. Using a row to row and plant to plant spacing of 25 cm and 5 cm, respectively, the seeds of each genotype were sown in three rows, each row one meter long. The experimental field under the conventional production system was well-prepared and the recommended fertilizer doses of 50 kg N, 40 kg P<sub>2</sub>O<sub>2</sub>, and 20 kg K<sub>2</sub>O per hectare were applied. The other half of the nitrogen was top-dressed after 45 days after seeding and the full amounts of P<sub>2</sub>O<sub>5</sub> and K<sub>2</sub>O were applied as basal fertilizer. The test field was routinely weeded to maintain it free of weeds. In the current study, weeds in linseed were controlled using the herbicide Vesta (post-emergence). Under ZBNF, seed treatment was carried out using beejamrit which was freshly made at the Department of Organic Agriculture and Natural Farming CSKHPKV, Palampur. At the time of sowing Ghanjeevamrit (microbial mix) was applied @ 250 kg/ha whereas, Jeevamrit (soil inoculants), a 10% microbial mix, was applied once 21 days after sowing and once repeated every 15 days till harvest. Beejamrit is a mixture of cow dung and urine, whereas *jeevamrit* is an *in-situ* culture of water, cow manure and urine, unrefined cane sugar, legume flour, and virgin soil (Bharucha 2020). Ghanjeevamrit is a dry version of jeevamrit, which could be used when access to water is limited. It once prepared, can be used for a year. All these components contain beneficial bacterias which also show plant protective qualities and stimulate plant growth (Khadse and Rosset 2019). Mulching, hand weeding and hoeing were carried out to keep the fields weed-free. There was no use of farmyard manure or other chemicals.

#### Data Analysis

The phenotypic data collected from sixteen environments were subjected to two stability models viz., Eberhart and Russell model 1966 and AMMI model using R software. The stability parameters were used to identify stable genotypes as per Eberhart and Russell model, whereas AMMI model was used to assesss the stability and adaptability of the linseed genotypes across sixteen environments. According to Eberhart and Russell model, a stable genotype is one with high mean value, unit regression coefficient and deviation not significantly differing from zero ( $S^2$  di = 0). Whereas, according to the AMMI model, cultivars characterized by means greater than grand mean and the IPCA score nearly zero are considered generally adaptable to all environments. AMMI stability value (ASV) and Genotype stability index (GSI) values were calculated as per the method described by Purchase et al. 2000 using R software to identify the most stable and high yielding genotypes. ASV is the distance from zero in a two-dimensional scattergram of IPCA1 (interaction principal component analysis axis 1) scores against IPCA2 scores (Farshadfar 2011). Hence, it takes into account both IPCA1 and IPCA2 which justify most of the variation in the GE interaction. It ranks the genotypes as per their yield stability. Low ASV value corresponds to higher stability and vice-versa (Bocianowski et al. 2019).

 $AMMI \text{ Stability Value (ASV)} = \sqrt{\left[\frac{IPCA1SumofSquares}{IPCA2SumofSquares} \right]^2 + \left[IPCA2score\right]^2}$ 

Where,  $\frac{IPCA1SumofSquares}{IPCA2SumofSquares}$  is the weight given to the IPCA1

**Table 1.** Experimental linseed elite lines and checks evaluated in sixteen environments

| S.No | Genotype | Source/Pedigree            | S.No. | Genotype                  | Source/Pedigree        |
|------|----------|----------------------------|-------|---------------------------|------------------------|
| 1    | KL-236   | Jeevan × Janki             | 16    | Giza-7                    | Exotic collection      |
| 2    | KL-244   | (RLC 29 × Jeevan) × RLC-29 | 17    | Giza-8                    | Exotic collection      |
| 3    | KL-257   | LC-2323 × KLS-1            | 18    | Nagarkot                  | New River × LC-216     |
| 4    | KL-269   | EC-21741 × LC-216          | 19    | Himani                    | DPL-20 × KLS-1         |
| 5    | KL-278   | Giza-5 × Aayogi            | 20    | Jeewan                    | Sumit × LC-216         |
| 6    | KL-279   | Mariena × Giza-5           | 21    | Baner                     | EC-21741 × LC-214      |
| 7    | KL-280   | Giza-7 × Belinka           | 22    | Bhagsu                    | RL-50-3 × Surbhi       |
| 8    | KL-284   | Rajeena × Him Alsi-2       | 23    | Himalini                  | K2 × Kangra Local      |
| 9    | KL-285   | Binwa × Him Alsi-2         | 24    | Him Alsi-1                | K2 × TLP-1             |
| 10   | KL-309   | Canada $	imes$ Nagarkot    | 25    | Janki                     | Palampur               |
| 11   | KL-311   | Giza-6 × Nagarkot          | 26    | Surbhi                    | LC-216 × LC-185        |
| 12   | KL-315   | TL-27 × Flak-1             | 27    | Binwa                     | Flak-1 × SPS 47/7-10-3 |
| 13   | KL-314   | Belinka 60 × Nagarkot      | 28    | Him Palam Alsi-1 (KL-241) | Giza-7 × KLS-1         |
| 14   | KL-317   | Him Alsi-1 × Binwa         | 29    | Him Palam Alsi-2 (KL-263) | KL-223 × KL-224        |
| 15   | Canada   | Exotic collection          | 30    | Him Alsi-2                | EC-21741 × LC-216      |

value by dividing the IPCA1 sum squares by the IPCA2 sum of squares.

Genotype stability index (GSI) proposed by Farshadfar (2011) also referred to as simultaneous selection index or yield stability index (YSI) was computed by adding the ranks of stability index/parameter and mean yields. The least SSI is considered the most stable with high yield, whereas high SSI is the least stable with low yield.

$$GSI_i = RY_i + RASV$$

In AMMI 2 interaction, the genotypes and environments with the same sign on the PCA axis show positive interaction and are positioned close to each other on the biplot. This reflects the specific adaptation of that genotype in the respective environment. However, the most discriminating environment is the one showing the longest spoke length.

In the present study  $G \times E$  interaction effects, representativeness and discriminating ability of environments was determined for only trait seed yield where as stability of genotypes was assessed for seed yield as well as other attributing traits such as primary branches per plant, secondary branches per plant, capsules per plant, seeds per capsule, biological yield, harvest index and 1000 seed weight.

#### **Results and discussion**

#### Mean yield performance

For seed yield per plant the mean performance of the genotypes evaluated ranged from 4.06g to 6.57 g. Genotype KL-269 was the lowest seed yielder (4.06), whereas Nagarkot was the highest yielder among all. Only two genotypes *viz.*, Nagarkot and Surbhi showed significant above-average performance. When compared with the best check *viz.*, KL-263 (5.95) none of the genotypes showed significant higher mean seed yield whereas genotypes KL-311, KL-315, KL314, KL-317, KL-236, KL-241, KL-244, KL-278, KL-285, Giza-7, Nagarkot, Himani, Baner, Bhagsu, Himalini and Surbhi were found to be at par with the best check KL-263. Seed yield was lower under ZBNF in comparison to the conventional farming system across all four locations (Fig. 2). However, at locations Kangra and Dhaulakuan seed yields were higher

under both the systems of production as compared to Palampur and Bajaura. Location Bajaura showed the lowest yields among all.

## Combined analysis of variation and joint regression analysis of variation

Pooled analysis of variance for 4 locations over two years at both production systems (Table 2) revealed highly significant differences for grain yield among genotypes. For the mean seed yield, under the conventional system all genotypes and genotype  $\times$  year  $\times$  location, differed considerably (p 0.01), whereas for ZBNF system genotypes, location  $\times$  year and genotype  $\times$  year  $\times$ location differed considerably (p 0.01), indicating that genotypes responded to environments differently over each year. As genotypes responded differently at various sites throughout the years, the data suggested a significant genotype  $\times$  environment interaction in this region.

Highly significant differences were revealed among the deviations of genotypes when tested against pooled error). Highly significant differences were also observed among the genotypes when the genotype mean sum of squares was tested against pooled error. Variance due to environments was also found significant. The environment + (genotype x environment) was also significant for the trait studied. The significant GEI indicated that the genotypes were suitable for applying stability parameters. Therefore, the prediction of the performance of genotypes based on stability parameters would be feasible and reliable. Significant differences among the genotypes for linear response to environments as per Eberhart and Russell model indicated that the behavior of the genotypes could be predicted over environments more precisely and G × E interaction was outcome of the linear function of environmental components. The significant GEI indicated that the genotypes were suitable for applying stability parameters.

## Individual regression analysis and estimation of stability parameters of individual genotype for seed yield per plant and attributing traits

The stability analysis revealed that genotypes KL-309, KL-263, KL-284, Him Alsi-2, Nagarkot, Himani, Jeewan, Himalini, Him Alsi-1, Surbhi and Binwa showed stable (s<sup>2</sup>d=0) performance



Fig. 1. Average rainfall distribution and temperature for four experimental locations for year 2019-20 and 2020-21



**Fig. 2.** Mean performance for seed yield under conventional and ZBNF farming systems across four locations

across environments (Table 3). However, the most stable (significant high mean performance, bi=1, s<sup>2</sup>d=0) of all the genotypes was Surbhi whereas Him Alsi-2, Jeewan, Himalini and Binwa showed comparatively low mean performance than the population mean. Genotypes KL-244, Nagarkot, Himani and KL-263 showed above-average performance with below average stability (bi>1) and could achieve maximum performance in favorable environments. For the various yield attributing traits none was the genotypes were stable for primary branches per plant, secondary branches per plant, capsules per plant and 1000 seed weight. Whereas, for seeds per capsule KL-315, Him Alsi-2, Janki and Binwa had a significant high mean performance than the population mean and also showed non-significant regression coefficient with least deviation from regression. Therefore, these

| Table 2. Combined three factor analysis of variance for conventional |
|--|
| and ZBNF production system over locations and years                  |

| Source of variation          | DF  | MSS<br>(Conventional) | MSS (ZBNF) |  |
|------------------------------|-----|-----------------------|------------|--|
| Replicates                   | 2   | 1.379                 | 3.04       |  |
| Genotypes                    | 29  | 988.002 ***           | 381.747*** |  |
| Location                     | 3   | 6.456                 | 1.898      |  |
| Genotypes *Location          | 87  | 29.309 ***            | 16.006***  |  |
| Year                         | 1   | 10.397                | 34.035***  |  |
| Genotypes *Year              | 29  | 10.800 **             | 3.768***   |  |
| Location *Year               | 3   | 12.678                | 3.641*     |  |
| Genotypes *Location<br>*Year | 87  | 9.081 ***             | 4.179***   |  |
| Error                        | 478 | 6.144                 | 1.078      |  |
| Total                        | 719 | 49.113                | 18.787     |  |

genotypes were the most stable. For biological yield per plant KL-263 and for harvest index Giza-7 and Himani were the most stable genotypes.

## AMMI analysis of variance

For seed yield per plant highly significant differences were observed for environments, genotypes and GE interactions (Table 4). The maximum contribution of 89.74% to total

| Traits               | Genotypes observed as most stable | Mean<br>(x̄) | Regression<br>coefficient (bi) | Mean square<br>deviations (S <sup>2</sup> di) | Grand<br>mean | CD (5%) |
|----------------------|-----------------------------------|--------------|--------------------------------|---|---------------|---------|
| Seed yield/plant     | KL-309                            | 4.84         | 0.86**                         | -0.16   | 5.32          | 0.81    |
|                      | KL-263                            | 5.95         | 1.24**                         | -0.47   |               |         |
|                      | KL-284                            | 4.65         | 0.83**                         | 0.30  |               |         |
|                      | Him Alsi-2                        | 5.15         | 0.93                           | 0.59  |               |         |
|                      | Nagarkot                          | 6.57*        | 1.23**                         | -0.22   |               |         |
|                      | Himani                            | 5.79         | 1.16**                         | 0.38  |               |         |
|                      | Jeewan                            | 4.74         | 0.88**                         | 0.32  |               |         |
|                      | Himalini                          | 5.26         | 1.00                           | 0.39  |               |         |
|                      | Him Alsi-1                        | 4.88         | 0.88**                         | 0.48  |               |         |
|                      | Surbhi                            | 6.24*        | 0.99                           | 0.44  |               |         |
|                      | Binwa                             | 4.88         | 0.72**                         | 1.49  |               |         |
| Seeds per capsule    | KL-315                            | 8.17*        | 1.63                           | -0.012  | 7.99          | 0.15    |
|                      | Him Alsi-2                        | 8.23*        | 1.65                           | 0.054   |               |         |
|                      | Janki                             | 8.15*        | 1.05                           | 0.055   |               |         |
|                      | Binwa                             | 8.16*        | 0.68                           | 0.033   |               |         |
| Biological yield (g) | KL-263                            | 24.04*       | 1.161                          | 0.68  | 21.83         | 1.46    |
| Harvest index (%)    | Giza-7                            | 30.71*       | 0.89                           | 2.46  | 29.22         | 0.87    |
|                      | Himani                            | 30.13*       | 1.01                           | 3.81  |               |         |

| Table 3. Individual red | pression analysis an | d estimation of stabil | ity parameters for see | d yield attributing traits |
|-------------------------|----------------------|------------------------|------------------------|----------------------------|
|                         |                      |                        |                        |                            |

\*\*Significance at 1% level of significance (p <0.01) \* Significance at 5% level of significance (p < 0.05)

| Source of variation                           | DF   |          | Seed yield/ | plant                                       |
|---|------|----------|-------------|---|
|   |      | SSS      | MSS         | Proportion<br>of variation<br>explained (%) |
| Trials  | 479  |          |             |   |
| Environments                                  | 15   | 44585.35 | 2972.35**   | 89.74                                       |
| $\operatorname{Rep} 	imes \operatorname{Env}$ | 32   | 675.679  | 21.11**     | 1.36  |
| Genotypes                                     | 29   | 405.4949 | 13.98**     | 0.81  |
| Genotype ×<br>Environment                     | 435  | 4015.001 | 9.22**      | 8.08  |
| PC1   | 43   | 1570.294 | 36.51**     | 39.1  |
| PC2   | 41   | 1126.057 | 27.46**     | 28  |
| PC3   | 39   | 736.7606 | 18.89**     | 18.4  |
| PC4   | 37   | 191.0627 | 5.16**      | 4.8   |
| PC5   | 35   | 150.0377 | 4.29**      | 3.7   |
| Residuals                                     | 928  | 2775.734 | 2.99        | 5.6   |
| Total   | 1874 | 56472.27 |             |   |

**Table 4.** Pooled analysis of variance over environments as per AMMI model for seed yield and oil content

\*Significance at 5% level of significance (p < 0.05), \*\*Significance at 1% level of significance (p < 0.01)

variation was shown by environment sum of squares followed by GE interaction component (8.08%) also reported by <u>Adugna</u> et al. 2002 and Cerda et al. 2014. A low contribution of 0.81% was shown by genotype sum of squares. All the differences were found significant at 1% level of significance. Out of the total GEI contribution, 39.1% was explained by IPCA1, while IPCA2, IPCA3, IPCA4 and IPCA5 explained 28, 18.4, 4.8 and 3.7%, respectively. The first two IPCAs collectively captured 67.2% of total GEI. In contrast, first three IPCA's captured more than 70% (85.5%) of the total GEI. A large contribution of the environment indicated that the environments were diverse, with large differences among environmental means causing most of the variation (Tadesse et al. 2017). The largest mean sum of squares due to environment shows that environmental conditions have the most control over seed yield (Philanim et al. 2022), whereas significant genotypes suggested that broad range of diversity existed among cultivars. The larger magnitude sum of squares of GEI compared to the effects of genotypes indicated larger differences in genotypic response across environments (Rezene et al. 2014). The strong environmental influence suggests the need or MET data generation that may help identify stable, top-performing genotypes with widespread adaptation as well as for genotype selection with good adaptation to specific agro-ecologies such as ZBNF.

#### Genotype by environment interaction

Based on the AMMI study the average seed yield in each environment, the IPCA1 and IPCA2 scores, and the top four genotypes for seed yield in each environment are shown in <u>Table 5</u>. Low IPCA1 values indicate a high contribution to genotype stability and a low contribution to the G E interaction. The IPCA 1 scores indicated that environment E13 was the main contributor to the stability of genotypes in terms of seed yield per plant. Genotype G30 was consistently

| Table 5. AMMI analysis based mean phenotypic and IPCA values and four top-ranking genotypes for seed | yield in each environment |
|--|---------------------------|
|--|---------------------------|

| /          |     | <i></i>  |        | 1 33   | <i></i> |     |     |     |
|------------|-----|----------|--------|--------|---------|-----|-----|-----|
| Trait      | Env | Mean (g) | IPCAe1 | IPCAe2 | 1       | 2   | 3   | 4   |
| Seed yield | E1  | 11.41    | -1.13  | 0.60   | G23     | G6  | G13 | G18 |
|            | E2  | 4.18     | 1.29   | 2.82   | G1      | G29 | G30 | G8  |
|            | E3  | 5.84     | -0.73  | -0.12  | G30     | G27 | G22 | G19 |
|            | E4  | 1.31     | -0.61  | 0.00   | G28     | G22 | G15 | G27 |
|            | E5  | 15.09    | 1.94   | -3.08  | G5      | G21 | G4  | G8  |
|            | E6  | 13.21    | -0.51  | -0.99  | G16     | G4  | G9  | G8  |
|            | E7  | 17.50    | 3.45   | 0.74   | G2      | G23 | G6  | G7  |
|            | E8  | 6.52     | 0.74   | -0.09  | G2      | G20 | G7  | G26 |
|            | E9  | 1.52     | -0.49  | 0.04   | G27     | G1  | G28 | G19 |
|            | E10 | 1.30     | -0.55  | 0.08   | G8      | G14 | G20 | G27 |
|            | E11 | 0.94     | -0.60  | 0.03   | G30     | G8  | G28 | G19 |
|            | E12 | 0.84     | -0.55  | -0.04  | G4      | G27 | G20 | G26 |
|            | E13 | 1.80     | -0.41  | -0.07  | G21     | G14 | G20 | G7  |
|            | E14 | 1.17     | -0.60  | 0.09   | G19     | G3  | G18 | G24 |
|            | E15 | 1.27     | -0.68  | -0.03  | G11     | G30 | G28 | G16 |
|            | E16 | 1.16     | -0.56  | 0.01   | G18     | G20 | G11 | G19 |

| Table 6. ASV, | GSV and     | combine | d mean pe | rforman | ce of the top f | ive and | d bottom three | genotype  | es for see | d yield an | d its cor | ntributing trai | ts  |
|---------------|-------------|---------|-----------|---------|-----------------|---------|----------------|-----------|------------|------------|-----------|-----------------|-----|
| Primary brar  | nches per   | plant   |           |         |                 |         | Secondary b    | ranches p | er plant   |            |           |                 |     |
| Genotypes     | Means       | IPCA1   | IPCA2     | ASV     | ASV Rank        | GSI     | Genotypes      | Means     | IPCA1      | IPCA2      | ASV       | ASV Rank        | GSI |
| G27           | 6.75        | 0.06    | -0.07     | 0.09    | 1               | 8       | G10            | 18.23     | 0.52       | -0.08      | 0.69      | 2               | 5   |
| G4            | 6.50        | 0.07    | 0.27      | 0.28    | 4               | 15      | G27            | 17.39     | 0.05       | -0.71      | 0.71      | 3               | 13  |
| G6            | 6.35        | 0.03    | 0.30      | 0.31    | 5               | 19      | G9             | 16.88     | -0.10      | 0.14       | 0.19      | 1               | 22  |
| G9            | 6.21        | -0.02   | 0.19      | 0.19    | 2               | 21      | G17            | 16.96     | -0.23      | -0.82      | 0.87      | 4               | 24  |
| G14           | 5.78        | 0.07    | -0.24     | 0.26    | 3               | 31      | G12            | 15.11     | 0.54       | 0.60       | 0.93      | 5               | 35  |
| G30           | 7.98        | 0.32    | 1.67      | 1.70    | 28              | 29      | G30            | 19.22     | -1.03      | 2.96       | 3.25      | 28              | 30  |
| G2            | 6.58        | 1.58    | 0.11      | 1.71    | 29              | 38      | G28            | 20.44     | -2.42      | -0.93      | 3.29      | 29              | 30  |
| G5            | 7.16        | 1.67    | 0.46      | 1.86    | 30              | 33      | G8             | 18.03     | -2.19      | 1.69       | 3.31      | 30              | 36  |
| Mean          | 6.40        |         |           |         |                 |         | Mean           | 17.19     |            |            |           |                 |     |
| Capsules pe   | r plant     |         |           |         |                 |         | Seeds per ca   | psule     |            |            |           |                 |     |
| Genotypes     | Means       | IPCA1   | IPCA2     | ASV     | ASV Rank        | GSI     | Genotypes      | Means     | IPCA1      | IPCA2      | ASV       | ASV Rank        | GSI |
| G29           | 50.94       | 0.78    | -0.40     | 1.30    | 3               | 19      | G13            | 8.22      | 0.23       | 0.28       | 0.40      | 4               | 6   |
| G1            | 49.11       | -0.32   | 1.15      | 1.26    | 2               | 24      | G30            | 8.16      | -0.07      | 0.26       | 0.27      | 3               | 9   |
| G12           | 49.21       | -0.69   | -0.73     | 1.31    | 4               | 24      | G19            | 8.06      | 0.10       | -0.07      | 0.14      | 1               | 12  |
| G24           | 47.23       | 0.75    | 0.20      | 1.20    | 1               | 26      | G5             | 7.97      | 0.11       | -0.13      | 0.19      | 2               | 19  |
| G22           | 49.17       | -0.88   | -0.57     | 1.50    | 5               | 26      | G17            | 7.94      | 0.31       | -0.16      | 0.42      | 5               | 25  |
| G10           | 52.24       | -3.73   | 2.50      | 6.40    | 28              | 41      | G10            | 7.83      | -0.87      | 0.15       | 1.09      | 28              | 53  |
| G18           | 49.22       | 4.38    | 0.86      | 6.98    | 29              | 48      | G4             | 7.76      | -0.80      | 0.44       | 1.09      | 29              | 58  |
| G27           | 58.10       | -5.88   | 1.20      | 9.37    | 30              | 31      | G16            | 8.10      | -0.89      | -0.14      | 1.12      | 30              | 38  |
| Mean          | 51.18       |         |           |         |                 |         | Mean           | 7.99      |            |            |           |                 |     |
| Biological yi | eld per pla | ant     |           |         |                 |         | Harvest Index  |           |            |            |           |                 |     |
| Genotypes     | Means       | IPCA1   | IPCA2     | ASV     | ASV Rank        | GSI     | Genotypes      | Means     | IPCA1      | IPCA2      | ASV       | ASV Rank        | GSI |
| G22           | 22.21       | 0.61    | -0.36     | 0.81    | 4               | 16      | G26            | 29.72     | -0.37      | -0.45      | 0.67      | 1               | 12  |
| G24           | 21.22       | -0.51   | 0.39      | 0.73    | 2               | 22      | G6             | 29.79     | 0.69       | 0.32       | 0.97      | 3               | 13  |
| G14           | 21.33       | 0.16    | -0.81     | 0.84    | 5               | 24      | G9             | 28.20     | 0.51       | 0.52       | 0.85      | 2               | 27  |
| G19           | 20.82       | -0.11   | 0.53      | 0.55    | 1               | 27      | G27            | 28.26     | -0.76      | 0.09       | 1.01      | 5               | 28  |
| G5            | 20.69       | 0.49    | -0.46     | 0.75    | 3               | 30      | G22            | 26.35     | -0.47      | 0.77       | 0.99      | 4               | 34  |
| G27           | 21.96       | -1.79   | 2.84      | 3.57    | 28              | 42      | G28            | 31.15     | 1.92       | -1.28      | 2.83      | 28              | 31  |
| G11           | 19.66       | 2.89    | 0.87      | 3.60    | 29              | 57      | G19            | 30.88     | 1.76       | -2.40      | 3.34      | 29              | 33  |
| G4            | 19.15       | 3.74    | -0.47     | 4.54    | 30              | 59      | G2             | 29.98     | -2.57      | -0.47      | 3.42      | 30              | 37  |
| Mean          | 21.83       |         |           |         |                 |         | Mean           | 29.22     |            |            |           |                 |     |
| Seed yield p  | er plant    |         |           |         |                 | -       | 1000 seed w    | /t        |            |            |           |                 |     |
| Genotypes     | Means       | IPCA1   | IPCA2     | ASV     | ASV Rank        | GSI     | Genotypes      | Means     | IPCA1      | IPCA2      | ASV       | ASV Rank        | GSI |
| G18           | 5.62        | -0.13   | 0.16      | 0.24    | 1               | 10      | G30            | 7.49      | 0.12       | 0.05       | 0.15      | 2               | 4   |
| G16           | 5.55        | -0.30   | 0.09      | 0.42    | 2               | 13      | G26            | 7.40      | 0.25       | -0.02      | 0.30      | 3               | 6   |
| G1            | 5.41        | 0.36    | 0.25      | 0.57    | 3               | 16      | G16            | 7.20      | 0.04       | -0.31      | 0.32      | 5               | 17  |
| G28           | 5.43        | -0.41   | -0.12     | 0.58    | 4               | 16      | G2             | 7.08      | -0.09      | -0.09      | 0.14      | 1               | 19  |
| G25           | 5.26        | 0.43    | 0.21      | 0.63    | 5               | 21      | G7             | 7.08      | -0.15      | -0.25      | 0.31      | 4               | 23  |
| G23           | 6.03        | 1.16    | 1.39      | 2.13    | 28              | 31      | G10            | 7.37      | 0.34       | 0.78       | 0.88      | 28              | 34  |
| G11           | 4.06        | -1.55   | -0.63     | 2.24    | 29              | 59      | G9             | 7.17      | -0.60      | 0.71       | 1.01      | 29              | 43  |
| G30           | 4.88        | -1.65   | -0.33     | 2.32    | 30              | 52      | G22            | 7.10      | 0.84       | -0.12      | 1.01      | 30              | 47  |
| Mean          | 5.32        |         |           |         |                 |         | Mean           | 7.16      |            |            |           |                 |     |

top ranker across both years in the same environment (E3 and E11) while genotypes G1, G27, G21 and G20 consistently ranked in top four across years in the same environment

(Table 5). However, a comprehensive analysis of each environment and genotype as per AMMI biplots is described below.

## AMMI1 biplot

#### Performance of environments

For site Palampur AMMI1 biplot analysis revealed that variability for interaction and main effects was higher in the first year between the two production systems (E1 and E2) as compared to the second year (E9 & E10) (Fig. 3). E1 showed the highest positive PC score and also the highest mean performance, whereas E2, E9 and E10 showed negative PC scores with below-average performance. Instability in the interaction effects and main effects was observed across years for both production systems. However, ZBNF system was consistent in its below average performance in both years. Regarding site Bajaura as revealed by AMMI1 biplot analysis, less variation was observed among the test environments (E3, E4, E11, E12) for interaction effects compared to main effects. This showed that similar magnitudes of GE interactions were observed in both years for both systems of production. E3 was the only test environment that showed above average performance. Mean performances of the remaining environments was below average. The mean performances of the ZBNF system was found to be same across the years and was lower than that of a conventional system in both years whereas, it differed for a conventional production system. For site Kangra (Fig. 3), the PC scores for most of test environments (E6, E13, E14) were similar but negative. Only E5 showed positive interaction effects. The interaction effects varied for conventional production systems (E5 and E13) over the years, whereas it remained the same for ZBNF systems (E6, E14). For main effects, it was observed that first year favored higher mean performance in seed yield under both production systems (E5 and E6) but seed yield was lower under ZBNF conditions. However, the mean performance reduced in the second year to below average under both production systems but still the ZBNF system was poorer than the conventional system. Site Dhaulakuan AMMI1 biplot (Fig. 3) revealed that variability in main and interaction effects was observed among all test environments (E7, E8, E15, E16). However, variation was high under conventional systems (E7 & E15) than ZBNF systems (E8 and E16). For the main effects, seed yield was higher than mean in the first year for both production systems with the highest in the conventional system (E7). Variation in the mean performance in the first year was very large between the two production systems. However, in the second year less variation was observed for main effects across production systems but ZBNF environment was still poorer than a conventional system.

Overall, the highest interaction was observed for locations Dhaulakuan and Kangra. The highest mean performances were also favored in these locations. Variation in the interaction and main effects was observed across the years. The various climatic conditions that prevailed each year of the study could be the cause of the differences in genotypic yield between the years (Panigrahi et al. 2022). This suggested that the genotypes for seed yield should be evaluated on many test environments. It is suggested that at least 8 trials within each mega-environment are necessary for reasonably reliable estimates of stability (Annicchiarico 2002; Gauch 2013). The year to year variation in the environment means and the genotype response indicated the importance of seasonal climatic variations. Since, yield is a complex quantitative character and is greatly influenced by environmental fluctuations, the selection for superior genotypes based on yield per seat a single location in a year may not be very effective. It was also suggested by Kang (1993) that the genotypes discarded in the early stages of the breeding programmes might have the potential to perform better in another location or in another year. Hence, limited testing could result in the loss of potentially useful genes. This indicates that two or more seasons of testing are better than a single year. Therefore, it is suggested that the genotypes should be evaluated for more years over the environments for clear and better identification of the most representative environment.

With respect to seed yield it was also found that ZBNF production system was consistently poor yielder than the conventional production system under all four locations which was contrary to the studies of Khurana et al. (2022) who found consistently higher yields in linseed under organic approach than the inorganic in a nine-year trial. Hence, ZBNF could be considered as non-favourable environment for seed yield. However, an initial yield reduction is common when converting from conventional to organic/ZBNF farming. Yields under the ZBNF conditions may improve with time as soil health improves and is able to provide ecological processes required for crop growth (Duddigan et al. 2022). Locations Dhaulakuan and Kangra have shown consistently high mean seed yield under ZBNF across years in comparison to the other two locations. Also, the difference in the seed yields between conventional and ZBNF system was not very large as compared to the other two locations. Therefore they are best suited for linseed cultivation under ZBNF. Both these locations also showed higher mean yields under conventional production system compared to Palampur and Bajaura, which are comparatively colder regions. Hence, environmental factor viz., high temperature, could possibly affect seed yield. The highest mean performance was observed in Dhaulakuan under the conventional production system (E7) in the first year. However, the lowest was observed in Bajaura under ZBNF production system (E12).

Performance of genotypes for seed yield and its attributing traits

Considering the IPCA1 scores the genotypes showing least interaction with the environment for seed yield were G13, G19, G27, G12 and G18. They had PC score close to zero (Fig. 3), indicating that they are the most stable genotypes. Among them genotype G18 showed high mean performance whereas G12 and G19 were average in performance and G13 and G27 were below average. However, when IPCA2 scores were also considered, genotypes G24, G10, G15, G16 and G20 were identified as the most stable. This implies that the two IPCAs have various meanings and values. Hence, it is suggested to calculate ASV which estimates values between both IPCA1 and IPCA2 scores. Including phenotypic trait and stability in a single selection index is also necessary as a stable genotype may not necessarily be a high yielder (Nagaraja et al. 2023). Hence, GSI was also estimated for identifying stable and high yielding genotypes ASV is believed to produce a measurement that balanced the two IPCA scores (Adugna and Labuschagne 2003). As per the ASV values the genotype identified as most stable for seed yield was G18 followed by G16, G1, G28 and G25. G18, G16, G1 and G28 had high stability and grain yield based on their GSI and, therefore should be selected. Whereas, genotype G25, though stable should not be selected as it showed lower yields (Table 6). The most unstable genotypes were G23, G30 and G11 as they had the highest ASV values. The genotypes studied varied more for their interaction effects than main effects. The mean performances of most genotypes were close to average. G20 had the highest mean performance but high PC score viz., unstable and G11 had the lowest seed yield across environments but was unstable in its performance.

For other yield contributing traits such as primary branches per plant stable and high yielding genotypes as per their ASV and GSI values were G27 and G4, for secondary branches per plant genotypes G10 and G27 and for seeds per capsule genotypes G13, G30 and G19 could be selected (Table 6). For trait biological yield per plant genotype G22, for harvest index G6 and G26 and for 1000 seed weight genotypes G26 and G16 could be selected.

Genotype Surbhi has been identified as stable according to Eberhart and Russell model for seed yield, whereas, as per the AMMI model considering the ASV and GSI values the most stable and high yielding genotypes identified for seed yield are G18, G16, G1 and G28. Surbhi (G28) was identified stable by both models. Among these genotypes, G16 had high stability in seed yield as well as high 1000 seed weight. The utilization of these genotypes in linseed breeding projects should, therefore be given priority in order to further improve grain yield and other desirable traits. Different genotypes were identified as stable using two different stability approaches *viz.*, univariate and multivariate approaches also observed by <u>El Mohsen</u> and Amein (2016) and <u>Fisseha</u> et al. (2017) in linseed and <u>Abate</u> (2015) and <u>Misganaw</u> et al. (2017) in sesame.

As per the genotype ranks, G20 was the winner under conventional and ZBNF with mean performances of 7.53 and 4.70 g, respectively, pooled over locations and years. Whereas, under the conventional system G7 (7.42), G5 (7.39) and G6 (7.38) were among the top four best performing genotypes under the conventional system. However, G16 (4.44), G4 (4.30) and G18 (4.25) were best performing after G20 under ZBNF system.

## AMMI2 biplot anlaysis

#### Performance of environments

For site Palampur no correlation (90° angle) was observed between the two production systems in the first year (E1 and E2); however, they were positively correlated in the second year (E9 and E10) (Fig. 4). For conventional production system negative correlation was found across years (E1 and E9) but for ZBNF production system positive correlation was observed (E2 & E10). Higher interactions were observed in the first year (E1 and E2) compared to the second year, with inconsistent discriminating ability of the environments across years. E1 was the most discriminating of all. Site Bajaura AMMI2 biplot analysis revealed that all four test environments (E3, E4, E11, E12) were positively correlated. For site Kangra the two production systems (E5 & E6) were positively correlated in the first year however, both differed for their discriminating ability. E5 showed greater interaction than E6. In the second year, a positive correlation was observed between the two production systems (E13 & E14) with less variation in interaction effects. For the conventional production system, a negative correlation was observed across years (E5 and E13), whereas, for ZBNF production system, a positive correlation was observed across years (Fig. 4). Higher GE interactions were observed in the first year for both production systems (E5 and E6) as compared to the second year (E13 & E14). E5 showed the longest spoke viz., highly informative whereas E13 showed the shortest spoke length. Regarding location Dhaulakuan as per AMMI2 biplot analysis, a positive correlation was found between the two productions systems for both years viz., between E7 and E8 and E15 and E16. However, negative correlation was observed for both production systems across the years, viz., between E7 and E15 and E8 and E16. E7 showed the highest interaction. The conventional system showed higher interaction in the first year compared to second, however, in case of ZBNF system of production, not much difference was observed in the discriminating ability across years.

The significant test environments were determined based on the result's reproducibility across years and production systems. For site Palampur the results signified the use of two different production systems as two different test environments for seed yield estimation. While it is crucial to repeat the experiment under ZBNF system of production, it is not necessary to do so under conventional. However, with regard to the location Bajaura all the test environments were equally informative in determining the seed yield performances of the genotypes studied. As a result, any of the environments might be used as a test environment without having to repeat over time. Additionally, all four environments may be viewed as a single mega-environment because of how similar they were in terms of their capacity for discrimination. A negative correlation was observed across years for the conventional production system (E5 & E13) with respect to site Kangra, indicating them as two test environments, whereas, for ZBNF production system, a positive correlation was observed across years, signifying them as one test environment. Also, inconsistency in the discriminating ability of the production systems was observed across years. For site Dhaulakaun the analysis revealed that the two production systems as two different test environments were not as informative as the test environments across years. Additionally, it was observed that ZBNF systems exhibited less interaction than conventional systems.

Overall, a positive correlation was seen in the second year between all site test conditions. In contrast, the first year's test environments had different effects on correlation and interaction. Hence, it was concluded that the environments should be evaluated for a greater number of years to provide a more accurate validation of the most representative environment and a clear understanding of the grouping of environments into different mega-environments with respect to the trait seed yield per plant. Also, the highest interactions were observed for test environments belonging to conventional production systems while consistently low interactions were seen for ZBNF system. As a result, two production systems' interaction impacts were distinct; as a result, they ought to be treated differently as test environments.

Specific adaptation of genotypes as per AMMI model

Genotypes and environments that are positioned close to each other on the biplot have a positive association (Fig. 4). For location Palampur under the conventional system the winner for first year (E1) was G23 while none were responsive in the second year (E9). Genotype G17 was best performing under ZBNF system (E2) in the first year but G16 replaced it in the next year (E10). For location Bajaura it was observed that G15 was responsive to all the test environments, whereas, at location Kangra, for the first year the winners under the conventional production system (E5) was G5 and under ZBNF system (E6) were genotypes G9, G12, G22 and G3. But in the second year genotypes G16 and G28 showed the most interaction with E14 and E13, respectively. With respect to site Dhaulakuan for the first year the genotypes that showed maximum interaction under the conventional system (E7) were G6 and G2 whereas, under ZBNF system (E8) the most responsive genotypes were G24 and G20. However, in the second year G15 was the most interactive with both the systems of production (E15 and E16).



Fig. 3. AMMI1 biplot display of genotypes between mean and PC1

Rabi 2019-2020: Palampur: E1 (Conventional), E2 (ZBNFI), Bajaura: E3 (Conventional), E4 (ZBNF), Kangra: E5 (Conventional), E6 (ZBNF), Dhaulakuan: E7 (Conventional), E8 (ZBNFI)

Rabi 2020-2021: Palampur: E9 (Conventional), E10 (ZBNF), Bajaura: E11 (Conventional), E12 (ZBNF), Kangra: E13 (Conventional), E14 (ZBNF), Dhaulakuan: E15 (Conventional), E16 (ZBNF)

Genotypes: G1 (KL-311), G2 (KL-315), G3 (KL-309),G4 (KL-314), G5 (KL-317), G6 (KL-236), G7 (KL-241), G8 (KL-244), G9 (KL-257), G10 (KL-263), G11 (KL-269),G12 (KL-278), G13 (KL-279), G14 (KL-280), G15 (KL-284), G16 (KL-285), G17 (Giza-8), G18 (Giza-7), G19 (Him Alsi-2), G20 (Nagarkot), G21 (Himani), G22 (Jeewan),G23 (Baner), G24 (Bhagsu), G25 (Himalini), G26 (Him Alsi-1), G27 (Janki), G28 (Surbhi), G29 (Canada), G30 (Binwa)

In plant breeding crossover interactions are more important than non-cross-over interactions because they help in identifying genotypes with specific adaptations (Tena et al. 2019); however, the frequency of crossover interactions is also prime (Singh et al. 1999). If the crossover interactions are repeatable over years, this would help in identifying the environments with minimized crossover interactions over time, and hence cultivar recommendation could be done (Russell et al. 2003). Overall, line G15 (KL-284) was responsive to all the test environments of Bajaura and, hence, could be recommended for specific adaptation under both production systems with respect to location Bajaura. However, changes in the genotype ranks over years and across production systems were seen for other test environments. This indicates that genotypes did not perform consistently, and therefore no specific recommendations. However, the genotypes identified as most stable and high yielding under all sixteen environments i.e., G18, G16, G1 and G28 would perform better in both the production systems as they are least affected by environmental fluctuations.



**Fig. 4.** AMMI2 biplot display of genotypes between PC1 and PC2

High GE interactions have been associated with high mean performance because interactions are predicted to change the amount of genotype performance. Most of the test environments were similar in their PC scores and main effects and could be grouped as one. However, few environments showed high interaction effects as well. AMMI 2 biplots helped in identifying different mega environments. As per the AMMI analysis the mega-environments were the set of environments that constantly shared the best set of genotypes across years. Also, the most discriminative test environments based on the spoke length were also identified. This helped in evaluating the importance of different test environments in determining GE interactions for various traits. If the test environments are consistently non-discriminating and non-informative, they should not be used as test environments (Yan and Tinker 2006). Therefore, if the test environments cannot discriminate or provide enough information about the genotype performance, it is important to identify more test environments for extracting maximum information. The environments showing greater interaction were more discriminating whereas the others were poor contributors. When a cultivar's rank changes from one environment to another, it is called crossover interaction. The genotypes showing the same PC scores as the environment or the genotypes present in the same direction as the environment were the genotypes showing most of the interaction with that particular environment. This indicated that the genotype interacting most with a particular environment was the most responsive in that environment and hence, could be concluded as specifically adapted to that environment. This study reveals the paucity of high yielding linseed cultivars in the linseed germplasm evaluated that could perform better even under less favorable conditions. Hence, there is room to breed better and higher yielding linseed genotypes for the ZBNF agricultural system, which could benefit the State's farmers.

## Authors' contribution

Conceptualization of research (SP and GT); Designing of the experiments (SP and GT); Contribution of experimental materials (SP); Execution of field/lab experiments and data collection (GK, UC and GT); Analysis of data and interpretation (SP and GT); Preparation of manuscript (SP, GK, UC and GT).

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