Identification of high-yielding and stable lentil genotypes under cold dryland conditions

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ABSTRACT

To clarify the effects of genotype-by-environment interaction (GEI) and to develop high-yielding, cold-tolerant lentil lines suitable for cold rainfed conditions, a two-year study was conducted across four locations in Iran: Maragheh, Ardabil, Zanjan, and Kurdistan. Nine lentil landraces and one promising line (Pardis) were evaluated alongside the check varieties Bilehsavar and Sana using a randomized complete block design with three replications. Each plot consisted of four rows, each four meters long. The results indicated that the main effects of environment (E), genotype (G), and their interaction (GEI) were highly significant. Parametric and non-parametric stability analyses, together with GGE biplot analysis, were employed to interpret GEI patterns. The GGE biplot showed that the first two principal components explained 52.2% and 29.4% of the total variation in grain yield, respectively. Mean comparisons revealed that genotype G3 produced the highest average yield (781.5 kg/ha), followed by G5 (762.5 kg/ha). Genotype G10 achieved an average yield of 704.4 kg/ha, representing a 43% yield advantage over the check variety Bilehsavar and 6% over Sana. Overall, G3 and G5 emerged as the most promising genotypes, exhibiting superior performance across multiple evaluation methods. These findings underscore the importance of employing a multifaceted approach to genotype evaluation, enabling breeders to make informed decisions that enhance resilience and productivity in cold, dryland lentil production systems.

Key words: Cold tolerant, Dryland, Lentil, Yield.

INTRODUCTION

Lentil (*Lens culinaris* Medik.) is an edible, herbaceous, self-pollinated, diploid (2n=14) annual legume belonging to the Fabaceae family (Sardar *et al.*, 2025). In addition to being rich in protein, lentil seeds contain substantial amounts of carbohydrates, minerals, vitamins, and dietary fiber. Moreover, symbiotic bacteria in the root system facilitate biological nitrogen fixation (Ramirez and ValenciaCantero, 2024). These characteristics make lentil a suitable crop for rotation with wheat in Iran (Amiri *et al.*, 2021). Despite the extensive cultivation of lentils in the cold, dryland regions of the country, only a limited number of varieties have been developed specifically for these environments.

In cold regions, the largest share of lentil cultivation occurs in spring. However, high soil moisture in March often prevents machinery from entering fields for land preparation, delaying sowing until April in most years (Sedaghatkhani *et al.*, 2011). In dryland areas, flowering typically begins around May 15. During flowering and pod formation, lentils have their highest water requirement, yet in colder regions, effective rainfall is usually absent during the second half of May. The combination of insufficient rainfall during this critical growth stage and rising temperatures toward the end of the season intensifies drought stress and increases the formation of infertile flowers. Autumn sowing can enhance yield by extending the growing season, improving the use of latewinter and earlyspring precipitation, synchronizing flowering and pod setting with adequate soil moisture, and ultimately avoiding terminal drought (Tabrizivand Taheri *et al.*, 2022).

A key requirement for successful autumn cultivation is the availability of high-yielding, cold-tolerant varieties that maintain desirable traits across diverse environmental conditions. To identify superior genotypes for specific locations or to determine stable genotypes across environments, cultivars and breeding lines are evaluated through multi-environment field trials (Baxevanos *et al.*, 2024). The yield potential of genotypes depends on their interaction with environmental factors, and their responses can vary considerably. Therefore, studying genotype by environment interaction (GEI) is essential for assessing performance, stability, and concepts such as mega-environments, specific adaptation, and broad adaptability. Understanding GEI and stability is crucial for selecting high-performing lentil varieties (Ceritoglu *et al.*, 2025). Breeding programs commonly use multi-environment trials (METs) in the final stages of cultivar development to identify genotypes that are high-yielding, stable, and widely adaptable (Gerrano *et al.*, 2022). GGE biplot analysis provides a graphical approach to simultaneously evaluate yield performance and stability across environments (Mullualem *et al.*, 2024). The primary objectives of GGE biplot analysis include: (i) classifying target environments into meaningful megaenvironments; (ii) identifying genetic and environmental covariates contributing to GEI; (iii) determining representative and discriminating test environments; and (iv) identifying superior genotypes with high and stable performance within megaenvironments (Das *et al.*, 2025). Numerous studies have successfully applied GGE biplot, AMMI, regression models (Wricke, 1962; Eberhart and Russell, 1996), coefficient of variation (Francis and Kannenberg, 1978), and stability variance (Shukla, 1972) to identify superior lentil genotypes (Sarker *et al.*, 2010; Subedi *et al.*, 2020; Beggar *et al.*, 2022; Hossain *et al.*, 2023).

The additive main effects and multiplicative interaction (AMMI) model is widely used to interpret GEI in multi-environment experiments (Olivoto *et al.*, 2021). However, Olivoto *et al.* (2019) highlighted limitations of the AMMI model, including sensitivity to outliers and insufficient integration with linear mixedeffects models (LMM). To address these issues, they combined AMMI with the best linear unbiased prediction (BLUP) method. The WAASB index, which integrates AMMI and BLUP, calculates the weighted average of absolute scores derived from the singular value decomposition of the matrix that best estimates GEI effects under LMM (Sharifi, 2020). WAASB enables the simultaneous selection of genotypes based on mean performance and stability. Several studies have reported significant GEI effects in lentil using this approach (Abbas *et al.*, 2019; Shobeiri *et al.*, 2021; Tadesse *et al.*, 2021; Shobeiri *et al.*, 2023).

The objectives of the present study were to analyze GEI affecting grain yield using parametric, non-parametric, GGE biplot, and WAASB methods; to assess the representativeness and discriminating ability of test environments; and to identify ideal genotypes with superior yield and stability for potential cultivation in the cold regions of Iran and for use in future breeding programs.

MATERIALS AND METHODS

In this study, nine pure lines derived from local landraces (previously selected using the plant-to-row

method), one advanced lentil genotype (Pardis), and two check cultivars (Bilesavar and Sana) were evaluated during 2022–2024 across four locations using a randomized complete block design with three replications (Table 1). Field preparation at all sites followed the technical recommendations of the Dryland Agricultural Research Institute. Fertilization included the application of 35 kg/ha triple superphosphate and 45 kg/ha urea. Seeds were sown in four rows, each four meters long, with 0.25 m spacing between rows. A plant density of 200 seeds per square meter was maintained.

The Maragheh research station (37°15′ N, 46°15′ E) is located at 1720 m above sea level. In Zanjan province, the Khodabandeh research station (36°9′ N, 48°49′ E) is situated at 1875 m. The Alarough research station in Ardabil province (38°15′ N, 48°20′ E) lies at 1390 m, while the Kurdistan research station (35°42′

N, 48°8′ E) is located at 2120 m above sea level. Climatic characteristics of the experimental sites are presented in Table 2.

In the second year at the Maragheh station and the first year in Kurdistan, the experiment produced no usable results due to poor seedling emergence. Before conducting ANOVA, data normality, experimental errors, and homogeneity of variances were assessed. The results indicated heterogeneity of variances; therefore, a combined analysis of variance was performed using data from five environments, excluding the secondyear results from Kurdistan.

To assess the impact of GEI on grain yield, GGE biplot analysis was conducted using the metan package (Olivo and Lucio, 2020) in R software. Parametric, non-parametric, and GGE biplot analyses were also performed in R.

Table 1. Description of the evaluated lentil genotypes and geographic characteristics of the four experimental locations used in the 2022–2024 multienvironment trials.

Code	Genotype/Originated location	Longitude	Latitude	Altitude (m)
G1	Varzeghan- Oarangh	46°-51'-43"	37°-28'-33"	1632
G2	Varzeghan- Somedel	48°-43'-16"	38°-31'-6"	1644
G3	Ardebil- Nir-Dashbolagh	48°-32'-58"	37°-58'-6"	2492
G4	Ardebil- Nir-Chehrebargh	48°-2'-33"	37°-58'-35"	1724
G5	Boukan-Darbesar	46°-5'-20"	36°-32'-13"	1543
G6	Naghadeh- Balighchi	45°-22'-4"	36°-55'-44"	1328
G7	Ardebil-Khalkhal	48°-52'-46"	37°-62'-13"	1787
G8	Ardebil-Khangah	48°-56'-40"	37°-61'-94"	1907
G9	Ardebil-Namin-Nanekaran	48°-31'-17"	38°-22'-15 "	1336
G10	Pardis			
G11	Bilesavar			
G12	Sana			

Table 2. Meteorological characteristics of the four experimental sites during the 2022–2024 lentil growing seasons, including temperature and precipitation patterns relevant to cold dryland conditions.

Location	Year	Temperature (°C)		Precipitation (mm)
		Min	Max	
Maragheh	2022-2023	-18	30	290.5
	2023-2024	-11.5	34	383.9
Zanjan	2022-2023	-15	30.4	286.7
	2023-2024	-10.6	31.2	265.3
Ardebil	2022-2023	-18.6	32.4	217.3
	2023-2024	-16.8	33.6	259.5
Kurdistan	2022-2023	-26.1	29.2	311.2
	2023-2024	-16.7	38.4	376.8

RESULTS AND DISCUSSION

The analysis of variance revealed highly significant effects of genotype, environment, and genotype×environment interaction (GEI) on grain yield at the 1% probability level (Table 3). The significant genotype effect highlights the presence of substantial genetic variability among the evaluated materials, while the significant environmental effect indicates that the genotypes responded differently to the climatic conditions across the two experimental years. The pronounced GEI further demonstrates that the performance of genotypes varied considerably across environments, suggesting that environmental factors played a major role in influencing yield expression. The magnitude of the environmental effect relative to the GEI implies the existence of distinct environmental groupings, which may promote specific adaptation. Similar findings of significant GEI in lentil have been reported by several researchers (Jeberson *et al.*, 2019; Gupta *et al.*, 2021; Beggar *et al.*, 2022).

Mean grain yield across genotypes ranged from 492.6 to 781.5 kg/ha. Mean comparisons showed that genotypes G3, G5, G10, and G7 produced the highest yields, with 781.5, 762.5, 704.4, and 702.6 kg/ha, respectively. These genotypes demonstrated superior performance and are therefore identified as promising candidates for further evaluation and potential use in lentil improvement programs (Table 4).

GGE-biplot analysis
The GEI heat map, generated based on the mean yield of genotypes across five environments, displays high values in brown and low values in white (Figure 1). The highestyielding group consisted of genotypes G3,

Table 3. Combined analysis of variance (ANOVA) for grain yield of 12 lentil genotypes evaluated across five cold dryland environments during the 2022–2024 growing seasons.

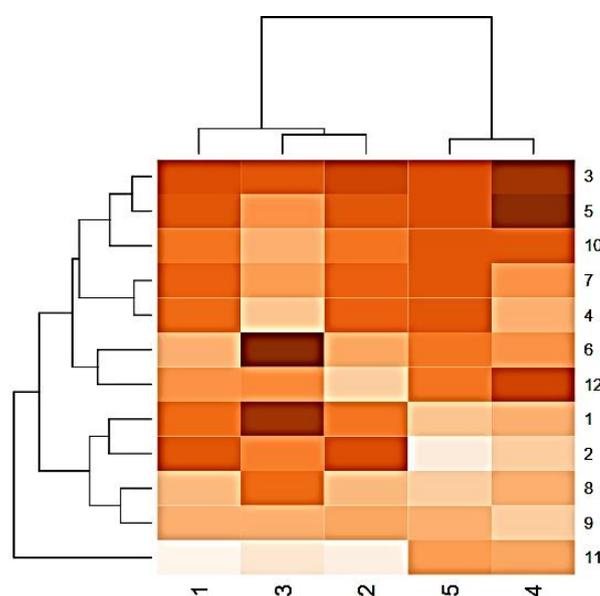| Source	df	SS	MS
Environment (E)	4	31593769	7898442**
Rep/E	10	63462	6346
Genotype (G)	11	1098167	99833**
GxE	44	927141	21071**
Error	110	431981	3927
Total	179	34114519	

**: Significant at 1%.

Figure 1. Heat map illustrating the grain yield performance of 12 lentil genotypes across five cold dryland environments during the 2022–2024 growing seasons.

Table 4. Mean grain yield (kg/ha) of 12 lentil genotypes tested across five cold rainfed environments during the 2022–2024 growing seasons.

No.	Maragheh	Zanjan	Ardebil	Total mean	CH% Sana	CH% Bilesavar
1	374.6	546.8	1098.6	673.3[cd]	102	137
2	407.5	523.7	1006.7	645.9[de]	97	131
3	418.2	558.5	1367.8	781.5[a]	118	159
4	373	457.9	1219	683.3[cd]	103	139
5	407.6	501	1379	762.5[ab]	115	155
6	225.9	514	1218.5	652.8[cd]	98	133
7	382.1	482.4	1243.5	702.6[c]	106	143
8	236.7	441.3	1077	585[e]	88	119
9	247.3	398.7	1077.8	574.6[e]	87	117
10	351.2	448.9	1313.3	704.4[bc]	106	143
11	82.6	249.4	1146	492.6[f]	74	100
12	295	391.7	1303.1	663.2[cd]	100	135
LSD5%				59.15		

G5, G10, and G7. The moderate yield group included G4, G6, G2, G1, and G12, while the lowest yielding group comprised G8, G9, and G11.

Analyzing genotype×environment interaction (GEI) is essential prior to releasing new high-yielding cultivars. Such analysis enables breeders to identify either widely adapted and stable genotypes suitable for diverse environments or specifically adapted genotypes optimized for particular target environments, thereby minimizing the negative effects of GEI. The GGE biplot analysis showed that the first two principal components (PC1 and PC2) explained 81.6% of the total variation in grain yield (52.2% and 29.4%, respectively). Similar contributions of the first two PCs have been reported in lentil GEI studies, including 61.6% and 31.2% (Dehghani *et al.*, 2008), 49.2% and 49.2% (Karimizadeh *et al.*, 2013b), and 48.41% and 41.0% (Karimizadeh *et al.*, 2013a). The polygon view of the GGE biplot is considered one of the most effective tools for evaluating genotype adaptation to specific target environments (Yan *et al.*, 2010).

Figure 2 presents the polygon view of 12 genotypes across five environments. Genotypes located farthest from the origin form the vertices of the polygon, representing those with either the highest or lowest performance in specific environments. In this study, genotypes 2, 3, 5, 12, and 11 were positioned at the polygon vertices, indicating strong or weak performance depending on the environment. The remaining genotypes were positioned within the polygon. Two mega-environments were identified: Mega-environment 1 included Environments 4 and 5 (both from Ardabil), with genotypes 5 and 10 performing best. Mega-environment 2 included Environments 1 and 2 (Maragheh 2023 and Zanjan 2023), where genotypes 3, 7, and 4 showed superior performance.

Figure 3 illustrates the ranking of genotypes based on yield and stability across environments. The line connecting the biplot origin to the ideal genotype represents the average test coordinate (ATC) (Yan and Kang, 2003). Genotypes positioned farther from the ATC axis contribute more to GEI and are therefore less stable (Yan, 2002). The results showed that G3, G5, and G10 had the highest average yields. Genotypes 4 and 1 exhibited performance closest to the overall mean. Genotypes 1 and 2 showed considerable instability, whereas genotypes 7 and 4 demonstrated the highest stability. Genotype G3 combined high yield with acceptable stability. Numerous studies have reported similar differential responses of lentil genotypes across
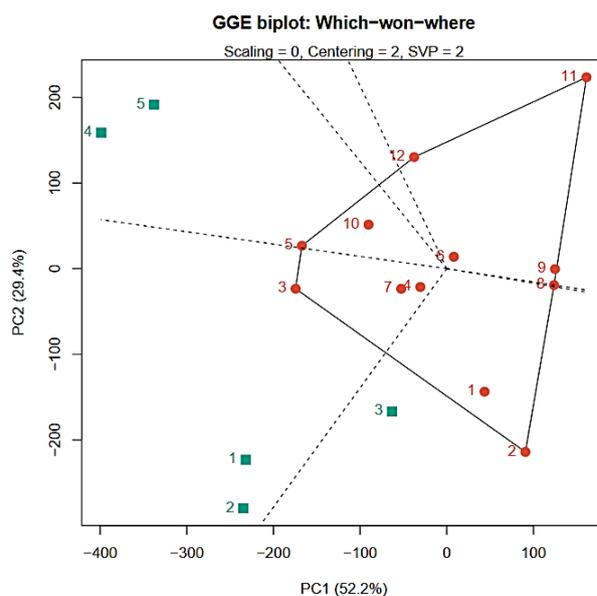

Figure 2. GGE biplot polygon view ("Which-Won-Where") showing the interaction patterns and mega-environment delineation for 12 lentil genotypes evaluated across five environments.
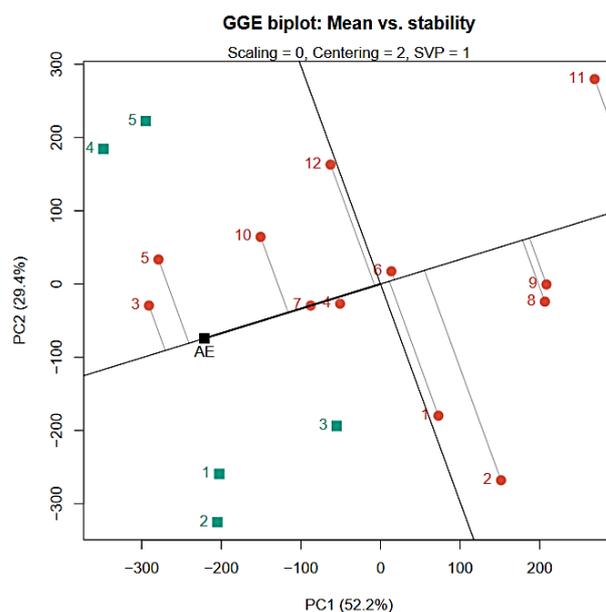

Figure 3. GGE biplot ranking of 12 lentil genotypes based on mean grain yield and stability across five cold dryland environments.

environments (Abbas *et al.*, 2019; Pezeshkpour and Mirzaii, 2021; Namdari *et al.*, 2022; Poudel *et al.*, 2023).

An ideal genotype is defined as one that exhibits both high mean yield and high stability across environments (Yan, 2002). In the ideal genotype biplot

(Figure 4), this is represented by the genotype closest to the center of the concentric circles. In the present study, genotypes G3 and G5 were closest to the ideal genotype, indicating their superior performance and stability.

The GGE biplot method is widely recognized as one of the most effective multivariate tools for evaluating adaptability and stability in crop species. Numerous studies have confirmed its effectiveness in identifying superior lentil genotypes in multi-environment trials (Moradi *et al.*, 2021; Gaffar *et al.*, 2023; Rizal *et al.*, 2024; Choukri *et al.*, 2025).

The results of the parametric and non-parametric stability analyses are presented in Table 5. Parametric methods provide a comprehensive assessment of genotype stability across environments, enabling the identification of genotypes with consistent performance. In contrast, non-parametric methods offer a robust evaluation of genotype performance without relying on strict distributional assumptions (Huehn, 1990; Meena *et al.*, 2025).

Kang's Yield and Stability Index (YSi) integrates mean yield with Shukla's stability variance to generate a combined selection criterion. Kang and Pham also proposed a non-parametric ranksum statistic that incorporates both yield and Shukla's variance. Genotypes with YSi values greater than the overall mean are considered desirable. Based on YSi, genotypes G3, G5, G7, and G10 were selected. Shobeiri *et al.* (2023) reported the effectiveness of TOP and YSi statistics in identifying stable lentil genotypes, and several studies have confirmed the reliability of these methods (Sabaghnia *et al.*, 2013; Namdari *et al.*, 2022; Namdari *et al.*, 2025).
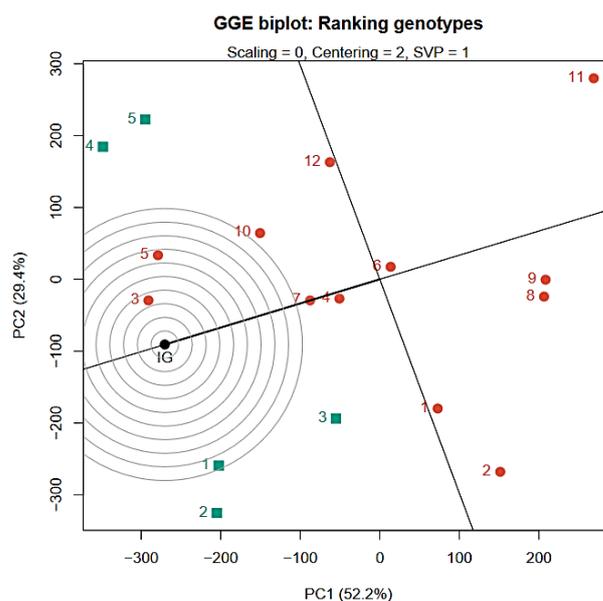

Figure 4. Ideal-genotype GGE biplot identifying the proximity of 12 lentil genotypes to the theoretical ideal genotype based on combined yield performance and stability across five environments.

Fox *et al.* (1990) introduced a non-parametric method to assess cultivar superiority based on general adaptability. Genotypes ranking predominantly in the upper third of environments are classified as widely adapted. According to this criterion, G3 and G5 were identified as broadly adapted and well-suited to the tested environments.

Nassar and Huehn (1987) proposed two non-parametric stability statistics, S1 and S2, which emphasize genotype ranking across environments. Genotypes with minimal rank fluctuations are considered more stable. Using these indices, genotypes

Table 5. Parametric and nonparametric stability statistics for 12 lentil genotypes evaluated across five environments, including yield stability indices and ranking based measures.

No.	Pi_a	Pi_f	Pi_u	WAASB	YS	TOP	S1	S2	S3	S6
1	16784.5	39437.9	1682.2	6.74	-2	1	5.2	20.3	5.41	1.6
2	30093.3	69316.3	3944.7	4.52	-5	2	6.6	31.5	13.31	3.1
3	879.9	81.4	1412.2	5.83	15	5	3	6.5	0.25	0.3
4	15576.3	22368.1	11048.4	6.59	1	1	5.2	20.3	7.06	2.1
5	3157.2	0.0	5262.1	9.74	10	4	4.2	12.8	2.43	0.9
6	12150.8	15605.8	9847.4	2.77	0	1	4.8	15.5	5.39	1.6
7	10108.3	15285.3	6657.0	3.21	10	0	3.2	7.3	1.47	0.8
8	27402.1	45752.2	15168.7	5.18	-3	0	3.4	7.7	9.16	2.7
9	30833.8	48379.7	19136.5	3.33	0	0	2.2	3.8	2.00	1.3
10	7264.5	2689.6	10314.4	2.23	9	0	3.2	7.2	2.30	1.2
11	48085.9	28904.7	60873.3	4.40	-10	0	5.8	21.5	8.86	3.9
12	12213.3	2941.5	18394.5	2.52	-1	1	5.4	18.7	5.67	1.7

G3, G5, G7, and G10 were identified as stable. Additionally, the Linn and Binns (1986) superiority index and the WAASB index confirmed the high stability of genotypes G3 and G5. Similar findings have been reported in lentil stability studies (Mohebodini *et al.*, 2006; Rajput *et al.*, 2022; Pezeshkpour *et al.*, 2024).

The comparative evaluation of autumn-sown lentil genotypes using parametric, non-parametric, and GGE biplot methods highlighted their performance and adaptability across diverse environments. Parametric analyses identified G3 and G5 as the most stable genotypes. Non-parametric methods further supported the stability of G3, G5, G7, and G10. The GGE biplot provided additional insights through its "WhichWonWhere" and "Discriminativeness vs. Representativeness" views, indicating that genotypes G3, G5, and G10 exhibited strong discriminative ability and consistent performance.

Overall, G5 and G10 appear suitable for cultivation in the cold climate of Ardabil province, while G3, G4, and G7 are recommended for the cold dryland regions of Maragheh and Zanjan.

CONCLUSION

The findings of this study clearly demonstrated substantial effects of genotype, environment, and genotype×environment interaction on lentil grain yield under cold rainfed conditions. The significant GEI emphasized the importance of evaluating genotypes across multiple environments to reliably identify stable and high-performing lines. The combined application of parametric and non-parametric stability statistics, together with GGE biplot analysis, proved to be an effective and complementary strategy for dissecting GEI and assessing both yield performance and stability.

Among the evaluated genotypes, G3 and G5 consistently exhibited superior grain yield and high stability across environments, while G10 showed a notable yield advantage over the check cultivars, highlighting its potential for further improvement and possible release. These results underscore the value of integrating multiple analytical approaches in breeding programs aimed at developing cold-tolerant, high-yielding lentil cultivars. The promising genotypes identified in this study can contribute to enhancing productivity and yield stability in cold rainfed lentilgrowing regions and support the development of resilient and sustainable lentil production systems.

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