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Intra-population Variability of the Main Morphological Traits of *Agropyron intermedium* Species

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ABSTRACT

***Agropyron intermedium* (*Thinopyrum intermedium*), a perennial forage and grain grass, is valued for its high tolerance to abiotic and biotic stresses and its potential use in sustainable agriculture and cereal breeding. Understanding the intra-population morphological variability of this species is essential for selecting superior genotypes for yield, resilience, and agronomic performance. This study evaluated the morphological variability of 25 *Agropyron intermedium* genotypes under the environmental conditions of 2023-2024. The genotypes were evaluated for plant height, spike number per plant, spike length, flag leaf length and width, vegetative and generative tiller, and heading. Results showed moderate variation in plant height (120-150 cm), with most genotypes exhibiting a tall and erect growth habit, while reproductive traits such as spike number and spike length displayed greater variability. Correlation analysis indicated that most traits were largely independent, except for a significant positive relationship between flag leaf length and width ($r = 0.54$, $p \leq 0.01$). Principal component analysis (PCA) identified five components explaining 100% of total variance, with the first two components accounting for 56.7% of variation: PC1 was primarily associated with leaf morphology and photosynthetic capacity, and PC2 with reproductive traits and tillering ability. Variable contribution analysis confirmed that plant stature, vegetative growth, and reproductive development represent independent dimensions of variability. The findings highlight the multidimensional trait structure of *A. intermedium*, providing valuable information for selection and breeding programs aimed at improving yield, plant architecture, and phonological performance.**

Keywords: genotypes, morphological measurements, *Agropyron intermedium*.

List of Abbreviations: PC - Principal Component, PC1, PC2… PC5 - Principal Components 1-5 in PCA analysis, r - Pearson correlation coefficient, p - Statistical significance of correlation, °C - Degrees Celsius, mm - Millimeters (precipitation), Statistica 7 - Software used for statistical analysis, N - Nitrogen (related to nitrogen-use efficiency), *Th. intermedium* - *Thinopyrum intermedium* (abbreviated species name), MT, AA, DM, BL - Initials of authors contributing to different stages (Conceptualization, Methodology, etc.), ± - Standard deviation or deviation from long-term average, Height - Plant height, Spikelet/plant - Number of spikelets per plant, Spike length - Length of spike, Flag leaf length - Length of the flag leaf, Flag leaf width - Width of the flag leaf, Generative tillers - Number of reproductive tillers, Vegetative tillers - Number of vegetative tillers, Heading - Date of inflorescence emergence.

INTRODUCTION

Agropyron intermedium, sin. *Thinopyrum intermedium*, known commonly as intermediate wheatgrass, because of the similarity of their seed heads or ears to common wheat, is a cool season perennial grass, native to central Europe and Western Asia. Its represents one of the most valuable genetic resources used in modern cereal breeding programs, due to its high tolerance to abiotic and biotic stresses. The species' ability to withstand drought, low temperatures and salinity, as well as its natural resistance to major pathogens, have supported the inclusion of its genetics in wheat breeding and the development of perennial cereals (Liu et al., 2023; Qi et al., 2023).

In this context, studies conducted on wheat under controlled drought conditions have demonstrated the importance of evaluating genotypic variability based on yield components such as grain number per spike, thousand kernel weight and overall productivity, in order to identify tolerant genotypes suitable for breeding programs. Such approaches highlight the relevance of systematic phenotypic assessment under

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stress conditions as a key step in the selection process (Racz et al., 2020).

In addition to its genetic potential, *T. intermedium* attracts agronomic interest due to its central position in the development of perennial cereals for sustainable agriculture. Recent studies on the performance of the species in different agroecological environments have demonstrated high nitrogen use efficiency, superior water use capacity and production stability under variable pedoclimatic conditions, including prolonged drought situations (Mårtensson et al., 2022). These qualities give it a strategic role in low-input production systems and in contexts of accelerated climate change. Recent research highlights both its forage value and adaptability in mixed cropping systems, especially when cultivated with legumes, demonstrating improved nutritional potential and evaluation through modern techniques such as near-infrared spectrometry (Fagnant et al., 2024). Also, molecular and proteomic studies have provided insights into its response to drought stress, emphasizing its role in improving wheat through chromosomal translocations and stress-resilience mechanisms (Lu et al., 2022). At the same time, long-term research in Romania has highlighted a consistent genetic progress in wheat breeding programs, emphasizing the role of continuous selection and evaluation of genetic resources in increasing yield potential under variable environmental conditions. The annual genetic gain obtained in multi-environment trials reflects the efficiency of breeding strategies and supports the importance of integrating valuable genetic resources, such as wild relatives, into breeding programs (Marinciu et al., 2025). In parallel, recent studies on the seed microbiome have suggested that the breeding process can influence the microbial communities associated with the species, an emerging aspect that may indirectly contribute to the agronomic performance of the improved material (Michl et al., 2024). Thus, *T. intermedium* is of interest not only through its intrinsic genetic characteristics, but also through its ecological and microbiological interactions that may influence the stability of agronomic phenotypes. Cultivation of *Agropyron intermedium* will provide not only ecological, but also social and economic benefits. This is also important due to challenges associated with the climate warming, the necessity to reduce the greenhouse effect, in agricultural production as well (Pototskaya, 2022). In this context, the aim of the present scientific work is to evaluate the agronomic value and potential for breeding of 25 *Agropyron intermedium* genotypes, by analyzing their performances under specific experimental conditions, from Romania. The objectives include characterizing phenotypic variability, identifying genotypes with high potential for use in breeding programs and evaluating agronomic traits relevant for productivity, resilience and sustainability. Considering the characteristics of *Agropyron intermedium* species, as a valuable forage-grain crop, which could provide also environmental benefits in soil protection, research was initiated for the evaluation of the main morphological traits and to determine the correlations between them, with the aim of introducing the species into the institute's breeding program. In Romania, there are no varieties created for this species and it is important that, in the context of global warming, we have varieties of resistant species that can successfully supplement the feed during the dry months.

MATERIAL AND METHODS

The present research was conducted in 2023-2024, in a field trial, at the Grassland Research Institute - Braşov, a region with a typical continental climate. The meteorological conditions in 2023-2024 (Table 1) indicate a drier and warmer period compared to the multiannual average. In both years, the temperature exceeded the multiannual average, with 0.6°C in 2023 with 2.7°C in 2024.

Considering the precipitation values, compared to the multiannual average both years were droughty, 2023 recording a total deficit of -182.8 mm and of -121,3 mm during the vegetation period, and 2024 a total deficit of -22.6 mm and of -43,2 mm in the

vegetation period. Deviations in air temperature and precipitation were assessed relative to the corresponding long-term averages.

The biological material was represented by 25 genotypes belonging to a wild population collected from the tailings dumps in the Sânpetru area, located at 5 km distance from Braşov.

The plants were obtained by sowing in March 2023, in the greenhouse, in rows in trays, followed by individual transplanting into small plastic pots. Maintenance work was applied: watering, weeding, cutting. When the seedlings were sufficiently well developed, with vigorous shoots and a well-developed root system, they were transplanted into the field, as individual plants, at equal distances of 50 cm, 25 rows with 10 plants/row.

The observations and measurements were carried out in 2024, on 250 plants, at the beginning of July when the plants were fully mature. The morphological traits, including plant height, spike count /plants, spike length, flag leaf length, flag leaf width, bush shape, major components of plant yield, as selection criteria in breeding, were determined, in table 2 it is presented the average of them.

The obtained data were statistically processed using the Statistica 7 software package.

Table 1. Meteorological conditions from Braşov stationary 2023-2024

Year	Annual average I-XII	Deviation	Vegetation period IV-IX	Deviation
Temperature (°C)				
2023	10,1	+2,3	14,8	+0,6
2024	11,4	+3,6	17,6	+2,7
Average 59 years	7.8	0	14.2	0
Precipitation (mm)				
2023	570,4	-182,8	407,8	-121,3
2024	730,6	-22,6	485.9	- 43,2
Average 59 years	753.2	0	529.1	0

RESULTS AND DISCUSSION

The analysed *Agropyron intermedium* accessions exhibited moderate phenotypic variability for most of the evaluated morphological traits (Table 2). Plant height ranged from 120 to 150 cm, indicating relatively uniform vertical growth among genotypes. In contrast, the number of spikelet per plant showed substantial variation, with values ranging from 136 to 350, suggesting pronounced differences in reproductive potential.

Table 2. Morphological traits of *Agropyron intermedium*

Nr. crt.	Plant height (cm)	Nr of spikets/plant	Spike length (cm)	Flag leaf length (cm)	Flag leaf width (mm)	Bush shape
1	130	292	28	28.5	5	Erect
2	130	253	30	30	7	Lax
3	140	255	27.5	22.5	6	Lax
4	130	282	26	16.5	5	Erect
5	140	241	30	28.5	6	Lax
6	130	235	31	24.5	6	Lax
7	140	226	31.5	21.5	5	Erect
8	140	350	30.5	31	6	Lax
9	130	287	23.5	16.5	5	Erect
10	140	268	32	29	7	Erect
11	130	212	31	23	7	Erect
12	120	266	25	29	5	Lax
13	140	220	31	30	7	Erect
14	140	289	24	27	7	Erect

15	140	255	32	31	5	Lax
16	130	164	33	33.5	7	Erect
17	140	151	29	16	5	Erect
18	130	136	26.5	18.5	5	Erect
19	120	276	33.5	18.5	6	Erect
20	130	198	30	32	6	Erect
21	130	189	21	29.5	7	Lax
22	150	267	30	21.5	6	Erect
23	140	250	29.5	30	6	Lax
24	130	213	31	30	7	Erect
25	140	258	30.5	31	8	Erect

Spike length varied between 21.0 and 33.5 cm, while flag leaf length ranged from 16.0 to 33.5 cm, reflecting variability in photosynthetically active surface. Flag leaf width showed a narrower range (5-8 mm), indicating comparatively lower variability for this trait. Regarding plant architecture, most genotypes displayed an erect growth habit, while fewer exhibited a lax habit, indicating structural diversity within the studied material. It is important that when choosing the plants that will be selected to continue the breeding process, there are phenotypically similar plants with high disease resistance. They will not choose phenotypically similar plants, and with high productive capacity, but with low disease resistance (Figure 1).

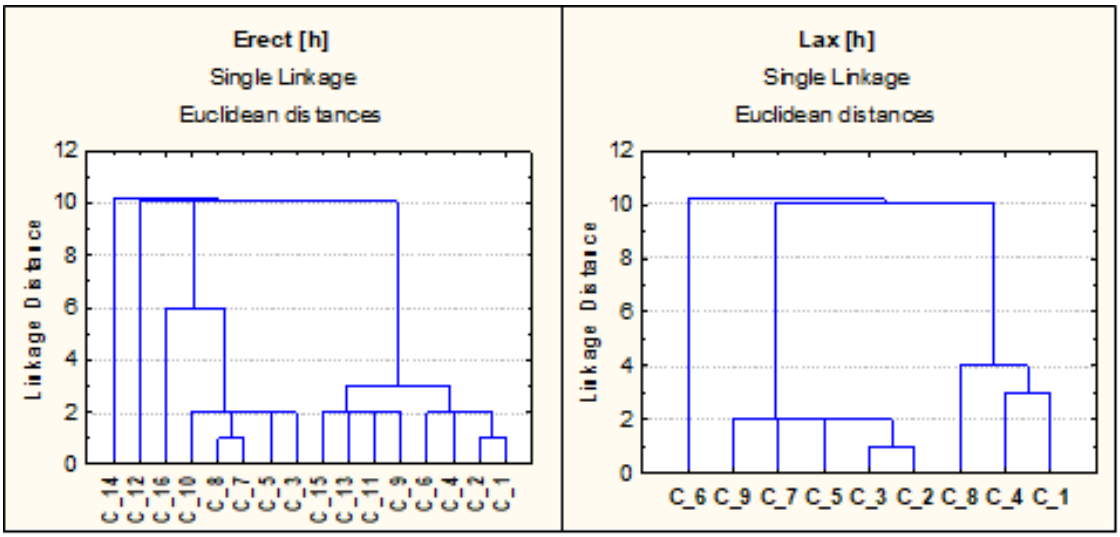

Figure 1. Cluster analysis of 25 genotypes based on quantitative traits

The Pearson correlation analysis (Table 3) revealed generally weak relationships among most morphological traits. Plant height was weakly and positively correlated with the number of spikelet per plant (r = 0.12), spike length (r = 0.17), flag leaf length (r = 0.08), and flag leaf width (r = 0.14), with none of these correlations reaching statistical significance.

The number of spikelet per plant showed no meaningful association with spike length (r = -0.04), flag leaf length (r = 0.08), or flag leaf width (r = -0.05), indicating that spikelet number varies independently of plant size and spike morphology in the analyzed genotypes (Figure 2).

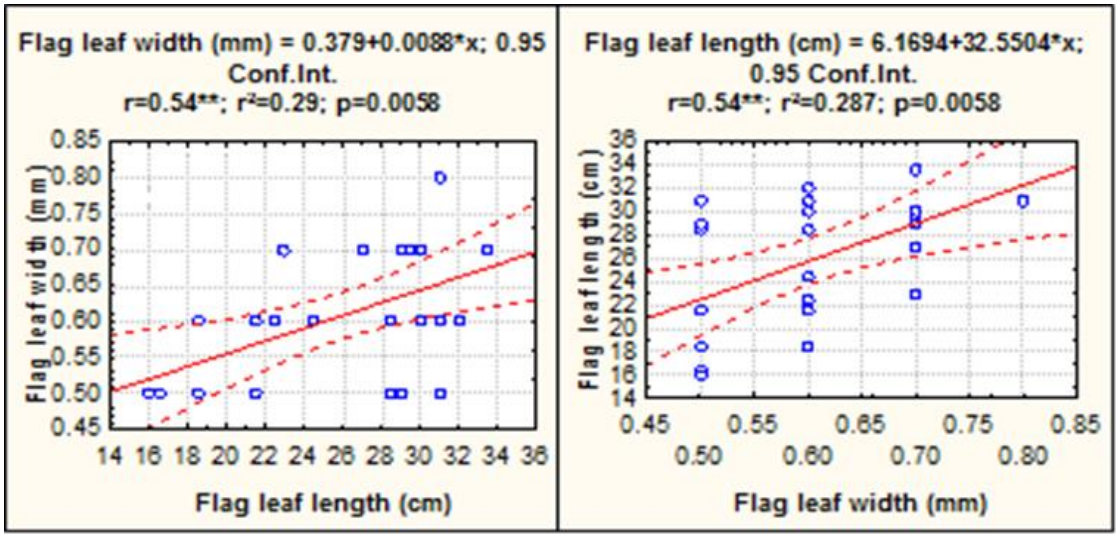

Figure 2. The correlation between leaf length and leaf width

A statistically significant positive correlation ($r = 0.54$, $p \leq 0.01$) was observed between flag leaf length and flag leaf width, suggesting coordinated development of flag leaf dimensions. This relationship indicates that genotypes with longer flag leaves tend to also develop wider leaves, potentially increasing the photosynthetic capacity of the upper canopy.

Overall, the results indicate that most morphological traits in *Agropyron intermedium* are largely independent, with limited interdependence among plant stature, spike characteristics, and reproductive components. The significant association between flag leaf length and width highlights a potential trait combination of interest for selection aimed at improving photosynthetic efficiency.

Table 3. Eigenvalues of correlation matrix, and related statistics

Component	Eigenvalue	% Total variance	Cumulative Eigenvalue	Cumulative %
1	1.7416	34.8326	1.7416	34.8326
2	1.0943	21.8856	2.8359	56.7182
3	0.9544	19.0880	3.7903	75.8062
4	0.7713	15.4256	4.5616	91.2318
5	0.4384	8.7682	5.0000	100.0000

Table 4. Corelation matrix

	Plant height (cm)	Nr of spikets/plant	Spike length (cm)	Flag leaf length (cm)	Flag leaf width (mm)
Plant height (cm)	1.00	0.12	0.17	0.08	0.14
Nr of spikets/plant		1.00	-0.04	0.08	-0.05
Spike length (cm)			1.00	0.24	0.21
Flag leaf length (cm)				1.00	0.54**
Flag leaf width (mm)					1.00

$r_{5\%} = 0.38$; $r_{1\%} = 0.49$; $r_{0.1\%} = 0.60$.

Based on the eigenvalue structure (Table 3) and the correlation relationships among traits (Table 4), the first principal component (PC1), which explains 34.83% of the total variance, can be interpreted as a leaf size and photosynthetic surface component. This interpretation is supported by the moderate and significant positive correlation between flag leaf length and flag leaf width ($r = 0.54$, $p \leq 0.01$), suggesting that variation in PC1 is

primarily associated with coordinated development of flag leaf dimensions. Therefore, PC1 likely reflects differences among genotypes in leaf morphology and potential photosynthetic capacity.

The second principal component (PC2), accounting for 21.89% of the total variance, appears to be associated mainly with reproductive traits, particularly the number of spikelet per plant and spike length. Given the weak correlations between these traits and vegetative parameters, PC2 likely represents an independent axis of variation related to reproductive architecture, distinguishing genotypes based on their yield-related structural components rather than plant size.

The third principal component (PC3) explains an additional 19.09% of the variance and may reflect overall plant stature and spike morphology, integrating minor variations in plant height and spike length. Although plant height showed limited variability across genotypes, its contribution to PC3 suggests that subtle differences in vertical growth still play a role in overall morphological differentiation.

The remaining components (PC4 and PC5), which together explain less than 25% of the total variance, likely capture residual and genotype-specific variation, with limited biological relevance for trait differentiation and selection purposes.

Overall, the PCA indicates that morphological variability in *Agropyron intermedium* is structured primarily along two major biological dimensions: leaf development and photosynthetic potential (PC1) and reproductive structure and spike architecture (PC2). This separation suggests a relative independence between vegetative and reproductive traits, which may be advantageous in breeding programs by allowing the simultaneous improvement of photosynthetic efficiency and reproductive performance (Figure 3).

The analysis of variable contributions (Table 3) indicates that the extracted factors represent distinct morphological and phonological dimensions in *Agropyron intermedium*.

Factor 1 (PC1) is mainly influenced by heading (0.3690), with moderate contribution from plant height (0.1806). This suggests that PC1 reflects a phenology-related axis, particularly the timing and development of the reproductive spike (Figure 4).

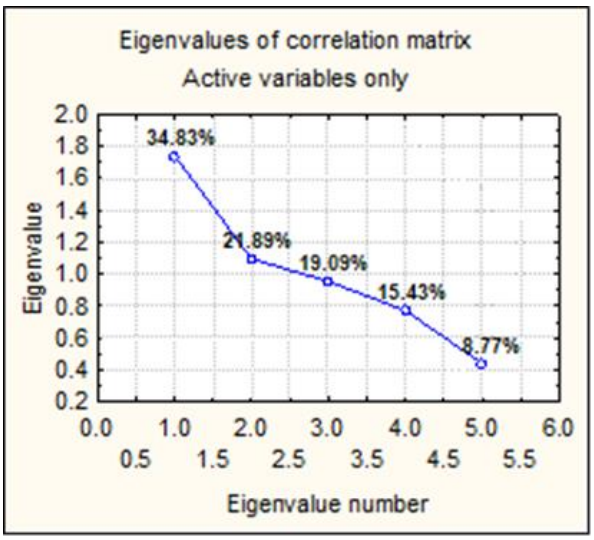

Figure 3. Eigenvalue graph

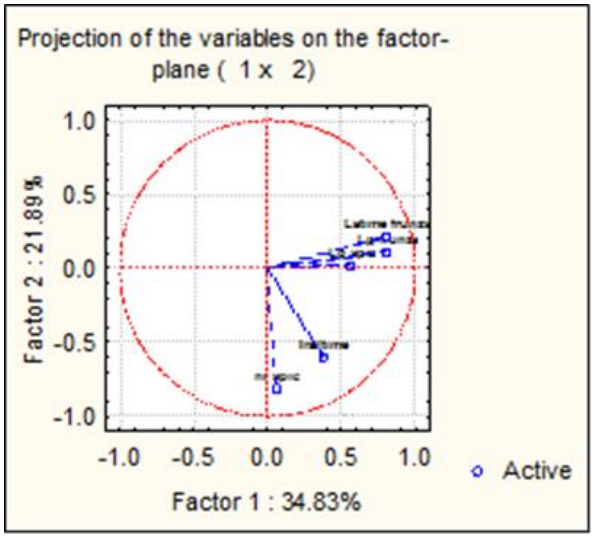

Figure 4. Projection of variables in the plane of the factorial axes of the two principal components

Factor 2 (PC2) is dominated by generative tillers (0.6097) and, to a lesser extent, vegetative tillers (0.3365). Therefore, PC2 represents shoot production and reproductive capacity, distinguishing genotypes with high tiller potential from those with lower productivity.

Factor 3 (PC3) shows contributions from plant height (0.3075), vegetative tillers (0.2726), and generative tillers (0.2311). This indicates that PC3 captures overall plant stature and biomass allocation, integrating both vertical growth and shoot development.

Factor 4 (PC4) is largely determined by plant height (0.5024), highlighting variation in vertical growth architecture among genotypes. Vegetative tillers (0.2934) also contribute, reflecting minor structural differences.

Factor 5 (PC5) is mainly influenced by heading (0.4565), representing a secondary phonological axis, possibly independent of plant size and tilling capacity.

In summary, the PCA reveals that reproductive development (heading and generative tillers), vegetative growth (vegetative tillers), and plant stature (height) are largely independent dimensions of variability. This multidimensional trait structure is useful for breeding, as it allows selection for one trait group without negatively affecting others (Figure 5).

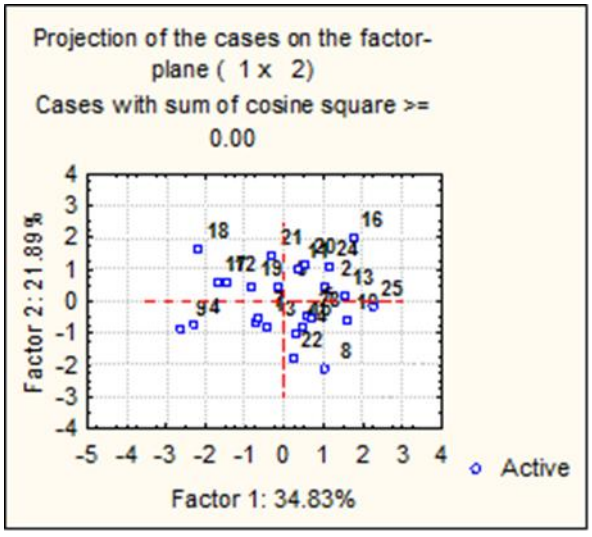

Figure 5. Projection of genotypes in the plane of the factorial axes of the two principal components

CONCLUSIONS

Agropyron intermedium genotypes exhibited moderate variability in morphological traits, with plant height ranging from 120 to 150 cm and most genotypes showing a tall, generally erect growth habit. Reproductive traits, such as number of spikelet per plant and spike length showed higher variation, indicating differences in reproductive potential.

Correlation analysis revealed that most traits were largely independent, with a significant positive correlation observed between flag leaf length and width ($r = 0.54$, $p \leq 0.01$), suggesting coordinated development of the photosynthetic surface.

Principal component analysis (PCA) identified five components explaining 100% of the variance, with the first two components accounting for 56.7% of the total variation. PC1 was associated mainly with leaf morphology and photosynthetic potential, while PC2 reflected reproductive traits and tilling capacity. Subsequent components captured plant stature, biomass allocation, and phonological characteristics.

Variable contribution analysis confirmed that reproductive traits (generative tillers, heading), vegetative growth (vegetative tillers), and plant height represent distinct dimensions of variability, largely independent from one another. This multidimensional structure provides opportunities for targeted selection in breeding programs. Overall, the study demonstrates that *Agropyron intermedium* exhibits diverse morphological and phenological traits, with separable axes of variation in vegetative, reproductive, and phenological characteristics, which can be exploited for improveyield potential, plant architecture, and disease resistance.

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