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GGE biplot and AMMI analysis of advanced breeding lines of linseed

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Abstract
Ten advanced breeding lines of linseed were assessed in four environments *viz*; two locations each during 2016-17 and 2017-18. Analysis of variance exhibited a significant variation for genotype, environment and G X E effects for all the traits. The GGE biplot analysis showed that genotypes LCP 87, LC 2063, LDCP 13 and M 6 are suitable for seed yield for the present locations. Combined analysis for stability through GGE biplot and AMMI revealed that the genotypes LC 2063 & LCP 87 can further be utilized in the future breeding programme for higher seed yield and LC 2063 & LDCP 1 for better stability.

Key words
Linseed, stability, GGE biplot, AMMI, G X E interaction

INTRODUCTION

Linseed or flax is one of the oldest fibre crops in the world. It is an important oilseed crop raised during *Rabi* season and next in importance to rapeseed-mustard in area as well as production. During 2017, globally it was cultivated on an area of 2.77 m. ha with a production of 2.79 m. t. productivity of 1006 kg/ha, while in India it was cultivated on 0..3 m. ha with a production of 0.18 m. t. and productivity of 613 kg/ ha in contrast to the world average of 1006 kg /ha (FAO 2019). The productivity of the crop is lower in India because of its cultivation mainly confined to rainfed conditions.

Linseed is cultivated for dual purpose both for seed as well as fibre. Every part of linseed plant is utilized commercially, either directly or after processing. Linseeds (also known as flaxseeds) are power houses of nutrition. Seed contain a good percentage of oil that ranges from 33 to 45 per cent (Singh *et al*., 2011). Linseed offers various health benefits such as lowering of serum cholesterol, blood pressure, risk of cardiovascular diseases, diabetes, cancer etc. (Singh *et al*., 2011 ; Zuk *et al.,* 2015). The linseed seeds are extremely good source of health-promoting omega-3 fatty acid, alpha linolenic acid (Singh *et al*., 2011). Keeping in view, the health benefits of linseed, cultivation of this crop should be taken on large scale to ensure the nutritional security to the poor masses but the productivity of the crop in India is lower than the productivity in the world. So, to increase the productivity of linseed concreted breeding efforts are needed. The multi-location testing of new cultivars plays an important role in any breeding programme (Yan and Tinker, 2006).
Seed yield is a complex trait influenced by many components which may have positive or negative effect. Evaluation of cultivar based on multiple traits is an important objective of plant breeding as these leads to identification of the traits having greater influence on the target trait. Apart from identifying high ranking genotypes with wide adaptation, the additive mean effects and multiplicative interaction (AMMI) (Gauch, 1992) and genotype main effect plus GE interaction (GGE) biplot analyses are the methods commonly used for analysing GE interactions in MET data. For visualizing the genotype-by-trait data, genotype–trait (GT) biplot is an excellent tool (Deghani *et al.,* 2008). These GT biplots graphically

summarize the genotype-by-trait matrix (Yan and Kang 2003) and can be used to visualize correlations among traits, identification of specific combinations useful for indirect selection and performance of genotypes based on different traits which may held in the selection of the parents for breeding programmes (Yan and Tinker, 2005).

The present investigation was conducted to evaluate 10 genotypes of linseed across two location Gurdaspur and Ballowal Saunkhri region of Punjab during *Rabi*, 2016-17 and 2017-18, to identify superior genotypes that can be used in the development of breeding populations and to understand trait associations in linseed.

MATERIALS AND METHODS

Nine advanced breeding lines of linseed and one check (LC 2063) (**Table 1**) were analysed in randomized complete block design with three replications at two location Gurdaspur and Ballowal Saunkhri region of Punjab during *Rabi,* 2016-17 and 2017-18. The test locations were selected based on variation in weather parameters, geography and other agro-climatic factors among the area under the crop in Punjab. The soil of experimental site at Gurdaspur was clay loam medium in organic carbon and phosphorus and low in potassium, while at Ballowal Saunkhri was sandy loam low in organic carbon and phosphorus and medium in potassium. The plot size for each genotype was 9 square metre at both the location with row-row spacing 30 cm. Sowing was done manually and management practices as per package of practices of Punjab Agricultural University, Ludhiana. Plot mean data were used for further analysis. Plot seed yield data were converted to quintal per acre. The data were subjected to analysis of variance. AMMI models were used to GE interaction (Zobel *et al.,* 1988). The basic model of the analysis is given below:

$$Y_{ij} = \mu + \beta_j + \sum \lambda_{in} \xi_{in} \eta_{in+} \varepsilon_{ij}$$

Where Yij is the mean of genotype i in environment j; μ is the grand mean; βj is the environment j main effect; n is the singular value; λ_{in}, $\xi_{in}^{n=1}$ and η_{in} are, the singular vectors for genotype and environment for n = 1, 2, ..., respectively; and ε_{ij} is the residual effect. GGE biplots were created using the first two symmetrically scaled Principal Components (PC) for an average tester coordinate and polygon view biplots. To visualize associations between locations, a vector view biplot was constructed. These graphic analyses were done using the PBTools, version 1.4. (STAR 2014).

RESULTS AND DISCUSSION

The general picture of the relative magnitude of G, E, and G X E interaction for all the traits are presented in **Table 2** in the form of ANOVA. Analysis of variance revealed that the genotype, environment and G X E effects were significant (P<0.01) for all the traits studied. Environment was noticed to be the main controlling source of variation for all the traits and its proportion ranged from 29.68 to 87.15 per cent for various traits. For seed yield, environment accounted for 29.68 per cent, genotypes 18.74 per cent and G X E 39.76 percent. For 1000 seed weight environment accounted for 39.68 per cent, genotypes 39.88 per cent and G X E 10.58 per cent. For days to maturity environment accounted for 87.15 percent, genotype 2.68 per cent and G X E 7.63 per cent. The environment accounted for 83.34 per cent, genotype 7.51 per cent and G X E 5.01 per cent for days to 50% flowering.

Table 1. Information on the breeding lines and environments used in the study

S. No	Advanced Breeding Lines	Code	Pedigree
1	LDCP 25	G1	LCK 88062 X EC 322651
2	LCP 87	G2	GS 134 X LC 2023
3	M 6	G3	LC 2063 (Mutant)
4	LCPY 35	G4	JRF 5 X EC 322651
5	JSB 1	G5	Acc no. 2251 X EC 322651
6	LDCP 1	G6	Belley X LCK 88062
7	LCP 1	G7	LC 2179 X LC 2023
8	LCP 3	G8	LC 2013 X T 397
9	LDCP 13	G9	Belley X Acc no. 2251
10	LC 2063	G10	1509 X LC 54
Environments			
E1	Gurdaspur rabi 2016-17		
E2	Ballowal Saunkhri rabi 2016-17		
E3	Gurdaspur rabi 2017-18		
E4	Ballowal Saunkhri rabi 2017-18		

Successful cultivars are better adapted to a range of environments and yield stability is the main feature for their acceptance. G X E interaction is observed as the differential ranking of genotypes across environments based on their performance and GEI for seed yield and other traits in linseed have been reported earlier (Mohammadi and Amri, 2013). In the present investigation, GGE biplot analysis was used to study the complexity of the GEI in linseed MLT data. Highly significant mean sum of squares reported for seed yield and other traits in the present investigation emphasized on the sensitivity of the genotypes in terms of their responses to environments (**Table 2**).

The environment contributed 29.68 – 87.15 per cent of the variation in the data, however the contribution of genotype and their interaction with location was less (**Table 2**). The MET data of the environments account for about 80 per cent of the total variation (Gauch and Zobel 1997; Sood *et al.,* 2017). Significant genotype by environment interaction for days to flower, days to maturity and seed per capsule has been reported earlier by Gabiana (2005). Other researches also reported significant GEI for days to flower and days to maturity (Adugna and Labuschange, 2003 ;Choferie, 2008).

The mean seed yield (**Table 3**) of the genotypes over environments was 5.96 q/acre. The average seed yield of genotypes was highest in E3 and was least in E4. Among the genotypes, G10 gave the highest yield followed by G2 and G9 in all the environments. Variations for seed yield among the genotypes across the environment indicated the presence of G X E interaction e.g. the genotype G2 performing best in E1 (8.49 q/acre) among the genotypes but poor in E2 and E4.

The mean 1000-seed weight of the genotypes across the environments was 6.994 (gm). The genotype G4 was having the highest seed weight (8.507 gm) followed by G3 (7.721 gm) and G1 (7.375 gm). The overall days

Table 2. Mean squares and per cent variation explained by genotype (G), Location (L) and GL interaction for various traits

SV	df	Type IV SS	Mean squares	F Value	Pr>F	Explained %
Seed yield						
Rep	2	0.01517	0.00758	0.02	0.9785	
Env.	3	68.2076	22.7359	65.33	<.0001	29.68
Genotype	9	43.0654	4.78505	13.75	<.0001	18.74
GXE	27	91.3804	3.38446	9.72	<.0001	39.76
Error	78	27.1468	0.34804			
Corr. Total	119	229.815				
1000 seed weight						
Rep	2	0.32356	0.16178	1.06	0.3504	
Env.	3	49.1048	16.3683	107.53	<.0001	39.68
Genotype	9	49.3548	5.48386	36.03	<.0001	39.88
GXE	27	13.0897	0.4848	3.18	<.0001	10.58
Error	78	11.873	0.15222			
Corr. Total	119	123.746				
Days to 75% Maturity						
Rep	2	8.75	4.375	4.07	0.0209	
Env.	3	3171.43	1057.14	982.61	<.0001	87.15
Genotype	9	97.375	10.8194	10.06	<.0001	2.68
GXE	27	277.658	10.2836	9.56	<.0001	7.63
Error	78	83.9167	1.07586			
Corr. Total	119	3639.13				
Days to 50% Flowering						
Rep	2	14.8167	7.40833	1.55	0.2185	
Env.	3	7797.09	2599.03	544.2	<.0001	83.34
Genotype	9	702.875	78.0972	16.35	<.0001	7.51
GXE	27	468.492	17.3515	3.63	<.0001	5.01
Error	78	372.517	4.77586			
Corr. Total	119	9355.79				

Table 3. Mean values of different traits at 4 environments

Genotype	Seed Yield(q/acre)					1000-seed weight (g)					Days to 75 % Maturity					Days to 50% Flowering				
	E1	E2	E3	E4	Mean	E1	E2	E3	E4	Mean	E1	E2	E3	E4	Mean	E1	E2	E3	E4	Mean
G1	4.83	6.04	8.07	4.74	5.92	6.824	8.413	6.744	7.520	7.375	158	157	154	145	154	107	87	98	97	97
G2	8.49	5.65	7.85	4.61	6.65	5.433	5.973	5.033	7.127	5.892	156	155	149	146	152	100	79	97	95	93
G3	3.63	6.46	6.89	5.43	5.60	7.547	7.933	7.551	7.853	7.721	158	158	156	145	154	102	84	108	98	98
G4	3.50	6.02	6.56	5.11	5.30	7.788	8.613	7.672	8.507	8.145	159	157	154	144	154	103	88	107	97	99
G5	6.27	5.00	4.36	4.06	4.92	6.378	7.853	6.245	7.687	7.041	161	153	151	144	152	108	88	104	97	99
G6	6.64	6.24	7.58	4.69	6.29	5.783	8.027	5.811	7.707	6.832	160	157	153	146	154	106	87	110	97	100
G7	6.07	4.20	7.19	5.72	5.79	6.300	7.333	6.300	7.433	6.842	158	158	156	144	154	108	89	111	97	101
G8	5.96	4.31	7.10	5.35	5.68	6.515	7.387	6.379	7.387	6.917	157	155	152	143	152	105	82	104	93	96
G9	5.91	6.96	7.70	5.28	6.46	5.022	7.027	4.873	7.407	6.082	158	152	158	145	153	107	89	108	98	101
G10	7.50	7.43	7.87	5.06	6.96	6.564	8.053	6.352	7.393	7.091	159	156	155	144	154	107	89	110	97	101
Mean	5.88	5.83	7.12	5.01	5.96	6.416	7.661	6.296	7.602	6.994	158	156	154	145	153	105	86	106	97	98

to maturity for the genotypes over the environments was 153 days and showed not much variation among the genotypes except in E4 which shows early trends because of very less rainfall in that environment during the season. Mean day to flowering for the genotypes over the environments was 98 days and the least was recorded for G2 (93 days) and the maximum for G9 and G10 in all the environments.

The mean performance and stability of the genotypes was visualized using average environment coordination (AEC) through GGE biplot (Yan, 2001). The genotype-metric (SVP=1) GGE biplots for seed yield, 1000-seed weight, days to 75% maturity and days to 50% flowering are presented in **Fig 1a-d**, respectively**.** The first two PCs explained 83 per cent variation for seed yield, 97.5 per cent for 1000-seed weight, 70.5 per cent for days to 75% maturity and 94.6 per cent for days to 50% flowering. The results are in agreement with Yang *et al.* (2009) and Yan *et al.* (2010) who stated that if the first two PC explain more than 60 per cent of the variability then the biplot adequately approximates the variability in G X E data. The line with single arrow head in **Fig 2a-d** is the axis of the AEC abscissa that passes through the biplot origin. This line is also the marker for average environment and points towards the higher mean values (Yan, 2001). The double arrowed line, in **Fig 1a-d**, perpendicular to the AEC passing through the biplot is AEC ordinate. The absolute length of the projection of a cultivar determines its stability across the environments. The stability decreases with the increase in the absolute length of projection and the average yield of genotypes is approximated by the projections of their markers to the AEC abscissa (Kaya *et al*., 2006). Genotypes G10, G2 and G9 showed the higher yield than average and G10 was the best performing genotype **(Fig.1a)**, while G4 & G5 were the worst performing genotypes in terms of yield. G10 and G6 were stable genotypes having the higher seed yield across the environments; however G3, G2, G4 and G5 were the least stable genotypes with higher projection from the AEC abscissa. For 1000-seed weight the genotypes G1, G10, G5, G8, and G7 showed relative stability, while G4 and G3 as the best genotypes for 1000-seed weight **(Fig.1b).** The genotype G7 and G10 were late in flowering as well as late maturing (**Fig.1c-d**), whereas G2 and G8 were early in flowering and maturing. The genotype G10 was a best yielder but was late in flowering and maturity, while genotype G2 ranked the second in term of yield but was early in flowering and maturity.

The 'which-won-where' biplot graphically addresses crossover of GE, ME differentiation, specific adaptation etc. (Rao *et al.,* 2011 ; Rakshit *et al.,* 2012) and are constructed by joining the farthest genotypes to form a polygon. The genotypes at the vertices of the polygon are the best or worst genotypes in one or more environments and the genotype at the vertex of the polygon is the best performing genotype in the environments falling within the sector (Yan and Tinker, 2006). Which-won-where biplot for seed yield, 1000-seed weight, days to 75% maturity and days to 50% flowering are presented in **Fig.2a-d.** The polygon for seed yield exhibited six, five, four and five vertex respectively, for seed yield, 1000 seed weight, days to 75% maturity and days to 50% flowering. The equality lines divided the biplot for seed yield into five sectors effectively of which three retained all the four environments; for 1000-seed weight into three sectors of which one retained all the four environments; for days to maturity into four sectors of which three retained all the four environments and for days to flowering into four sectors of which two retained all the four environments. The biplots for seed yield and days to maturity were more informative than the rest two as they were able to discriminate between the environments effectively. For seed yield, E1 fell in the sector in which G2 was the vertex cultivars, E2 & E3 in the sector in which G10 and G9 were at the vertex and E4 in the sector with G3 at vertex. This means G2 was the best genotype for E1, G10 & G9 were the best genotypes for E2 & E3 and G3 for E4 for seed yield. This highlighted the importance of G X E interaction

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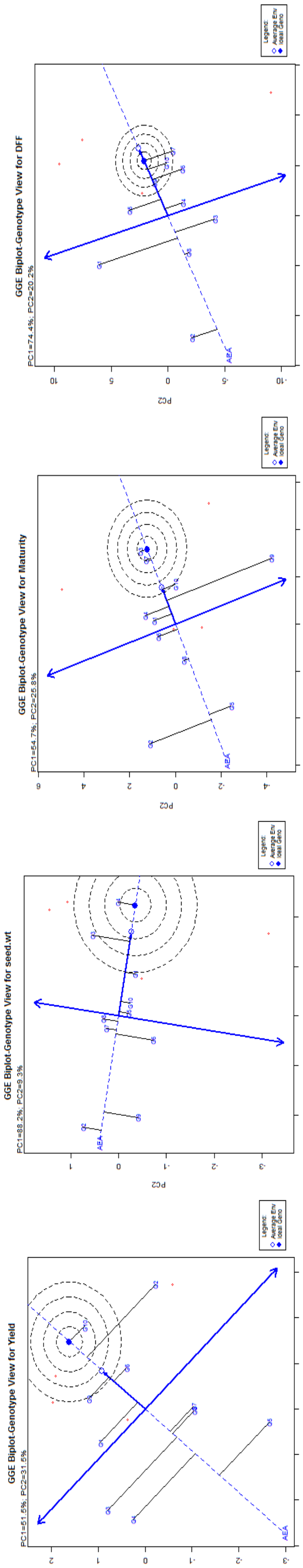


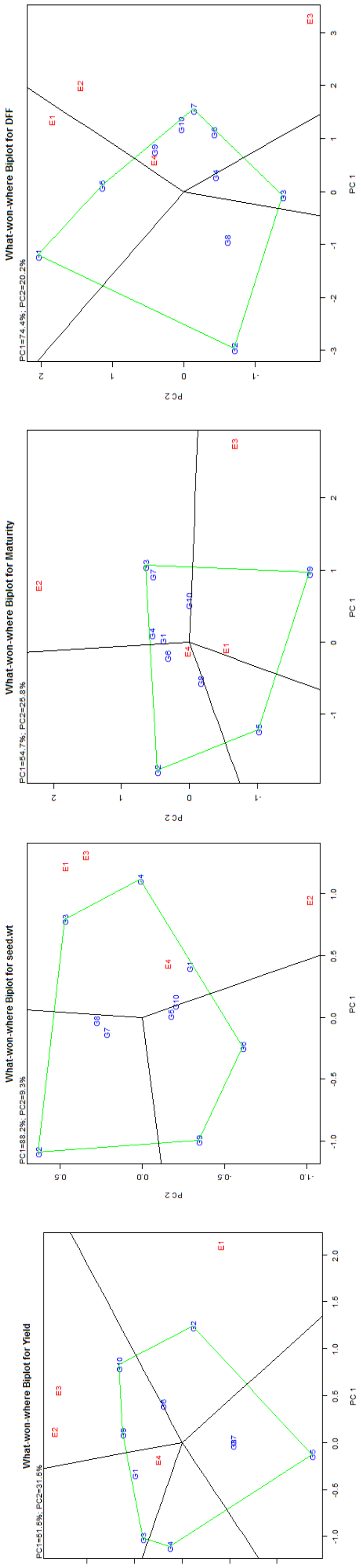
1a: Seed Yield

1b: 1000-seed weight

1c: Days to 75% maturity

1d: Days to 50% flowering

Fig.1. GGE biplots for various traits



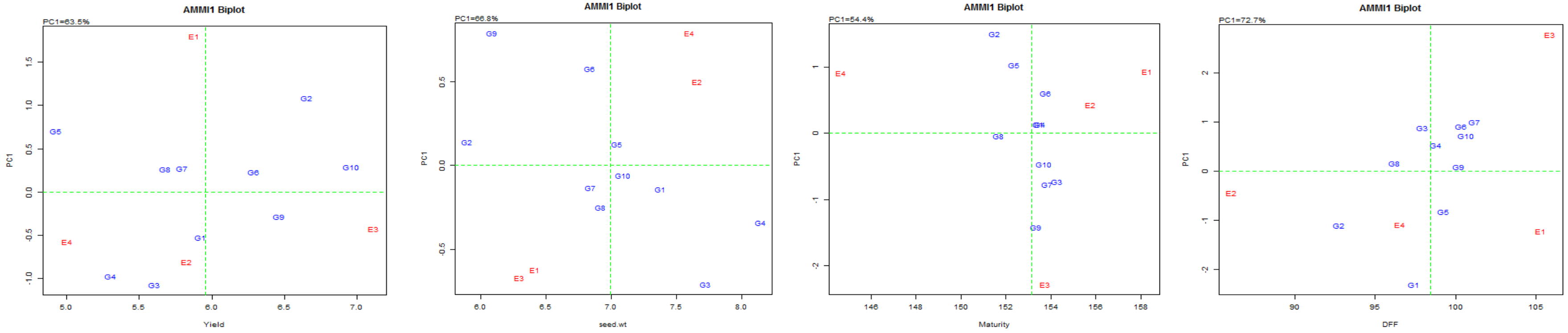
2a: Seed yield

2b: 1000-seed weight

2c: Days to 75% maturity

2d: Days to 50% flowering

Fig. 2. Which-won-where view of the GGE biplot for various traits



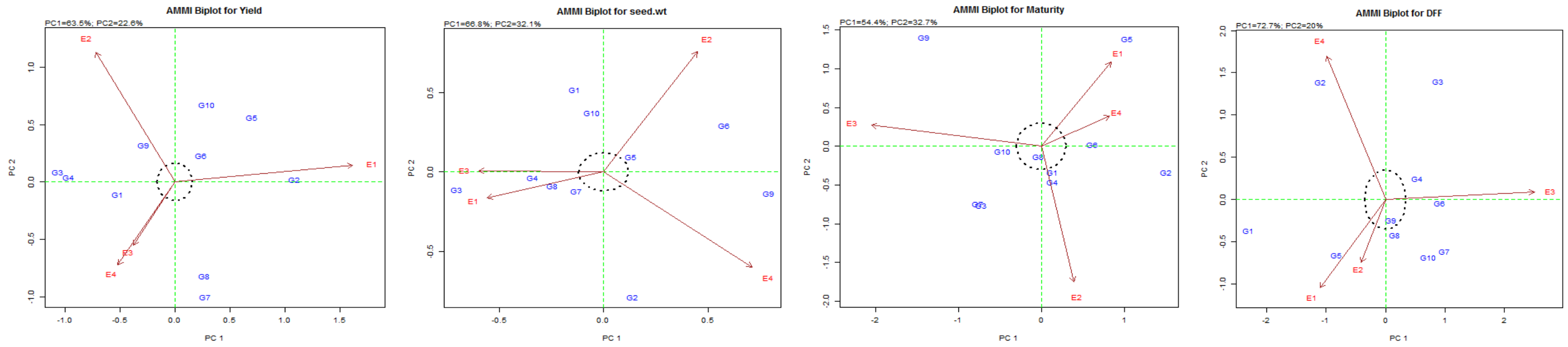
3a: Seed yield

3b: 1000-Seed weight

3c: Days to 75% maturity

3d: Days to 50% flowering

Fig. 3. The AMMI biplot (IPCA1 vs mean) for various traits



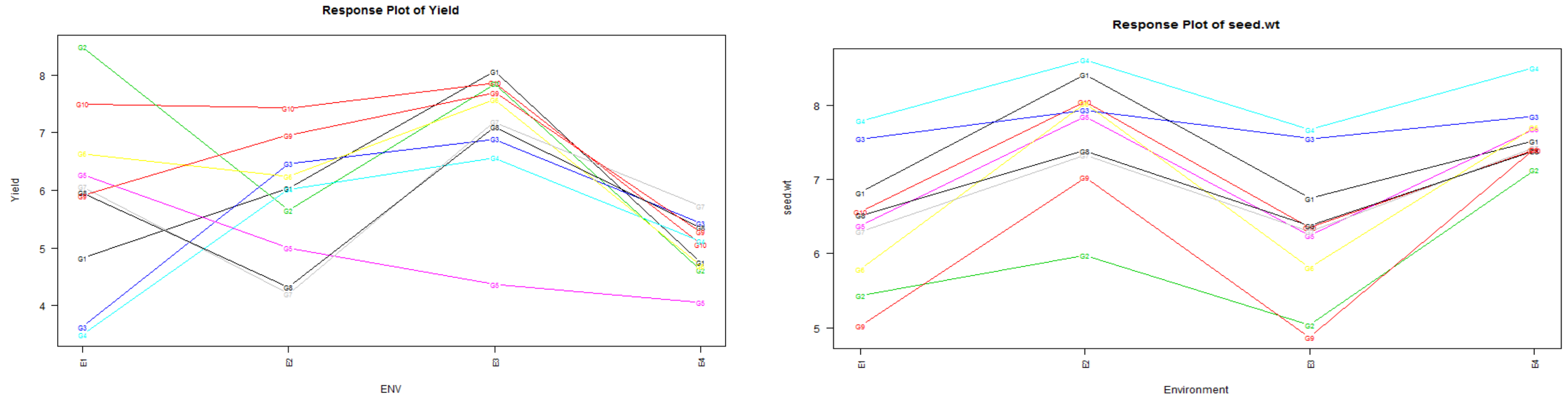
4a: Seed yield

4b: 1000-Seed weight

4c: Days to 75% maturity

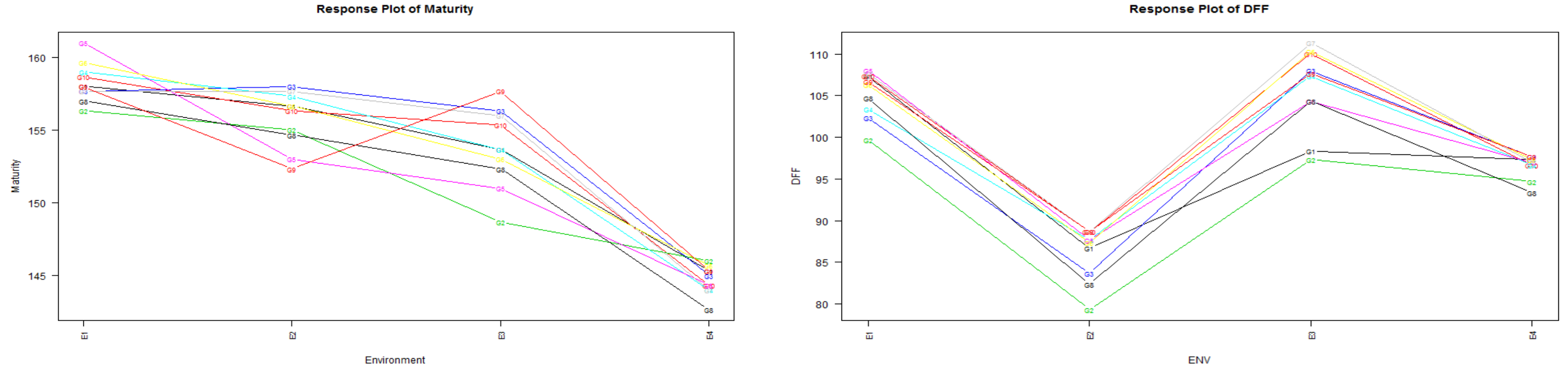
4d: Days to 50% flowering

Fig. 4. The AMMI biplot (IPCA1 vs IPCA2) for various traits.



5a: Seed yield

5b: 1000-Seed weight



5c: Days to 75% maturity

5d: Days to 50% flowering

Fig. 5. Interaction plot for various traits.

on the genotypes as no genotype was wining genotype for seed yield at a given locations during two years. For 1000-seed weight G3 & G4 were the winning genotypes in all the environments. For Days to 75% Maturity G9 was the winning genotype in E1 & E3 and G3 in E2 and G2 in E4. For Days to 50% flowering G7 was the winning genotype in E2, E3 & E4 and G1 and G5 in E1. Partitioning of the target environment into meaningful MEs and deploying different cultivars for different ME is the best way to exploit the positive GEI (Yan and Tinker, 2005). This suggests that some environment were better than others for genotypes evaluation and the uninformative location can be excluded without compromising the precision of evaluation (Chobe and Ararsa, 2018).

The AMMI biplot depicts genotypes and environments as points on a plane in **Fig.3a-d**. The Abscissa showed the main effects and the ordinate showed the first multiplicative axis term (PC1). The vertical dotted line showed the grand mean and horizontal dotted line indicated the interaction score of zero. In the AMMI biplot displacement along the ordinates indicated differences in interaction effects, while displacement along the abscissa indicated differences in main effects. Effects of G, E and G X E interaction on linseed seed yield were highly significant. AMMI biplots (**Fig.3a-d**) clearly indicated that the points for environment are more scattered compared to genotypes for seed yield and shows same pattern of scattering for 1000 seed weight, days to 75% maturity and days to 50% flowering. This emphasized that the variability is more due to environments than genotypes and similar results are depicted by ANOVA. The genotypes on the right side of vertical dotted line (high mean performance) and closer to the horizontal dotted line showing IPCA1=0 (negligible or no G X E interaction) are considered adaptable to all environment. Whereas, the genotypes with high mean performance and large value of IPCA1 score are having specific adaptability to a particular environment (Bhagwat *et al.,* 2018). G2, G6, G9, G10 were above average for seed yield in the E1 (**Fig.3a-d**) and out of these G10 & G6 were the most stable linseed genotypes based on IPCA1 scores. For 1000 seed weight the genotype G4, G3 showed the high seed weight but were less stable and G1 with less IPCA score IPCA=0 was adaptable to all environments. The genotypes G2, G5 and G8 showed earliness in maturity in E4 and were stable because their interaction is positive and for flowering G2 and G1 showed earliness but were unstable because of instability in E2 & E4.

For AMMI-2 Model, a biplot is generated using genotypic and environmental scores of the first two AMMI components (Vergas and Crossa, 2000). Furthermore, when IPCA1 is plotted against IPCA2, the genotypes with score closer to the centre of the biplot are more stable than the others (Purchase, 1997). In AMMI (**Fig.4a-d**) the location scores are joined to the origin by side lines. Locations with long vector might influence strong interactive forces. Those with short vector exert weak interaction. For seed yield the locations E3, E2 and E1 had long vectors, for 1000 seed weight E2 and E4 had long vector, for earliness in maturity E3 and E2 and earliness in flowering E4 and E3 had long vectors, thus they exert strong interactive forces.

IPCA1 component accounted for only 22.6 per cent. Distribution of genotype points in the AMMI-2 biplot revealed that the genotypes, G6, G9 and G1 are closer to the origin, indicating minimal interaction of these genotypes with environment for seed yield. For 1000-seed weight IPCA1 accounted for 66.8 and IPCA2 32.1 per cent. Genotypes G5, G7 and G8 show minimal interaction with environment for 1000 seed weight. For earliness in maturity G8 and G1 shows minimal interaction with environment, thus can be exploited for earliness in maturity. For earliness in flowering G9 was the most stable genotype.

The average grain yield distribution in the examined environments as follow: E1>E3>E2>E1 (**Fig.5a-d**). The 1000-seed weight distribution in the examined environment E2>E4>E1>E3, for earliness in maturity E4>E2>E3>E1 and for earliness in flowering E2>E4>E1>E3.

The results of the present investigation revealed the existence of large and complex G X E interaction, which imply the repressiveness of some genotypes to different environments. The GGE biplot analysis showed that the genotypes G2 (LCP 87), G10 (LC 2063), G9 (LDCP 13) and G3 (M6) were vertex genotypes and suited to particular environments for seed yield. According to AMMI analysis for stability, genotypes G10 (LC 2063) and G6 (LDCP 1) were shown to have higher stability for seed yield. The genotypes G2 (LCP 87), G5 (JSB 1) and G8 (LCP 3) showed the stability for earliness in maturity. The breeding lines G10 (LC 2063) and G2 (LCP 87) are further recommended for its inclusion in the breeding programme due to its high seed yield and breeding lines G10 (LC 2063) and G6 (LDCP 1) for stability.

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