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Identification of Associations between SSR Markers and Quantitative Traits of Maize (*Zea mays* L.)

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Abstract: Microsatellite or simple sequence repeat (SSR) markers have wide applicability for genetic analysis in crop plant improvement strategies. Marker-assisted selection is an important tool for plant breeders to increase the efficiency of a breeding process, especially for multigenic traits, highly influenced by the environment. In this paper, the relationships between SSR markers and 26 quantitative traits of hybrid maize varieties (*Zea mays* L.) were analyzed. Association analyses were performed based on 30 SSR primers in a set of thirteen hybrid maize varieties. A total of 112 SSR markers were detected in these genotypes. The number of alleles per locus ranged from 1 to 17, with the average number of alleles per locus equal to 3.7. The number of molecular markers associated with observed traits ranged from 1 (for the number of kernels in row, ears weight and fresh weight of one plant) to 14 (for damage of maize caused by *P. nubilalis*) in 2016 as well as from 1 (for soil plant analysis development—SPAD, the number of grains in ear and fresh weight of one plant) to 12 (for carotenoids content) in 2017. The sum of statistically significant associations between SSR markers and at least one trait was equal to one hundred sixty in 2016 as well as one hundred twenty-five in 2017. Marker trait associations (MTAs) were found on the basis of regression analysis. The proportion of the total phenotypic variances of individual traits explained by the marker ranged from 24.4% to 77.7% in the first year of study and from 24.3% to 77.9% in 2017. Twenty-two SSR markers performed a significant effect on at least one tested trait in both years of experiment. The three markers (phi021/4, phi036/3, and phi061/2) can be a good tool in marker-assisted selection because they allow simultaneous selection for multiple traits in both years of study, such as the number of kernels in row and the number of grains in ear (phi021/4), the number of plant after germination, the number of plants before harvest, and the number of ears (phi036/3), as well as moisture of grain and length of ears (phi061/2).

Keywords: maize; microsatellite markers; quantitative traits; regression

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1. Introduction

Maize (*Z. mays* L.) belongs to the group of crops in which significant breeding progress has been observed in recent years. It is manifested not only by a large number of new hybrid varieties but also by changes in the selection of different types of breeding [1]. All maize varieties offered for cultivation are hybrid varieties, characterized by exuberance, high vigor, and good alignment [2]. Progress in maize breeding is very high, and the average period of use of varieties in production rarely lasts more than 10 years [1,3]. New maize varieties are characterized by better tolerance to a cold weather and a vigor of initial growth, as well as higher yield and lower moisture content during harvesting [4–6].

Older varieties are therefore quickly replaced by new hybrid varieties with better utility traits [7,8].

Currently, there are over 160 hybrid varieties in the Polish register (Research Centre for Cultivar Testing—COBORU, www.coboru.pl), which have undergone relevant research and can be grown in Poland. In the light of EU regulations, other varieties are legally permitted for cultivation in our country. The cultivation of such varieties is, however, less secure, because they are not tested in Poland, hence the risk of their cultivation falls on the farmer. There are more and more of such varieties on the market. Some of them are currently tested, and only they should be taken into consideration. Therefore, this justifies the advisability of testing new varieties for yield stability under agro-climatic conditions in Poland. In terms of the area under cultivation, maize occupies third place in the world, after wheat and rice, while in terms of the harvest volume, it is the first crop plant in the world [1]. A whole range of roughage and concentrate, with different composition and concentration of energy, needed in every type of animal production can be obtained from maize [9,10]. In this century, industrial maize processing has been intensively developed, and the products obtained from it are a raw material useful for further processing into confectionery, baking, brewing, oil, and feed industries. Moreover, the maize is also used as a raw material in the paper industry and has great potential in bioenergy production (e.g., biomass).

Microsatellites or SSRs (simple sequence repeats) are DNA stretches consisting of short, tandemly repeated di-, tri-, tetra-or penta-nucleotide motifs. SSRs have been found in all eukaryotic species that were scrutinized for them [11]. SSRs can be used to identify and verify varieties of plants [12]. The information provided by molecular markers can be used in breeding programs to better estimate the genetic value of individuals subjected to selection [13].

Information about genetic variation and diversity within breeding materials is critical to accelerating the effect of biological progress. In the literature, there are comparisons of the efficiency of marker types in determining the genetic diversity of maize breeding materials showing a high output of SSR markers [14]. Thanks to their codominant nature, high number of alleles per locus, easiness of automation, and the ability to differentiate inbred lines, SSR markers are very useful [15].

The aim of this study was detection of relationships between DNA marker profiles and quantitative traits of maize. Molecular markers were compared with observations of 26 quantitative traits in selected varieties. Possible associations between SSR markers and some Quantitative Trait Locus (QTLs) in order to furnish elements are very important for breeders to perform marker-assisted selection in order to speed up the selection process to obtain varieties with improved characteristics and to contribute to the genotyping.

2. Materials and Methods

2.1. Plant Material

Thirteen hybrid maize varieties (NK Cooler, Delitop, Gazele, NK Ravello, ES Palazzo, ES Paroli, SY Cooky, Drim, Clarica, PR 39 G12, SY Mascotte, ES Fortran, PR 39 K 13) of fodder maize, grown for grain and purchased from different seed production companies, were compared.

2.2. Field Experiment

The experiment was carried out at the Department of Agronomy, the Poznań University of Life Sciences, in 2016 and 2017 in the fields of the Agricultural Experimental Station in Swadzim (52°26′ N; 16°45′ E). The experiment was established in a one-factor randomized block design with four field replications. It was carried out in typical grey-brown podzolic soil composed of coarse sandy soil, shallowly deposited on light loam. The same *nitrogenium-phosphorus-kalium* (NPK) fertilization was applied throughout the experimental field: 100 kg N ha^{-1} in the form of urea, 80 kg P_2O_5 ha^{-1} in the form of granular triple superphosphate 46% P_2O_5, and 120 kg K_2O ha^{-1} in the form of 60% potassium salt. All

the agricultural and cultivation measures were performed following the guidelines of good agricultural practices. Overall, the temperature and rainfall were favorable for maize growth and development (Table S1). Gross plot size was 24.5 m^2 (length—8.75 m, width—2.8 m). The net plot area for harvesting was 12.25 m^2. The maize sowing in each of the study years was performed in the third decade of April, while the harvest was performed when the maize grain was mature. Ten ears were collected from each experimental plot, with ten kernels collected for genetic marker analyses after their manual threshing.

2.3. Quantitative Traits

The plant density on the surface unit was determined twice: after full growth of plants and before harvesting. Double determination of the quantitative status of plants enabled the disappearance of plants during their vegetation to be determined. During the time of the experiment, plants infection was determined by diseases and damage by pests. The occurrence of fusarium diseases (*Fusarium* ssp.), Nodular bladder (*Ustilago maydis* Corda), and the pest European maize borer (*Pyrausta nubilalis* Hbn.) was recorded. In the cases both of diseases and of the pest, only the number of plants that were attacked or infested by the given pathogen was recorded, and the result was expressed as a percentage of infection. The degree of disease and pest infestation was not taken into account. Ten ears were used for length and diameter measurements, which were collected from the plot at harvest time. Then, the length of the ears was determined using a ruler. The diameter of the ear was measured in half of the length using a caliper. The measurement of chloroplast pigment content was performed in the 5–6 leaf phase Biologische Bundesanstalt, Bundessortenamt und CHemische Industrie (BBCH 15/16). In the case of the direct method, the leaf weights were cut into 2–3 mm sections and filled with 5 mL dimethyl sulfoxide. The samples were left for about 1 h in the dark at room temperature and then incubated at 65 °C (water bath) for 30 min. In the obtained extract after cooling, the chlorophyll *a* and *b* content were spectrophotometrically determined. The content of chlorophyll dyes was determined by means of a spectrophotometer (Spekol type) at the appropriate wavelength. For chlorophyll *a*, measurement of the absorbance of the extract was made at 663 nm, for chlorophyll *b*, it was made at 645 nm. The amount of chlorophyll *a* and chlorophyll *b*, and the sum of chlorophyll *a* + *b* and carotenoids were calculated by using the formulas contained in the paper [16]. In the indirect method of determining the nutritional status of maize with nitrogen, an optical device known in Europe as the Hydro N-Tester was used. The apparatus works by measuring the light absorption of a leaf at two wavelengths: 650 nm and 940 nm. The quotient of these differences is an indicator of the chlorophyll content and is referred to as the SPAD (soil and plant analysis development) unit. High determination coefficient (R^2) was demonstrated depending on the species, between the indications of the apparatus and the extracted amount of chlorophyll [17]. Ten plants were cut from each plot during the flowering phase of the maize. The leaves, ears, and stems were separated and then weighed. Knowing the weight of a single plant and the weight of the leaves, the share of leaves in the mass of a single plant was calculated. Grain moisture content was determined on random samples from threshing grain mass from each plot, with the Super Matic electronic moisture meter. The weight of the sample taken for the assays was 250 g. The maize harvest was done with a plotter harvester from Wintersteiger, and the yield of grain was converted to a constant humidity of 15%. The thousand-grain weight was calculated from the sum of two random samples of 500 kernels each. The ears were counted before harvest on each plot in two rows destined for harvesting. The number of ears per 1 m^2 was determined by dividing the sum of the obtained ears from the plot by its surface. The number of grains in the ear was obtained from the product of the number of rows and the number of grains in a row of a given ears.

2.4. DNA Extraction

Genomic DNA was extracted from 8–12 days old maize leaves. The tissue was placed in tubes, and the plant tissue was ground to a powder in liquid nitrogen. Genomic DNA

from plants was isolated by phenol extraction. The concentration of extracted DNA was estimated with the NanoMasterGen MN-913. The final concentration of each DNA sample was adjusted to 30 ng μL^{-1}. The 30 microsatellite sequences (Table S2), available in the MaizeGDB database (www.maizegdb.org), were used for analysis. PCR was performed in a volume of 15 µL containing 5.9 µL H_2O, 1.5 µL buffer × 10 (Thermo, Waltham, USA), 2.5 µL $MgCl_2$ (25 mM, Thermo), 1 µL dNTP (10 mM each, mix, Thermo), 1 µL forward (F) and reverse (R) primers (primers concentration 0.28 mM), 0.2 µL of polymerase (5 µ μL^{-1}, Thermo), and genomic DNA (30 ng μL^{-1}). The PCR amplification was performed using a TProfessional gradient thermal cycler (Biometria GmbH, Goettingen, Germany). The PCR amplification conditions were 94 °C for 3 min, 35 cycles at 94 °C for 30 s, 55 °C (differentiated according to the temperature requirements of the primer hybridization temperatures in the Table 1) for 45 s, and 72 °C for 1 min, then final elongation at 72 °C for 10 min, and then the reaction mixture was cooled to 4 °C. The amplification products were separated using Qiaxel capillary electrophoresis system (Qiagen, Hilden, Germany), and the size of the products was determined using ScreenGel software (Qiagen, Hilden, Germany).

Table 1. Code and sequences of primers tested, with annealing temperature and the polymorphic information content (PIC).

Primer Code	Left End	Right End	Temp (°C)	PIC
phi001	TGACGGACGTGGATCGCTTCAC	AGCAGGCAGCAGGTCAGCAGCG	71	0.357
phi002	CATGCAATCAATAACGATGGCGAGT	TTAGCGTAACCCTTCTCCAGTCAGC	66	0.401
phi008	CGGCTACGGAGGCGGTG	GATGGGCCCACACATCAGTC	65	0.051
phi015	GCAACGTACCGTACCTTTCCGA	ACGCTGCATTCAATTACCGGGAAG	67	0.080
phi021	TTCCATTCTCGTGTTCTTGGAGTGGTCCA	CTTGATCACCTTTCCTGCTGTCGCCA	63	0.387
phi026	TAATTCCTCGCTCCCGGATTCAGC	GTGCATGAGGGAGCAGCAGGTAGTG	70	0.373
phi036	CCGTGGAGAGACGTTTGACGT	TCCATCACCACTCAGAATGTCAGTGA	66	0.129
phi041	TTGGCTCCCAGCGCCGCAAA	GATCCAGAGCGATTTGACGGCA	64	0.007
phi042	ATGTGGCCATCATTCAATGCTGTAGAC	ACACATGCAGGTGCAGCCAGA	68	0.397
phi047	GGAGATGCTCGCACTGTTCTC	CTCCACCCTCTTTGACATGGTATG	63	0.255
phi049	GATTGCGATAACATTGCGGCAAGTTGT	CTTCTGTTCCGCCATCCAGTATGTT	69	0.390
phi054	AGAAAAGAGAGTGTGCAATTGTGATAGAG	AATGGGTGCCTCGCACCAAG	66	0.401
phi056	ACTTGCTTGCCTGCCGTTAC	CGCACACCACTTCCCAGAA	63	0.447
phi061	GACGTAAGCCTAGCTCTGCCAT	AAACAAGAACGGCGGTGCTGATTC	69	0.497
phi064	CCGAATTGAAATAGCTGCGAGAACCT	ACAATGAACGGTGGTTATCAACACGC	68	0.394
phi068	GTACACACGCTCCGACGATTAC	TCTTCTCCACCAGAGCCTTGTAAG	62	0.257
phi070	GCTGAGCGATCAGTTCATCCAG	CCATGGCAGGGTCTCTCAAG	64	0.269
phi072	ACCGTGCATGATTAATTTCTCCAGCCTT	GACAGCGCGCAAATGGATTGAACT	70	0.110
phi073	GTGCGAGAGGCTTGACCAA	AAGGGTTGAGGGCGAGGAA	63	0.028
phi076	TTCTTCCGCGGCTTCAATTTGACC	GCATCAGGACCCGCAGAGTC	65	0.381
phi079	TGGTGCTCGTTGCCAAATCTACGA	GCAGTGGTGGTTTCGAACAGACAA	68	0.473
phi080	CACCCGATGCAACTTGCGTAGA	TCGTCACGTTCCACGACATCAC	64	0.308
phi085	AGCAGAACGGCAAGGGCTACT	TTTGGCACACCACGACGA	64	0.333
phi112	TGCCCTGCAGGTTCACATTGAGT	AGGAGTACGCTTGGATGCTCTTC	66	0.240
phi113	GCTCCAGGTCGGAGATGTGA	CACAACACATCCAGTGACCAGAGT	63	0.197
phi116	TCCCTGCCGGGACTCCTG	GCATACGGCCATGGATGGGA	68	0.165
phi119	GGGCTCCAGTTTTCAGTCATTGG	ATCTTTCGTGCGGAGGAATGGTCA	68	0.180
phi120	TGATGTCCCAGCTCTGAACTGAC	GACTCTCACGGCGAGGTATGA	63	0.199
phi127	ATATGCATTGCCTGGAACTGGAAGGA	AATTCAAACACGCCTCCCGAGTGT	69	0.197
phi129	TCCAGGATGGGTGTCTCATAAAACTC	GTCGCCATACAAGCAGAAGTCCA	65	0.317

2.5. Microsatellite Markers Analysis

Primers were selected from the database on the basis of previous studies, the selection criterion was high polymorphism obtained in preliminary studies on maize genotypes. All the hybrid maize varieties were scored for the presence and absence of the SSR bands. Data were entered into a binary matrix as discrete variables, with "1" for the presence and "0" for the absence of alleles. The polymorphic information content (PIC) for each marker was calculated using the formula described by Wolko et al. [18]. PIC values ranged from 0 (in the case of fixation of one allele) to 0.5 (when the frequencies of both alleles were equal).

2.6. Statistical Analysis

The normality of residuals from the regression model was tested by using Shapiro–Wilk's normality test [19]. A two-way analysis of variance (ANOVA) was carried out to determine the effects of variety, year, and variety-by-year interaction on the variability of studied traits.

The association between molecular markers and observed traits of 13 hybrid maize varieties was estimated using regression analysis by the following formula:

$$y_k = \mu + a_k \cdot m_k + e_k \quad (1)$$

where y_k is the mean values of y trait for k-th hybrid maize variety, a_k is the effect of k-th SSR marker, m_k is k-th SSR marker, and e_k is a random residual. The molecular marker observations were treated as independent variables and considered in individual models. We used the critical significance level equal to 0.05, resulting from a Bonferroni correction, for each regression model in a year. Data analyses were performed by using the statistical package GenStat 18th edition (Hemel Hempstead, UK).

3. Results

A total of 112 SSR markers were detected with the set of 30 SSR primers. The number of alleles per locus ranged from 1 to 17, with the average number of alleles per locus equal to 3.7. The set of SSR primers used in this study generated highly informative loci with PIC values ranging from 0.077 (for phi041) to 0.497 (for phi061), with the mean 0.274. The size of PCR products ranged from 63 to 267 bp.

The analysis of variance shows that the genotypes and years differed with regard to all the 26 traits of study. The genotype-by-year interaction was also significant for all the observed quantitative traits. The differences of average values between the years were large ($p < 0.001$), therefore the marker trait association (MTA) analyses between particular SSR markers and quantitative traits were made separately for each year.

The number of molecular markers associated with observed traits ranged from 1 (for number of kernels in row, ears weight, and fresh weight of one plant) to 14 (for damage of maize caused by *P. nubilalis*) in 2016 as well as from 1 (for SPAD, the number of grains in ear, and fresh weight of one plant) to 12 (for carotenoids content) in 2017 (Table 2, Tables S3–S28). The total of significant MTAs of 160 SSR markers with at least one trait in 2016 as well as 125 in 2017 was found on the basis of regression analysis (Table 2, Tables S3–S28). The different number of MTAs in the first and the second year of study was caused by an environmental effect. The proportion of total phenotypic variance of individual trait explained by the marker ranged from 24.4% for phi079/2 SSR marker for yield of grain to 77.7% for phi083/3 SSR marker for infection of maize by *Fusarium* spp. in 2016 (Table 2, Tables S7 and S20), and from 24.3% for phi068/3 marker for chlorophyll *a/b* to 77.9% for phi076/2 marker for the number of plants before harvest in 2017 (Table 2, Tables S5 and S27).

Twenty-two SSR markers performed a significant effect on at least one tested trait in both years of experiment (Tables 2 and 3). Most of all, four MTAs (phi127/3, phi083/2, phi054/3, and phi058/1) were significant for weight of 1000 grains (Table 3, and Table S8) as well as four MTAs (phi070/2, phi076/3, phi041/1, and phi120/3) determined the diameter of ears (Table 3, and Table S10). The three markers (phi021/4, phi036/3, and phi061/2) significantly determined at least two traits in both years (Table 3). However, fourteen traits (SPAD, the number of rows in ear, stems weight, ears weight, fresh weight of one plant, share of leaves in the mass of the plant, damage of maize caused by *P. nubilalis*, infection of maize by *Fusarium* spp., infection of maize by *U. maydis* Corda, plant density, content of chlorophyll *a*, content of chlorophyll *b*, content of chlorophyll $a + b$ and content of chlorophyll a/b) had no marker trait associations that would determine them in both years (Table 2).

Table 2. The number of molecular markers associated with 26 observed traits of maize (*Zea mays* L.) in two years of study.

Trait	2016		2017		The Number of Common Markers
	The Number of Significant Markers	Range of the Proportion of Total Phenotypic Variance Explained by the Marker	The Number of Significant Markers	Range of the Proportion of Total Phenotypic Variance Explained by the Marker	
Soil plant analysis development (SPAD)	8	25.5–36.1	1	25.7–25.7	0
The number of plants after germination	8	26.1–44.8	6	29.3–76.7	1
The number of plants before harvest	5	26.4–53.6	6	29.4–77.9	1
Moisture of grain	4	25.9–51.0	3	25.4–29.3	2
Yield of grain	10	24.4–42.8	3	27.1–35.3	1
Weight of 1000 grains	6	26.6–43.5	5	38.3–42.0	4
Length of ears	3	28.4–50.2	3	27.8–32.7	2
Diameter of ears	11	24.8–60.7	5	25.3–45.6	4
The number of rows in ear	4	24.4–47.1	3	25.9–41.8	0
The number of kernels in row	1	31.6–31.6	3	24.9–34.8	1
The number of grains in ear	5	27.5–45.6	1	24.9–24.9	1
Leaf weight	4	28.9–34.4	2	25.1–25.9	1
Stems weight	10	26.0–60.1	2	25.9–29.1	0
Ears weight	1	48.5–48.5	11	25.5–46.6	0
Fresh weight of one plant	1	43.9–43.9	1	37.8–37.8	0
Share of leaves in the mass of the plant	5	25.3–40.6	7	24.4–47.5	0
Damage of maize caused by *P. nubilalis*	14	25.1–62.3	4	24.5–52.7	0
Infection of maize by *Fusarium* spp.	10	28.8–77.7	4	27.1–32.3	0
Infection of maize by *Ustilago maydis* Corda	12	25.2–62.0	7	32.0–75.4	0
The number of ears	12	25.1–56.5	6	26.9–68.3	3
Plant density	10	26.0–50.4	10	29.0–42.7	0
Content of chlorophyll *a*	3	42.5–64.7	5	30.1–34.5	0
Content of chlorophyll *b*	3	40.1–66.2	5	25.7–35.7	0
Content of chlorophyll *a* + *b*	3	42.0–65.1	7	24.7–34.1	0
Content of chlorophyll *a*/*b*	2	45.3–58.5	3	24.3–44.7	0
Carotenoids content	5	26.1–57.0	12	24.5–44.7	1

Table 3. Molecular markers associated with observed traits in both years of study.

Trait	Marker Symbol	2016			2017		
		Estimates of Regression Coefficients	*p*-Value	The Proportion of Total Phenotypic Variance Explained by the Marker	Estimates of Regression Coefficients	*p*-Value	The Proportion of Total Phenotypic Variance Explained by the Marker
The number of plants after germination	phi036/3	0.394	0.007	44.8	1.488	0.028	31.2
The number of plants before harvest	phi036/3	0.363	0.007	45.1	1.476	0.025	32.3
Moisture of grain	phi073/5	−2.052	0.010	41.5	−1.399	0.038	27.4
	phi061/2	1.993	0.044	25.9	1.676	0.033	29.3
Yield of grain	phi047/4	16.97	0.033	29.2	9.00	0.019	35.3
Weight of 1000 grains	phi127/3	−60.70	0.008	43.5	−66.20	0.014	38.3
	phi083/2	−60.70	0.008	43.5	−66.20	0.014	38.3
	phi054/3	−60.70	0.008	43.5	−66.20	0.014	38.3
	phi058/1	−60.70	0.008	43.5	−66.20	0.014	38.3
Length of ears	phi061/2	−1.15	0.004	50.2	−1.722	0.029	30.5
	phi061/5	−0.866	0.016	36.8	−1.424	0.037	27.8
Diameter of ears	phi070/2	0.349	0.016	37.1	0.210	0.046	25.3
	phi076/3	0.327	0.002	57.0	0.190	0.016	36.9
	phi041/1	0.336	0.001	60.7	0.207	0.007	45.6
	phi120/3	0.336	0.001	60.7	0.207	0.007	45.6
The number of kernels in row	phi021/4	−2.750	0.027	31.6	−2.570	0.043	26.0
The number of grains in ear	phi021/4	−86.40	0.012	39.8	−66.30	0.048	24.9
Leaf weight	phi001/3	24.60	0.032	29.5	19.41	0.047	25.1

Table 3. *Cont.*

Trait	Marker Symbol	2016			2017		
		Estimates of Regression Coefficients	*p*-Value	The Proportion of Total Phenotypic Variance Explained by the Marker	Estimates of Regression Coefficients	*p*-Value	The Proportion of Total Phenotypic Variance Explained by the Marker
The number of ears	phi076/2	−1.011	0.041	26.7	−1.345	<.001	64.8
	phi036/3	1.380	0.039	27.3	1.388	0.022	33.8
	phi116/2	0.896	0.010	42.1	0.788	0.016	37.1
Carotenoids content	phi073/2	−1.188	0.043	26.1	−0.915	0.049	24.5

For all twenty-two molecular markers associated with quantitative traits in both years of study (Table 3), the signs of regression coefficients agreed; this includes the SSR markers increasing the value of the observed trait in both years of study (positive sign of regression coefficient) or the SSR markers decreasing the value of the observed trait in the first and second years of study (negative sign of regression coefficient).

4. Discussion

Knowledge of maize genetic diversity is important to understand the genetic structure, which helps the breeder to select required parents for breeding programs. SSR markers have been reported to be a powerful tool for detecting the genetic diversity of maize populations [20]. These markers could be applied in line characterization at the molecular level; they could also assist maize breeders in an efficient assigning of lines to heterotic groups and in indicating a proper selection of parents for the development of new hybrid varieties [21]. Genetic diversity testing is crucial to select individual genotypes from closely related groups in order to start new breeding experiments. Genotyping is one of the most reliable methods to establish such phylogenetic relationships among a set of inbred lines [22]. The polymorphic information content (PIC) average in the current study was 0.274. This parameter indicates the informativeness of the SSR loci and their ability to detect differences between the genotypes on the basis of their genetic relationships [23]. The phi061 primer was found to be the most appropriate for testing genetic diversity, as its PIC value was the highest (0.497). Genetic distance measures the degree of relatedness between individuals in a given population [24,25].

The results of the statistical analysis identified several molecular markers associated with quantitative traits. Some of these marker trait associations affected more than two analyzed traits, which is not surprising considering their polygenic background. In our study, 22 SSR marker trait associations, which were genotyped in both years of field trials, proved to be the most promising markers for subsequent breeding programs. Three of these SSR markers (phi021/4, phi036/3, and phi061/2) have become candidates for marker-assisted selection for further experiment, because they allowed simultaneous selection for multiple traits in both years of study, such as the number of kernels in row and the number of grains in ear (phi021/4), the number of plant after germination, the number of plants before harvest, and the number of ears (phi036/3), as well as moisture of grain and length of ears (phi061/2).

The regression method for selecting SSR markers associated with quantitative traits presented in this article was rather straightforward. This approach has already been applied previously in different plant species [26–28]. The use of molecular markers significantly improves the selection process. Marker-assisted selection is an important technique applied by plant breeders, enabling them to increase the efficiency of the breeding process, particularly in case of multigenic traits that are strongly affected by the environment. SSR markers that have significant effects on quantitative traits are probably linked with the QTLs that determined these traits. Microsatellite markers detected in both years of study

should contribute to improved understanding of the genetic of yield and other quantitative traits.

5. Conclusions

The relationships between molecular markers and phenotypic traits, such as grain yield, 1000-grains weight, grain moisture, and carotenoid content, can be a significant diagnostic tool in maize (*Z. mays* L.) selection breeding. The efficiency of such markers in different genetic backgrounds as well as their usefulness in breeding programs for the development of hybrid maize varieties with different features need to be demonstrated.

Supplementary Materials: The following are available online at http://www.mdpi.com/xxx/s1, Table S1: Average monthly air temperatures and monthly total precipitation for the growing season, Table S2: Simple sequence repeat (SSR) markers motifs (https://www.maizegdb.org/), Table S3: Molecular markers associated with SPAD, Table S4: Molecular markers associated with the number of plants after germination, Table S5: Molecular markers associated with the number of plants before harvest, Table S6: Molecular markers associated with moisture of grain, Table S7: Molecular markers associated with yield of grain, Table S8: Molecular markers associated with weight of 1000 grains, Table S9: Molecular markers associated with length of ears, Table S10: Molecular markers associated with diameter of ears, Table S11: Molecular markers associated with the number of rows in ear, Table S12: Molecular markers associated with the number of kernels in row, Table S13: Molecular markers associated with the number of grains in ear, Table S14: Molecular markers associated with leaf weight, Table S15: Molecular markers associated with stems weight, Table S16: Molecular markers associated with ears weight, Table S17: Molecular markers associated with fresh weight of one plant, Table S18: Molecular markers associated with share of leaves in the mass of the plant, Table S19: Molecular markers associated with damage of maize caused by *P. nubilalis*, Table S20: Molecular markers associated with infection of maize by *Fusarium* spp., Table S21: Molecular markers associated with infection of maize by *U. maydis* Corda, Table S22: Molecular markers associated with the number of ears, Table S23: Molecular markers associated with plant density, Table S24: Molecular markers associated with content of chlorophyll *a*, Table S25: Molecular markers associated with content of chlorophyll *b*, Table S26: Molecular markers associated with content of chlorophyll $a + b$, Table S27: Molecular markers associated with content of chlorophyll a/b, Table S28: Molecular markers associated with carotenoids content.

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