Bangladesh J. Bot. **54**(4): 957-966, 2025 (December) DOI: https://doi.org/10.3329/bjb.v54i4.86569

GENOTYPE-ENVIRONMENT INTERACTIONS OF SOWING DATES ON MUSTARD USING AMMI AND GGE BIPLOT ANALYSIS

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Keywords: Mustard, Sowing dates, G×E interactions, AMMI analysis, GGE biplot, Stability index

Abstract

Globally, breeding programs focus on selecting stable genotypes to ensure consistent crop yields across varying climatic conditions. In this study, seven mustard genotypes (*Brassica juncea*) were evaluated at three environments (early, mid and late sowing) on ten yield-contributing traits to determine the best genotypes and their optimal growing periods. Significant differences among the three environmental conditions were noted through a combined analysis of variance. The overall seed yield performance at late sowing was considerably lower than the early and mid-sowing conditions. The genotype ranking through AMMI and GGE biplots revealed that Daulat, BARI-11 and BARI-16 were the highest yield-producing stable genotypes across the environments. Additionally, correlation coefficient analysis indicated seed yield was positively correlated with siliquae per plant, thousand-seed weight and harvest index under all the environmental conditions, providing a sound basis for future mustard breeding programs.

Introduction

Brassica oilseeds, including *Brassica juncea* or rai sarisha, constitute a major source of edible oil globally, with mustard oil production (27 MMT) ascending to third place behind soybean and palm oil (FAO 2022). The rising consumption of mustard edible oils is attributed to their high oil (30-48%) and protein (28-36%) content (Saikia *et al.* 2018). However, climatic variability, suboptimal agronomic management, and the narrow genetic base of cultivated varieties reduce the average yield production that remains far below its potential. Among the agronomic factors, sowing date plays a critical role in determining crop performance, as it regulates the photoperiod exposure and incidence of biotic and abiotic stresses during growth and reproductive phases (Alam *et al.* 2014). Additionally, global warming has led to shorter winter seasons, particularly in tropical and subtropical regions, such as Southeast Asia, threatening mustard oilseed production. Consequently, Bangladesh faces a considerable deficit in mustard oil production, with domestic output only meeting 10% of demand (BBS 2022), highlighting the introduction of robust and high-yielding mustard genotypes capable of withstanding environmental stresses

The unpredictable climate change driven by globalization exerts significant adverse effects on agricultural sectors worldwide, leading to a decline in production by 7-23% (Rezaei *et al.* 2023). Interestingly, these climatic disruptions disproportionately affect low-income nations and developing countries like Bangladesh (Arshad *et al.* 2017). Bangladesh faces a projected temperature rise of 1.28-2.04°C by 2060 and 3.39-4.47°C by 2100, endangering sustainable agriculture and contributing to up to 30% crop yield losses (Miah *et al.* 2016, Hossain *et al.* 2019). Environmental factors trigger biochemical alterations and hinder physiological development (Sharma *et al.* 2022). Early sowing might extend vegetative growth and reduce profitability; conversely, delayed sowing reduces germination (%) and pollen viability while increasing sterility due to low temperatures and accelerating maturity. This forced maturity hampers post-fertilization

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processes, shortens the grain-filling period, and ultimately results in poor yield (Akhter *et al.* 2015, Sakpal *et al.* 2023). Therefore, identifying optimal growing periods is crucial for cultivating resilient mustard varieties and ensuring stable yields.

Genotype-environment interactions (GEI) are crucial for improving crop productivity in the face of climate variability. Seed yield, influenced by genetic and environmental factors, varies significantly under different ecological conditions (Tayade *et al.* 2023). Identifying stable genotypes performing consistently across diverse environments is essential. Statistical models like AMMI and GGE biplots have proven effective for analyzing GEI and identifying suitable genotypes. AMMI integrates variance analysis with principal component analysis (PCA) to interpret GEI, while GGE biplots analyze both genotypic and GEI effects, providing insights into genotype ranking, environmental associations, and genetic contributions (Yan and Tinker 2006). Understanding yield-attributing traits through correlation coefficient analysis enables breeders to compute the direct and indirect contributions of traits, facilitating the development of high-yielding varieties (Saroj *et al.* 2021). These studies establish a foundation for breeding programs to enhance yield stability and adaptability under dynamic climatic conditions.

In this context, seven widely cultivated varieties of *Brassica juncea* under three distinct growing conditions based on yield-attributing traits were evaluated. The investigation sought to pinpoint optimal sowing periods that enhanced yield production and to distinguish top-performing genotypes utilizing AMMI and GGE biplot analyses. Additionally, a correlation study on quantitative traits to develop an efficient selection protocol for future mustard breeding endeavors was executed.

Materials and Methods

Seven genotypes of *B. juncea, viz.*, BINA-7, Rye-5, Daulat, BARI Sarisha-10, BARI Sarisha-16, SAU Canola-1 (SC-01), and BARI Sarisha-11, were evaluated at the agronomy field of Sher-e-Bangla Agricultural University, Dhaka, during the 2020-2021 rabi season. The genotypes were sown on (1^{st}, 15^{th}, and 30^{th} November) to assess genotype × environment interactions. A randomized complete block design with three replications was used, maintaining 0.4 m plant-to-plant and 0.5 m line-to-line spacing. Standard agronomic practices were followed by the guidelines of the handbook of BARI to ensure proper growth (Azad *et al.* 2020). Thirty plant samples were randomly selected for each genotype under study to evaluate ten yield and yield-attributing traits including days to siliquae maturity (DAS), plant height (cm), number of primary branches, number of secondary branches, number of siliquae per plant, siliquae length (cm), number of seeds per siliquae, thousand seed weight (g), yield per plant (g) and harvest index (%). Analysis of variance (ANOVA) was performed using Statistix-10 software. Genotype-environment interaction (GEI) effects were assessed using AMMI and GGE biplot models, while Pearson correlation coefficients were computed using R software.

Results and Discussion

The variance for joint regression analysis under different environmental conditions is depicted in Table 1. The environmental interaction effects with genotypes exhibited considerable variation for all traits except days to siliquae maturity, plant height and seeds per siliquae. Moreover, the linear interaction between genotype and environment also showed similar variation except for the number of secondary branches and siliquae per plant, indicating that the existing variation can be employed in the superior genotype selection strategies. Previously, Oladosu *et al.* (2017) and Mondal *et al.* (2023) reported similar statements. Additionally, both AMMI components showed significant variance for all yield-related traits except plant height and seeds per siliquae. The findings revealed inconsistent performance of the genotypes across environments, highlighting the need for cultivars with stable yields. Therefore, it is essential to account for variations caused by genetic and environmental factors in selecting the most suitable genotypes. The mean performance of seven selected genotypes at three sowing dates is illustrated in Table 2. For days to maturity, SC-01 (116.30 DAS) tends to have the highest maturity duration at 1st sowing, whereas BINA-7 (96.70 DAS) requires the lowest duration under late sowing. Collectively, genotypes matured an

Table 1. Joint regression analysis of variance by partitioning the genotype and environmental interaction of *Brassica juncea* genotypes.

Sources of variation	df	Mean sum of squares									
		DM	PH	NPB	NSB	SPP	SL	SPS	TSW	YPP	HI
Genotype (G)	6	127.37**	293.10*	2.43**	41.37**	7.88**	1.05**	8.48*	1.82**	6.98**	47.55**
Environment (E)	2	24.70	200.90	1.23**	1.04**	1.77**	0.32**	7.43**	0.29**	0.44**	19.21**
Interaction (G×E)	12	9.41	96.42	2.24**	10.83**	2.50**	0.20**	1.70	0.15**	0.74**	5.37**
AMMI comp. 1	7	14.85*	102.27	2.88**	12.77**	1372.88**	0.27**	2.58	0.19**	1.17**	4.49**
AMMI comp. 2	5	8.96*	88.26	1.79**	8.12**	840.91**	0.11**	0.48	0.10**	0.13**	6.07**
G × E (Linear)	6	647.09*	118.24*	2.49**	12.80	1323.89	0.31**	2.81*	0.23**	1.28**	5.07**
Polled deviation	7	11.80	64.00	1.96	7.58	840.01	0.08	0.52	0.07	0.17	1.86
Polled error	36	32.41	104.32	0.01	1.64	460.15	0.03	1.54	0.01	0.35	0.53

*, ** indicate 1% and 5% significance, respectively. DM = Days to siliquae maturity (DAS), PH = Plant height (cm), NPB = Number of primary branches, NSB = Number of secondary branches, SPP = Siliquae per plant, SL = Siliquae length (cm), SPS = Seeds per siliquae, TSW = 1000 seeds weight (g), YPP = Yield per plant (g) and HI = Harvest index (%).

Table 2. Mean comparison among the three environments of *Brassica juncea* for the yield-attributing traits.

Genotypes	Conditions	DM	PH	NPB	NSB	SPP	SL	SPS	TSW	YPP	HI
BARI-11	Early	107.30	165.70	8.20	12.60	278.70	4.30	15.10	4.30	8.10	31.80
	Mid	109.00	158.20	6.30	11.90	239.70	4.30	14.80	4.30	7.80	29.30
	Late	101.70	146.30	6.40	10.20	204.00	4.10	10.70	3.70	6.00	25.50
SC-01	Early	116.30	172.20	6.80	9.50	211.70	3.60	11.50	3.20	5.20	24.80
	Mid	115.30	166.20	5.90	11.70	201.00	3.60	12.30	3.10	5.00	21.60
	Late	108.00	168.30	6.30	11.30	194.70	3.40	9.90	2.70	4.70	19.70
BARI-16	Early	113.70	170.40	9.10	13.50	264.70	4.50	16.10	4.10	7.80	28.50
	Mid	108.30	162.70	5.80	16.50	242.30	4.40	15.00	4.50	7.10	30.10
	Late	102.70	149.70	6.00	9.60	218.70	4.00	11.10	3.50	6.10	26.60
BARI-10	Early	109.30	160.60	9.30	19.80	213.00	4.10	15.00	3.30	6.90	27.40
	Mid	104.70	159.70	6.40	17.50	251.70	4.40	14.00	3.40	7.00	25.20
	Late	100.00	163.50	6.20	13.60	170.00	4.30	10.90	3.30	5.10	23.50
Daulat	Early	106.00	155.90	8.90	13.30	266.70	4.00	14.80	3.20	6.70	29.70
	Mid	103.30	160.30	8.40	16.70	269.30	4.10	15.20	3.50	6.60	27.10
	Late	99.00	151.60	5.80	9.70	177.70	4.20	12.10	3.20	4.90	25.30
Rye-5	Early	105.70	167.33	7.90	16.60	219.70	3.90	13.80	3.50	5.70	28.10
	Mid	107.70	156.43	8.00	17.00	203.70	3.90	13.00	3.20	5.20	28.70
	Late	101.70	160.60	6.60	10.30	151.50	4.00	11.10	3.00	4.00	21.70
BINA 7	Early	103.30	149.30	6.80	10.50	248.00	4.40	15.20	4.30	7.40	30.40
	Mid	102.70	157.70	7.30	13.50	249.70	5.30	14.30	4.30	7.50	27.10
	Late	96.70	145.10	5.40	10.60	169.30	4.20	9.70	3.20	4.30	23.10

DM = Days to siliquae maturity (DAS), PH = Plant height (cm), NPB = Number of primary branches, NSB = Number of secondary branches, SPP = Siliquae per plant, SL = Siliquae length (cm), SPS = Seeds per siliquae, TSW = 1000 seeds weight (g), YPP = Yield per plant (g) and HI = Harvest index (%).

average of 7 days earlier in late sowing compared to early and mid-sowing. Turning to the yield-attributing traits, BARI-11 (278.70) produced the highest number of siliquae per plant at early sowing, whereas Rye-5 (151.30) had the lowest production under late sowing. Late sowing resulted in an average siliquae reduction of 24.53 and 22.44% compared to early and mid-sowing, respectively. Thousand seed weight was highest in mid-sowing, with late sowing showing reductions of 13.10 and 14.79% relative to early and mid-sowing. Moreover, the highest thousand seed weights tend to be BARI-16 (4.50 g) and the lowest value for SC-01 (2.72 g). Similarly, seed yield was highest in early and mid-sowing, whereas it was reduced by 26.93 and 24.17% seed yield in late sowing. BARI-11 (8.12 g) achieved the highest yield at early sowing, while Rye-5 (4.0 g) had the lowest under late sowing, likely due to a shorter vegetative phase and poor grain filling. Reduced performance under late sowing was also reported by Tomar *et al.* (2022). Additionally, late-sowing populations showed a lower harvest index, potentially due to higher dry matter accumulation, but reduced seed set caused by forced maturity from rising temperatures. Therefore, the mustard genotypes should be sown before mid-November to get a satisfactory yield.

To further assess the stability and adaptability of these genotypes across environments, an Additive Main Effects and Multiplicative Interaction (AMMI) analysis was employed, providing valuable insights into genotype-environment interactions (Fig. 1). The proximity to the origin in AMMI1 indicates high stability (Islam *et al.* 2021). Daulat was a stable genotype for both the maturity period and high yield across the environment. Therefore, it will perform almost the same in all environments for maturity periods and yield. Genotypes SC-01 and BINA-7 emerged as the most stable genotypes for the maturity period. However, their contrasting maturity values provide a deeper understanding of their adaptability to specific conditions (Fig. 1a). Genotype SC-01 required a longer maturity period, which may be advantageous in regions where extended growing seasons are allowed. On the other hand, BINA-7 exhibited the lowest maturity duration, marking it as a fast-maturing genotype, particularly beneficial in areas with shorter growing seasons. Genotypes BARI-11, BARI-10 and Daulat were identified as highly stable for yield (Fig. 1b). BARI-16 showed considerable seed yield production, but with potentially less focus on stability compared to others. BARI-11 followed closely, balancing high yield with stability, making it a strong candidate for environments where both traits are essential. Genotypes BARI-10 and Daulat displayed moderate yield levels. Collectively, Daulat and BARI-11 are ideal for achieving a balance between high yield and stability across diverse environments, whereas SC-01 and BINA-7 could be used in specific regions, making them useful for breeding programs tailored to certain climates.

The stability analysis and genotype rankings based on important yield-attributing traits across three environments are presented in Fig. 2. This genotype-by-environment interaction (GEI) analysis highlights the disparities between performance and stability among genotypes. PC1 explained the majority of variation (88.34%), highlighting the mean performance of the genotypes, whereas PC2 (11.22% of variation) reflected stability and genotype-environment interactions (Fig. 2a). The optimal genotype demonstrating the best stability across the environments should be positioned in the tiny circle on the AEC abscissa line (Kona *et al.* 2024). Considering this, genotypes SC-01 and BINA-7 demonstrated the highest stability, whereas genotype SC-01 required the maximum maturation period, but BINA-7 matured within the shortest period, indicating that these two performed well under diverse environmental conditions. In contrast, BARI-11 and BARI-16 were positioned furthest to the AEA, indicating lower stability with moderate maturity duration, which may show stability at specific locations. According to nearest to farthest position, the ranking of early-matured and stable genotypes is SC-01 > BARI-16 > BARI-11 > Rye-5 > BARI-10 > Daulat > BINA-7. Moreover, the biplot analysis for yield per plant revealed that PC1 reflected the most variability (86.34%), whereas PC2 accounted for 12.58% of variation only (Fig. 2b). Genotypes like Daulat and BARI-10, positioned near the center of origin and exhibiting higher stability, are suitable for diverse environments, followed by BARI-11 and BARI-16. Conversely, SC-01 exhibited low performance and low stability. Overall, the ranking is BARI-11 > BARI-16 > BARI-10 > BINA-7 > Daulat > Rye-5 > SC-01. Therefore, selecting genotypes based on stability with desirable performance will be advantageous for future breeding programs.

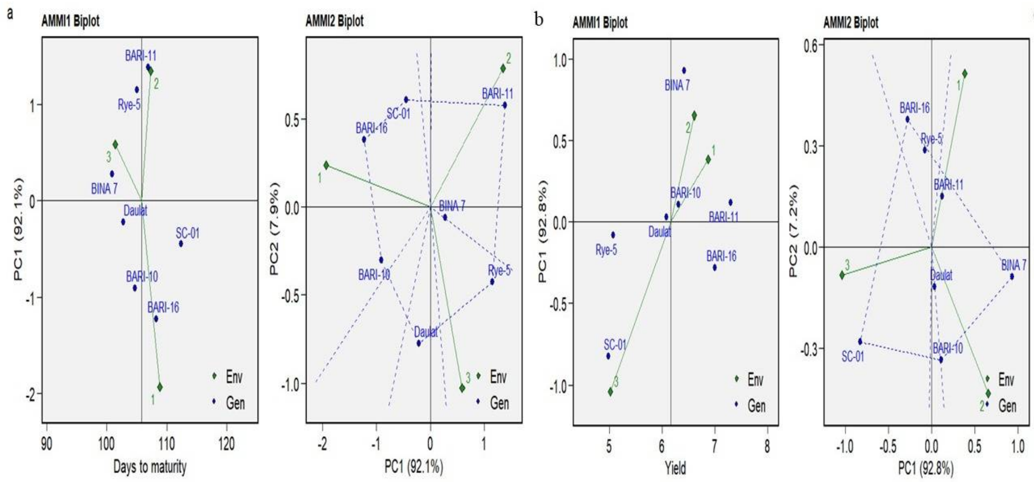

Fig. 1. Additive main effects and multiplicative interaction (AMMI 1 and 2) biplots based on PC1 illustrating G × E interactions of the seven genotypes of *B. juncea* for days to siliquae maturity (a) and yield per plant (b), respectively.

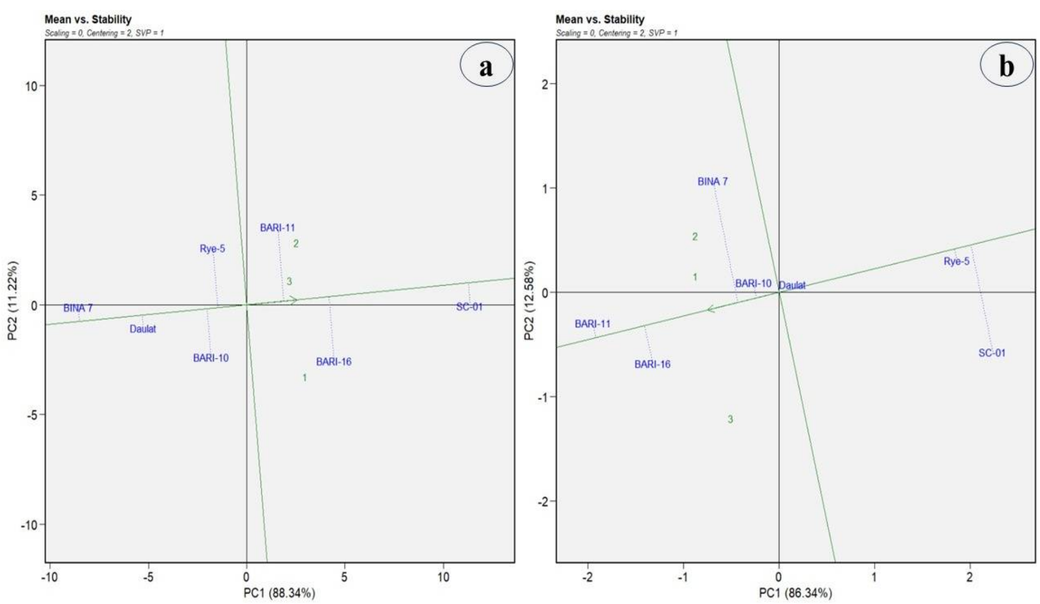

Fig. 2. Mean vs. stability based on PC1 and PC2 showing G × E interactions of the seven *B. juncea* genotypes for days to siliquae maturity (a) and yield per plant (b), respectively.

In addition, the computed GGE biplot illustrated the discriminating and representative performance of early maturity and yield per plant for selecting the ideal environment **(**Fig. 3**).** The principal component (PC1) interpreted 88.34% of the total 99.56% of the variation and left 11.22% interpreted by PC2 for days to siliquae maturity (Fig. 3a). In contrast, PC1 accounted for 86.34% and PC2 accounted for 12.58% of the cumulative 98.92% variation for yield per plant (Fig. 3b). The environment's standard deviation or discriminating ability is proportional to the radial distance, where the lower angle indicates the more representativeness of the individual environment (Yan and Tinker 2006). E3 exhibited the shortest radial distance for both days to siliquae maturity and yield per plant, indicating its low discriminatory ability. This suggests that E3 does not effectively differentiate among genotypes regarding these traits. On the other hand, E1 demonstrates a high discriminatory ability specifically for days to siliquae maturity, highlighting its potential to distinguish genotypes based on this trait. However, for yield per plant, both E1 and E2 produce nearly identical results, reflecting comparable performance in their ability to discriminate. Because, under optimal growing conditions, genotypes exploit their full potential based on their genetic heredity, which helps to measure the genotypes accurately. Regarding representativeness, E3 performs the best for days to siliquae maturity, as it aligns closely with the ideal environmental conditions for evaluating this trait. Among the genotypes, SC-01 and BINA-7 showed the maximum representativeness. Conversely, E1 emerges as the most representative environment for yield per plant. This conclusion is based on its minimal angle with the Average Environmental Coordinate (AEC) axis, indicating proximity to the ideal environment and better reflection of average genotype performance for these traits (Adham *et al.* 2023). Therefore, selection of genotypes *viz.*, Daulat, BARI-10, BARI-11 and BARI-16 based on representativeness will be valuable to advance them in multi-location trials for more robust evaluation in the future.

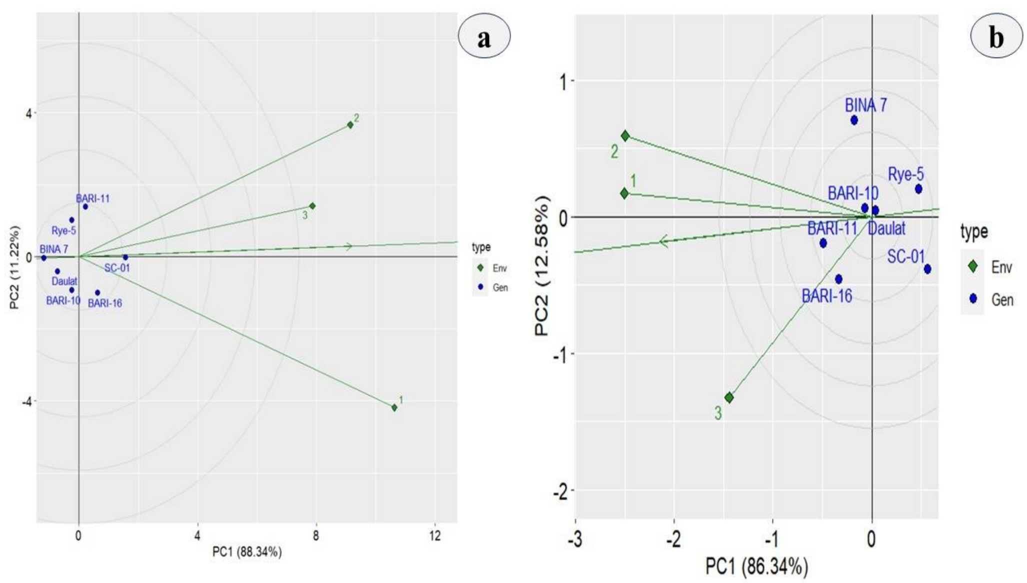

Fig. 3. Discriminating ability and representativeness of the test environments for days to siliquae maturity (a) and yield per plant (b), respectively.

The polygon view of which-won-where is a critical GGE biplot component that illustrates the genotype and environmental interaction patterns as presented in Fig. 4. The uppermost genotypes exhibited either a positive or negative extreme interaction. The polygon sides were separated into distinct environmental areas by crossing several perpendicular lines (Oladosu *et al.* 2017). For maturity, a single mega-environment (ME) combined all sowing conditions (E1, E2, and E3), suggesting this trait's genotype response for this trait was constant and had no significant effect on the maturity period (Fig. 4a). In contrast, the environments were split into two distinct mega-environments for yield per plant. E1 and E2 were grouped into one ME, likely due to their similar environmental factors influencing genotype performance (Fig. 4b). Conversely, E3 formed a separate ME, indicating that the environmental conditions in E3 had a markedly different impact on yield. Genotypes SC-01 and BARI-16 emerged as the most responsive genotypes for days to siliquae maturity and showed strong adaptability and stability across diverse sowing conditions. Similar observations were noted by Sadhu *et al.* (2024). For yield per plant, BARI-11 and BARI-16 were the most promising genotypes as they were highly responsive in environments E1 and E2. This highlights their ability to exploit the favorable conditions of these environments to achieve higher yields. However, none of the genotypes under study exhibited desirable yield performance in E3, indicating that these genotypes didn’t receive favorable conditions at late sowing for optimal yield production. Therefore, the studied varieties must be cultivated within a favorable environment, or breeding efforts should be given for developing more improved varieties that can withstand more environmental fluctuations.

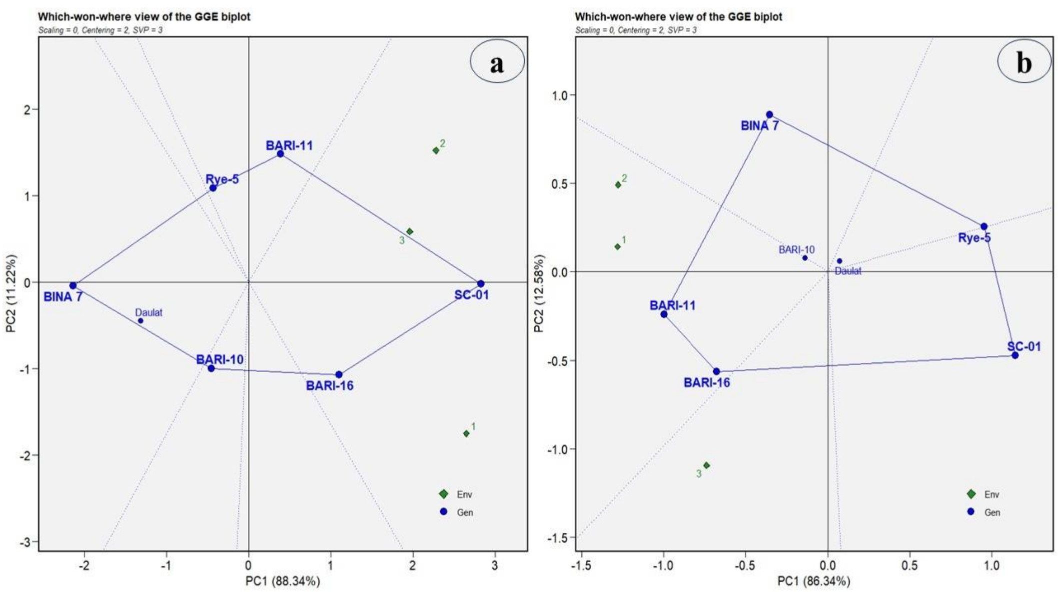

Fig. 4. Polygon views of GGE biplot for which-won-where analysis of the seven *B. juncea* genotypes under the effects of genotype-by-environment interactions for days to siliquae maturity (a) and yield per plant (b), respectively.

The Pearson correlation matrix provided insights into the association among ten yield-attributing traits of *B. juncea* under three sowing conditions (early, mid and late). Under early sowing conditions, siliquae per plant (0.71***), seed per siliquae (0.70***), siliquae length (0.69***), thousand seed weight (0.65**) and harvest index (0.64**) showed a strong and positive significant interaction with seed yield, suggesting that improvement of these traits proportionally enhance the seed yield performance and *vice versa* (Fig. 5). In contrast, plant height (-0.34) and days to siliquae maturity (-0.18) exhibited a negative non-significant relationship with seed yield, highlighting that shorted maturity reduces the yield production. Similarly, under mid-sowing conditions, seed yield had a positive and significant association with thousand seed weight (0.73***), siliquae length (0.69***), siliquae per plant (0.53*) and harvest index (0.50*) (Fig. 5b). Conversely, days to siliquae maturity (-0.24), number of secondary branches (-0.08), and number of primary branches (-0.04) had a non-significant negative direction with yield per plant. Saroj *et al.* (2021) and Yadav *et al.* (2021) also noted a similar association for the Brassica accession. On the other hand, siliquae per plant (0.75***), thousand seed weight (0.65**) and harvest index (0.61**) demonstrated a positive and significant relationship with yield per plant under the late-sowing environment (Fig. 5c). However, plant height (-0.20) and the number of secondary branches (-0.11) showed a negative non-significant relation with seed yield which was undesirable for higher seed yield production. Collectively, the results underscore the critical role of siliquae per plant, thousand seed weight, and siliquae length in determining seed yield, while fewer primary and secondary branches and shorter maturation periods were detrimental to yield performance. Moreover, late sowing was associated with reduced plant vigor and a shortened maturation period, which ultimately had negative impacts on yield performance. Therefore, facilitating optimum growth conditions is an urgent need to ensure enhanced mustard seed yield production.

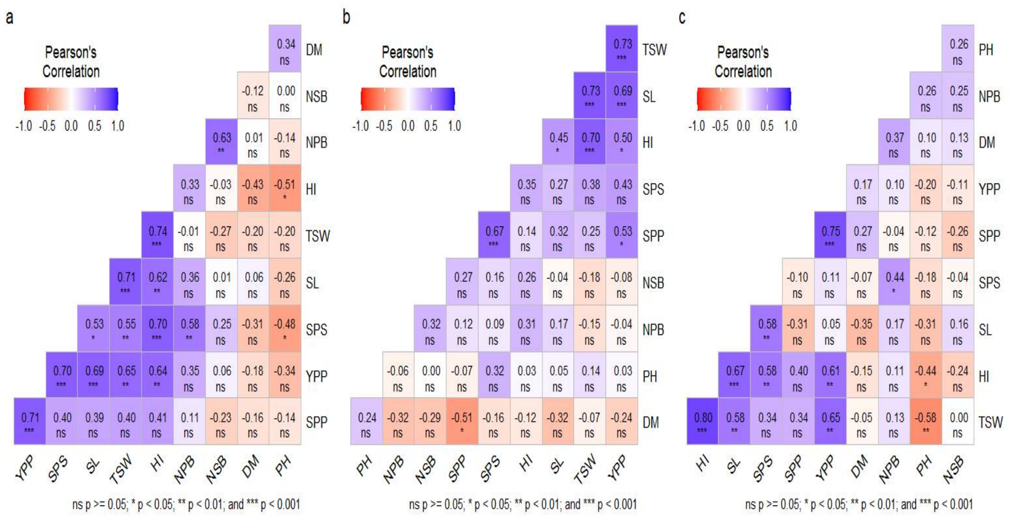

Fig. 5. Pearson correlation coefficient among the yield-attributing traits of *B. juncea* across three environments. (a) early sowing, (b) mid sowing and (c) late sowing. DM = Days to siliquae maturity (DAS), PH = Plant height (cm), NPB = Number of primary branches, NSB = Number of secondary branches, SPP = Siliquae per plant, SL = Siliquae length (cm), SPS = Seeds per siliquae, TSW = 1000 seeds weight (g), YPP = Yield per plant (g) and HI = Harvest index (%).

Based on the aforementioned findings, it is obvious that environmental factors associated with varying sowing conditions exert significant effects on the genotype's performance. The genotypes under study produced markedly lower yields under late sowing conditions. Moreover, in terms of overall performance, genotypes Daulat, BARI-11 and BARI-16 exhibited maximum seed yield and stability. Conversely, BINA-7 and genotype SC-01 could be considered for the early and late maturity index, respectively. Consequently, interspecific hybridization among these genotypes, considering their contrasting phenotypes, holds the potential for the development of high-yielding, early-maturing and stable mustard cultivars in the future.

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(Manuscript received on 10 March, 2025; revised on 11 September, 2025)