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Original Article

Evaluation of genotype × environment interaction and stability analysis for seed yield and oil content in *Brassica juncea* L.

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Abstract

Brassica juncea is one of the major oilseed crops in the subcontinent and worldwide, with its performance affected by environmental conditions; therefore, understanding the genotype × environment interaction (GEI) is essential for identifying high-yielding and stable genotypes. Forty-five *B. juncea* genotypes were evaluated across four diverse locations in Khyber Pakhtunkhwa over two years to assess stability and high yield. For this purpose, additive main effects and multiplicative interaction (AMMI) analysis was applied to seed yield and oil content, following a significant GEI. Significant ($P \leq 0.01$) GEI was observed for the studied traits, indicating substantial differences among genotypes across tested environments. Overall, higher seed yield was recorded for genotypes AUP-619 and AUP-641, and elevated oil content was found for AUP-1800, as compared to the check cultivars. The AMMI-1 biplot analysis identified relatively stable and high-yielding genotypes for seed yield and oil content. The biplot also revealed that the Kohat and Bannu environments showed limited discriminatory ability for both traits among genotypes, likely due to uniform environmental stress and restricted genetic variation for both traits. Additionally, the AMMI-2 biplot assessment detected two possible mega-environments for seed yield and oil content, along with their associated genotypes, highlighting the significance of environment-specific allocation of genotypes. Moreover, the genotype selection index identified comparatively stable and high-yielding genotypes, such as AUP-618 for seed yield and AUP-625 for oil content. These findings demonstrate the effectiveness of AMMI-based approaches in identifying relatively stable and specifically adapted *B. juncea* genotypes for diverse agro-climatic conditions.

Keywords: Genotype × Environment interaction (GEI), Stability analysis, AMMI analysis, AMMI stability value (ASV), Genotype selection index (GSI)

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Introduction

The genus Brassica (Brassicaceae) comprises several economically important oilseed crops, and their genetic relationships are explained by the classical U-triangle model proposed by Nagaharu (1935). According to this model, the allotetraploid *Brassica juncea* (AABB, 2n = 36) originated through natural hybridization between *B. rapa* (AA, 2n = 20) and *B. nigra* (BB, 2n = 16). *B. juncea* (Indian mustard) is extensively cultivated in South Asia due to its wide adaptability, short growth duration, and tolerance to heat, drought, major diseases, and insect pests. Its seeds contain 44–46% high-quality oil, whereas the residual meal possesses 38–40% protein with a balanced amino acid composition, making it valuable for both human consumption and livestock feed (Yılmaz et al., 2024).

The worldwide productivity of rapeseed-mustard ranges from 86.0 to 92.3 million tonnes, with an average yield of 2070 kg ha^{-1}, whereas Pakistan contributed only 0.796 million tonnes, with a yield of 1335.57 kg ha^{-1} (USDA, 2024; MNFSR, 2023). Khyber Pakhtunkhwa province merely added 7.50 thousand tonnes, with a mean yield of 561 kg ha^{-1}, showing less attention towards this crop. Additionally, the importation of 2.681 million tonnes edible oil cost about US$3.56 billion, whereas local production accounted for only 0.496 million tonnes, indicating a heavy burden on the Pakistan's economy (MNFSR, 2023). These data emphasize the need for the development of mustard cultivars with high yield and stability, so that the burden on economy can be reduced.

An effective cultivar development program is directed by the amount of genetic variability and the genotype × environment interaction (GEI) (Begna and Teressa, 2024). Specifically, quantitative traits like seed yield, oil content, and their associated traits are highly affected by genotype, environment, and GEI, making the selection process difficult (Bascuñán-Godoy et al., 2023). Therefore, an analysis of GEI is crucial for selecting widely and specifically adapted genotypes (Qamar et al., 2025). Commonly, pooled analysis of variance (ANOVA) is used to dissect GEI, but it does not identify stable genotypes across distinct environments (El-Hashash and El-Absy, 2025). Consequently, several statistical models have been proposed to explain GEI effects, among which the additive main effects and multiplicative interaction (AMMI) model is widely used (Lin et al., 2025).

The AMMI approach integrates ANOVA for main effects with interaction principal component analysis (IPCA) for interaction effects, thereby providing a more precise interpretation of GEI patterns (Fonsêca et al., 2025). Furthermore, Purchase et al. (2000) proposed the AMMI stability value (ASV) based on the AMMI model's IPCA1 and IPCA2 scores for each genotype. A genotype with the lowest ASV value is considered more stable across distinct environments. However, stability alone does not mean that the genotype will be high-yielding. Therefore, ASV and the genotype selection index (GSI) allow for the simultaneous selection of high-yielding and stable genotypes across environments (Farshadfar et al., 2011; Wang et al., 2023).

A major reason for the low yield of mustard in Khyber Pakhtunkhwa is the lack of a complete understanding of the performance of genotypes across different environmental conditions. To date, GEI and stability studies of *Brassica juncea* have been limited in this region, making it difficult to identify locally adapted and stable genotypes. Addressing this research gap, the aim of this study was to understand the GEI effects for seed yield and oil content, explain interaction patterns using the AMMI model, and identify genotypes that are relatively stable and high-performing under different agro-ecological conditions. These findings will assist in providing a better foundation for future breeding programs and regional cultivation.

Material and Methods

Plant material

A set of 45 *Brassica juncea* genotypes was evaluated, including two check cultivars: UCD-10/8 (University of California, Davis, USA) and NIFA-Raya (Nuclear Institute for Food and Agriculture, Peshawar, Pakistan) (Table 1). Forty accessions were collected from diverse agro-ecological regions of Pakistan, whereas five exotic lines were obtained from the University of California, Davis, USA. Prior to field evaluation, all genotypes were advanced for two generations through selection and seed multiplication to ensure genetic uniformity.

Table-1. Forty-five *Brassica juncea* L. genotypes collected across Pakistan.

Line	Genotype	Line	Genotype	Line	Genotype
1	AUP-600	16	AUP-623	31	AUP-653
2	AUP-601	17	AUP-624	32	AUP-654
3	AUP-602	18	AUP-625	33	AUP-655
4	AUP-604	19	AUP-626	34	AUP-656
5	AUP-605	20	AUP-627	35	AUP-657
6	AUP-606	21	AUP-628	36	AUP-658
7	AUP-609	22	AUP-630	37	AUP-659
8	AUP-610	23	AUP-632	38	AUP-663
9	AUP-611	24	AUP-633	39	AUP-1800
10	AUP-614	25	AUP-634	40	AUP-2727
11	AUP-615	26	AUP-641	41	UCD-635
12	AUP-616	27	AUP-642	42	UCD-636
13	AUP-617	28	AUP-645	43	UCD-638
14	AUP-618	29	AUP-649	44	UCD-10/8 (check)
15	AUP-619	30	AUP-652	45	NIFA-Raya (check)

UCD = University of California, Davis, United States of America. AUP = Agriculture University Peshawar, Pakistan

Experimental sites and trial design

The field trails were conducted over two consecutive cropping seasons (2016-17 and 2017-18) at four locations in Khyber Pakhtunkhwa, Pakistan: (i) The University of Agriculture, Peshawar; (ii) Amir Muhammad Khan Campus, Mardan; (iii) Barani Agricultural Research Station, Kohat; and (iv) Agricultural Research Station, Serai Naurang (Bannu) (Table 2). These locations represent the major mustard-growing agro-ecological zones, ranging from subtropical semi-arid to sub-humid environments. Peshawar is characterized by warm semi-arid conditions with relatively mild winters. Mardan lies in a sub-humid zone with slightly higher precipitation. Kohat is situated in a dry, moderately elevated valley with greater diurnal temperature variation, while Bannu represents a warmer low-elevation environment typical of the southern plains (Khan and Hasan, 2019).

Table-2. Description of the tested locations in Khyber Pakhtunkhwa, Pakistan.

Site	Latitude (N)	Longitude (E)	Altitude (m)
Peshawar	34.008°	71.580°	331
Mardan	34.200°	72.033°	311
Kohat	33.583°	71.433°	489
Bannu	32.985°	70.604°	376

At each location during both cropping seasons, the experiment was laid out in a randomized complete block design with two replications. Each genotype was sown in five-row plots of 5 m length, maintaining a 75 cm row spacing and a 30 cm plant-to-plant distance. Sowing was carried out during the third week of October in both years. Standard agronomic practices were uniformly applied across all locations. Seeds were dibbled at a depth of 1-2 cm in well-prepared seedbeds. Irrigation was applied at critical growth stages, and weeds were controlled manually and mechanically.

Oil content determination

Seed oil content (%) was estimated at the Crop Breeding Section, Biochemical Laboratory, Nuclear Institute for Food and Agriculture, Peshawar, Pakistan, using near-infrared reflectance spectroscopy. Clean and well-dried samples of each genotype were scanned three times, and the mean value was used for subsequent analyses.

Statistical analysis

Pooled ANOVA was used for the pods plant^{-1}, seeds pod^{-1}, 1000-seed weight, seed yield, and oil content, as described by Steel et al. (1997). The sources of variation for this model consisted of genotype, location, year, and their interactions. The analysis was conducted using SAS software (SAS Institute, 2004), and genotype means were compared using the least significant difference (LSD) test at a probability level of 5%.

Genotype and environment coding

Each genotype listed in Table 1 was coded from Line 1 to Line 45 for graphical analysis. For better assessment of the GEI, each location × year interaction was considered a separate environment, as significant genotype × location × year interaction was observed in the pooled ANOVA, resulting in variable genotypic performance. Accordingly, environments were denoted as E-01 (Peshawar 2016-17), E-02 (Peshawar 2017-18), E-03 (Mardan 2016-17), E-04 (Mardan 2017-18), E-05 (Kohat 2016-17), E-06 (Kohat 2017-18), E-07 (Bannu 2016-17), and E-08 (Bannu 2017-18). Following the detection of a significant GEI, AMMI analysis was applied to seed yield and oil content.

AMMI Analysis

The AMMI model was employed using the GEA-R version 4.1 software (Pacheco-Gil et al., 2015). The model is expressed as:

$$Y_{ij=\mu+G_i+E_j+\sum_{k=1}^{n}\lambda_k\alpha_{ik}\gamma_{jk}+\varepsilon_{ij}}$$

where Y_{ij} is the mean response of the i^{th} genotype in the j^{th} environment, μ is the grand mean, G_i and E_j are the genotype and environment deviations from the grand mean, respectively, λ_k is the singular value of the k^{th} IPCA, α_{ik} and γ_{jk} are the genotype and environment eigenvectors for the k^{th} IPCA, and ε_{ij} is the residual error. Biplots were generated using the first two IPCAs to visualize genotype performance and stability.

AMMI stability value

Genotype stability was assessed using the ASV, as proposed by Purchase et al. (2000):

$$\mathbf{ASV} = \sqrt{\left[\frac{\mathbf{SS_{PCA1}}}{\mathbf{SS_{PCA2}}}(\mathbf{IPCA1\ Score})\right]^2 + (\mathbf{IPCA2\ Score})^2]}$$

where SS_{PCA1}/SS_{PCA2} represents the weighting factors derived from the sums of squares of IPCA1 and IPCA2.

Genotype selection index

To facilitate the simultaneous selection of both high mean yield and stability, the GSI was calculated by integrating the rank of mean yield (RY_i) with the rank of ASV ($RASV_i$), as described by Farshadfar et al. (2011):

$$\mathbf{GSI = RY_i + RASV_i}$$

Genotypes with lower GSI values were considered desirable.

Variance component analysis

Linear mixed model analysis was performed to estimate the variance components of genotype and GEI using SPSS version 23 (West, 2009). In this model, genotype and GEI were treated as random effects, while environment was considered fixed effect. Genotype and GEI effects were treated as random to accurately estimate their variance components and to quantify the variability in genotypic performance across environments. In contrast, environment effect was considered fixed because tested environments represented specific locations in particular years. Consequently, the software only estimated variance components of the random effects (genotype and GEI). This approach makes it possible to understand the magnitudes of genotypic and GEI variances, while environmental effect had already been calculated through the AMMI ANOVA.

Results

Pooled ANOVA and mean performance of genotypes

The pooled ANOVA revealed highly significant ($P \leq 0.01$) differences among genotypes (G), locations

(L), years (Y), and their first- and higher-order interactions (L × Y, G × L, G × Y, and G × L × Y) for most of the tested traits (Table 3). However, the effects of location, G × Y, and G × L × Y interactions were non-significant for seeds pod^{-1} and 1000-seed weight.

Table-3. Mean squares for studied traits of 45 *Brassica juncea* genotypes evaluated across four locations over two years.

	DF	Pods plant^{-1}	Seeds pod^{-1}	1000-seed weight	Seed yield	Oil content
Location (L)	3	1481.27**	5.63^{NS}	0.105^{NS}	2319325**	547.31**
Year (Y)	1	18617.71**	11.67**	0.392**	8206980**	666.82**
L×Y	3	2786.21**	1.28**	0.541**	3338250**	26.46**
Rep w/n (L×Y)	8	182.53	4.14	0.071	49749	34.5
Genotype (G)	44	138026.37**	8.39**	0.237**	4472284**	13.13**
G×L	132	676.87**	2.58**	0.033**	51485**	2.00**
G×Y	44	459.27**	0.34^{NS}	0.005^{NS}	121004**	0.98**
G×L×Y	132	502.52**	0.29^{NS}	0.005^{NS}	107999**	1.02**
Error	288	136.76	0.30	0.006	13471	0.8
CV (%)		2.46	4.63	2.06	5.62	2.03

** = significant at the 1% probability level and NS = non-significant.

Averaged over two years at each location, genotype AUP-619 recorded the highest number of pods plant^{-1} at Peshawar (729) and Mardan (718), and the maximum seed yield at Peshawar (4318 kg ha^{-1}) and Bannu (3994 kg ha^{-1}), whereas AUP-641 was superior for pods plant^{-1} at Kohat (721) and Bannu (734), and for seed yield at Mardan (4297 kg ha^{-1}) and Kohat (4087 kg ha^{-1}) (Table 4). Higher oil content was recorded for AUP-628 at Bannu (44.9%) and AUP-626 at Mardan (48.4%), whereas AUP-1800 exhibited superior oil content at Peshawar (47.6%) and Kohat (46.3%).

Among the tested locations, pods plant^{-1} and seed yield were higher at Peshawar (478; 3134 kg ha^{-1}) than at Mardan, Kohat, and Bannu. However, oil content was greater at Peshawar and Mardan (45.7% at both locations) compared to Kohat and Bannu (Table 4). Across all tested environments, pods plant^{-1} reached a maximum of 722 (AUP-619), seed yield attained up to 4141 kg ha^{-1} (AUP-641), oil content increased to 46.3% (AUP-1800) (Table 4). The overall mean seed yield and oil content of the tested genotypes exceeded those of the two check cultivars (2140 kg ha^{-1} and 44.2%, respectively).

Table-4. Mean performance of 45 *Brassica juncea* genotypes evaluated across four locations over two years. The codes (e.g., AUP- and UCD-) shown in parentheses denote the genotypes exhibiting the minimum and maximum mean values at the tested locations for the respective traits.

Location		Pods plant^{-1}	Seeds Pod^{-1}	1000-seed weight (g)	Seed yield (kg ha^{-1})	Oil content (%)
Peshawar	Min	370 (AUP-604)	10 (AUP-653)	3.6 (AUP-630)	2460 (AUP-630)	42.6 (UCD-636)
	Max	729 (AUP-619)	14 (AUP-649)	4.3 (AUP-653)	4318 (AUP-619)	47.6 (AUP-1800)
	Check	357	12	4.0	2163	45.7
	Mean	478	12	3.9	3134	45.7
Mardan	Min	353 (AUP-1800)	10 (AUP-627)	3.7 (AUP-649)	2262 (AUP-627)	42.9 (AUP-633)
	Max	718 (AUP-619)	14 (AUP-614)	4.2 (AUP-653)	4297 (AUP-641)	48.4 (AUP-626)

	Check	362	11	3.8	2245	45.5
	Mean	476	12	3.9	2998	45.7
Kohat	Min	354 (AUP-606)	10 (AUP-616)	3.6 (AUP-649)	1922 (AUP-614)	40.6 (AUP-606)
	Max	721 (AUP-641)	14 (AUP-654)	4.3 (AUP-653)	4087 (AUP-641)	46.3 (AUP-1800)
	Check	341	12	3.9	2095	42.3
	Mean	472	12	3.8	2922	42.8
Bannu	Min	355 (AUP-655)	10 (UCD-635)	3.5 (AUP-649)	1976 (AUP-616)	40.1 (UCD-636)
	Max	734 (AUP-641)	14 (AUP-624)	4.2 (AUP-653)	3994 (AUP-619)	44.9 (AUP-628)
	Check	335	12	3.9	2056	43.5
	Mean	474	12	3.9	2873	42.6
Environments	Min	374 (AUP-606)	10 (AUP-642)	3.6 (AUP-649)	2365 (AUP-605)	42.0 (UCD-636)
	Max	722 (AUP-619)	13 (AUP-654)	4.3 (AUP-653)	4141 (AUP-641)	46.3 (AUP-1800)
	Check	349	12	3.9	2140	44.2
	Mean	475	12	3.9	2982	44.2

AMMI analysis for seed yield

The AMMI ANOVA showed highly significant (P≤0.01) effects of genotypes, environments, and GEI for seed yield (Table 5a). The genotype main effect explained the largest proportion of the total variation (79.24%) for the studied trait, followed by GEI (10.62%) and environment (10.14%). Similarly, the mixed model analysis also revealed that genotypic variance (84.59%) was greater than GEI variance (11.01%) for seed yield (Table 5b).

Furthermore, partitioning of GEI for the tested trait into IPCAs indicated that the first two axes (IPCA1 = 28.95% and IPCA2 = 21.88%) were highly significant and together explained 50.83% of the GEI sums of squares (Table 5a).

Table-5a. AMMI analysis of variance for selected traits of 45 *Brassica juncea* genotypes.

		Seed yield			Oil content		
SOV	**DF**	**SS**	**Var (%)**	**MS**	**SS**	**Var (%)**	**MS**
Genotype (G)	44	98380829	79.24	2235928**	289	16.94	6.56**
Environment (E)	7	12589048	10.14	1798435**	1193	70.03	170.42**
GEI	308	13189244	10.62	42822**	222	13.03	0.72**
IPCA1	50	11454950	28.95	229099**	179	26.95	3.59**
IPCA2	48	8657794	21.88	180371**	156	23.50	3.26**
IPCA3	46	6775105	17.12	147285**	115	17.22	2.49**

Table-5b. Genotypic and GEI variance estimates from the mixed model analysis for seed yield and oil content.

Trait	Genotypic (G) variance	GEI variance	G (%)	GEI (%)
Seed yield	274165.49	35679.59	84.59	11.01
Oil content	0.73	0.00	32.67	0.00

The AMMI-1 biplot separated genotypes and environments based on mean seed yield and IPCA1 scores (Figure 1a). Genotypes AUP-610 (Line 8), AUP-619 (Line 15), AUP-641 (Line 26), and AUP-649 (Line 29) were positioned on the right side of the abscissa with relatively low IPCA scores, showing both stability and above-average seed yield. Genotypes AUP-632 (Line 23), UCD-10/8 (Line 44), and AUP-614 (Line 10) were found to be relatively stable, with low IPCA scores for the tested trait, while AUP-604 (Line 4), AUP-645 (Line 28), and AUP-659 (Line 37) exhibited high IPCA values. Environments E-02 (Peshawar 2017-18), E-03 (Mardan 2016-17), and E-04 (Mardan 2017-18) had long vectors for seed yield, whereas E-06 (Kohat 2017-18) and E-08 (Bannu 2017-18) were located closer to the origin (Figure 1a).

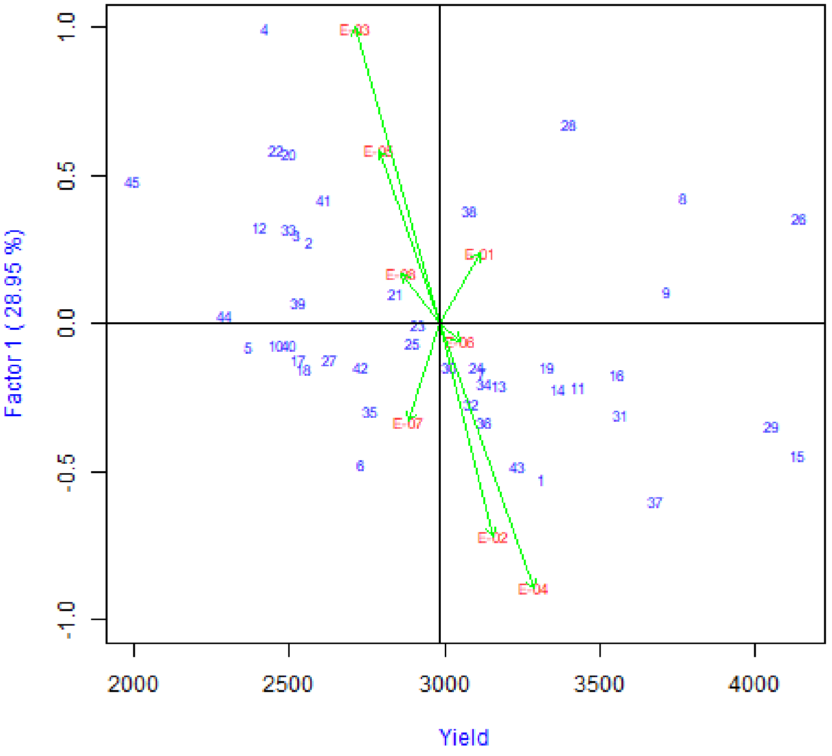

Figure-1a. AMMI-1 biplot for seed yield (kg ha^{-1}).

The AMMI-2 biplot, combining IPCA1 and IPCA2 scores, explained almost half (≈ 50%) of the total GEI variance for seed yield (Figure 1b). Genotypes AUP-604 (Line 4), AUP-645 (Line 28), AUP-659 (Line 37), and AUP-655 (Line 33) were located farther from the origin, whereas AUP-605 (Line 5), AUP-614 (Line 10), and AUP-624 (Line 17) were positioned closer. Environments E-03 (Mardan 2016-17), E-04 (Mardan 2017-18), E-07 (Bannu 2016-17), and E-08 (Bannu 2017-18) exhibited long vectors, while E-01 (Peshawar 2016-17) was positioned close to the origin. The biplot delineated two mega-environments for seed yield: the first comprised E-02 (Peshawar 2017-18) and E-04 (Mardan 2017-18) with AUP-600 (Line 1) and AUP-619 (Line 15) as the vertex genotypes; the second consisted of E-05 (Kohat 2016-17) and E-08 (Bannu 2017-18), with AUP-634 (Line 25) and AUP-645 (Line 28) positioned at the polygon vertices (Figure 1b).

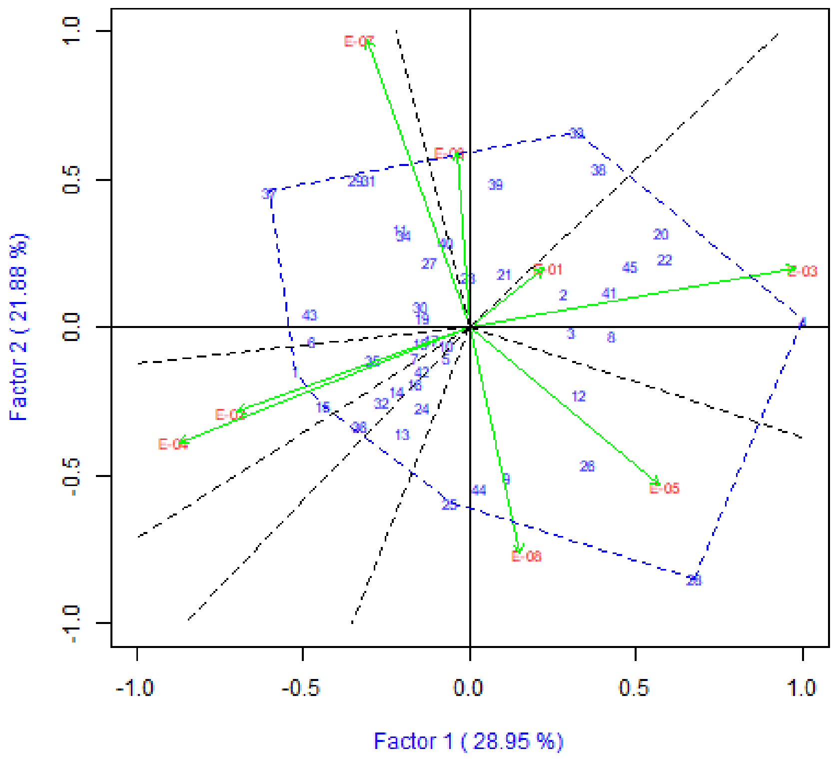

Figure-1b. AMMI-2 biplot for seed yield.

Based on the GSI, genotype AUP-626 ranked first (GSI = 17) for seed yield, followed by AUP-623 and AUP-618 (Table 6). These genotypes combined relatively high yield (3327–3553 kg ha^{-1}) with low ASVs (0.19–0.36). The check cultivars UCD-10/8 and NIFA-Raya ranked 28th and 32nd, respectively.

Table-6. Top ten *Brassica juncea* genotypes based on the genotype selection index for seed yield (kg ha^{-1}).

Genotype	Mean	SE	Mean rank	ASV	ASV rank	GSI	GSI rank
AUP-626	3327	75	12	0.19	5	17	1
AUP-623	3553	73	8	0.29	11	19	2
AUP-618	3367	71	11	0.36	14	25	3
AUP-609	3123	65	18	0.24	9	27	4
AUP-632	2911	69	23	0.18	4	27	4
AUP-611	3714	80	5	0.53	23	28	5
AUP-615	3428	66	9	0.44	19	28	5
AUP-652	3013	73	22	0.21	7	29	6
AUP-610	3770	41	4	0.57	26	30	7
AUP-619	4137	82	2	0.64	29	31	8
AUP-641	4141	61	1	0.66	30	31	8
AUP-633	3105	73	19	0.33	13	32	9
AUP-628	2841	64	25	0.23	8	33	10
UCD-10/8 (check)	2287	85	42	0.54	24	66	28
NIFA-Raya (check)	1993	34	43	0.67	31	74	32

SE = Standard error.

AMMI analysis for oil content

The AMMI ANOVA for oil content revealed significant (P≤0.01) effects of genotypes, environments, and GEI (Table 5a). The environment main effect contributed the largest proportion of variation (70.03%), followed by genotype (16.94%) and GEI (13.03%). Furthermore, mixed model analysis showed genotypic variance of 32.67%, while GEI variance became negligible (0.00%) for the studied trait (Table 5b).

Partitioning of the GEI showed that the first two IPCAs were significant, with IPCA1 and IPCA2 explaining 26.95% and 23.50% of the GEI variation, respectively, and together accounting for 50.45% of the total interaction sums of squares (Table 5a).

The AMMI-1 biplot distributed genotypes and environments based on mean oil content and IPCA1 scores (Figure 2a). Comparatively stable genotypes with higher oil content, including AUP-626 (Line 19), AUP-653 (Line 31), and AUP-1800 (Line 39), were located on the positive side of the abscissa with lower IPCA scores. Genotypes AUP-628 (Line 21), AUP-652 (Line 30), and AUP-1800 (Line 39) were identified as relatively stable due to their lower IPCA scores, while AUP-632 (Line 23), AUP-633 (Line 24), and AUP-645 (Line 28) showed higher IPCA values. Environments E-02 (Peshawar 2017-18) and E-04 (Mardan 2017-18) displayed long vectors, whereas E-06 (Kohat 2017-18) and E-08 (Bannu 2017-18) were positioned near the center of the biplot (Figure 2a).

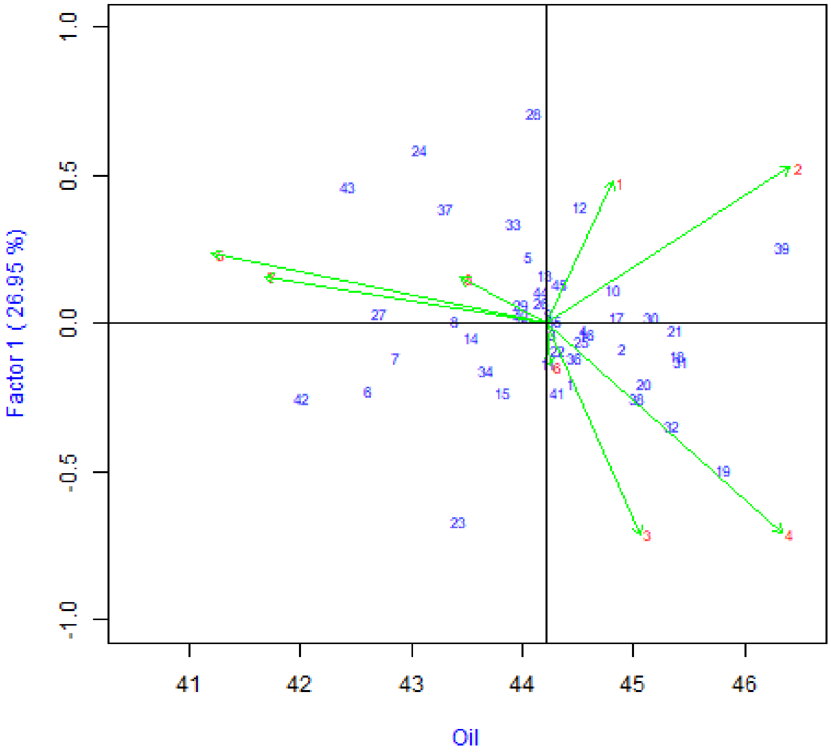

Figure-2a. AMMI-1 biplot for oil content (%).

The AMMI-2 biplot, using the first two IPCAs (IPCA1 and IPCA2), accounted for approximately 50% of the total GEI variance for oil content (Figure 2b). Genotype AUP-641 (Line 26) was located farther from the origin, followed by AUP-645 (Line 28) and AUP-632 (Line 23), whereas AUP-623 (Line 16), AUP-642 (Line 27), and AUP-657 (Line 35) were clustered around the origin. Environments E-02 (Peshawar 2017-18) and E-05 (Kohat 2016-17) showed long vectors, while E-07 (Bannu 2016-17) and E-08 (Bannu 2017-18) were close to the origin. Two mega-environments were identified for oil content: The first included E-01 (Peshawar 2016-17) and E-02 (Peshawar 2017-18) with AUP-617 (Line 13) and AUP-645 (Line 28) as vertex genotypes, and the second contained E-03 (Mardan 2016-17) and E-04 (Mardan 2017-18) with AUP-645 (Line 28) as the winning genotype (Figure 2b).

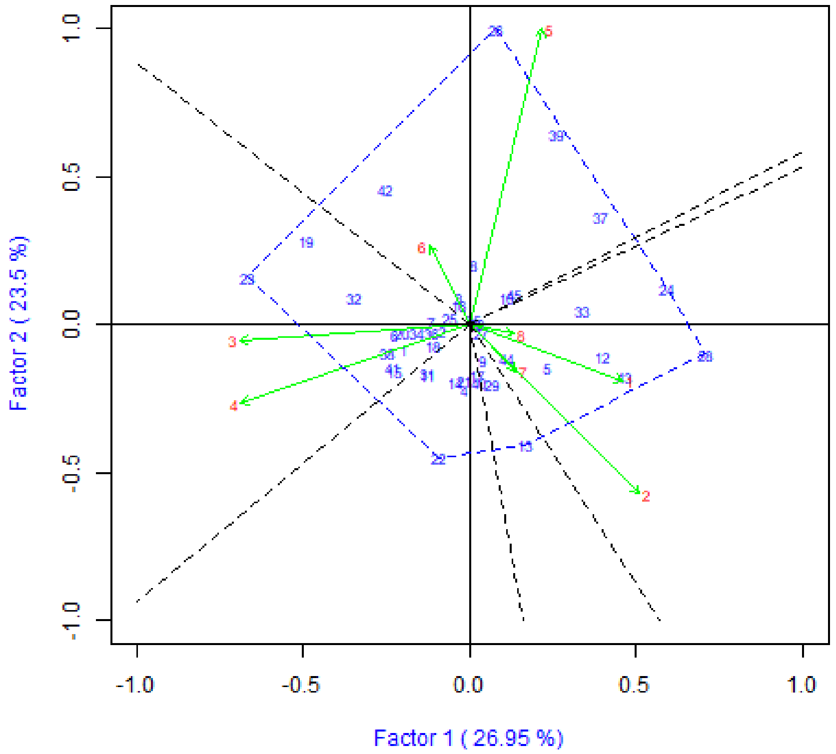

Figure-2b. AMMI-2 biplot for oil content.

The lowest GSI for oil content was recorded for genotype UCD-636 (GSI = 6; rank = 1), followed by AUP-625 and AUP-649 (Table 7). These genotypes exhibited relatively high oil content (44.3%–45.1%) with low ASVs (0.02–0.07). Compared to the check cultivars, UCD-10/8 ranked 9th with a GSI score of 24, and NIFA-Raya ranked 16th with a GSI score of 32, showing below-average performance relative to most of the tested genotypes (Table 7).

Table-7. Top ten *Brassica juncea* genotypes based on the genotype selection index (GSI) for oil content (%).

Genotype	Mean	SE	Mean rank	ASV	ASV rank	GSI	GSI rank
UCD-636	45.1	0.50	5	0.02	1	6	1
AUP-625	44.5	0.57	9	0.07	3	12	2
AUP-649	44.3	0.54	11	0.02	1	12	2
AUP-610	44.5	0.57	9	0.08	4	13	3
AUP-614	45.4	0.50	3	0.15	10	13	3
AUP-630	44.9	0.71	7	0.1	6	13	3
AUP-618	45.3	0.67	4	0.18	12	16	4
AUP-652	44.2	0.54	12	0.09	5	17	5
UCD-638	45.4	0.56	3	0.22	15	18	6
AUP-600	44.2	0.56	12	0.12	7	19	7
AUP-601	44.8	0.51	8	0.17	11	19	7
AUP-611	44.8	0.55	8	0.17	11	19	7
AUP-653	44.4	0.58	10	0.14	9	19	7

AUP-617	45.1	0.67	5	0.23	16	21	8
AUP-658	44.3	0.63	11	0.19	13	24	9
AUP-659	44.5	0.47	9	0.22	15	24	9
AUP-633	42.7	0.50	23	0.05	2	25	10
UCD-10/8 (check)	44.1	0.57	13	0.17	11	24	9
NIFA-Raya (check)	43.4	0.43	19	0.19	13	32	16

Discussion

Several stable *Brassica juncea* genotypes and suitable environments for cultivation were identified in this study for seed yield and oil content. The significant differences among genotypes (G), locations (L), years (Y), and their lower-order (G × L and G × Y) and higher-order (G × L × Y) interactions for most of the tested traits indicated that the genetic makeup and environmental conditions considerably affected the expression of traits in *B. juncea*. Conversely, seeds pod^{-1} and 1000-seed weight showed non-significant differences among locations, G × Y, and G × L × Y, suggesting comparatively stable traits across environments. These parameters can serve as selection criteria for high-yielding genotypes (Gupta et al., 2025). Corresponding results have been reported by Sur et al. (2022) and Yadav and Lal (2023) in *B. juncea* and other oilseed crops, identifying significant effects of diverse climatic conditions on seed yield, oil content, and their contributed traits.

Genotypes that produced higher numbers of pods plant^{-1}, maximum seed yield, and elevated oil content may have been influenced by stable gene expression across the tested locations over two years. Among the locations, such as Peshawar and Mardan were identified as more favorable for higher seed yield, oil content, and associated traits, indicating that the climate in Peshawar and Mardan was suitable for productive growth and oil formation due to optimal temperature and moisture availability in the soil. Compared to the check cultivars (UCD-10/8 and NIFA-Raya), the overall means of yield and associated traits of the tested genotypes were higher, illustrating the outstanding performance of several tested genotypes over the checks.

The AMMI ANOVA also validated the significant effects of genotypes, environments, and their interaction on seed yield and oil content, suggesting the complicated and quantitative nature of these traits in *B. juncea*. The AMMI analysis showed greater genotypic variance than environmental variance for seed yield, while mixed model analysis also indicated higher genotypic variance then GEI variance, highlighting the polygenic nature of the trait. Adžić et al. (2023) and Łopatyńska et al. (2023) also reported higher genotypic effects for seed yield in Brassica, highlighting the significance of multi-environment trials in resolving variable genotypic responses. On the other hand, environmental variance was higher for oil content, illustrating the vulnerability of oil biosynthesis to agro-climatic conditions. Therefore, overall oil content level of the tested genotypes may vary across locations and years. The dominant role of environmental variance in oil content has also been documented in Brassica by Secchi et al. (2022). Mixed model analysis further indicated that the GEI effects for oil content were very low, indicating that the genes expressing oil content in genotypes were relatively stable across environments. Zhang et al. (2023) also reported low GEI effects, supporting the stability of genetic expression for oil content across diverse environments.

The AMMI model efficiently summarized a large proportion of the GEI for seed yield and oil content using the first two IPCAs, supporting the suitability of AMMI analysis for explaining the complex GEI patterns. These IPCAs were highly significant and were selected for analysis because they collectively accounted for approximately 50% of the total GEI for the studied traits, making them appropriate for the graphical representation of genotypes and environments. Several researchers, including Ghosh et al. (2023) and Yadav et al. (2025), have also documented the significance of the first two IPCAs, and these sufficiently explained the major portion of complex GEI patterns.

The AMMI-1 biplot utilizes both mean yield and IPCA1 scores for seed yield and oil content to identify stable and high-yielding genotypes. Genotypes with both low IPCA scores and high yields for both traits indicated the strong genetic potential of the genotypes across diverse environments, whereas the environments of Peshawar and Mardan (both in 2017-

18) exhibited strong discrimination toward genotypes for both traits, suggesting relatively favorable and less stressful agro-climatic conditions that allow for a clearer expression of genotypic differences. In contrast, the Kohat and Bannu (both in 2017-18) environments showed weak discrimination toward genotypes for seed yield and oil content, reflecting a limited potential to differentiate among genotypes. This may be due to relatively uniform environmental stress or limited genetic variability among the evaluated genotypes at those locations. Genotypes with higher IPCA scores, such as AUP-604, AUP-645, and AUP-659 for seed yield, and AUP-632, AUP-633, and AUP-645 for oil content, were comparatively unstable and showed environment-specific responses. These interpretations align with previous studies by Qasemi et al. (2022) and Datta et al. (2024).

The magnitude and direction of the GEI for seed yield and oil content were further simplified by the AMMI-2 biplot analysis, which contains both IPCA1 and IPCA2 scores. Genotypes that were distantly located from the origin for both traits demonstrated strong interaction and specific adaptation, while those found near the origin indicated relative stability and wide adaptability across environments. Environments such as Mardan and Bannu across both years for seed yield, and Peshawar (2017-18) and Kohat (2016-17) for oil content, displayed long vectors, suggesting comparatively higher discriminatory power toward genotypes, whereas Peshawar (2016-17) for seed yield and Bannu across both years for oil content were positioned near the origin, illustrating minimal genotypic differentiation. Furthermore, two separate mega-environments and their corresponding genotypes were identified for both traits, emphasizing the importance of allocating specific genotypes to these regions to achieve higher productivity. These explanations corroborate the findings of Matin et al. (2025), who demonstrated similar interpretations related to specifically and broadly adapted genotypes, environmental discrimination toward genotypes, and mega-environments in Brassica. However, the mega-environments identified for the studied traits should be considered empirical, as they were based on only eight environments (four locations over two years); therefore, these groupings may change if more locations or years are included.

The GSI combined both high mean and high stability to select top-ranking genotypes for seed yield and oil content. Genotypes exhibiting the lowest GSI values were considered high-yielding and stable across locations over the years. This study identified various relatively superior genotypes based on GSI ranking, such as AUP-618, AUP-623, and AUP-626 for seed yield, and AUP-625, AUP-649, and UCD-636 for oil content, which possess an optimal combination of high yield and stability across diverse environments. These results validate the guidelines documented by Liersch et al. (2024), who highlighted that genotypes having low GSI scores are more favorable for cultivation under diverse agro-climatic environments. However, the GSI is a rank-based index that can be affected by tied ranks, as well as variations in mean performance and stability estimates. Moreover, stability of the genotypic mean was estimated based on the consistency of genotype performance across environments, as reflected by the IPCAs of the AMMI model and the derived ASV and GSI indices. Collectively, these indices effectively summarized the variability among genotypes across environments, making it possible to identify relatively stable and unstable genotypes.

In general, the analyses efficiently selected relatively superior *Brassica juncea* genotypes that were relatively stable across diverse environments, along with identifying possible mega-environments. However, variance components for heritability estimation were not included in this study. Therefore, future studies might integrate these estimates to complement the GEI interpretation. Furthermore, the high-yielding genotypes that were stable and environment-specific could be evaluated in multi-location trials and utilized in breeding programs focusing on diverse agro-climatic conditions. Such approach would facilitate the development and release of mustard varieties suitable for cultivation across a wide range of environments, while genotypes exhibiting superior performance in specific environments may be recommended for cultivation in their respective zones.

Conclusion

The combined and AMMI ANOVAs showed significant differences among genotypes, environments, and GEI for seed yield and oil content in *Brassica juncea*, validating the inconsistent performance of genotypes across environments. Mixed model analysis further indicated that genotypic variance in seed yield was greater than GEI variance, while the effect of GEI on oil content was much less, suggesting the relative stability of this trait. Mean performance indicated that the tested genotypes, including AUP-619 and AUP-641 for seed yield, and AUP-626, AUP-653, and AUP-1800 for oil content, were better than the check cultivars. The AMMI-1 biplot identified AUP-610, AUP-619, AUP-641, and AUP-649 for seed yield, and AUP-626, AUP-653, and AUP-1800 for oil content, as relatively stable and widely adapted genotypes. Moreover, the biplot indicated that Kohat and Bannu were low-discriminating environments for both traits, possibly due to similar environmental stresses and narrow genetic variability among the evaluated genotypes. The AMMI-2 analysis further partitioned the environments into two mega-environments for both traits, indicating the presence of environment-specific adaptation among the genotypes. The GSI highlighted AUP-618, AUP-623, and AUP-626 for seed yield, and AUP-625, AUP-649, and UCD-636 for oil content as promising genotypes. However, as these findings were based on four locations over two years, they should be considered preliminary; therefore, further testing of the genotypes is required for future breeding programs.

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