

Article

Assessment of the Degree of Relatedness of Some Inbred Lines Created at ARDS Turda

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Abstract: Knowledge of relatedness among maize elite breeding genotypes is useful in planning crosses for hybrid and inbred line improvement and assigning them into heterotic groups. This study investigated phenotypic and genetic similarities or diversities between 7 flint inbred lines (of common origin) adapted to the early maize growing regions created at ARDS Turda-Romania. They were studied for per se traits and in two crossing systems: a diallel crossing system, resulting in 21 hybrids and a cyclic system using four testers, thus resulting in 28 maize hybrids. Both parental inbred lines and hybrids were evaluated for several traits over three years. The level of relatedness/diversity was determined by various methods, grouped into: phenotypic methods and genetic methods. Similarities were identified between certain lines and a degree of differentiation between others. The analysis of the hybrid combinations from the diallel crossings between the seven sister lines highlighted the TD236 inbred line as the only one that differs genetically from the other. Following the analysis of the data obtained in the cyclic system, some inbred lines can be sources of favorable traits: TD236 and TD233 for yield, ear weight, grain/ear weight; TD233 and TD234 for the number of kernels/row, TD237 for plant breaking resistance.

Keywords: hybrid; inbred lines; heterosis; relatedness



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1. Introduction

Maize (*Zea mays* L.) is one of the most complex crop plants, with wide genetic and phenotypic variation. Its genetic diversity has been characterized by numerous researchers [1–4] in studies regarding morphological traits, pedigree analysis, heterosis data, and analysis using biochemical and molecular markers [5–11]. Due to an unknown mechanism of genetic control and a strong influence of environmental conditions on character expression, phenotypic markers are often considered reliable indicators of genetic relationships for the analyzed material.

For maize, knowledge of germplasm diversity and the relationship between genotypes is crucial for planning hybridization formulas and creating inbred lines [12], genotypes protection [13], and germplasm conservation management.

During the phenotypic characterization of maize genotypes, an objection was raised about the subjective assessment of some traits. However, the method is effective for a large volume of unknown material whose genetic origin is being studied [13].

Most researchers [4,14,15] believe that to obtain an effective heterosis, diversity must exist either due to the phylogenetic genealogy or as a result of the breeding process, and its manifestation depends on the genetic diversity of the parents, meaning that more different they are from a phenotypic point of view, more intense the heterosis could be in the first hybrid generation.

Maize breeders are often in a quandary in verifying the genetic value of an inbred line by selecting a tester or a pair of testers when the inbred line has been created from a hybrid of unknown genetic formula or crosses between parents belonging to different heterotic groups.

The first phases of knowing the new inbred lines are realized mainly based on the phenotypic traits, according to the UPOV descriptor [3]. However, in many cases, the phenotypic characterization of maize germplasm does not accurately reproduce its genetic variability due to the existing interactions between genotype and environment, and the pedigree method is not always followed correctly or is not available for most of the breeding sources [6]. Furthermore, the limitations of the measurements of the morphological characters are reflected by the low level of polymorphism, the low heritability, and the restricted possibilities of the ability to discriminate or determine the relationship and the genetic similarity [16]. Therefore, the use of molecular markers in the evaluation of genetic differences between inbred lines of unknown origin seems a much more appropriate method [6].

The effects of the general (GCA) and specific (SCA) combining ability are essential indicators of the potential value of the inbred lines used in hybrid combinations. The use of diallel crosses is an excellent evaluation method, where the contribution of each inbred line can be determined [17]. These crosses have been used in plant breeding to investigate the combining ability of the inbred lines, to identify superior parents for hybrid creation [18] and select better crosses for further breeding work [19].

Using the best combination of inbred lines among the heterotic groups is crucial for creating maize hybrids [20]. Assigning an inbred line to a heterotic group is influenced by the classification method. Two primary methods are widely used: SCA with inbred lines with pedigree information; and molecular markers to identify genetic similarity or distance [20,21].

Analyses assisted by molecular markers can provide maize breeders with valuable and quick information regarding the classification of new lines into heterotic groups, supporting the elaboration of the most appropriate hybridization formulas and the choice of parental genotypes to create new hybrids.

Identifying a new class of genetic markers, respectively molecular markers, represents a revolutionary event in plant breeding. Molecular markers are used due to their unlimited number. They come from different types of DNA mutations, molecular markers not influenced by environmental conditions or the stage of plant development. The most critical applications of molecular markers in plant breeding are represented by marker-assisted selection, accelerating backcross, genetic characterization, detecting diversity and genetic differences between different populations, respectively determining the relationship between different genotypes and classifying them into heterotic groups [7–9,20,22–25].

Classifying elite germplasm into heterotic groups and analysis of new lines to assign them into heterotic groups are significant decisions in any maize breeding program [4,22,23,26]. For this reason, maize breeders have a particular interest in characterizing the genetic diversity within existing heterotic groups, as well as the relationships between important inbred lines in current use as well as older ones.

In a study [27] that included 90 lines studied using 80 molecular markers, the inbred lines were classified into six heterotic groups: B73 (BSSS); Mo17 and C103 (Lancaster); TB329 (Iodent); Fv2 and Lo3 (Flint European); Oh43; W153R. The lines used in this study came from the ARDS Turda maize inbred line collection, and 11 of these were analyzed in detail in the present study.

A study on the phenotypic and genetic diversity (intra-population) between inbred lines obtained from the same initial material was initiated at the Agricultural Research and Development Station (ARDS) Turda. This study, of inbred lines, with a common origin was initiated to clarify whether the existing phenotypic differentiation between the studied inbred lines also exists at the level of genetic determinism of some traits of agronomic interest. Another objective of the study was to identify some genotypes that can be used to improve some sister lines that can be used as parental forms of some maize hybrids.

2. Materials and Methods

Following the selection process, two inbred lines, genetically different, TC177 and TB329, resulted from one commercial maize hybrid characterized by superior yield capacity. The two lines were crossed, and then self-pollination was carried out, followed by the selection of several lines. Seven “sister” lines resulted: TD233, TD234, TD235, TD236, TD237, TD238, TD239.

The seven lines were studied for per se traits but crossed in a diallel system $p(p - 1)/2$, resulting in 21 hybrids.

The seven sister lines were also crossed with four testers and studied in a trial with 28 hybrids in a cyclic crossbreeding ($m \times n$) system. The four testers are the two parental lines of the hybrid from which the sister lines originate: TB329 (Iodent) and TC177 (European Flint), and two inbred lines that are genetically different, belonging to other germplasm groups: TC344 (BSSS) and TA367 (Min. 13 \times SSS).

The diversity estimation was carried out by several methods grouped into: phenotypic and genetic methods. The results were also confirmed by SSR analysis.

Eighteen traits related to production elements and traits of the plant were analyzed: yield, dry matter, unbroken plants, thousand kernel weight, shelling percentage, hectoliter mass, ear weight, grains/ear weight, ear length, no kernels/row, kernel row number, kernels depth, plant height, ear height, total no leaves, leaves above the ear, leaf area, no tassel branches. The data were obtained during 2019–2021 at the Agricultural Research and Development Station Turda. Both inbred lines and hybrids were tested in comparative trials arranged according to the method of randomized blocks with four replications. Each genotype was sown in 2 rows, with a length of 5 m, at a sowing density of 60,000 plants/ha.

The experimental field was fertilized by applying 400 kg/ha of complex fertilizer of type NPK 27:13.5:0, together with the preparation of the germination bed. Herbicide was performed pre-emergence with 1.2 L/ha, using dimetamid-p as the active substance (720 g/L) and post-emergence with 2 L/ha using tembotrione (44 g/L) and isoxadiphenethyl (22 g/L) as active substances.

Phenotypic methods were based on biometric analysis, analysis of variances, and analysis of correlations for some traits. The characterization of a genotypic structure as part of a population or family by biometry and summing the quantitative and/or qualitative multivariable data. The phenotypic variance was calculated using ANOVA, both in lines and in simple hybrids.

The heterosis was estimated using the formula mentioned by Hallauer and Miranda [14] for the heterosis value compared to the average parent:

$$H\% = \frac{F_1 - \frac{P_1 + P_2}{2}}{\frac{P_1 + P_2}{2}} \times 100$$

The genetic methods were based on the analysis of crossbreeding systems with the determination of genetic variances, genetic effects (additive, non-additive), the intensity of heterosis manifestation and the analysis of correlations at the level of genetic parameters. Complete evaluation of the genetic diversity that conditions the expression of quantitative traits was achieved by simultaneously considering both the diversity of expression of genes with additive actions (homozygous loci) and the differences in the expression of intra- and interallelic interactions (dominance, epistasis).

At the level of quantitative traits, the genetic diversity was estimated based on the GCA (additive actions) and the SCA (non-additive interactions) characteristic of each line, each interaction between the lines, and each crossing system (cyclic and diallel).

For the diallel crossing system, the additive and non-additive effects were calculated based on the method used by Griffing [28], Gardner and Eberhart [29]:

$$X_{ij} = \mu + \hat{g}_i + \hat{g}_j + \hat{s}_{ij} + e$$

where X_{ij} = the value of $i \times j$ genotype; μ = experimental mean; \hat{g}_i/j = GCA effect of parent i/j ; \hat{s}_{ij} = SCA effect of $i \times j$ cross; e = experimental error.

For the cyclic system, the additive and non-additive effects were calculated using the formula proposed by Cabulea [30,31]:

$$X_{mn} = \mu + \hat{g}_m + \hat{g}_n + \hat{s}_{mn} + e$$

where X_{mn} = the mean of $m \times n$; μ = experimental mean; \hat{g}_m = GCA effects of the tested inbred lines; \hat{g}_n = GCA effects of the tester lines; \hat{s}_{mn} = SCA effects of the $m \times n$ crossings; e = experimental error.

The additive effects were calculated based on the formula:

$$\hat{g}_m/\hat{g}_n = X_{m/n} - \bar{X}$$

where $X_{m/n}$ = mean of the m or n parent; \bar{X} = the mean of all values of the factorial system.

The non-additive effects were calculated using the formula:

$$\hat{s}_{mn} = X_{mn} - X - (\hat{g}_m + \hat{g}_n)$$

3. Results and Discussions

3.1. Results Regarding the Per Se Traits of the 7 Sister Inbred Lines

3.1.1. Per Se Phenotypic Traits of the Inbred Lines

To evaluate the yield potential, the resistance to breaking, the vegetation period expressed by the dry matter in grains at harvest, and other agronomic traits, a series of determinations were carried out in the plots (Table 1).

Table 1. Some per se traits of the seven inbred lines.

Trait	Lines p(p – 1)/2								LSD 5%
	TD233	TD234	TD235	TD236	TD237	TD238	TD239	Average	
Yield (kg/ha)	4026 ^{ns}	2049 ⁰	3945 ^{ns}	4576 [*]	4169 ^{ns}	4553 [*]	3135 ⁰	3779	608
Dry matter (%)	82.4 ^{ns}	87.1 [*]	82.6 ^{ns}	80.3 ⁰	82.7 ^{ns}	83.9 ^{ns}	82.2 ^{ns}	83.0	1.6
Unbroken plants (%)	78.5 ^{ns}	73.4 ^{ns}	82.9 ^{ns}	78.8 ^{ns}	80.1 ^{ns}	79.7 ^{ns}	77.7 ^{ns}	78.7	6.1
Thousand kernel weight (g)	233 ^{ns}	178 ⁰	227 ^{ns}	221 ^{ns}	243 [*]	216 ^{ns}	277 [*]	228	14
Shelling percentage (%)	79.4 ^{ns}	73.4 ^{ns}	73.3 ^{ns}	80.1 ^{ns}	77.6 ^{ns}	81.0 ^{ns}	75.7 ^{ns}	77.2	4.2
Hectoliter mass (kg/hl)	62.4 ^{ns}	60.3 ⁰	61.0 ⁰	67.6 [*]	63.3 ^{ns}	66.9 [*]	66.2 ^{ns}	64.0	2.9
Ear weight (g)	80.3 ^{ns}	47.0 ⁰	109.3 [*]	100.5 [*]	90.6 [*]	84.4 ^{ns}	72.7 ⁰	83.5	6.0
Grains/ear weight (g)	66.8 ^{ns}	37.2 ⁰	88.2 [*]	85.8 [*]	74.6 [*]	69.9 ^{ns}	58.2 ⁰	68.7	3.9
Ear length (cm)	12.9 ⁰	11.8 ⁰	15.6 [*]	15.2 [*]	14.2 [*]	13.7 ^{ns}	12.0 ⁰	13.6	0.5
No kernels/row (no.)	25.5 ^{ns}	20.8 ⁰	27.7 [*]	27.8 [*]	28.3 [*]	27.0 [*]	21.7 ⁰	25.5	1.3
Kernel row number (no.)	13.9 ^{ns}	11.2 ⁰	16.7 [*]	17.0 [*]	12.7 ⁰	13.8 ^{ns}	12.3 ⁰	13.9	0.5
Kernel depth (cm)	0.72 ^{ns}	0.47 ⁰	0.74 ^{ns}	0.67 ^{ns}	0.70 ^{ns}	0.68 ^{ns}	0.66 ^{ns}	0.70	0.04

^{ns}, ^{*}, ⁰: not significant, significant at 5% probability level, positive and negative values.

The yield had values between 2049 kg/ha for the inbred line TD234 and 4576 kg/ha for TD236, the average of the genotypes being 3779 kg/ha. Other sister lines with a high yield capacity are TD238 (4553 kg/ha) and TD237 (4169 kg/ha).

Regarding the vegetation period, expressed by the dry matter in grains at harvest, there were significant differences between the inbred lines: TD236 (80.3%) was significantly later than the average, and the line TD234 (87.1%) was significantly earlier.

Resistance to breaking, expressed by the percentage of unbroken plants at harvest, had values between 73.4% (TD234) and 82.9% (TD235). Between the seven related lines, there were no statistically significant differences in resistance against breaking.

The analysis of the data regarding the diversity of the inbred lines, regarding the production elements, reveals that some inbred lines are related phenotypically for the studied traits (TD233, TD234, TD238, TD239), while others differ significantly from the average through several production traits (TD235, TD236 and TD237). Some inbred lines that differ significantly from the average through the following production elements are ear and grain/ear weight (TD235, TD236, TD237); ear length (TD235, TD236, TD237); no kernel rows (TD235, TD236); no kernels/row (TD237, TD236, TD235); thousand kernel weight (TD239, TD237).

Among the seven inbred lines of common origin, TD235 and TD236 differ significantly from the average for most phenotypic traits regarding yield and production elements.

3.1.2. Variability of the Phenotypic Per Se Traits of the Inbred Lines

The analysis of phenotypic variances for the inbred lines with common origin is presented in Table 2. The data analysis over the three years of experimentation resulted that between the seven related lines studied, there were significant differences for each of the traits analyzed.

Table 2. Variances for some per se traits of inbred lines.

Trait	Source of Variation		Variance (s ²)					
	DF		Years (Y)		Lines (L)		Y × L	Error
			2	8	16	72		
Production traits								
Yield (kg/ha)	7,620,960	**	7,774,270	**	4,854,916	**	559,772	
Dry matter (%)	433.01	**	42.77	*	8.95	**	3.77	
Unbroken plants (%)	762.24	**	130.07	*	52.73	ns	56.05	
Thousand kernel weight (g)	9515.33	**	8513.99	**	913.47	**	312.42	
Shelling percentage (%)	44.28	ns	137.50	**	49.79	*	26.98	
Hectoliter mass (kg/hl)	147.62	**	78.25	**	65.25	**	12.61	
Ear weight (g)	6105.36	**	3930.87	**	472.58	**	38.54	
Grains/ear weight (g)	4558.92	**	2983.08	**	365.43	**	22.54	
Ear length (cm)	3.36	*	30.64	**	2.91	**	0.44	
No kernels/row (no.)	92.78	**	132.79	**	19.49	**	2.38	
Kernel row number (no.)	0.15	ns	45.64	**	0.91	**	0.33	
Kernels depth (cm)	0.05	**	0.08	**	0.005	**	0.002	
Plant traits								
Plant height (cm)	12,966.39	**	3344.26	**	667.23	**	42.27	
Ear height (cm)	283.98	*	1755.10	**	216.89	**	21.30	
Total no leaves (no.)	7.18	**	23.27	**	4.61	**	0.38	
Leaves above the ear (no.)	1.61	*	9.27	**	0.42	**	0.08	
Leaf area (cm ²)	15,306.26	**	47,565.71	**	2511.65	*	1281.55	
No tassel branches (no.)	4291.93	**	105.05	**	54.41	**	2.81	

ns, *, **: not significant, significant for 5% and 1%.

The influence of the experimental years was significant for most of the traits, except the shelling percentage and the number of kernel rows (traits with additive genetic determinism). For the other analyzed traits, significant or distinctly significant differences were recorded from one year to another.

The influence of the genotypes on the manifestation of the analyzed traits was higher in the case of thousand kernel weight, shelling percentage, ear and grain/ear weight, ear length and the number of kernel rows.

Analyzing the variances of the interactions between years and genotypes, it is found that most traits were distinctly significant, except for one trait, namely the percentage of unbroken plants, which, under the given experimental conditions, showed smaller, insignificant oscillations.

3.2. Results Regarding the $p(p - 1)/2$ Diallel Crossing System

3.2.1. Expression of the Degree of Relatedness between the Sister Inbred Lines by the Intensity of Heterosis

The intensity level of heterosis could indicate the degree of diversity between the parental forms to the extent that the phenotypic expression is not altered by the interaction with the environmental conditions. The expression of heterosis is one of the most sensitive genetic criteria for evaluating the diversity between the crossed parental forms [32]. The diversity analysis based on the expression intensity of reproductive heterosis (Hr%) reflects a manifestation with quite different intensity values (Table 3).

Table 3. Expression of heterosis (%) in the diallel crossing system.

Parental Lines		TD233	TD234	TD235	TD236	TD237	TD238	TD239
TD233	1		14	86	55	32	31	10
	2	-	24	28	89	32	27	32
	3		25	29	92	35	32	32
TD234	1			83	53	28	31	74
	2		-	29	90	32	26	33
	3			29	91	37	33	34
TD235	1				47	33	15	91
	2			-	79	32	12	12
	3				79	34	13	15
TD236	1					154	74	40
	2				-	97	89	88
	3					100	88	87
TD237	1						29	21
	2					-	26	34
	3						29	35
TD238	1							22
	2						-	14
	3							15
Hr (%)		Grouping of lines based on heterosis:						
1—yield		(a) similar—Hr% < 30%;						
2—ear weight		(b) genetically closed—Hr% = 30–70%;						
3—grains weight/ear		(c) genetically distant—Hr% = 70–90%;						
		(d) from alternative groups—Hr% > 90%						

The high heterosis levels within the diallel crossing system can be considered the crosses between TD236 and all other sister lines studied. The high values of heterosis, respectively >70%, express the genetic distance [33,34] between the parental inbred lines. The inbred line TD236 is the most genetically distant compared to the other sister lines.

The most discriminating trait can be considered the heterosis of yield, and the most differentiated line can be regarded as TD236. This confirms that, generally, phenotypic

differences cannot indicate the intensity of heterosis, only for some traits and only in the case of certain lines.

3.2.2. Analysis of Genetic Variances between the Sister Inbred Lines

The variance analysis for the 21 direct, simple hybrids, resulting after crossing the seven lines of common origin in the diallel system, are presented in Table 4. The data analysis of the two years of experimentation found significant differences between the lines used for each of the analyzed traits.

Table 4. Variances of some traits in the diallel crossing system.

Trait	Source of Variation		Variance (s ²)			
	DF	Years	Genotypes	Years × Genotypes	DF	DF
		1	20	20	20	20
Production traits						
Yield (kg/ha)		5.22 ns	60.61 **		1.60 ns	
Dry matter (%)		632.55 **	10.49 **		2.84 ns	
Unbroken plants (%)		45.32 **	3.37 **		2.22 ns	
Thousand kernel weight (g)		2.29 ns	28.91 **		2.76 **	
Hectoliter mass (kg/hl)		368.26 **	5.57 **		2.21 **	
Ear weight (g)		76.18 **	100.60 **		2.74 *	
Grains/ear weight (g)		21.32 *	96.02 **		2.89 *	
Ear length (cm)		337.09 **	34.79 **		9.76 **	
No kernels/row (no.)		720.70 **	34.56 **		10.15 **	
Kernel row number (no.)		2.47 **	7.64 **		1.06	
Plant traits						
Plant height (cm)		1.00 ns	32.51 **		0.001 ns	
Ear height (cm)		1.25 ns	24.99 **		0.54 ns	
Leaf area (cm ²)		0.81 ns	22.95 **		1.49 ns	
No tassel branches (no.)		3.31 ns	49.84 **		4.41 **	

ns, *, **: not significant, significant for 5% and 1%.

In the experimental years, we have significantly influenced some production elements, such as dry matter at harvest, hectoliter mass, ear length, and plant resistance to breakage.

The yield, the ear and grain/ear weight, the thousand kernel weight, the number of kernel rows and, in general, the vegetative traits were strongly influenced by the studied genotypes.

3.2.3. Analysis of Additive Genetic Effects

The assessment of polygenic diversity was carried out at the level of homozygous loci by calculating the additive gene effects (\hat{g})—for the diallel system. Genetic diversity at the additive level was estimated based on the effects of the GCA of each line. The additive effects for the analyzed traits, corresponding to the seven parental inbred lines, from the system of $p(p - 1)/2$ diallel crossings, are presented in Table 5.

Table 5. Additive genetic effects (\hat{g}) in the diallel crossing system for production traits.

Trait	Inbred Lines $p(p - 1)/2$							LSD 5%
	TD233	TD234	TD235	TD236	TD237	TD238	TD239	
Yield (kg/ha)	−61.8 ^{ns}	−241.1 ^{ns}	−619.9 ⁰	2097.3 [*]	−309.6 ^{ns}	−303.7 ^{ns}	−561.2 ⁰	505.7
Dry matter (%)	0.0 ^{ns}	0.34 ^{ns}	0.30 ^{ns}	−0.63 ⁰	−0.33 ^{ns}	−0.10 ^{ns}	0.35 ^{ns}	0.54
Thousand kernel weight (g)	−6.12 ^{ns}	−12.59 ⁰	−3.17 ^{ns}	29.20 [*]	2.68 ^{ns}	−2.95 ^{ns}	−7.06 ^{ns}	10.7
Hectoliter mass (kg/hl)	0.16 ^{ns}	0.70 ^{ns}	1.16 ^{ns}	−1.26 ^{ns}	−0.77 ^{ns}	−0.24 ^{ns}	0.25 ^{ns}	1.61
Ear weight (g)	5.25 ^{ns}	−2.22 ^{ns}	−13.23 ⁰	28.98 [*]	−0.13 ^{ns}	−8.9 ⁰	−9.73 ⁰	5.97
Grains/ear weight (g)	5.05 [*]	−0.99 ^{ns}	−11.35 ⁰	23.73 [*]	1.50 ^{ns}	−6.62 ⁰	−8.32 ⁰	5.04
Ear length (cm)	0.64 [*]	−0.02 ^{ns}	−0.94 ⁰	0.45 [*]	0.29 ^{ns}	−0.19 ^{ns}	−0.23 ^{ns}	0.39
No kernels/row (no.)	1.86 [*]	0.49 ^{ns}	−1.53 ⁰	1.25 [*]	−0.93 ^{ns}	−0.15 ^{ns}	−0.98 ^{ns}	1.15
Kernel row number (no.)	−0.07 ^{ns}	0.10 ^{ns}	−0.24 ^{ns}	0.60 ^{ns}	−0.07 ^{ns}	−0.24 ^{ns}	−0.07 ^{ns}	0.80

^{ns}, ^{*}, ⁰: not significant, significant at 5% probability level, positive and negative values.

Considering the high degree of relatedness of the seven inbred lines studied, the lack of heterotic reaction at the level of the hybrids resulting from the crossing of the seven sister lines was expected. TD236 is the only inbred line that accumulates a very large series of favorable genes with the additive transmission, significantly positive, in interaction with the sister lines, for yield ($\hat{g} = 2097.3^{***}$), ear weight ($\hat{g} = 28.98^{***}$), grain/ear weight ($\hat{g} = 23.73^{***}$), thousand kernel weight ($\hat{g} = 29.2^{***}$).

Two sister lines studied showed completely different reactions compared to the TD236 line. The inbred lines transmitted a low yield potential in the hybrid combinations in which they participated: TD235 ($\hat{g} = -619.90$), respectively TD239 ($\hat{g} = -561.20$), as well as significant negative values of the additive effects for the production elements.

Therefore, the genetic analysis of the hybrid combinations from the diallel crossings between the seven sister lines highlighted the TD236 line as the only one that differs genetically from the other.

3.2.4. Analysis of Non-Additive Genetic Effects (\hat{s}_{ij})

Since related lines were involved in the diallel crossing system, the non-additive effects (\hat{s}_{ij}) were calculated to determine the degree of relatedness and genetic differentiation between the seven parental sister lines.

Higher (positive) values of the non-additive effects mean that at the level of these interactions, there are apparent differences. Conversely, when they are negative and lower, the degree of relatedness is clearer. The values of the non-additive gene interactions for some traits of agronomic importance of the seven sister lines in the $p(p - 1)/2$ crossing system are included in Table 6.

Considering the relativity of the interactions of the seven analysed sister lines, it can be stated based on the value of the non-additive effects that there are:

- positive differentiations between: TD236 \times TD238 ($\hat{s}_{ij} = 689.36^{*}$); TD236 \times TD235 ($\hat{s}_{ij} = 448.73^{*}$)—for yield; TD238 \times TD237 ($\hat{s}_{ij} = 5.26^{*}$)—for plant breaking resistance.
- relatedness between: TD239 \times TD235 ($\hat{s}_{ij} = -548.29^{0}$)—for yield; TD239 \times TD237 ($\hat{s}_{ij} = -0.66^{0}$), TD236 \times TD233 ($\hat{s}_{ij} = -0.57^{0}$)—for dry matter.

Table 6. Non-additive genetic effects (\hat{s}_{ij}) in the diallel crossing system.

m Inbred Line		TD233	TD234	TD235	TD236	TD237	TD238
TD234	a	−486.69 ^{ns}					
	b	−0.37 ^{ns}	-				
	c	3.19 ^{ns}					
TD235	a	63.64 ^{ns}	−214.46 ^{ns}				
	b	0.20 ^{ns}	0.29 ^{ns}	-			
	c	0.44 ^{ns}	−2.11 ^{ns}				
TD236	a	53.00 ^{ns}	350.27 ^{ns}	448.73 ^{ns}			
	b	−0.57 ⁰	0.01 ^{ns}	−0.30 ^{ns}	-		
	c	−0.15 ^{ns}	1.86 ^{ns}	2.21 ^{ns}			
TD237	a	216.05 ^{ns}	147.44 ^{ns}	−247.98 ^{ns}	277.87 ^{ns}		
	b	0.47 ^{ns}	0.09 ^{ns}	−0.09 ^{ns}	0.14 ^{ns}	-	
	c	−2.10 ^{ns}	−4.20 ^{ns}	4.96 ^{ns}	−1.70 ^{ns}		
TD238	a	−63.23 ^{ns}	−217.71 ^{ns}	−121.50 ^{ns}	689.36 [*]	−333.61 ^{ns}	
	b	0.09 ^{ns}	0.04 ^{ns}	0.03 ^{ns}	−0.09 ^{ns}	−0.29 ^{ns}	-
	c	−3.80 ^{ns}	0.01 ^{ns}	−1.57 ^{ns}	−2.01 ^{ns}	5.26 [*]	
TD239	a	155.48 ^{ns}	180.00 ^{ns}	−548.29 ⁰	278.06 ^{ns}	−369.40 ^{ns}	−257.04 ^{ns}
	b	0.27 ^{ns}	0.28 ^{ns}	0.17 ^{ns}	0.18 ^{ns}	−0.66 ^{ns}	0.11 ^{ns}
	c	2.56 ^{ns}	1.78 ^{ns}	−5.43 ⁰	1.75 ^{ns}	−2.05 ^{ns}	1.09 ^{ns}
a = yield		LSD 5% = 505.76					
b = % dry matter		LSD 5% = 0.54					
c = % unbroken plants		LSD 5% = 5.07					

^{ns}, ^{*}, ⁰: not significant, significant at 5% probability level, positive and negative values.

3.3. Results Regarding the $m \times n$ Cyclic Crossing System (7×4)

In the cyclic crossing system ($m \times n$), the sister inbred lines were tested to know the degree of differentiation or relatedness in interaction with the two tester lines (used as initial material for the breeding of the sister lines): TB329 (Iodent heterotic group) and TC177 (European Flint group heterotic), and also finding out the genetic value of the seven sister lines in interaction with two other tester lines TC344 (BSSS heterotic group) and TA367 (Minn.13xFlint), as well as determining the degree of relatedness or differentiation to the two heterotic groups.

3.3.1. Analysis of Genetic Variances between the Inbred Lines

Through the analysis of the genetic variances for the genotypes included in the $m \times n$ cyclic crossing system (Table 7), additive and non-additive genetic variances that control the genetic determinism of the studied traits and their significance were separated.

Regarding the influence of the additive actions of the seven sister lines (m), they were significant for the thousand kernel weight, the number of kernel rows, the kernel depth, the plant and ear height and the leaf area. The influence of the additive actions of the tester lines (n) was much higher, contributing distinctly significantly to the manifestation of most traits.

For most traits, the non-additive variances exceed the value of the additive variances' " m " or " n ", which attests to both genetic control and the significant involvement of environmental conditions on the manifestation of most analyzed traits.

Table 7. Variances for some traits in the cyclic crossing system.

Trait	Source of Variation		Years (Y)	Genotypes (G)	Additive Actions—Inbred Lines (Am)		Additive Actions—Testers (An)		Non-Additive Actions (NA)		Y × G		
	DF				(6)	(3)	(18)	54					
Yield (kg/ha)		1937.7	**	65.2	**	0.2	ns	46.1	**	90.1	**	6.45	**
Dry matter (%)		126.8	**	3.6	**	0.1	ns	1.8	ns	5.1	**	0.87	ns
Unbroken plants (%)		149.9	**	2.8	**	0.1	ns	1.8	ns	3.9	**	1.41	*
Thousand kernel weight (g)		848.5	**	27.6	**	2.1	**	15.0	**	38.2	**	5.63	**
Hectoliter mass (kg/hl)		236.6	**	16.4	**	0.4	ns	10.9	*	22.7	**	2.40	**
Grains/ear weight (g)		38.9	**	107.5	**	0.9	ns	76.5	**	98.0	**	6.66	**
Ear length (cm)		1.6	ns	106.2	**	0.3	ns	75.4	**	147.6	**	9.68	**
No kernels/row (no.)		7.9	**	42.3	**	1.3	ns	26.5	**	58.7	**	7.03	**
Kernel row number (no.)		15.6	**	72.1	**	4.5	**	38.3	**	100.9	**	2.80	**
Kernel depth (cm)		1.4	**	0.1	**	0.001	**	0.1	**	38.6	**	0.008	**

ns, *, **: not significant, significant for 5% and 1%.

3.3.2. Analysis of Additive Genetic Effects (\hat{g})

The additive effects corresponding to the “m” parental lines, for the analyzed traits, from the “m × n” cyclic crossing system are presented in Table 8.

Table 8. Additive genetic effects (\hat{g}_m) in the cyclic crossing system.

Traits	m Lines							LSD 5%
	TD233	TD234	TD235	TD236	TD237	TD238	TD239	
Yield (kg/ha)	218.1 ^{ns}	72.1 ^{ns}	−94.7 ^{ns}	101.3 ^{ns}	25.4 ^{ns}	−36.9 ^{ns}	−285.3 ⁰	259.7
Dry matter (%)	−0.05 ^{ns}	0.55 ^{ns}	−0.60 ^{ns}	−0.30 ^{ns}	−0.17 ^{ns}	0.33 ^{ns}	0.25 ^{ns}	1.76
Unbroken plants (%)	0.16 ^{ns}	−0.29 ^{ns}	−1.04 ^{ns}	0.93 ^{ns}	3.13 ^{ns}	−0.84 ^{ns}	−2.04 ^{ns}	7.22
Thousand kernel weight (g)	−3.28 ^{ns}	−15.18 ⁰	3.72 ^{ns}	10.02 [*]	5.40 ^{ns}	5.10 ^{ns}	−5.78 ^{ns}	9.46
Shelling percentage (%)	0.53 ^{ns}	0.23 ^{ns}	−0.44 ^{ns}	−0.67 ^{ns}	−0.87 ^{ns}	1.01 ^{ns}	0.21 ^{ns}	2.29
Hectoliter mass (kg/hl)	0.22 ^{ns}	0.84 ^{ns}	−0.11 ^{ns}	−0.98 ^{ns}	−0.03 ^{ns}	−0.13 ^{ns}	0.19 ^{ns}	1.46
Ear weight (g)	6.94 ^{ns}	−1.51 ^{ns}	4.09 ^{ns}	8.97 ^{ns}	−4.76 ^{ns}	−4.63 ^{ns}	−9.11 ^{ns}	11.95
Grains/ear weight (g)	5.95 ^{ns}	−0.41 ^{ns}	3.23 ^{ns}	4.48 ^{ns}	−7.59 ^{ns}	−0.44 ^{ns}	−5.21 ^{ns}	8.56
Ear length (cm)	0.13 ^{ns}	−0.19 ^{ns}	−0.19 ^{ns}	−0.17 ^{ns}	0.26 ^{ns}	0.13 ^{ns}	0.03 ^{ns}	0.58
No kernels/row (no.)	1.77 [*]	1.22 ^{ns}	−0.13 ^{ns}	−1.40 ^{ns}	−1.45 ^{ns}	0.15 ^{ns}	−0.15 ^{ns}	1.69
Kernel row number (no.)	−0.08 ^{ns}	0.45 ^{ns}	−0.23 ^{ns}	1.55 ^{ns}	−1.08 ^{ns}	−0.40 ^{ns}	−0.20 ^{ns}	1.72
Kernel depth (cm)	0.01 ^{ns}	−0.01 ^{ns}	0.00 ^{ns}	0.01 ^{ns}	−0.02 ^{ns}	0.01 ^{ns}	−0.01 ^{ns}	0.05

^{ns}, *, ⁰: not significant, significant at 5% probability level, positive and negative values.

All the additive genetic level information can reflect the degree of differentiation regarding the breeding value of some of the parental lines as sources of favorable or unfavorable genes corresponding to each trait. From the analysis of these data, the following inbred lines were noted:

- TD236—for the hereditary transmission of yield ($\hat{g}_m = 101.3$), ear weight ($\hat{g}_m = 8.97$), grain/ear weight ($\hat{g}_m = 4.48$), TKW ($\hat{g}_m = 10.02$) and for the kernel row number ($\hat{g}_m = 1.55$);

- TD233—for the hereditary transmission of yield ($\hat{g}_m = 218.1$), ear weight ($\hat{g}_m = 6.94$), grain/ear weight ($\hat{g}_m = 5.95$) and for the number of kernels/row ($\hat{g}_m = 1.77$);
- TD234—the positive value of additive effects for precocity ($\hat{g}_m = 0.55$), higher hectoliter mass ($\hat{g}_m = 0.84$) and a number of kernels/row.
- TD237—the source of improvement for plant breaking resistance.

The analysis of the GCA noted both genetically valuable lines in the breeding process, such as those presented previously, but also lines that do not seem to be endowed with favorable genes or lines that seem genetically related to the other parental lines due to the lack of heterosis for yield (TD239, TD235, TD238).

3.3.3. Analysis of the Non-Additive Genetic Effects (\hat{s}_{mn})

For the genetic evaluation, the non-additive effects (\hat{s}_{mn}) of the analyzed traits were calculated. A positive and higher value indicates more pronounced differences at the level of these interactions, and in the case of negative and lower values, the degree of relatedness is obvious.

The values of the non-additive effects are presented in Table 9. The analysis of these data shows that the significantly positive values of the non-additive effects were estimated in the case of a small number of hybrid combinations. For example, the hybrid combination TB329 × TD236 stood out for the SCA of most traits regarding production elements: yield ($\hat{s}_{mn} = 815^{**}$), ear weight ($\hat{s}_{mn} = 17.39^{**}$), grain/ear weight ($\hat{s}_{mn} = 13.45^{**}$), ear length ($\hat{s}_{mn} = 1.45^{**}$), kernel row number ($\hat{s}_{mn} = 1.65$), no kernels/row ($\hat{s}_{mn} = 2.95^{**}$).

Table 9. Non-additive genetic effects (\hat{s}_{mn}) in cyclic crossing system.

Inbred Lines n	Trait	Inbred Lines m						
		TD233	TD234	TD235	TD236	TD237	TD238	TD239
TB329	a	−369 ^{ns}	85 ^{ns}	−502 ^{ns}	815 [*]	−190 ^{ns}	−69 ^{ns}	230 ^{ns}
TC177		9 ^{ns}	−61 ^{ns}	292 ^{ns}	−473 ^{ns}	167 ^{ns}	−136 ^{ns}	201 ^{ns}
TC344		709 [*]	59 ^{ns}	−44 ^{ns}	105 ^{ns}	−415 ^{ns}	−65 ^{ns}	−348 ^{ns}
TA367		−349 ^{ns}	−83 ^{ns}	254 ^{ns}	−447 ^{ns}	438 ^{ns}	270 ^{ns}	−83 ^{ns}
TB329	b	−6.19 ^{ns}	−0.74 ^{ns}	−8.84 ^{ns}	17.39 [*]	2.91 ^{ns}	−1.21 ^{ns}	−3.34 ^{ns}
TC177		−1.94 ^{ns}	0.91 ^{ns}	3.21 ^{ns}	−17.87 ⁰	0.26 ^{ns}	6.83 ^{ns}	8.61 ^{ns}
TC344		11.49 ^{ns}	−0.56 ^{ns}	2.44 ^{ns}	6.36 ^{ns}	−13.01 ⁰	1.76 ^{ns}	−8.46 ^{ns}
TA367		−3.36 ^{ns}	0.39 ^{ns}	3.19 ^{ns}	−5.88 ^{ns}	9.84 ^{ns}	−7.38 ^{ns}	3.19 ^{ns}
TB329	c	−0.97 ^{ns}	0.39 ^{ns}	−5.76 ^{ns}	13.45 [*]	−0.94 ^{ns}	−2.43 ^{ns}	−3.74 ^{ns}
TC177		−0.37 ^{ns}	1.31 ^{ns}	2.91 ^{ns}	−13.12 ⁰	3.23 ^{ns}	1.60 ^{ns}	4.44 ^{ns}
TC344		6.50 ^{ns}	−2.41 ^{ns}	2.34 ^{ns}	5.38 ^{ns}	−11.15 ⁰	−0.01 ^{ns}	−0.65 ^{ns}
TA367		−5.15 ^{ns}	0.70 ^{ns}	0.51 ^{ns}	−5.70 ^{ns}	8.86 [*]	0.84 ^{ns}	−0.06 ^{ns}
TB329	d	−0.15 ^{ns}	0.28 ^{ns}	−0.52 ^{ns}	1.45 [*]	−0.47 ^{ns}	−0.25 ^{ns}	−0.35 ^{ns}
TC177		0.42 ^{ns}	0.05 ^{ns}	0.35 ^{ns}	−1.28 ⁰	0.10 ^{ns}	0.22 ^{ns}	0.13 ^{ns}
TC344		−0.08 ^{ns}	−0.15 ^{ns}	−0.15 ^{ns}	−0.48 ^{ns}	0.10 ^{ns}	0.22 ^{ns}	0.52 ^{ns}
TA367		−0.20 ^{ns}	−0.18 ^{ns}	0.32 ^{ns}	0.30 ^{ns}	0.27 ^{ns}	−0.20 ^{ns}	−0.30 ^{ns}
a = yield (kg/ha)		LSD 5% = 527.21		c = grain/ear weight (g)		LSD 5% = 8.56		
b = ear weight (g)		LSD 5% = 11.95		d = ear length (cm)		LSD 5% = 0.58		

^{ns}, ^{*}, ⁰: not significant, significant at 5% probability level, positive and negative values.

High values of the non-additive positive effects were noted in the hybrid combinations: TC344 × TD233: for yield ($\hat{s}_{mn} = 709^{**}$), ear weight ($\hat{s}_{mn} = 11.49$), grain/ear weight ($\hat{s}_{mn} = 6.50$), as well as TD237 × TA367: for yield ($\hat{s}_{mn} = 439$).

The high values of the non-additive effects representing the degree of genetic differentiation between the seven sister lines studied. The tester lines for some of the ear traits are presented as follows: for ear and grain/ear weight, the highest values were obtained in the hybrid combinations: TD236 × TB329 ($\hat{s}_{mn} = 17.39^{*}$; $\hat{s}_{mn} = 13.45^{*}$), TD233 × TC344 ($\hat{s}_{mn} = 11.49$; $\hat{s}_{mn} = 6.50$), TD237 × TA367 ($\hat{s}_{mn} = 9.84$; $\hat{s}_{mn} = 8.86$).

It should be mentioned that some of the higher values of the non-additive effects were achieved in the crossing between TD236 and one of the lines obtained from the original hybrid, a fact that also explains the initiation of a comparative study for the other sister lines (TD236 \times TB329).

These examples come to emphasize the idea supported by some researchers [26,35–37], according to which F2 populations from simple or trilinear hybrids can become initial sources for creating valuable inbred lines, the breeding being achieved based on agro-economic traits through recurrent selection methods. When hybrid populations are used as initial material in the breeding of lines, one requirement is mandatory, namely, that of working with a sufficiently large number of plants, which ensures the possibility of recombining complementary characters.

3.4. Discussions Regarding the Study on Genetic Diversity/Relatedness of Maize Inbred Lines Using SSR Molecular Markers

The inbred lines used in the present study are also part of a research [27] through which 90 inbred lines from the ARDS Turda collection were analyzed with the help of 80 SSR molecular markers. The genetic distance values confirm the pedigree information for lines TD233, TD234, TD235, TD237, TD238 and TD239. All these lines come from the same original breeding material. Most inbred lines are part of the same family, so they are registered with a very little differentiated pedigree and are part of the Iodent heterotic group. Only one line, TD236, differs genetically from the rest of the sister lines and is genetically similar to the tester line TC177, belonging in proportion to 40% to the Flint European heterotic group, and 60% of the genotype belongs to the Iodent group. This genetic differentiation of the TD236 line, highlighted with the help of SSR molecular markers, confirms the results obtained through the analysis by classical genetic methods through the high level of heterosis intensity when crossing the TD236 line with the sister lines.

4. Conclusions

The phenotypic traits of the TD236 inbred line differed significantly from those of TD234, TB329 and TC177 but are close to those of TD233, TD235, TD237 and TD238, while TD239 was phenotypically differently compared to most of the sister lines while presenting some traits close to tester lines TB329 and TC177.

The highest value of heterosis (>70%) was obtained with the combination TD236 \times TD237 ($H_r = 154\%$). These lines seem to be the most genetically distant.

In the diallel crossing system, from the analysis of the interactions between the seven sister lines, there were observed positive differences at the level of non-additive effects for the yield. The significantly highest value was achieved with the hybrid combination TD236 \times TD238.

In the cyclic system, the seven sister inbred lines presented a more pronounced positive degree of genetic differentiation, expressed by the value of non-additive genetic effects, as follows: tester line TB329 differentiated at a non-additive level from lines TD236 and TD234; line TD235 non-additively different from tester lines TC177 and TA367; tester line TC344 differentiated at a non-additive level from lines TD233 and TD236.

The levels of genetic similarity from the point of view of the value of non-additive effects are presented as follows: tester line TB329—similarity at a non-additive level with lines TD233 and TD235; tester line TC177—non-additive similarity to line TD236; tester line TA367—non-additive similarity with lines TD233 and TD236.

The presentation of the similarity between the sister lines and testers highlighted that the TD236 line is genetically closest to the TC177 parental line, and the TD235 line is genetically related to TB329. In all the other five lines studied, through the selection process, the recombination of the two parental lines was achieved in different proportions.

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References

- Babic, M.; Babic, V.; Prodanovic, S.; Filipovic, M.; Andjelkovic, V. Comparison of Morphological and Molecular Genetic Distances of Maize Inbreds. *Genetika* **2012**, *44*, 119–128. [[CrossRef](#)]
- Lauer, S.; Hall, B.D.; Mulaosmanovic, E.; Anderson, S.R.; Nelson, B.; Smith, S. Morphological Changes in Parental Lines of Pioneer Brand Maize Hybrids in the U.S. Central Corn Belt. *Crop Sci.* **2012**, *52*, 1033–1043. [[CrossRef](#)]
- Babic, V.; Nikolic, A.; Andjelkovic, V.; Kovacevic, D.; Filipovic, M.; Vasic, V.; Mladenovic-Drinic, S. UPOV Morphological versus Molecular Markers for Maize Inbred Lines Variability Determination. *Chil. J. Agric. Res.* **2016**, *76*, 417–426. [[CrossRef](#)]
- Oyetunde, O.A.; Badu-Apraku, B.; Ariyo, O.J.; Alake, C.O. Efficiencies of Heterotic Grouping Methods for Classifying Early Maturing Maize Inbred Lines. *Agronomy* **2020**, *10*, 1198. [[CrossRef](#)]
- Benchimol, L.L.; de Souza, C.L., Jr.; Garcia, A.A.F.; Kono, P.M.S.; Mangolin, C.A.; Barbosa, A.M.M.; Coelho, A.S.G.; de Souza, A.P. Genetic Diversity in Tropical Maize Inbred Lines: Heterotic Group Assignment and Hybrid Performance Determined by RFLP Markers. *Plant Breed.* **2000**, *119*, 491–496. [[CrossRef](#)]
- Patto, M.C.V.; Satovic, Z.; Pêgo, S.; Feveireiro, P. Assessing the Genetic Diversity of Portuguese Maize Germplasm Using Microsatellite Markers. *Euphytica* **2004**, *137*, 63–72. [[CrossRef](#)]
- Abegunde, O.I.; Idehen, E.O.; Oduwaye, O.A.; Fabunmi, T.O.; Kehinde, O.B. Genetic Characterization of Drought-Tolerant Maize Genotypes Based on SSR Markers. *Nig. J. Biotechnol.* **2023**, *39*, 97–105. [[CrossRef](#)]
- Bocianowski, J.; Nowosad, K.; Wróbel, B.; Szulc, P. Identification of Associations between SSR Markers and Quantitative Traits of Maize (*Zea mays* L.). *Agronomy* **2021**, *11*, 182. [[CrossRef](#)]
- Mathiang, E.A.; Sa, K.J.; Park, H.; Kim, Y.J.; Lee, J.K. Genetic Diversity and Population Structure of Normal Maize Germplasm Collected in South Sudan Revealed by SSR Markers. *Plants* **2022**, *11*, 2787. [[CrossRef](#)]
- Xia, X.C.; Reif, J.C.; Melchinger, A.E.; Frisch, M.; Hoisington, D.A.; Beck, D.; Pixley, K.; Warburton, M.L. Genetic Diversity among CIMMYT Maize Inbred Lines Investigated with SSR Markers: II. Subtropical, Tropical Midaltitude, and Highland Maize Inbred Lines and Their Relationships with Elite U.S. and European Maize. *Crop Sci.* **2005**, *45*, 2573–2582. [[CrossRef](#)]
- Xia, X.C.; Reif, J.C.; Hoisington, D.A.; Melchinger, A.E.; Frisch, M.; Warburton, M.L. Genetic Diversity among CIMMYT Maize Inbred Lines Investigated with SSR Markers: I. Lowland Tropical Maize. *Crop Sci.* **2004**, *44*, 2230–2237. [[CrossRef](#)]
- Senior, M.L.; Murphy, J.P.; Goodman, M.M.; Stuber, C.W. Utility of SSRs for Determining Genetic Similarities in Relationships in Maize Using an Agarose Gel System. *Crop Sci.* **1998**, *38*, 1088–1098. [[CrossRef](#)]
- Pejic, I.; Ajmone-Marsan, P.; Morgante, M.; Kozumplick, V.; Castiglioni, P.; Taramino, G.; Motto, M. Comparative Analysis of Genetic Similarity among Maize Inbred Lines Detected by RFLPs, RAPDs, SSRs, and AFLPs. *Theor. Appl. Genet.* **1998**, *97*, 1248–1255. [[CrossRef](#)]
- Hallauer, A.R.; Miranda, J.B. *Quantitative Genetics in Maize Breeding*; Iowa State University Press: Ames, IA, USA, 1981.
- Haş, I. Heterozisiz La Porumb. In *Porumbul—Studiu Monografic*; Editura Academiei Române: Bucureşti, Romania, 2004; Volume 1, pp. 311–362. ISBN 973-27-1056-X.
- Yadav, V.K.; Singh, I.S. Comparative Evaluation of Maize Inbred Lines (*Zea mays* L.) According to Dus Testing Using Morphological, Physiological and Molecular Markers. *Agric. Sci.* **2010**, *01*, 131–142. [[CrossRef](#)]
- Aliu, S.; Fetahu, S.; Salillari, A. Estimation of Heterosis and Combining Ability in Maize (*Zea mays* L.) for Ear Weight (EW) Using the Diallel Crossing Method. *Agron. Vestis* **2008**, *11*, 7–12.
- Aliu, S.; Rusinovci, I.; Fetahu, S.; Rozman, L. The Combining Ability of Maize (*Zea mays* L.) Inbred Lines for Grain Yield and Yield Components. *Agric. For.* **2016**, *62*, 295–303. [[CrossRef](#)]
- Murtadha, M.A.; Ariyo, O.J.; Alghamdi, S.S. Analysis of Combining Ability over Environments in Diallel Crosses of Maize (*Zea mays*). *J. Saudi Soc. Agric. Sci.* **2018**, *17*, 69–78. [[CrossRef](#)]
- Barata, C.; Carena, M.J. Classification of North Dakota Maize Inbred Lines into Heterotic Groups Based on Molecular and Testcross Data. *Euphytica* **2006**, *151*, 339–349. [[CrossRef](#)]
- Fan, X.M.; Zhang, Y.M.; Yao, W.H.; Chen, H.M.; Tan, J.; Xu, C.X.; Han, X.L.; Luo, L.M.; Kang, M.S. Classifying Maize Inbred Lines into Heterotic Groups Using a Factorial Mating Design. *Agron. J.* **2009**, *101*, 106–112. [[CrossRef](#)]
- Adu, G.B.; Awuku, F.J.; Amegbor, I.K.; Haruna, A.; Manigben, K.A.; Aboyadana, P.A. Genetic Characterization and Population Structure of Maize Populations Using SSR Markers. *Ann. Agric. Sci.* **2019**, *64*, 47–54. [[CrossRef](#)]

23. Kumar, S.; Das, A.K.; Naliath, R.; Kumar, R.; Karjagi, C.G.; Sekhar, J.C.; Vayas, M.; Yathish, K.R.; Singh, A.; Mukri, G.; et al. Potential Use of Random and Linked SSR Markers in Establishing the True Heterotic Pattern in Maize (*Zea mays*). *Crop Pasture Sci.* **2022**, *73*, 1345–1353. [[CrossRef](#)]
24. Zhao, M.; Shu, G.; Hu, Y.; Cao, G.; Wang, Y. Pattern and Variation in Simple Sequence Repeat (SSR) at Different Genomic Regions and Its Implications to Maize Evolution and Breeding. *BMC Genom.* **2023**, *24*, 136. [[CrossRef](#)] [[PubMed](#)]
25. Sa, K.J.; Park, H.; Jang, S.J.; Lee, J.K. Association Mapping of Amylose Content in Maize RIL Population Using SSR and SNP Markers. *Plants* **2023**, *12*, 239. [[CrossRef](#)]
26. Hallauer, A.R. Modern Methods in Maize Breeding. In Proceedings of the Maize Breeding and Maize Production; Maize Research Institute Zemun Polje: Belgrade, Serbia, 1988; pp. 1–20.
27. Şuteu, D.; Băcilă, I.; Haş, V.; Haş, I.; Miclăuş, M. Romanian Maize (*Zea mays*) Inbred Lines as a Source of Genetic Diversity in SE Europe, and Their Potential in Future Breeding Efforts. *PLoS ONE* **2013**, *8*, e85501. [[CrossRef](#)] [[PubMed](#)]
28. Griffing, B. Concept of General and Specific Combining Ability in Relation to Diallel Crossing Systems. *Aust. Jnl. Bio. Sci.* **1956**, *9*, 463. [[CrossRef](#)]
29. Gardner, C.O.; Eberhart, S.A. Analysis and Interpretation of the Variety Cross Diallel and Related Populations. *Biometrics* **1966**, *22*, 439. [[CrossRef](#)]
30. Căbulea, I. Metode Statistice Pentru Analiza Componentelor Genetice Ale Variabilităţii Continue [Statistical Methods for Analyzing the Genetic Components of Continuous Variability]. *Probl. Genet. Teor. Aplic.* **1975**, *7*, 391–420.
31. Căbulea, I. Genetica Porumbului. In *Porumbul—Studiu Monografic*; Editura Academiei Române: Bucureşti, Romania, 2004; Volume 1, pp. 207–310. ISBN 973-27-1056-X.
32. Li, Z.; Coffey, L.; Garfin, J.; Miller, N.D.; White, M.R.; Spalding, E.P.; De Leon, N.; Kaeppler, S.M.; Schnable, P.S.; Springer, N.M.; et al. Genotype-by-Environment Interactions Affecting Heterosis in Maize. *PLoS ONE* **2018**, *13*, e0191321. [[CrossRef](#)]
33. Musteaţă, S.I.; Mistreţ, S.I.; Brumă, S. Sravnitel'nyj Analiz Kriteriev Opredeleniâ Otlíčimosti u Rodstvennyh Linij Kukuzy. [Comparative Analysis of the Criteria for Determining the Distinctiveness of Related Maize Lines]. *Kukuzyza Sorgo* **2009**, *6*, 18–24.
34. Brumă, S. Aprecierea Comparativă a Diferitor Indici de Discriminare a Liniilor Înrudite de Porumb. *Ştiinţa Agric.* **2012**, *2*, 7–11.
35. Hallauer, A.R. Methods Used in Developing Maize Inbreds. *Maydica* **1990**, *35*, 1–16.
36. Troyer, A.F. Temperate Corn: Background, Behavior and Breeding. In *Speciality Corns*; CRC Press: Boca Raton, FL, USA, 2000; pp. 393–466.
37. Brumă, S. Evaluarea Capacităţii de Combinare şi a Distinctivităţii Liniilor Consangvinizate de Porumb Timpuriu. [Evaluation of Combining Ability and Distinctiveness of Early Maize Inbred Lines]. Ph.D. Thesis, Academy of Science of Moldova, Chişinău, Moldova, 2013.

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