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Screening of Romanian Maize Local Landraces to Identify Morpho-Productive Traits and Resistance to *Fusarium verticillioides* for Mitigating Fumonisin Contamination

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ABSTRACT

***Fusarium verticillioides* is a major mycotoxigenic pathogen of maize responsible for fumonisin contamination and yield losses under increasingly variable climatic conditions. This study evaluated 172 Romanian maize landraces conserved in the Suceava Gene Bank to identify germplasm combining agronomic performance with reduced susceptibility to fungal infection. Field experiments were conducted at ARDS Suceava during 2021–2022 using a randomized complete block design. Phenological, morphological, and yield traits were recorded, while resistance was assessed on 1,046 ears using the CIMMYT 1–6 scale. Fumonisin content was determined by ELISA method. Substantial phenotypic variation was observed among geographical regions. Southern landraces exhibited superior plant height, ear length, thousand-kernel weight, and grain yield (3.54 t ha^{-1}), whereas North-Eastern populations showed earlier flowering and higher variability. Fusarium incidence ranged from 6.95% to 8.89%, and fumonisin concentrations varied between 151 and 2,178 µg kg^{-1}.**

Principal component and cluster analyses revealed distinct germplasm groups, with several accessions combining moderate to high productivity and reduced fumonisin accumulation. These landraces represent valuable genetic resources for breeding programs aimed at improving maize resilience, yield stability, and food safety under changing environmental conditions.

Keywords: Fumonisin content, morphological traits, maize local landraces, pathogen micromycete, *Fusarium verticillioides* f. sp. *Moniliforme*.

INTRODUCTION

Fusarium verticillioides is a major fungal pathogen of maize, responsible for ear rot and the production of fumonisins, mycotoxins that pose serious risks to human and animal health (Headrick and Pataky, 1991; Munkvold et al., 1997a; 1997b; Czembor et al., 2015; Omotayo and Babalola, 2023). Infection often occurs without visible symptoms, facilitating contamination along the food and feed chain and complicating effective management strategies (Kedera et al., 1992; Munkvold, 2017). Consequently, fumonisin contamination represents a persistent challenge for global food security.

The interaction between *F. verticillioides* and maize is strongly influenced by environmental conditions. Drought stress, high temperatures, insect damage, and kernel characteristics favor fungal colonization and toxin biosynthesis (Betz et al., 2000; Burger et al., 2013; Chulze, 2010; Li et al., 2017). In recent decades, climate change has intensified these risk factors, promoting the expansion of toxigenic Fusarium species into regions previously considered less vulnerable (Kimanya et al., 2008; Bhatnagar et al., 2011; Deepa and Sreenivasa, 2014; Medina et al., 2015; Adiaha, 2018; Zingales et al., 2022). As a result, fumonisin contamination is increasingly reported in temperate areas, including Eastern Europe (Morales et al., 2019; Brown et al., 2001).

Recent studies conducted in Romania have emphasized the importance of both

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genetic and environmental factors in determining maize performance under variable agroecological conditions. Significant variability among maize genotypes in terms of yield potential and stability has been reported, highlighting the role of genotype × environment interactions (Horhocea et al., 2024). Agronomic factors such as plant density also influence crop development and stress responses, contributing to variation in productivity under field conditions (Vana et al., 2024). Fusarium ear rot remains a major constraint in maize production in Romania, with infection levels influenced by climatic conditions and insect damage. Differences in susceptibility among maize genotypes have been documented, confirming the importance of host genetic background in disease response (Tărău et al., 2024). More broadly, Fusarium species are recognized as major pathogens affecting cereal crops, contributing to both yield losses and mycotoxin contamination under favorable environmental conditions (Chiurciu et al., 2023).

Current management strategies, such as chemical control and post-harvest treatments, remain limited in their effectiveness and sustainability (Food and Drug Administration, 2000; Chulze, 2010). Among available approaches, the development of resistant maize hybrids represents one of the most promising long-term solutions. Recent advances in plant genetics and genomics have demonstrated that host genetic diversity can be exploited to enhance resistance to fungal infection and reduce mycotoxin accumulation without compromising agronomic performance (Santiago et al., 2015; Lanubile et al., 2017; Cao et al., 2022).

Local maize landraces represent an important reservoir of adaptive traits shaped by long-term selection under diverse environmental conditions (Eller et al., 2008; Mayer et al., 2022; Balconi et al., 2024). Actually, in Romania, local maize populations are conserved in Suceava Gene bank collections, but coming from heterogeneous agroecological regions, and their potential for resistance to *F. verticillioides* and fumonisin accumulation remains insufficiently explored.

In this context, the present study aimed to evaluate Romanian maize landraces for morpho-productive traits, resistance to *F. verticillioides* infection, and associated fumonisin contamination under field conditions. By integrating phenotypic assessment with multivariate statistical analyses, this study seeks to identify valuable germplasm combining agronomic performance with enhanced tolerance to fungal infection, thereby supporting breeding programs focused on food safety and climate resilience.

MATERIAL AND METHODS

Plant Material and Experimental Conditions

The study was conducted during two consecutive growing seasons (2021-2022) in the experimental field of the Agricultural Research and Development Station (ARDS) Suceava and in the phytopathology laboratory of the Suceava Gene Bank, Romania.

A total of 172 Romanian maize landraces conserved in the gene bank collection were evaluated, originating from four geographical regions: North-West (46 accessions), North-East (49), Center-West (46), and South (31).

Field experiments were arranged in a randomized complete block design (RCBD) with three replications. Each accession was planted in two rows per replicate, spaced 70 cm apart and 5 m long. Standard agronomic practices for maize cultivation were applied throughout the growing seasons.

Climatic conditions during the vegetation period are presented in Table 1. Both seasons were characterized by higher temperatures and reduced rainfall compared with long-term averages, reflecting moderately dry conditions.

Table 1. Air temperature and rainfalls during the experiment (Suceava 2021-2022)

Year	Month						Average
	May	June	July	August	September	October	
	Air temperature (^{0}C)						
2021	14.1	19	22.5	19.9	14.2	8.2	16.31
2022	14.1	20.4	21.5	21.4	14	11.6	17.16
MAA	13.7	16.9	18.4	18.3	14.2	8.4	14.98
	Amount of rainfall (mm)						Total
2021	44.3	35.2	11	35	24.2	1.6	151.3
2022	44.3	53.0	45	71.2	54	22	289.5
MAA	80.2	93.6	88.6	62.8	40	29.5	394.7

Phenotypic and Agronomic Evaluation

Phenological, morphological, and productivity traits were recorded in both experimental years following IBPGR descriptors (1991). Data were collected for days to tasseling, days to silking, plant height, ear length, kernel rows number, number of kernels per row, kernel type, 1000-kernel weight, and grain yield.

Biometric measurements were performed on representative plants, ears, and kernels from each accession and replication.

Evaluation of Fusarium Resistance

Resistance to *Fusarium verticillioides* infection was assessed after harvest in the phytopathology laboratory of the Suceava Gene Bank. Ear rot severity was evaluated using the macroscopic method and a standard 1-6 scoring scale, where 1 = 0% and 6 = 76-100% infected kernels, according to CIMMYT descriptors (IBPGR, 1991).

A total of 1,046 ears were examined across the two growing seasons.

Determination of Fumonisin Content

Fumonisin concentration in maize kernels was determined using an immunochemical ELISA method in an accredited laboratory, following the protocol of the ELISOFUMO Kit (PS-LR-05, Ed. VI) (Sokolovic, 2022; Di Pasquale et al., 2024).

For each analysis, approximately 100 g of kernel samples were processed. Measured concentrations ranged from the limit of detection (36 μg/kg) to the limit of quantification (52 μg/kg), in accordance with European Regulation (EU) No. 915/2023 and World Health Organization guidelines (2007).

Statistical Analysis

Data collected over the two growing seasons were combined for preliminary germplasm screening and analyzed using descriptive statistics, including mean, range, standard deviation, and coefficient of variation. Analysis of variance (ANOVA) was performed to assess differences among geographical regions.

Hierarchical cluster analysis was conducted using Ward's method and Euclidean distance to evaluate trait similarity among accessions. Principal Component Analysis (PCA) was applied to 11 descriptors to explore multivariate relationships.

Statistical analyses were performed using IBM SPSS Statistics version 26 and GraphPad Prism version 9.3.0.

RESULTS AND DISCUSSION

A total of 172 Romanian maize landraces were evaluated to identify germplasm sources combining agronomic performance with resistance to *Fusarium verticillioides* and reduced fumonisin contamination. Substantial phenotypic variability was observed among accessions originating from different geographical regions, reflecting genetic differentiation and local adaptation.

Phenotypic and Agronomic Variation

Significant regional differences were detected for most phenological and morphological traits (Table 2). Southern populations exhibited greater plant height, longer ears, higher kernel weight, and superior grain yield, whereas North-Eastern accessions were characterized by earlier flowering and

higher intra-regional variability. Early phenology observed in northern populations may represent an adaptive response to drought and pathogen pressure under less favorable climatic conditions.

Phenological traits such as days to tasseling and silking also varied significantly among regions. Southern and Central-West populations flowered later (average 74-75 days), whereas North-Eastern populations were earlier (71-72 days). Early flowering is likely an adaptive mechanism to escape late-season drought stress and fungal infection pressure, particularly *Fusarium* spp., under warmer and drier conditions typical of the North-East. This adaptive trend aligns with findings from *in situ* studies on maize landraces, where early phenology has been associated with resilience under stress environments (Mayer et al., 2022; Revilla et al., 2022).

Table 2. Statistical parameters and ANOVA test of phenotypical traits in maize populations from diverse geographical origins (2021-2022)

Geographical region	Northwest	Northeast	Center West	South
Number of local landraces tested	46	49	46	31
Descriptors	**Plant height (cm)**			
Average	183.30	178.57	187.32	197.93
Range	143.00- 236.00	127.00- 232.00	140.00- 232.00	150.00- 251.00
Standard deviation	25.02	24.17	22.52	21.50
Coefficient of variation (%)	13.64	13.53	12.02	10.86
F - Anova/ *p (0.05*)	F (3,171) = 4.53*; p- 0.004*			
Tukey HSD Test- *p (0.05)*	-14.63*	-19.36*	-	14.63*/ 19.36*
Descriptors	**Days to tasseling**			
Average	73.00	71.44	74.63	75.00
Range	66.00-79.00	66.00- 81.00	67.00- 81.00	68.00- 89.00
Standard deviation	3.20	3.64	2.71	3.97
Coefficient of variation (%)	4.38	5.09	3.63	5.29
F - Anova/ *p (0.05*)	F (3,171) = 10.08*; p- 0.000*			
Tukey HSD Test - *p (0.05)*	-	-3.18*/ -3.55*	3.18*	3.55*
Descriptors	**Days to silking**			
Average	73.43	71.77	75.08	75.54
Range	66.00-79.00	66.00- 81.00	67.00- 81.00	69.00- 90.00
Standard deviation	3.44	3.74	2.77	4.10
Coefficient of variation (%)	4.68	5.21	3.68	5.42
F - Anova/ *p (0.05*)	F (3,171) = 10.267*; p- 0.000*			
Tukey HSD Test- *p (0.05)*	-2.11*	-3.31*/-3.77*	3.31*	2.11*/ 3.77*
Descriptors	**Ear length (cm)**			
Average	17.21	14.86	17.44	17.76
Range	10.00- 21.40	14.86- 9.10	11.80- 24.60	12.60- 22.70
Standard deviation	2.39	3.17	2.43	2.32
Coefficient of variation (%)	13.88	21.33	13.93	13.06
F - Anova/ *p (0.05*)	F (3, 171) = 11.35*; p- 0.000*			
Tukey HSD Test- *p (0.05)*	2.34*	-2.34*/-2.57*/-2.89*	2.57*	2.89*
Descriptors	**Kernel rows number**			
Average	12.00	13.10	12.04	11.67
Range	8.00- 16.00	8.00- 20.00	8.00-16.00	10.00- 14.00
Standard deviation	1.68	2.12	1.60	1.64
Coefficient of variation (%)	14.00	16.18	13.28	14.05
F - Anova/ *p (0.05*)	F (3,171) =5.22*; p-0.002*			
Tukey HSD Test- *p (0.05)*	-1.10*	1.10*/1.05*/1.42*	-1.05*	-1.42*
Descriptors	**Number of kernels per row**			
Average	34.65	31.63	34.82	36.09
Range	16.00-43.00	21.00- 47.00	21.00-43.00	24.00-45.00
Standard deviation	5.03	6.26	4.93	4.76
Coefficient of variation (%)	14.51	19.79	14.15	13.18
F - Anova/ *p (0.05*)	F (3,171) =5.37*; p-0.001*			
Tukey HSD Test- *p (0.05)*	3.01*	-3.01*/-3.19*/ -4.46*	3.19*	4.46*

Geographical region	Northwest	Northeast	Center West	South
Descriptors	**Kernel type**			
Average	5.63	5.65	5.45	5.06
Range	3.00-6.00	2.00- 6.00	2.00- 6.00	2.00- 6.00
Standard deviation	0.79	0.99	1.06	1.34
Coefficient of variation (%)	14.03	17.52	19.44	26.48
F - Anova/ *p (0.05*)	F (3, 171) = 2.43*; p-0.067*			
Tukey HSD Test -*p (0.05)*	-	-	-	-
Descriptors	**1000-kernel weight (g)**			
Average	305.43	255.00	303.32	325.38
Range	140.00-434.00	114.00-391.00	152.00-432.00	152.00-467.00
Standard deviation	58.85	66.86	62.45	61.12
Coefficient of variation (%)	19.26	26.21	20.58	18.78
F - Anova/ *p (0.05*)	F (3, 171) = 2.43*; p-0.067*			
Tukey HSD Test -*p (0.05)*	-	-	-	-
Descriptors	**Grain yield (t/ha)**			
Average	3.16	2.85	3.41	3.54
Range	1.30-5.20	0.70-5.00	0.80-4.80	1.40-4.80
Standard deviation	0.83	0.92	0.93	0.76
Coefficient of variation (%)	26.26	32.28	27.27	21.46
F - Anova/ *p (0.05*)	F (3, 171) = 0.48*; p-0.069*			
Tukey HSD Test- *p (0.05)*	-	-	-	-

The comparative analysis of maize local landraces from the four geographical regions revealed significant differences for most of the evaluated agro-morphological traits. Plant height showed statistically significant variation among regions ($F = 4.53$; $p = 0.004$), with the Southern landraces recording the highest average plant height (197.93 cm), while the Northeast region presented the lowest mean value (178.57 cm). The coefficients of variation were relatively low (10.86-13.64%), indicating moderate phenotypic uniformity within regions.

Phenological traits, namely days to tasseling and days to silking, also differed significantly among regions ($p < 0.001$). Landraces originating from the South and Center West regions tended to exhibit later flowering, whereas those from the Northeast flowered earlier. The low coefficients of variation for these traits (3.63-5.42%) suggest high stability of flowering time within each geographical group.

Regarding ear-related traits, significant regional differences were observed for ear length, kernel rows number, and number of kernels per row. The Southern and Center West populations recorded the highest ear length averages (17.76 cm and 17.44 cm, respectively), while the Northeast landraces showed markedly shorter ears (14.86 cm). Similarly, the Northeast group presented the highest average number of kernel rows (13.10), whereas the South region recorded the greatest number of kernels per row (36.09). These traits exhibited moderate variability, especially in the Northeast populations, as reflected by higher coefficients of variation.

Kernel type did not differ significantly among geographical regions ($p = 0.067$), indicating a relatively similar distribution of kernel categories across the studied germplasm. In contrast, 1000-kernel weight displayed clear regional differentiation, with the Southern landraces showing the highest average value (325.38 g), followed closely by Northwest and Center West populations, while the Northeast accessions recorded substantially lower kernel weight (255.00 g). The variability for this trait was moderate to high, particularly in the Northeast region (CV = 26.21%).

Although grain yield ranged widely within all regions, differences among regional means were not statistically significant ($p = 0.069$). Nevertheless, the highest average grain yield was observed in the South region (3.54 t/ha), followed by Center West (3.41 t/ha), suggesting a tendency toward better

productive performance in these areas. Overall, the results highlight the existence of important phenotypic diversity among maize landraces according to their geographical origin, especially for morphological and phenological descriptors.

The ANOVA confirmed significant differences ($p < 0.05$) among regions for most traits, including plant height, ear length, kernel rows, and kernels per row, while kernel type, 1000-kernel weight and grain yield showed no statistically significant differentiation (Table 2). Coefficients of variation indicated substantial intra-regional diversity, particularly in the North-East, supporting the presence of heterogeneous local adaptation patterns.

Histograms of morphological traits (Figure 1) revealed continuous and near-normal distributions, consistent with polygenic inheritance and adaptation to regional ecological niches. Such distributions suggest that the traits studied are controlled by multiple loci with additive effects, which is favorable for selection in breeding programs (Suresh, 2019).

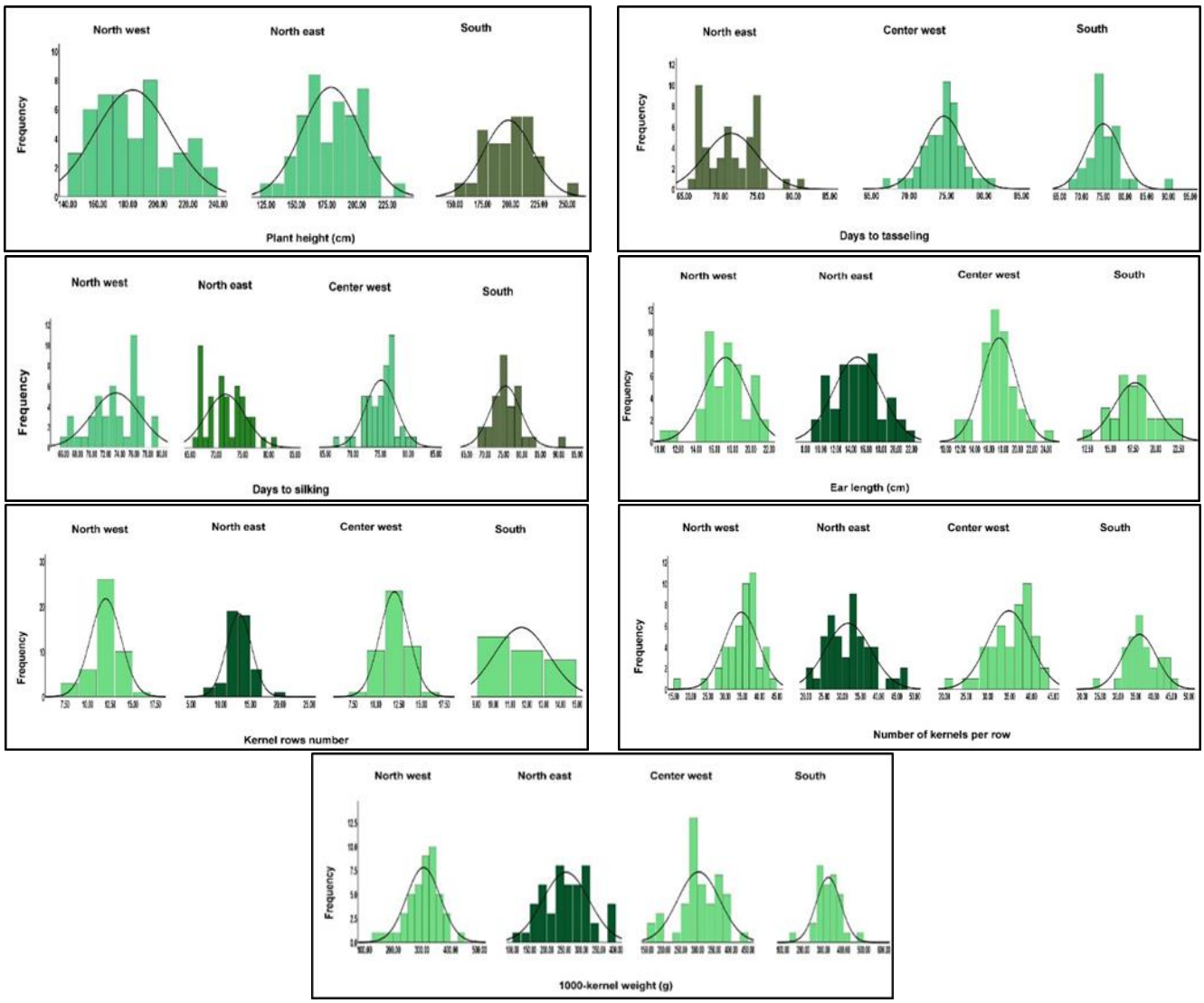

Figure 1. Histograms of phenotypical traits in 172 local maize populations, significantly differentiated by geographical origin

Overall, southern populations combined taller plants, prolonged vegetative phases, and higher productivity, whereas northern landraces were shorter, earlier-flowering, and more variable. The integration of morphological diversity with resistance screening against *F. verticillioides* offers promising opportunities to identify elite genotypes that combine productivity with

resilience to biotic and abiotic stress factors (Medina et al, 2015; Suresh, 2019).

Therefore, the results emphasize that southern maize landraces represent valuable germplasm for breeding programs targeting high yield and grain quality, while northern populations may serve as sources of early maturity and adaptation to stress-prone environments, contributing to a more sustainable maize improvement strategy under changing climatic conditions.

Variation of mycotoxin accumulation and Fusarium resistance among maize landraces from different geographical regions

The evaluated maize landraces exhibited important variation regarding mycotoxin accumulation and Fusarium resistance across the analyzed geographical regions. Mean values and variation intervals are presented in Table 3.

Table 3. Distribution of mycotoxin accumulation and Fusarium resistance in maize landraces from different geographical regions

Geographical region	No. of analyzed landraces	Mean mycotoxin level (μg/kg)	Mycotoxin range (μg/kg)	Mean fusarium resistance (%)	Resistance range (%)
Northwest	46	778.1	0-2178	6.7	0-18
Northeast	49	703.4	0-2178	5.2	0-40
Center	28	654.8	0-2178	4.0	0-18
West	18	812.6	0-2178	8.4	0-40
South	31	736.5	0-2178	5.1	0-18

Note: Mean values were calculated based on the analyzed maize landraces originating from different geographical regions. Considerable variability was observed for both mycotoxin accumulation and Fusarium resistance among the evaluated populations. Several landraces combined low mycotoxin contamination with increased Fusarium resistance, indicating their potential importance as valuable genetic resources for breeding programs focused on disease tolerance and grain quality improvement.

Comparable levels of variation have previously been reported by Santiago et al. (2015) and Gesteiro et al. (2021), confirming that local maize germplasm exhibits heterogeneous resistance patterns shaped by both genetic background and ecological adaptation.

Mean fumonisin concentrations showed distinct regional trends. The highest average mycotoxin level was recorded in the Western populations (812.6 μg/kg), followed by the Northwest region (778.1 μg/kg), while the Central region displayed comparatively lower contamination levels (654.8 μg/kg). These differences are likely associated with regional climatic variation, particularly humidity and temperature, which strongly influence fungal growth and fumonisin biosynthesis (Morales et al., 2019). The occurrence of the maximum fumonisin concentration (2178 μg/kg) in all geographical regions suggests that even moderately resistant genotypes may accumulate elevated toxin levels under favorable environmental conditions for fungal development.

Higher fumonisin contamination may also be associated with plant architecture and ear characteristics influencing pathogen infection, as previously reported by Cao et al. (2022). Interestingly, the increased fumonisin content observed in several productive southern landraces may indicate a potential trade-off between yield performance and resistance. Similar observations were discussed by Gesteiro et al. (2021), who suggested that genomic selection approaches directly targeting reduced fumonisin accumulation may represent an efficient strategy for improving resistance to Fusarium ear rot.

Fusarium resistance was generally low to moderate across the evaluated germplasm; however, several accessions originating from the West and Northeast regions exhibited increased resistance scores. Overall, the results highlight the importance of regional maize landraces as valuable reservoirs of adaptive traits associated with disease resistance and grain safety. Furthermore, the incorporation of genomic approaches,

including the identification of quantitative trait loci (QTLs) associated with Fusarium resistance and toxin accumulation, could significantly improve breeding efficiency for the development of safer and more resilient maize hybrids (Cao et al., 2022).

The first two principal components (PC1 and PC2) explained between 48.43% and 55.88% of the total variance in most regions, indicating that a few key variables captured most of the phenotypic differentiation among landraces. In the South, three components (PC1-PC3) were required to explain 69.02% of the total variance, suggesting a more complex multivariate structure in these populations. Comparable PCA structures were observed in previous maize diversity studies, where phenological and ear-related traits explained most of the variability among traditional germplasm (Kedera et al., 1992; Doko et al., 1995; Balconi et al., 2024) (Table 4).

Northern populations exhibited clearer separation between productivity-related and resistance-associated variables, whereas central and southern populations displayed more integrated multivariate structures, could indicate genotype × environment interactions. These results confirm that resistance to *Fusarium* ear rot and fumonisin accumulation is a complex and polygenic trait, only partially correlated with yield components.

Therefore, the PCA results demonstrate that phenological traits (days to tasseling and silking) and yield-related descriptors (ear length, kernels per row, and kernel weight) were the main drivers of genetic variability across all regions. In contrast, fumonisin content and kernel infection formed distinct and largely independent axes of variation, confirming that resistance to *Fusarium* ear rot and fumonisin accumulation are correlated with morphological development (Eller et al., 2008; Chen et al., 2023). Consequently, breeding programs aiming to combine high yield potential with enhanced resistance should adopt integrative strategies that merge phenotypic selection with molecular and genomic tools, enabling the simultaneous improvement of these partially independent trait complexes (Munkvold, 2017; Chen et al., 2023).

Table 4. Cumulative variation, vectors and eigenvalues of the principal components (PCs) for the correlation matrix based on the average of the values obtained in the case of maize populations coming from different geographical regions

Descriptors	Northwest		Northeast		Center west		South		
	PC1	PC2	PC1	PC2	PC1	PC2	PC1	PC2	PC3
Plant height (PH)	-0.691	0.241	-0.771	0.071	-0.784	0.232	0.559	-0.322	0.293
Days to tasseling (DT)	-0.785	0.460	-0.707	0.582	-0.652	-0.491	0.766	0.426	-0.207
Days to silking (DS)	-0.798	0.421	-0.727	0.530	-0.648	-0.495	0.788	0.399	-0.162
Ear length (EL)	-0.743	-0.189	-0.885	-0.185	-0.746	0.569	0.730	-0.189	0.342
Kernel rows number (NRG)	0.193	0.464	0.436	0.509	-0.081	-0.629	0.045	0.755	-0.335
Number of kernels per row (NKR)	-0.783	-0.006	-0.881	-0.154	-0.750	0.245	0.797	0.011	0.247
Kernel type (KT)	0.630	0.130	0.454	0.208	0.533	0.292	-0.521	-0.622	-0.173
1000-kernel weight (g) (KW)	-0.541	-0.511	-0.526	-0.429	-0.389	0.570	0.299	0.559	0.501
Grain yield (t/ha) (GY)	-0.513	-0.076	-0.313	0.129	-0.232	0.151	0.045	-0.333	-0.642
Fumonisin content (μg/ kg) (FE)	0.209	0.798	-0.019	-0.782	0.220	0.495	0.582	-0.493	-0.469
Damaged kernels by *F. verticillioides* (%) (FV)	0.087	0.755	-0.052	-0.677	0.134	0.681	0.450	-0.504	-0.552
Eigenvalue	3.936	2.187	3.958	2.255	3.14	2.462	3.595	2.335	1.663
Cumulative proportion of variance (%)	35.78	19.89	35.98	20.50	28.54	22.38	32.68	21.22	15.12

PCA Biplot Interpretation

PCA biplots illustrate consistent multivariate patterns across regions. In the North-West and North-East populations, agronomic traits tended to be negatively associated with fumonisin contamination and kernel damage, suggesting that extended vegetative growth and delayed flowering may contribute indirectly to tolerance mechanisms (Figures 2a and 2b).

In the Center-West region, infection and toxin accumulation formed a distinct cluster,

largely independent of phenological traits (Figure 2c). Southern populations displayed strong covariation among developmental and yield-related descriptors, while resistance-related variables showed weak associations with structural traits, indicating a stronger influence of environmental factors (Figure 2d).

The biplot analysis emphasizes that resistance expression varies across regions and is influenced by both genetic background and environmental conditions. Similar trends between phenological or morphological traits and fumonisin contamination have been reported previously (Cao et al., 2022; Santiago et al., 2015), supporting the hypothesis that plant architecture and flowering dynamics may contribute indirectly to tolerance mechanisms under field conditions.

Overall, the PCA results for the North-West region highlight general patterns of association between agronomic performance and resistance-related variables, while underscoring the complexity of these interactions.

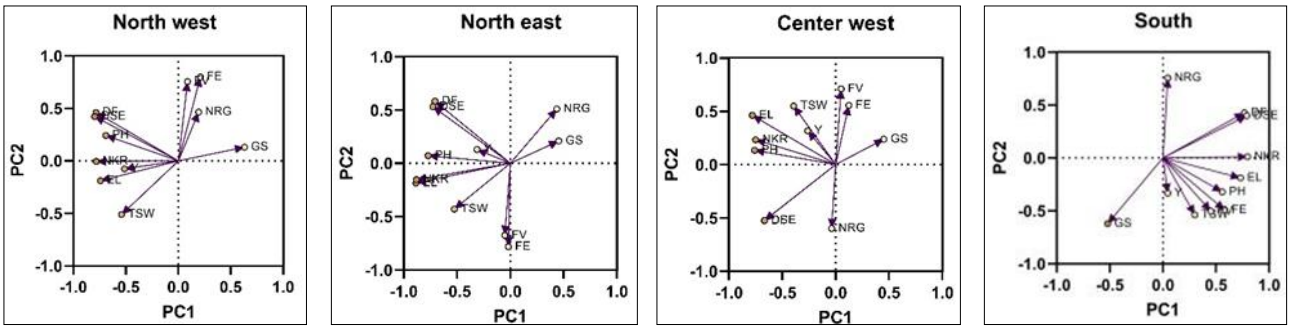

Figure 2. PCA biplot of morpho-physiological, productivity, and resistance-related traits in local maize populations from the North-West (a), North East (b), Center-West (c) and Southern (d) regions

Cluster Analysis

Hierarchical cluster analysis of the 172 local maize populations was performed using squared Euclidean distance and Ward's linkage method, based on 11 morpho-physiological, productivity-related, *Fusarium verticillioides* infection incidence, and fumonisin content. The analysis grouped the germplasm into four main clusters (A-D), which differed in size and trait composition: Cluster A (92 populations), Cluster B (27), Cluster C (29), and Cluster D (24) (Figure 3).

Cluster A represent the largest and most heterogeneous group, comprising accessions from all four regions. This cluster was characterized by intermediate values for yield-related traits and comparatively lower fumonisin accumulation, suggesting a broadly balanced agronomic and resistance profile. Cluster C was closely related to Cluster A but exhibited slightly higher mean values for plant height and grain yield, together with moderate resistance levels, indicating partially overlapping but distinct phenotypic characteristics.

Cluster D diverged from Cluster A at a higher rescaled distance and grouped accessions displaying elevated *Fusarium verticillioides* infection and higher fumonisin content. This cluster therefore represents a group with increased susceptibility, although internal variability suggests that resistance responses are not uniform across all accessions.

Cluster B was positioned at the greatest distance from the remaining clusters, forming a relatively compact group consisting of 27 accessions originating primarily from the North-West, North-East, and Center-West regions (Figure 4). This separation suggests a distinct multivariate profile, potentially reflecting regional adaptation or specific genotype × environment interactions. Despite reduced plant height and lower yield potential, the morpho-physiological variability observed within this cluster, together with moderate resistance levels, suggests potential value as a source of unique allelic

combinations for breeding programs targeting stability and stress tolerance.

Overall, the dendrogram highlights structured phenotypic and resistance-related variability among the studied maize populations. While the clustering patterns are consistent with trends observed in the PCA, they should be interpreted as complementary multivariate evidence rather than definitive confirmation of genetic differentiation. Together, these analyses provide a useful framework for identifying contrasting germplasm groups for further evaluation and better utilization in maize breeding programs.

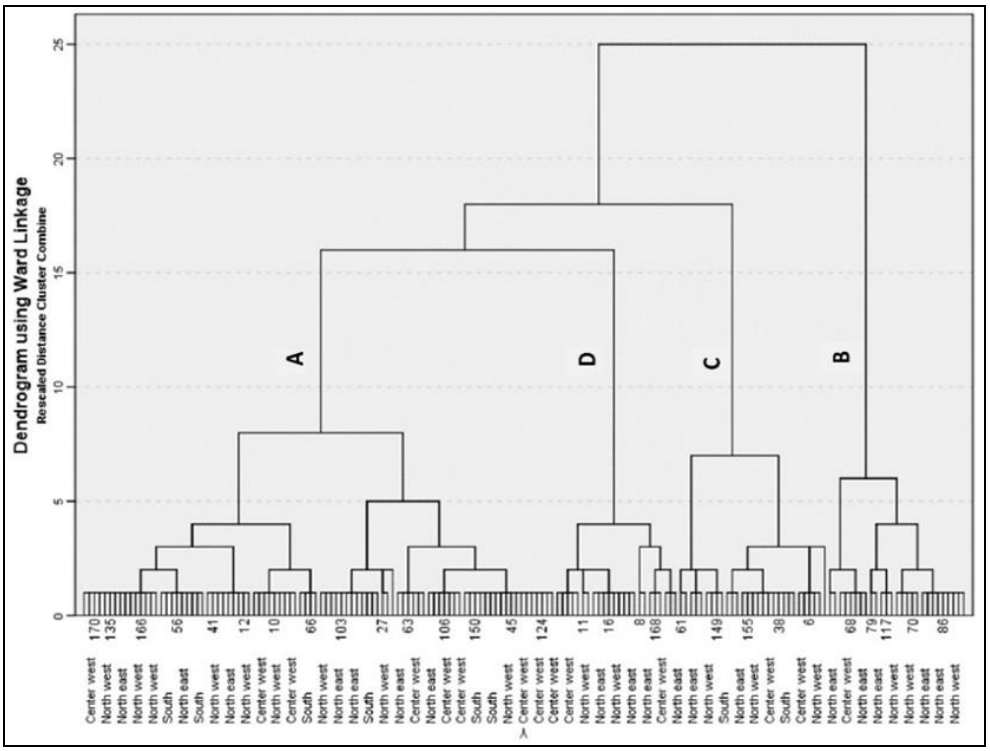

Figure 3. Hierarchical cluster dendrogram (Ward's method) of 172 local maize populations based on morpho-physiological traits, *Fusarium verticillioides* infection incidence (%), and fumonisin content (μg kg^{-1})

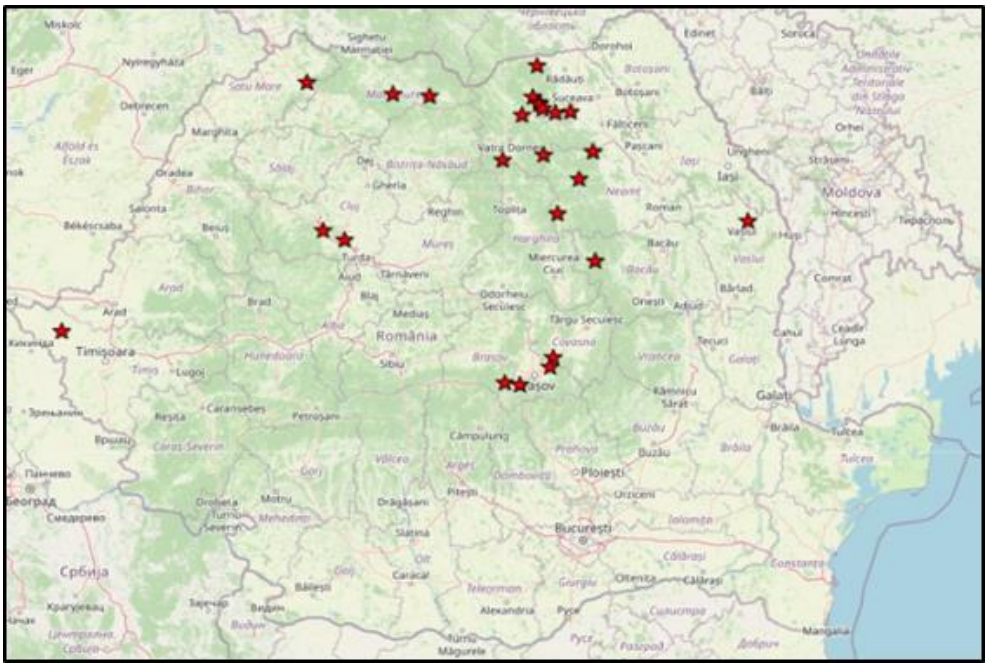

Figure 4. The origin sites of the 27 local maize populations grouped in cluster B

CONCLUSIONS

This study provides a comprehensive evaluation of 172 Romanian maize landraces originating from four agroecological regions, revealing substantial variability in morpho-physiological traits, agronomic performance, and susceptibility to *Fusarium verticillioides* infection and fumonisin accumulation.

Phenotypic and multivariate analyses demonstrated that agronomic and resistance-associated traits represent partially independent dimensions of variation. While phenological and yield-related traits accounted for most phenotypic differentiation, fumonisin content and kernel infection showed complex and region-dependent patterns, reflecting the polygenic and environmentally influenced nature of resistance.

The integration of PCA and hierarchical clustering identified distinct germplasm groups with contrasting agronomic and resistance profiles. Several clusters combined moderate to high productivity with reduced fumonisin contamination, whereas others exhibited increased susceptibility despite acceptable agronomic performance. These findings confirm that high yield potential does not necessarily coincide with enhanced resistance and highlight the importance of balanced trait selection.

Although evaluations were conducted over two growing seasons under natural field conditions, the results should be interpreted as indicative trends rather than definitive conclusions. Year-to-year and location-specific variability were not fully captured, emphasizing the need for further validation through multi-environment trials.

Among the evaluated germplasm, accessions SVGB-531, SVGB-1277, SVGB-8998, SVGB-912, and SVGB-570 emerged as the most promising candidates. These landraces combined favorable ear characteristics, low fumonisin contamination, and reduced susceptibility to *Fusarium verticillioides*, highlighting their potential value as donor genotypes for maize breeding programs.

Future research should integrate multilocational field experiments with genomic, metabolomic, and high-throughput phenotyping approaches to better elucidate genotype × environment interactions and identify quantitative trait loci associated with Fusarium resistance and fumonisin accumulation. The application of molecular breeding tools, including QTL mapping, genome-wide association studies, and genomic selection, will accelerate the incorporation of favorable alleles into inbreeding lines.

Overall, Romanian maize landraces represent a valuable genetic resource for developing hybrids with improved yield stability, resilience to climatic stress, and reduced mycotoxin risk. In particular, the accessions identified as promising candidates in the present study constitute valuable sources of favorable alleles for future breeding efforts. The integrated phenotypic and multivariate framework presented here provides a solid foundation for breeding strategies aimed at enhancing food safety and sustainability under changing environmental conditions.

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