



Evaluation of Grain Yield Potential and Environmental Stability of the Barley Genotypes Using an Augmented Randomized Complete Block Design

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ABSTRACT

In initial breeding field trials, insufficient seed quantity and limited land area frequently hinder complete replication, complicating the precise evaluation of genotypic potential due to environmental variability. This study aims to assess the grain yield performance of 336 unreplicated barley genotypes in conjunction with six replicated check varieties, employing an Augmented Randomized Complete Block Design. The experiment was carried out in 10 blocks for three consecutive years. To figure out how many environmental errors there were, six check varieties were repeated in each block. In contrast, 336 barley genotypes were tested without replication. The analysis result showed that the environment was different, with block mean yields for checks ranging from 2422 kg ha⁻¹ (Block 9) to 3407 kg ha⁻¹ (Block 6). Because of this change, genotypes in stress environments had to have their yields changed by as much as +585 kg ha⁻¹. Genotype AM-292 was the best line, with the highest adjusted yield (8392 kg ha⁻¹), showing that it did very well even though it was grown in the block with the least fertile soil. Genotype AM-159 (7902 kg ha⁻¹) and Genotype AM-185 (7206 kg ha⁻¹) were also chosen as the best performing genotypes. The check variety “V. Morales” was very stable, while the check variety “Alanda” showed a lot of phenotypic variation (2374–5755 kg ha⁻¹), which made it less useful as a stability reference. The improved design worked well to reduce environmental error. The top 10% of genotypes have been chosen (higher than 5000 kg ha⁻¹ yield potential) based on their adjusted yield performance.

Keywords: Augmented Block Design, Barley, Mid-Western, Genotype, Adjusted Yield

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INTRODUCTION

Barley productivity in Nepal is still low, even though it is important economically and socially (MoALD 2024). Several biotic, abiotic, and socio-economic factors contribute to this yield gap (Pokharel et al 2022). In the western midhills of Nepal, where barley is grown in larger areas, most of the landraces grown are low-yielding local ones (Sharma et al 1994, Pokharel et al 2016). These landraces have functional traits that help them adapt to their environment, but they are genetically prone to lodging and vulnerable to new pathogen. Barley in Nepal is often grown on the most marginal lands with little input management, such as limited chemical fertilizers or irrigation. This makes it even harder for it to reach its full yield potential.

For better genetic gain, it is essential to choose the best genotypes quickly in the early stages of a breeding program (Kumar et al 2020). But early-generation trials are often limited because there aren't enough seeds available, which makes it impossible to replicate hundreds or thousands of test lines. Testing unreplicated lines in extensive fields presents a considerable challenge: soil heterogeneity. Changes in soil fertility, moisture, and slope can hide the true genetic potential of plants (Rodriguez et al 2016). This can lead to the selection of bad lines because they were planted in good condition ("false positives") or the discarding of good lines because they were grown in bad soil ("false negatives").

Augmented Randomized Complete Block Design (ARCB) (Federer 1994) uses a set of standard check varieties repeated across all blocks to figure out experimental error. The current selection of barley varieties in Nepal is limited, especially those managed by the Hill Crops Research Program in Kabre, Dolakha. This shows how important it is to expand the genetic base of the crop. As a result, this study looked at a wide range of advanced barley genotypes in the western mid-hills of Nepal. Giving farmers these better choices directly help the country reach its goal of making sure that mountain agro-ecosystems have enough food and nutrition. The main goals of this field experiment were to: (1) look at how the environment changes in different parts of the trial site; (2) change the grain yield data to take into account block effects; and (3) find stable, high-yielding genotypes that can be tested in more than one environment in the future.

MATERIALS AND METHODS

Experimental Site

The field experiment was conducted during the winter seasons of 2017/2018, 2018/2019, and 2019/2020 at the research farm of the Agricultural Research Station (ARS), Dailekh, Nepal. The experimental site is geographically situated at 28.8443 latitude, 81.7101 longitude, with an altitude of 1240 meters above sea level (masl). The location represents the typical cool, subtropical climate of the western mid-hills (ARS Dailekh).

Table 1. Soil properties of the experimental field.

pH	OM (%)	Total N (%)	Available P ₂ O ₅ (mg kg ⁻¹)	Available K ₂ O (mg kg ⁻¹)	Sand (%)	Silt (%)	Clay (%)	Class
5.13	2.94	0.12	32.7	116.2	41.0	44.5	14.5	Loam

Source: National Soil Science Research Center, Khumaltar, Lalitpur, Nepal

Plant Materials

This study evaluated a total of 342 barley genotypes. 340 genotypes were sourced from the International Center for Agricultural Research in the Dry Areas (ICARDA), comprising 336 accessions from diverse origins and four popular international checks (Rihane-03, V. Morales, Arta, and Alanda). Additionally, two local Nepalese checks were included: Bonus (a two-rowed, hulled variety released in 1974) and Solu-Uwa (a six-rowed, hull-less variety released in 1990) (NARC 2020).

Experimental Design and Cultural Practices

The field trial was laid out in an Augmented Randomized Complete Block Design consisting of 10 blocks. Each block contained 40 plots totaling 400 experimental units. The following agronomic practices, including irrigation, fertilizer application and weed control, were applied uniformly in all three years to minimize non-experimental errors.

- Plot Size: Each experimental plot measured 3 m × 2 m (6 square meters), consisting of 8 rows.
- Spacing: Seeds were sown continuously in rows spaced 25 cm apart.
- Sowing Date: Sowing was done on November 07 for 2017/2018; November 16 for 2018/2019, and November 10 for 2019/20 planting. This is normal barley planting season in the region.
- Fertilizer Management: Fertilizers were applied at the recommended dose of 60:40:20 kg NPK per hectare. Half the dose of Nitrogen and the full doses of Phosphorus and Potash were applied as a basal dose at the time of sowing. The remaining half of the Nitrogen was top-dressed at the tillering stage (40 days after sowing) after the first irrigation.
- Weeding: Two manual weeding were performed at 35 and 70 days after sowing (DAS) to keep the plots weed-free.

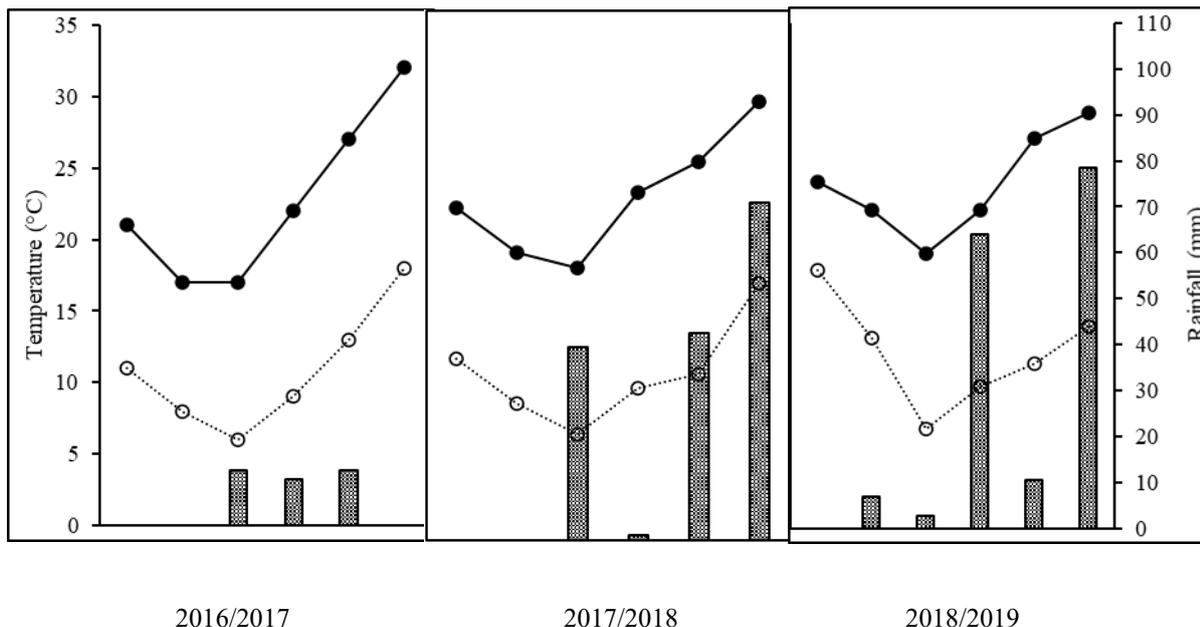


Fig. 1. Weather details of the experimental site [Source: Agricultural Research Station, Dailekh (Annual Report 2016/2017, 2017/2018, 2018/2019)]. The vertical column bar represents the total rainfall (mm) during the barley crop growing season. Likewise, the black line with round black spot represents the maximum temperature in degree Celsius. Similarly, the dotted line with round ring represents the minimum temperature in degree Celsius during the crop growing period of the experimental field.

Data Collection

Data were recorded on phenological traits, yield-contributing traits, and disease severity. Data were collected from the net plot area, excluding border rows, and from five randomly tagged plants where applicable. Phenological traits included Days to Heading (DH), defined as the number of days from sowing to the emergence of spikes in 50% of the plants in a plot, and Days to Maturity (DM), recorded as the number of days from sowing until 90% of the spikes turned yellow and grains reached hard dough stage. Morphological and yield-related traits measured were Plant Height (PH), recorded in centimetres from the ground level to the tip of the spike (excluding awns) on five randomly selected plants at maturity; Grains per Spike (GPS), calculated as the average number of grains from ten randomly selected spikes per plot; and Thousand Grain Weight (TGW), determined as the weight in grams of 1000 randomly counted grains at 12% moisture content. Grain yield (GY) was measured by manually harvesting the crop from the net plot area, followed by sun-drying, threshing, and cleaning. The grain yield per plot was weighed and expressed in kilograms per hectare (kg ha⁻¹) at 12% moisture content. Only grain yield data were considered for the analysis presented in this article.

All recorded data were compiled in Microsoft Excel and subjected to analysis of variance (ANOVA) using R Statistical Software (Version 4.2.2). For the augmented design, ANOVA was used to estimate block effects, and adjusted genotype yields were calculated using the formula:

$$\text{Adjusted Yield} = \text{Raw Yield} - (\text{Block Check Mean} - \text{Grand Check Mean}) \dots\dots\dots\text{Eq. 1 (Federer 1994)}.$$

This adjustment accounts for the specific advantage or disadvantage provided by the block, enabling accurate comparison of genotypic performance across heterogeneous field conditions.

RESULTS AND DISCUSSION

Environmental Variation and Block Effects

We used an Augmented Randomized Complete Block Design (ARCBD) to test 336 different test genotypes and six replicated check varieties across 10 blocks. The analysis showed that the experimental site had a lot of different environmental conditions. The block effect was significant, as demonstrated by the large difference in mean yields of the check varieties, which ranged from 2422 kg ha⁻¹ in Block 9 to 3407 kg ha⁻¹ in Block 6.

Because of this change in the environment, we need to use adjusted yield values to make valid genotypic comparisons. Block 6 and Block 10 were found to be environments with high productivity, which made the raw phenotypic expression of the genotypes grown there look better. On the other hand, Block 9 was a very stressful place to be, and it lowered phenotypic expression by an average of 585 kg ha⁻¹ compared to the grand mean. The adjustment process got rid of these confusing environmental errors, making it possible to find the true genotypic potential.

Performance and Plasticity of Check Varieties

The check variety “Arta” had the highest average performance (3782 kg ha⁻¹) and moderate stability among the check varieties. This makes it a good benchmark for high-yield potential. On the other hand, Solu Uwa was very sensitive to the environment (plasticity), and its yields ranged from 2374 to 5755 kg ha⁻¹, depending on how fertile the block was. This instability indicates that “Solu Uwa” may not be a dependable sole criterion for selection in fluctuating environments. Average performance of the check varieties is given in the table below.

Table 2. Mean grain yield of check varieties across 10 experimental blocks and the corresponding adjustment factors applied to the test genotypes.

Block	Check Mean (kg ha ⁻¹)	Environmental Status	Adjustment Factor (kg ha ⁻¹)
6	3407	Very High Fertility	-400
10	3330	High Fertility	-323
4	3304	High Fertility	-297
1	3251	Above Average	-245
5	3113	Above Average	-107
7	2892	Below Average	+115
3	2878	Below Average	+129
2	2875	Below Average	+132
8	2595	Low Fertility	+412
9	2422	Severe Stress	+585
Grand Mean: 3007			

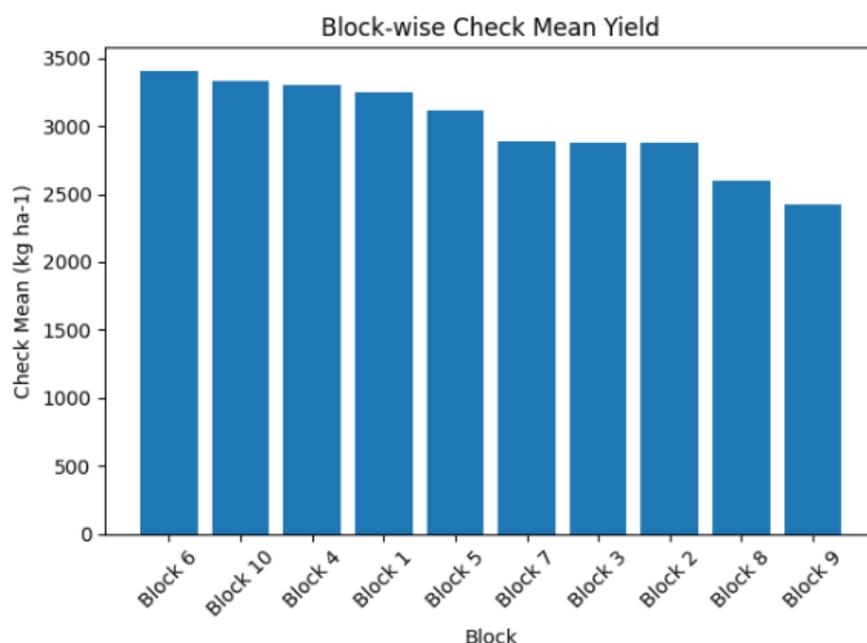


Fig. 1. Block-wise check mean grain yield (kg ha⁻¹) indicating environmental variability across experimental blocks.

Genotypic Performance and Stress Tolerance

The test genotypes showed a lot of variation in adjusted grain yield, which indicates that there is a lot of genetic diversity in the population and a lot of room for genetic improvement through selection. The analysis of adjusted yield led to a major re-ranking of the top genotypes when compared to their raw data. Genotype AM-292 stood out as the best line with the highest adjusted yield (8392 kg ha⁻¹). Genotype AM-292 had a lower raw yield (7807 kg ha⁻¹) than Genotype AM-159 (8009 kg ha⁻¹), but it did this in the block with the lowest performance (Block 9). This shows that Genotype AM-292 not only has a lot of potential for high yields, but it is also very resistant to stress from the environment.

Genotype AM-159 (Adjusted Yield: 7902 kg ha⁻¹) and Genotype AM-185 (Adjusted Yield: 7206 kg ha⁻¹) were also found to be top performers. It is important to note, though, that Genotype AM-185 was grown in the best

environment (Block 6). Its adjusted value correctly punishes it for this advantage, but it is still a top yielder genotype. A detail of the top ten performer genotypes is presented in the Table 3.

Table 3: Raw and adjusted grain yield (kg ha⁻¹) of the top 34 superior barley genotypes evaluated using Augmented Complete Randomized Block Design at the research field of Dailekh.

Rank	Genotype	Block	Raw Yield (kg ha ⁻¹)	Adjusted Yield (kg ha ⁻¹)	Selection Note
1	AM-292	9	7807	8392	Elite Performer. Exceptional yield Under high stress (Block 9).
2	AM-159	5	8009	7902	Highest raw yield, confirmed superior after adjustment.
3	AM-185	6	7606	7206	Strong performer in optimal conditions.
4	AM-334	10	7506	7183	Consistent high performer.
5	AM-102	3	6922	7051	Excellent performance in a below-average block.
6	AM-319	10	7345	7022	
7	AM-006	1	7244	6999	
8	AM-162	5	6821	6714	
9	AM-246	8	6238	6650	Stress Tolerant. High performance in a low-yield block.
10	AM-266	8	6077	6489	Stress-tolerant line.
11	AM-158	5	6580	6473	
12	AM-265	8	6016	6428	Stress-tolerant line.
13	AM-284	9	5835	6420	Moderate raw yield masked by severe block stress.
14	AM-156	5	6519	6412	
15	AM-316	10	6640	6317	
16	AM-190	6	6680	6280	
17	AM-242	8	5855	6267	
18	AM-039	2	6117	6249	
19	AM-243	8	5795	6206.7	
20	AM-268	8	5775	6187	
21	AM-164	5	6117	6010	
22	AM-320	10	6298	5975	
23	AM-296	9	5292	5877	Significant adjustment (+585) due to Block 9 stress.
24	AM-298	9	5252	5837	Significant adjustment (+585) due to Block 9 stress.
25	AM-10	1	6037	5792	
26	AM-305	9	5191	5776	
27	AM-259	8	5332	5744	
28	AM-125	4	5976	5679	Good yield despite negative adjustment for good soil.
29	AM-024	1	5916	5671	
30	AM-262	8	5211	5623	
31	AM-201	6	6016	5616	High penalty (-400) applied due to Block 6 advantage.
32	AM-175	6	5916	5516	
33	AM-244	8	5091	5503	
34	AM-326	10	5755	5432	

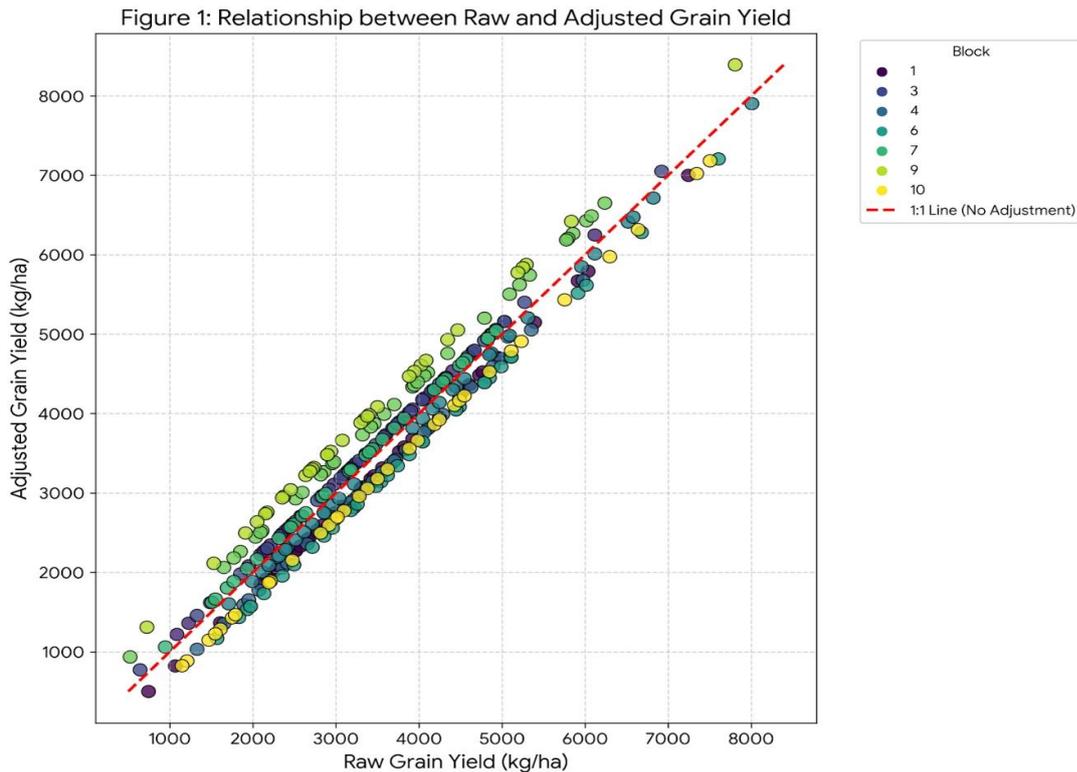


Fig. 2. Relationship between Raw Grain Yield and Adjusted Grain Yield for 342 barley genotypes

The solid diagonal line represents the 1:1 ratio where Raw Yield equals Adjusted Yield. Data points above the line (e.g., Block 9 genotypes) indicate upward adjustment due to environmental stress, while points below the line (e.g., Block 6 genotypes) indicate downward adjustment due to high soil fertility. "As illustrated in Figure 1, the adjustment process significantly altered the ranking of genotypes. The scatter plot reveals distinct clusters of genotypes corresponding to their block of origin. Genotypes from Block 9 (red points) are shifted upwards, highlighting their genetic potential masked by stress. Conversely, genotypes from Block 6 (green points) are shifted downwards, correcting for the environmental advantage they received." This scatter plot visualizes how the Augmented Design corrects for environmental variation.

- The Dashed Line (1:1): If there were no environmental differences between blocks, all points would fall on this line (Raw Yield = Adjusted Yield).
- Points Above the Dashed Line: These genotypes (mostly from Block 9, in dark purple/blue) had their yields adjusted upwards. The model recognized they were growing in poor soil and gave them "credit" for the stress.
- Points Below the Dashed Line: These genotypes (mostly from Block 6, in green/yellow) had their yields adjusted downwards. The model subtracted points because they had the unfair advantage of growing in the best soil.

DISCUSSION

The main goal of this study was to identify superior barley genotypes that could replace low-yielding landraces and varieties in the western mid-hills of Nepal. The significant differences observed in grain yield and yield-related traits among genotypes suggest considerable genetic diversity in the tested material, providing strong selection potential (Varshney et al 2022, Singh et al 2023). Similar diversity has been reported in other Himalayan barley germplasm collections, highlighting opportunities for improved cultivar development (Khan et al 2024).

Effectiveness of Enhanced Design in Managing Environmental Diversity

The wide range of block means (2422–3407 kg ha⁻¹) highlights the spatial heterogeneity that is typical of field studies (Rao and Prasad 2021). If environmental variation is not controlled, it can lower selection efficiency in early generation trials with limited seed and unrealistic replication for every entry (Pereira et al 2020). As advised by statistical breeding designs, the Augmented Randomized Complete Block Design (ARCBD) successfully corrected for this variation by estimating block effects through the performance of replicated

checks (Oyarzun et al 2021). In Block 9, where stress conditions reduced phenotypic expression by almost 17% relative to the grand mean, this modification was very crucial. Stress-tolerant genotypes in less advantageous blocks can be mistakenly eliminated in the absence of such adjustments (Zhang et al 2023).

Performance of Genotypes and Stress Tolerance

The finding that Genotype AM 292 is a promising line for low input or stress-sensitive situations was one of the study's main findings. Despite being planted in Block 9, AM 292 yielded the greatest adjusted yield in the population (8392 kg ha⁻¹) and the highest raw yield (7807 kg ha⁻¹). According to Hasan et al (2021) and Malik et al (2025), this implies inherent physiological systems for effective nutrition use and stress tolerance. The need of screening for physiological resilience has been demonstrated by comparable performance patterns observed in barley under nitrogen and water stress conditions (Verma et al 2022). Genotypes AM 159 and AM 185, on the other hand, performed better in more favourable conditions. Despite AM 185 producing high raw yield in Block 6 (fertile soil), adjustment analysis revealed that environmental factors rather than innate superiority contributed to some of its performance. This distinction is consistent with research on genotype-specific plasticity in different conditions (Kumar and Shrestha 2023).

Stability and Plasticity of the Check Varieties

Because of its high average yield and moderate stability, the check variety Alanda can be used as a standard reference for yield potential (Sharma et al 2023). Solu Uwa, on the other hand, displayed a very broad range (2374–5755 kg ha⁻¹), suggesting significant phenotypic plasticity but erratic performance across settings (Joshi and Thapa 2024). In favorable seasons, adaptability can be useful, but in uncertain conditions, it compromises dependability. In order to prevent bias from extreme reactions, this instability underscores the requirement for numerous check variations in enhanced architectures (Mehta et al 2021).

Consequences for Future Breeding Approaches

High genetic diversity in the breeding population is a necessary for the advancement of selection, as evidenced by the large range of adjusted yields among the 336 test genotypes (Dwivedi et al 2022). According to Singh et al (2023), the selection differential obtained by selecting the top 10% of lines—including Genotypes AM 292 and AM 159—indicates successful identifying of superior lines. To measure genotype × environment interactions (G×E) and verify stability across several ecological zones, these advanced lines should now go through Multi Environment Trials (METs) with full replication (Crossa et al 2024). In particular, AM 292 should be evaluated in stress screening nursery to confirm the mechanisms of tolerance that its performance in this investigation suggests (Gupta et al 2025).

CONCLUSION

In the case of grain yield, based on three years data, we found the top 10% of genotypes (34 lines) that should be moved forward based on their adjusted yield performance. Breeding programs that focus on stress tolerance and stability put a lot of emphasis on Genotype AM-292. Genotypes AM-159, AM-185, AM-334, and AM-319 are good candidates for advancement because they performed well in the best growing conditions. It is suggested that future multi-location trials be carried out to confirm that these lines are stable in a range of agro-ecological zones.

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AUTHORS' CONTRIBUTION

BBP – Preparation of research proposal, carried out field experiments, data collection, data analysis, interpretation of the results and manuscript preparation; HPT, KHG, SS, RB, BC – Contributed for manuscript preparation; PA – guided for field experimentation, soil sample collection, data analysis and manuscript preparation; JPU and DKRM – contributed for field data collection.

CONFLICT OF INTEREST

The authors declared that they have no conflict of interest regarding this manuscript.

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