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Evaluation of genetic diversity in Iranian rye accessions using agro-morphological traits in the Tabriz plain

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ABSTRACT

In this study, 13 rye populations—previously screened for superior performance over the “DANKO” control variety and high drought tolerance—were evaluated in the Tabriz Plain .The experiment was conducted at the Agricultural Research and Natural Resources Training Center of East Azerbaijan using a randomized complete block design (RCBD) with three replications. Following the assessment of agro-morphological traits, data were analyzed using analysis of variance (ANOVA), mean comparison tests, regression, path analysis, and cluster analysis. Results revealed significant differences ($p>0.01$) among the ecotypes for all studied traits. Mean comparisons indicated that ecotypes TN-243, 110027, TN-220, TN-3, and 110095 were high-yielding genotypes, whereas DANKO and TN-2 were classified as low-yielding under the regional conditions. Furthermore, the findings identified TN-220, TN-3, TN-243, 13139, TN-91, and 110085 as superior ecotypes .This study demonstrates that Iranian local rye accessions, particularly TN-3, TN-243, 13139, TN-91, and 110085, possess substantial genetic diversity and performance competitive with commercial varieties. These results highlight their potential as valuable germplasm for future breeding programs aimed at climate change adaptation. Overall, the observed high phenotypic diversity underscores the suitability of these ecotypes for diverse breeding objectives.

Key words: Cluster Analysis, Diversity, Genetic Resources, Growth, Rye.

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INTRODUCTION

Diversifying crop varieties and agricultural products is essential for strengthening sustainable production and ensuring national food security. In recent years, challenges driven by climate change, recurrent drought, and the growing demand for livestock fodder have intensified, turning forage shortages into a critical national concern. Integrating annual forage crops into fallow lands offers a practical strategy to improve the use of natural resources and partially address these shortages (Vahed Rezaei *et al.*, 2020).

Small-grain cereals such as barley, rye (*Secale cereale* L.), triticale, and wheat are widely cultivated worldwide as winter forage and play an important role in sustainable agricultural systems, particularly those supporting livestock production (Kim *et al.*, 2017). Among them, rye stands out as a multipurpose cereal used for grain, forage, and cover cropping. It is valued for its high crude protein content, favorable digestibility of organic matter, and broad adaptability to diverse soil conditions.

Rye's exceptional tolerance to drought and cold, along with its suitability for autumn sowing and efficient use of seasonal rainfall (green water), makes it especially well-suited for cultivation in marginal, arid, and semi-arid fallow drylands. In addition, rye forage is highly versatile and can be used for direct grazing, hay, dry fodder, or silage (Shahmoradi and Ghotbi, 2022).

In infertile, sandy soils with poor drainage, rye often produces higher yields than wheat, whereas wheat generally performs better in moderately to highly fertile soils (Schlegel, 2013). As a result, rye is predominantly grown on marginal lands where other cereals frequently fail to establish or produce acceptable yields (Miedaner *et al.*, 2012). Its remarkable drought tolerance is largely attributed to an extensive and highly branched root system that enables more efficient water uptake compared with wheat and triticale (Starzycki, 1976; Sheng and Hunt, 1991). Notably, rye requires 20–30% less water per unit of dry matter produced than wheat (Starzycki, 1976).

Rye also exhibits superior cold hardiness, outperforming both wheat and barley in its ability to withstand harsh winter conditions and improve nutrientpoor or stony soils (Abtahi and Bagherzadeh, 2014). From a nutritional standpoint, rye grain provides 85–90% of the energy value of maize and contains higher levels of digestible protein and nutrients than oats or barley (Thapa *et al.*, 2018).

Given that reduced crop diversity poses longterm risks to food security, promoting underutilized or native species such as rye is essential to avoid overdependence on a limited number of staple crops. Research further highlights rye's strong yield potential and adaptability, particularly in dualpurpose systems where winter cereals are grazed before stem elongation and later harvested for grain (Kim *et al.*, 2017).

In southwestern Saskatchewan, many rye producers routinely graze their winter rye crops in the fall or early spring. Under these conditions, the seed yield of the winter rye cultivar 'Puma' decreased by 10% with spring grazing and by 17% with fall grazing (Kilcher, 1982). However, other researchers reported a much smaller reduction in winter rye, with yield losses of less than 4%, compared with 9–12% reductions observed in winter wheat and triticale (Bishnoi and Hughes, 1979).

To compare winter forage yields, four species—barley, rye, triticale, and wheat—were evaluated across three growing seasons. Species, environment, and their interaction significantly affected both seasonal and average forage yields, with rye producing the highest average forage yield, followed by triticale, wheat, and oats (Kim *et al.*, 2017). In another study, four triticale genotypes, one winter wheat genotype, and one winter rye genotype were evaluated over three seasons. Spring forage harvesting at two different stages reduced final plant height and lodging incidence in winter cereals, although seed weight remained unaffected (Poysa, 1985). Spring forage harvesting (hay) in winter cereals is therefore considered an effective strategy for producing additional forage, reducing lodging, and improving seed yield in years with abundant spring growth.

According to Holliday (1956), the impact of forage harvesting on seed yield in cereals varied widely, with decreases reported in 24 cases and increases in 5 cases. Environmental conditions, management practices, soil moisture, soil fertility, and genotype all interact with forage production to influence final seed yield. As the most droughttolerant cereal, rye adjusts its maturation time according to moisture availability and requires 20–30% less water per unit of dry matter produced than wheat (Schlegel, 2013).

Several studies have examined the genetic diversity of forage plant collections preserved at the National Plant Gene Bank of Iran. Rye ecotypes collected from different regions of the country are maintained as distinct populations and referred to as ecotypes. Evaluations of agronomic and morphological traits, along with genetic diversity analyses of 271 rye ecotypes, revealed substantial variation in both quantitative and qualitative traits. Among the quantitative traits, yield and seed weight showed the greatest diversity (Gholizadeh Sarcheshmeh *et al.*, 2018). Similarly, an assessment of agronomic, morphological, and ecogeographical diversity in 108 rye ecotypes—based on climate and geographic origin—demonstrated wide variation in morphological, phenological, and agronomic traits, likely reflecting their evolutionary history (Shahmoradi, 2022).

Iranian rye lines represent a valuable indigenous genetic resource, exhibiting high levels of diversity in agronomic traits and strong tolerance to environmental stresses. In a study by Asadi *et al.* (2021), thirteen native lines from various regions of Iran—including *Kermanshah1*, *Hamedan2*, *Urmia4*, *Kurdistan3, Ardabil1*, *Lorestan2*, and *Mazandaran1*—were evaluated. The results showed that *Kermanshah1* had significantly higher grain yield and harvest index under drought stress compared with the other lines. *Urmia4* was identified as a suitable genotype for waterlimited environments due to its high drought tolerance and elevated chlorophyll content. In contrast, *Mazandaran1* performed best under nonstress conditions but was more sensitive to drought. These differences highlight the considerable potential of Iranian rye lines for breeding programs aimed at improving stress tolerance and enhancing performance across diverse climatic conditions.

This study aims to identify highpotential genetic resources within the rye collection of the National Plant Gene Bank of Iran to support the sustainable production of seed and forage for livestock, thereby contributing to national food security. Given rye's wellrecognized resilience and adaptability to harsh environmental conditions, the effective use of these genetic resources can play a key role in strengthening sustainable forage and seed production. In this context, the present research not only contributes to the conservation of biodiversity and indigenous genetic resources but also provides a scientific foundation for breeding and sustainable agriculture programs that directly influence the longterm stability of the nation's food and economic systems.

MATERIALS AND METHODS

This research was conducted at the Agricultural Research and Natural Resources Training Center of East Azerbaijan, located 2 km southwest of Tabriz at 46°03′ E longitude and 37°58′ N latitude, with an elevation of 1,349 meters above sea level. According to meteorological data, the region has an average annual temperature of 11.5 °C, with minimum and maximum temperatures of −7.7 °C in December and 32.5 °C in July, respectively. The average annual precipitation is 270 mm. Winter temperatures frequently fall below freezing, temporarily halting plant growth. The physical and chemical properties of the experimental field soil are presented in Table 1.

The plant materials used in this study consisted of 13 agricultural rye populations (Table 2) obtained from the National Plant Gene Bank of Iran (NPGBI). These populations had previously demonstrated superior performance compared with the control variety 'DANKO' and showed favorable drought tolerance under stress conditions (Shahmoradi, 2022; Shahmoradi and Ghotbi, 2022). The experiment was conducted using a randomized complete block design with three replications. Sowing was carried out in two 1-meter rows at a planting density of 350 seeds per square meter. Throughout the growing season, standard management practices—including fertilization, weed control, and irrigation—were applied according to crop water requirements.

At the end of the growing season, yield-related traits (seed and forage yields) were recorded along with other agronomic characteristics, including plant height, spike length, spike weight, number of seeds per spike, seed weight per spike, thousand-seed weight, dry forage weight, and fresh forage weight. After data collection, statistical analyses—including analysis of

Table 1. Physico-chemical properties of the experimental field soil at the Tabriz Plain research site.

Parameter	Unit	Value	Parameter	Unit	Value
pH	-	7.79	Nitrogen (N)	%	0.20
Phosphorus (P)	$mg.kg^{-1}$	19	Potassium (K)	$mg.kg^{-1}$	31
Calcium (Ca)	$mg.kg^{-1}$	8.36	Iron (Fe)	$mg.kg^{-1}$	1.86
Magnesium (Mg)	$mg.kg^{-1}$	1.71	Copper (Cu)	$mg.kg^{-1}$	1.11
Organic matter	%	1.57	Bulk density (T.N.V)	$g.cm^{-3}$	1.31
Soil texture	-	Clay-loam	Depth	cm	30

Table 2. Agromorphological and origin characteristics of the 13 Iranian rye ecotypes evaluated in the study.

Ecotypes number	Gene bank number	Cultivar / Ecotypes	Province	Country
1	TN-2	-	-	Iran
2	TN-22	46	East Azerbaijan	Iran
3	110027	-	-	Iran
4	TN-243	119	Hamedan	Iran
5	TN-220	108	Kerman	Iran
6	DANAKO	DANAKO	-	Germany
7	110085	26	-	Iran
8	110026	-	-	Iran
9	TN-172	-	-	Iran
10	TN-3	40	Fars	Iran
11	13139	35	-	Iran
12	TN-91	78	East Azerbaijan	Iran
13	110095	-	-	Iran

Table 3. Analysis of variance (ANOVA) for agromorphological traits measured in the Iranian rye ecotypes.

Source of variation	df	Mean of square							
		Forage yield	Thousand-seed weight	Spike weight	Number of seeds per spike	Seed weight per spike	Spike length	Seed yield	Plant height
Replication	2	158195.1*	0.023^{ns}	0.04^{ns}	20.10^{ns}	0.033^{ns}	0.057^{ns}	23643.8^{ns}	5.83^{ns}
Ecotype	12	185765.1**	0.164**	0.249**	126.47**	0.11**	3.07**	86609.6**	422.8**
Error	14	42171/1	0.04	0.03	9.01	0.02	0.71	7928.8	37.83

**, *, and ns indicate significance at the 1%, 5% probability levels, and non-significance, respectively.

variance, mean comparison tests appropriate for the experimental design, regression analysis, path analysis, and correlation analysis—were performed to evaluate differences among the ecotypes. These analyses were conducted using MSTAT-C and SPSS software. To classify the ecotypes, cluster analysis was performed in SPSS, and the resulting groups were validated through discriminant analysis. Genetic, phenotypic, and environmental variances were calculated according to Falconer (1967), and the corresponding coefficients of variation were estimated using the formulas of Singh and Chaudhury (1985).

RESULTS AND DISCUSSION

The analysis of variance for agro-morphological traits—including plant height, spike length, spike weight, number of seeds per spike, seed weight per spike, thousand-seed weight, dry forage weight, and fresh forage weight (Table 3)—showed significant differences among the ecotypes at the 1% probability level. This indicates the presence of considerable genetic diversity within the rye collection. Similar levels of variation among Iranian native rye ecotypes have been reported previously (Shahmoradi, 2022).

Mean comparisons for the evaluated traits are presented in Figure 1. For plant height, the ecotypes TN-243, 110085, and TN-3 recorded the highest values, whereas TN-2, 110026, and 110095 had the lowest. Seed yield was highest in TN-3 and TN-220 and lowest in TN-2 and TN-243. Spike length was greatest in TN-22, 110027, and TN-220, while DANAKO, TN-2, and 110085 showed the lowest values.

For seed weight per spike, the highest values were observed in 110027, TN-243, and 110095, whereas DANAKO and TN-3 had the lowest. The ecotypes TN-22, 110027, TN-243, TN-220, 110085, and 110095 produced the highest number of seeds per spike. Spike weight was greatest in 110027 and 110095 and lowest in TN-172, DANAKO, and TN-2. Thousand-seed weight was highest in 13139, 110095, and TN-243, while TN-2, TN-22, TN-220, 110085, TN-172, and TN-3 showed the lowest values. The highest forage yields were recorded in TN-220, 110027, DANAKO, and TN-172, whereas 110095, 13139, and TN-91 produced the lowest.

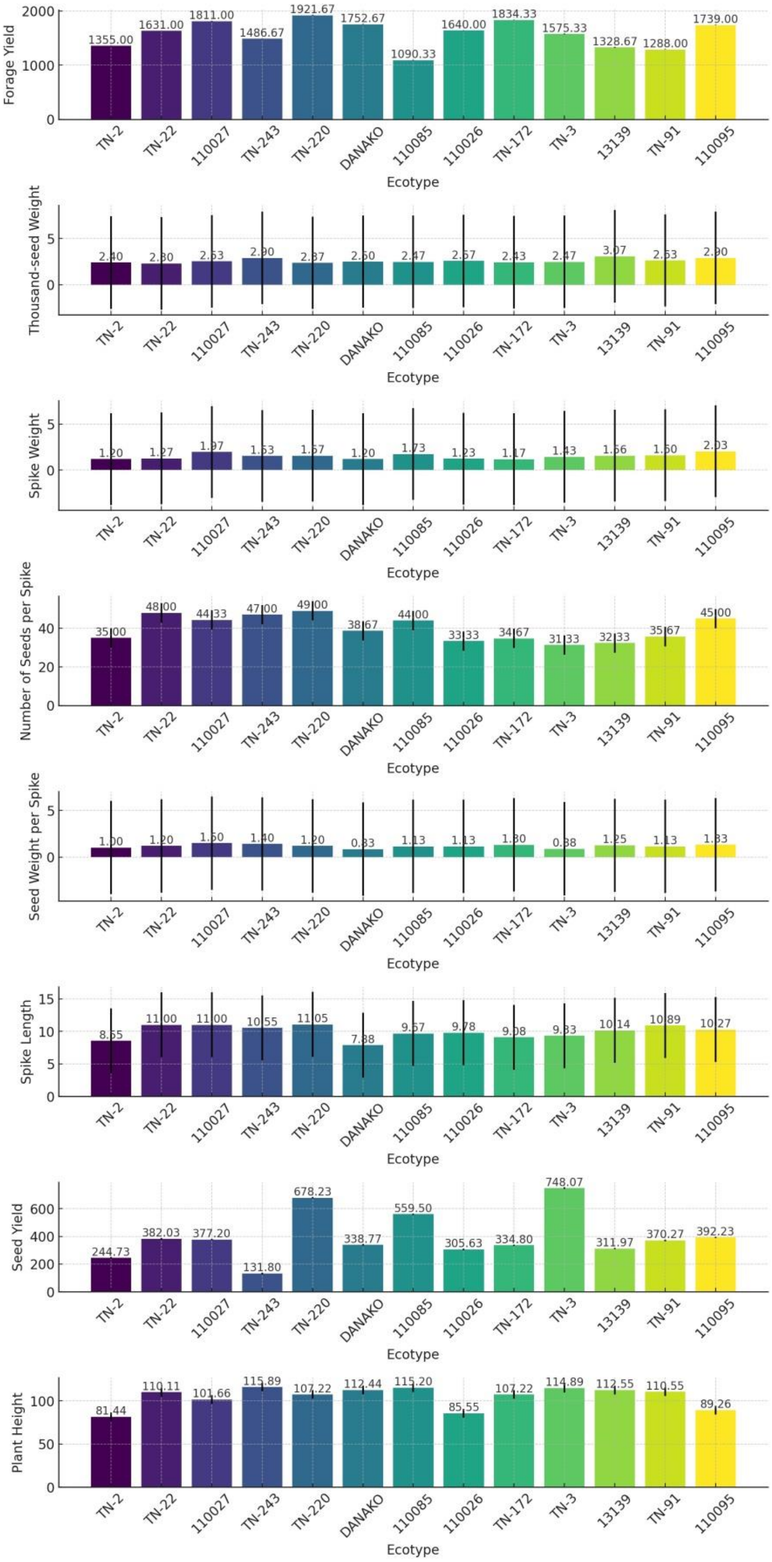

Figure 1. Mean comparison of agromorphological traits among the 13 Iranian rye ecotypes.

Overall, the ecotypes TN-243, 110027, TN-220, TN-3, and 110095 performed best across most traits and can be considered high-yielding genetic materials. In contrast, DANAKO and TN-2 consistently ranked among the lowest-performing ecotypes. These findings align with earlier studies reporting substantial agronomic and morphological diversity among Iranian rye collections (Shahmoradi *et al.*, 2011).

Diversity parameters for the evaluated traits are presented in Table 4. The highest genetic variances were observed for forage yield (35,899.2 g) and seed yield (19,670.22 g), while the lowest were recorded for seed weight per spike (0.02 g), thousand-seed weight (0.03 g), and spike weight (0.05 g). Phenotypic variance followed a similar pattern, with the highest values for forage yield (78,070.13 g) and seed yield (27,599.03 g), and the lowest for seed weight per spike (0.05 g), thousand-seed weight (0.08 g), and spike weight (0.09 g).

The genetic and phenotypic coefficients of variation were highest for seed yield and lowest for thousand-seed weight. The high coefficients for seed yield reflect the wide range of variations observed for this trait. The small differences between genetic and phenotypic coefficients of variation for number of seeds per spike and plant height suggest minimal environmental influence, indicating that these traits are largely genetically controlled. Therefore, they are suitable criteria for selecting parents in hybridization programs.

Cluster analysis grouped the ecotypes into three main clusters (Figure 2): Group 1 with six ecotypes (green), Group 2 with two ecotypes (yellow), and Group 3 with five ecotypes (red). The clustering pattern was consistent with the results of the analysis of variance and mean comparisons. Group means and their deviations from the overall mean (Table 5) showed that Group 1 contained ecotypes with the lowest values for all measured traits and can be classified as low-yielding. In contrast, Groups 2 and 3 included ecotypes with the highest values and represent high-yielding groups.

Discriminant analysis (Table 6), based on all measured traits, confirmed the three-group classification. Canonical correlations were significant for all functions, and Wilks' Lambda indicated that all discriminant functions were significant at the 5% probability level.

CONCLUSION

Rye is a native forage species adapted to the cold climates of Iran, and its ecotypes represent valuable genetic resources for improving adaptation to diverse environmental conditions. Evaluating the agro-morphological traits of Iranian native rye ecotypes is therefore essential, as the genetic diversity observed in this study revealed clear differences in their performance under the climatic conditions of the Tabriz Plain. Similar patterns of diversity in rye ecotypes have been reported by other researchers (Statkevičiūtė *et al.*, 2012; Shahmoradi, 2022).

The results of this study showed that although commercial varieties such as DANKO have high seed production potential, several native ecotypes—including TN-220, TN-3, TN-243, 13139, TN-91, and 110085—demonstrated strong performance and were competitive with the commercial variety under Tabriz Plain conditions. Mean comparisons indicated that TN-243, 110027, TN-220, TN-3, and 110095 had significantly higher values for

Table 4. Genetic, phenotypic, and environmental variance components and diversity parameters for the evaluated agromorphological traits.

Parameters	Traits							
	Plant height	Seed yield	Spike length	Seed weight per spike	Number of seeds per spike	Spike weight	100 seed weight	Forage yield
Total mean	104.92	398.09	9.94	1.18	39.87	1.50	2.58	1573.36
Maximum values	123.33	861.90	12.16	1.70	55.00	2.20	3.10	2202.00
Minimum values	79.00	67.10	6.83	0.80	30.00	1.00	2.10	716.00
Standard deviation	12.56	183.31	1.19	0.23	6.83	0.32	0.29	305.98
Genetic variance	96/26	19670.22	0.59	0.02	29.36	0.05	0.03	35899.02
Phenotypic variance	134.09	27599.03	1.30	0.05	38.38	0.09	0.08	78070.13
Environmental variance	37.83	7928/81	0.71	0.03	9.02	0.03	0.05	42171.10
Genetic coefficient of variation (%)	9.35	35.23	7.73	12.17	13.59	15.57	6.66	12.04
Phenotypic coefficient of variation (%)	11.04	41.73	11.48	18.71	15.54	19.50	10.65	17.76
Environmental coefficient of variation (%)	5.86	22.37	8.48	14.22	7.53	11.74	8.31	13.05

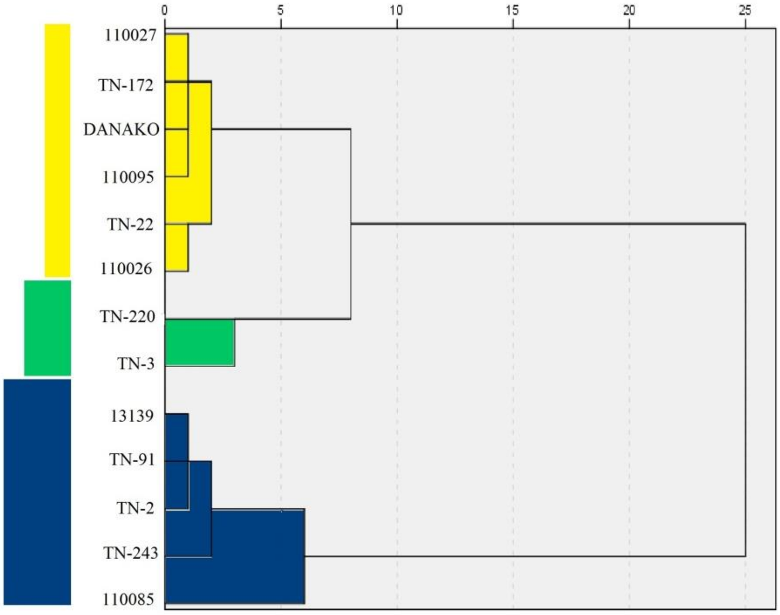

Figure 2. Dendrogram of the 13 rye ecotypes generated using hierarchical cluster analysis based on agromorphological traits**.**

Table 5. Cluster means and percentage deviation from the overall mean for agromorphological traits across the three ecotype groups.

Group	Cluster	Traits							
		Plant height	Seed yield	Spike length	Seed weight per spike	Number of seeds per spike	Spike weight	100 seed weight	Forage yield
1	Mean	107.12	323.65	9.95	1.18	38.80	1.52	2.69	1309.79
	Deviation from the total mean Percentage	2.1	-18.70	0.21	0.54	-2.68	1.70	4.41	-16.75
2	Mean	101.04	355.11	9.83	1.21	40.66	1.47	2.53	1734.66
	Deviation from the total mean Percentage	-3.70	-10.80	-1.03	3.37	1.99	-1.42	-1.57	10.25
3	Mean	111.05	713.15	10.19	1.04	40.16	1.50	2.41	1748.50
	Deviation from the total mean Percentage	84.5	79.14	2.56	-11.49	0.73	0.05	-6.30	11.13
	Total mean	104.92	398.09	9.93	1.17	39.87	1.49	2.57	1573.35

Table 6. Results of discriminant function analysis validating cluster separation among rye ecotypes based on agromorphological traits.

Group	Wilks' Lambda	Canonical Correlation	Cumulative Variance (%)	Variance (%)	Eigenvalues	Chi-Square	df
3	0.013	0.984	95.9	95.9	30.96	27.99	16*
	0.430	0.755	100	4.1	1.32	5.4	7^{ns}

* and ^{ns} indicate significance at the 5% probability level and non-significance, respectively.

most agro-morphological traits and were classified as high-yielding. In contrast, DANKO and TN-2 consistently showed lower values for several traits and were categorized as low-yielding.

These findings are consistent with previous studies (Shahmoradi, 2022; Shahmoradi and Ghotbi, 2022), which also highlighted the strong adaptability and performance potential of certain native rye genetic resources compared with commercial varieties. Such results have increased interest in these ecotypes as valuable breeding materials. Numerous studies have used agro-morphological traits to assess genetic diversity in rye and to identify high- and low-yielding genetic resources with potential for breeding (Shahmoradi and Ghotbi, 2022; Carrasco-Chilón *et al.*, 2023).

In this study, traits such as plant height, spike length, spike weight, number of seeds per spike, seed weight per spike, thousand-seed weight, dry forage weight, and fresh forage weight provided a comprehensive picture of genetic diversity and will be useful in future research. The application of multivariate statistical methods enabled reliable grouping of ecotypes and identification of superior materials. Ecotypes such as TN-3, TN-243, 13139, TN-91, and 110085, which showed the highest average values across traits, can be effectively used in breeding programs. Overall, the high phenotypic diversity observed among the rye ecotypes confirms their suitability for a range of breeding objectives.

The evaluation of traits under the environmental conditions of the Tabriz Plain also demonstrated that ecotype performance varies with climatic fluctuations. Multi-year studies are therefore recommended to obtain more accurate insights. Additionally, incorporating physiological and biochemical traits into future evaluations will provide a more comprehensive understanding of ecotype performance.

Based on our findings, we propose that future studies focus on long-term evaluations of the high-performing ecotypes—particularly TN-3, TN-243, 13139, TN-91, and 110085—under varying climatic conditions to better assess their stability and adaptability. Integrating physiological and biochemical traits will further enhance the understanding of their performance potential. The high genetic and phenotypic diversity observed indicates that these ecotypes are valuable resources for breeding programs aimed at improving yield, forage quality, and climate resilience in rye. Practical applications include selecting superior ecotypes for local adaptation and using them as parental material in hybridization programs to enhance productivity under environmental stress. For agronomists and farmers, prioritizing high-performing native ecotypes—especially TN-3, TN-243, 13139, TN-91, and 110085—is recommended, as they demonstrated strong agro-morphological performance and adaptability under Tabriz Plain conditions. Regular monitoring of plant height, spike traits, seed weight, and forage yield can help optimize management practices. Multi-year trials and expanded trait evaluations will further support targeted breeding and sustainable production.

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