Heredity of chloroplast pigments parameters of maize (*Zea mays* L.)

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Abstract

The environmental adaptation of maize germplasm is mainly controlled by certain genes. This factor is influenced by both the genotype and the environment itself. The gene is able to express itself in the germination and seedling vigor under the occurring conditions. The following 13 maize cultivars were used: NK Cooler, Delitop, Gazele, NK Ravello, ES Palazzo, ES Paroli, Clarica, PR 39 G 12, SY Cooky, Drim, SY Mascotte, ES Fortran and PR 39 K 13. The experimental design was completely randomized, with four replications. Selection for high and low chlorophyll content was performed in the field using the SPAD chlorophyll meter, and response to selection was measured in field evaluations of the populations. We evaluated the SPAD, content of: chlorophyll a, chlorophyll b, chlorophyll $a+b$, chlorophyll a/b and carotenoids. Data were submitted to analysis of variance. For comparison of means, we adopted the Tukey's honestly significant difference at the 0.05 level. The coefficients of phenotypic, genotypic and environmental variation as well as heritability and genetic gain were estimated. The results indicated genetic variability for the different traits of the studied chloroplast pigments, with high heritability and possible genetic gain with the potential to be used in breeding programs.

Abbreviations

ANOVA – analysis of variance
BBCH – Biologische Bundesanstalt, Bundessortenamt und CHemischeIndustrie
DMSO – dimethyl sulfoxide
ECV – environmental coefficient of variation
GCV – genotypic coefficient of variation
GG – genetic gain
h^2 – heritability
HSD – honestly significant difference
MS_e – mean squares error
MS_g – mean squares of genotypes
NPK – Nitrogen-Phosphorus-Potassium
PCV – phenotypic coefficient of variation
r – correlation coefficient
SPAD – Soil and Plant Analysis Development
V_e – environmental variance
V_g – genotypic variance
V_p – phenotypic variance

Introduction

Maize (*Zea mays* L.) is one of the world's three most widely cultivated crops. Maize has great adaptability to changes in ecological conditions, especially changes in climatic conditions (Leff *et al.* 2004; Ciampitti and Vyn 2012; Bocianowski *et al.* 2019). More vigorous, with higher germination speed, good adaptation and productive grains are characteristics desired by producers, and higher production can be attributed to the evolution of grain yield, which has a joint relationship with breeding techniques, adoption of supplies and different ways of managing and cultivation acquired by various cultures in which maize can be included (Walters *et al.* 1991; Ciampitti and Vyn 2012). Grains of local cultivars are considered components of agro biodiversity as they are of inestimable value for traditional populations.

Maize employs the highly efficient C4 type of photosynthesis, which compared with most C3 plants, has a higher capacity to absorb, metabolize and remobilize C and N metabolites (Cliquet *et al.* 1990; Oaks 1994). In addition, the number of leaves that emerge under specific growth conditions is genetically determineted (Fournier and Andrieu 1999).

The genotype can be seen through evaluations on the phenotype and its performance represents the genotypic value in occupied environment (Nowosad *et al.* 2016, 2017). In the literature, one can also find information on the different response of maize varieties to the applied herbicides (Jagła *et al.* 2020). The authors of the current study found that maize varieties used in the experiments showed different sensitivity to the applied herbicides. No relationship was found between the genetic similarity of individual maize cultivars and their response to the herbicides. Effective weed control without phytotoxic effects of plant protection products are factors that significantly affect maize yield (Jagła *et al.* 2020). Without studies comparing varieties in terms of genetic similarity, it is impossible to influence the yield progress of new maize varieties. In order to meet the nutritional and forage needs of the projected (predicted) human population by 2050, it is imperative that maize improvement programs focus on the development of maize varieties with climate-adapted traits (biometric features), in particular increased tolerance to drought stress (Dwivedi *et al.* 2016). Maize genetic resources are key to achieving this goal (Nelimor *et al.* 2020). Assessing genetic diversity using genotyping by sequencing provides robust estimates of diversity and is increasingly being accepted as a fast, high-throughput and inexpensive tool for analyzing genome-wide genetic diversity (Holtz *et al.* 2016). The phenotypic variance can be divided into: environmental produced variation, variation due to the different characteristics of heredity and variation acquired by the sum of the effects caused by environment and heredity. The variation can be calculated due to genetic differences between treatment or/and progeny, which is one of the favorable components to improvement, because it confers genetic gains. Genetic variability can be quantified by the coefficient of genetic variation, which expresses the genetic variation compared to the average evaluated character.

Gene expression is a result of the genetic additive, dominance and epistatic effect that may influence the expression of quantitative trait in a population (Bocianowski and Nowosad, 2015). Heritability is the result on the quotient between genotypic and phenotypic variances, which assesses the efficiency of selection in the application of genetic variability. This heritability is divided into wide or narrow, and may vary according to the kind, character, environmental conditions, and phenological stages.

The objective of this study was to estimate the genetic parameters of chloroplast related characters of 13 maize cultivars, so as to provide practical directions for their application in breeding programs.

Material and methods

Maize materials

The field experiment was carried out at the Department of Agronomy of the Poznań University of Life Sciences, in the fields of the Didactic and Experimental Center in Swadzim in 2016. The tested maize cultivars were: NK Cooler (FAO 240), Delitop (FAO 240), Gazele (FAO 220-230), NK Ravello (FAO 210), ES Palazzo (FAO 230-240), ES Paroli (FAO 250), SY Cooky (FAO 220-230), Drim (FAO 220), Clarica (280), PR 39 G12 (FAO 230), SY Mascotte(FAO 260), ES Fortran (210-220) and PR 39 K 13 (FAO 220).

Experimental plan and agronomical treatments

The experiment was established in a system of random blocks, in four replications, on typical brown soil made of light clay sands, shallowly deposited on light soil. The same NPK fertilization was used throughout the experimental field in the following amounts: 100 kg $N \cdot ha^{-1}$ in the form of urea, 80 kg $P_2O_5 \cdot ha^{-1}$ in the form of polyphonic 6 and 120 kg $K_2O \cdot ha^{-1}$ in the form of 60% potassium salt. The size of the plots was 30.8 m^2 (width 2.8 m, length 11.0 m), while the plot area for observation was 15.4 m^2 (after rejecting the extreme rows constituting the so-called sowings). The soil's abundance in phosphorus, potassium and magnesium in the testing year was at the following level: 129.0 mg $P \cdot kg^{-1}$ of soil; 64 mg $K \cdot kg^{-1}$ of soil; 79 mg $Mg \cdot kg^{-1}$ of soil. Measurement of the content of chloroplast pigments was carried out in the phase of 5-6 leaves (BBCH 15-16).

Chlorophyll and carotenoid analyses

Content of chlorophyll was determined by two methods: the direct and the indirect ones. In case of the direct method, the leaf weighed portion was cut into 2-3 mm sections and they were poured with 5 ml DMSO (dimethyl sulfoxide). The samples were kept in the dark at room temperature and then, they were incubated at 65°C (water bath) for 30 minutes. In the obtained extract, after cooling down, the content of chlorophyll a and band carotenoids were spectrophotometrically determined. The number of the particular pigments is quoted in μg g^{-1} of fresh matter, while the weight of carotenoids is given in mg g^{-1} of fresh weight. In case of the indirect method, maize nutritional status with nitrogen was defined using an optical apparatus known in Europe as Hydro N-Tester, while in the USA, it is known as SPAD-502 apparatus. This apparatus operates by measuring light absorption by a leaf at the wavelengths of 650 and 940 nm. The quotient of these differences indicates the chlorophyll contents and it is defined in SPAD (Soil and Plant Analysis Development) units (Scharf *et al.* 2006).

Statistical analysis

Statistical analysis was conducted using Bartlett's tests for comparison of the variances. A one-way analysis of variance (ANOVA) was carried out to determine the effects of cultivars on the variability of SPAD, content of: chlorophyll a, chlorophyll b, chlorophyll $a+b$, chlorophyll a/b and carotenoids. When critical differences were noted, multiple comparisons were carried out, using Tukey's honestly significant differences (HSDs) for each trait; based on this, homogeneous groups (not significantly different from each other) were determined for analyzed traits.

The phenotypic variation for each trait was partitioned into genetic and non-genetic factors and estimated by the following formulas (Sunday *et al.* 2007):

$$V_g = \frac{(MS_g - MS_e)}{r}$$

$$V_p = \frac{MS_g}{r}$$

$$V_e = MS_e$$

where V_g, V_p and V_e are genotypic variance, phenotypic variance and environmental variance, respectively, MS_g and MS_e are the mean squares of genotypes and mean squares error, respectively, and r is the number of replications.

The phenotypic variance is the total variance among phenotypes, the genotypic variance is the part of the phenotypic variance that can be attributed to genotypic effects and the error or environmental variance is the variance due to environmental effects. To compare the variations among traits, phenotypic coefficient of variation (PCV), genotypic coefficient of variation (GCV) and environmental coefficient of variation (ECV) were computed according to the method suggested by Allard (1999):

$$GCV = \frac{\sqrt{V_g}}{\overline{X}} \cdot 100$$

$$PCV = \frac{\sqrt{V_p}}{\overline{X}} \cdot 100$$

$$ECV = \frac{\sqrt{V_e}}{\overline{X}} \cdot 100$$

where $\overline{X}$ is the overall mean of each treatment.
Heritability was calculated by the formula:

$$h^2 = \frac{Vg}{(Vg + Ve)}$$

where V_g and V_p denote the genotypic and phenotypic

Table 1 - Mean and standard deviation values for different variables evaluated in maize cultivars

Cultivars	SPAD		Chlorophyll *a*		Chlorophyll *b*		Chlorophyll *a+b*		Chlorophyll *a/b*		Carotenoids	
	Mean#	s.d.	Mean	s.d.	Mean	s.d.	Mean	s.d.	Mean	s.d.	Mean	s.d.
NK Cooler	584b	34.2	2.27a	0.309	0.562a	0.094	2.84a	0.403	4.06d	0.159	9.87a	1.23
Delitop	551bc	23.8	1.88ab	0.645	0.44ab	0.161	2.33ab	0.806	4.3c	0.099	6.81c	1.282
Gazele	578b	13.6	1.29c	0.141	0.289c	0.043	1.58c	0.184	4.48abc	0.238	6.83bc	0.342
NK Ravello	494c	34	1.56bc	0.185	0.359bc	0.053	1.92bc	0.239	4.34bc	0.134	7.17bc	0.955
ES Palazzo	570b	10.4	1.38bc	0.05	0.301c	0.016	1.69c	0.063	4.61a	0.172	6.55c	0.63
ES Paroli	604ab	12.9	1.67bc	0.033	0.376bc	0.008	2.05bc	0.028	4.43abc	0.161	7.54bc	0.827
Clarica	601ab	8.4	1.4bc	0.154	0.306c	0.031	1.71bc	0.186	4.59a	0.035	6.25c	1.2
PR 39 G 12	621ab	27.6	1.63bc	0.214	0.371bc	0.062	2bc	0.277	4.4abc	0.15	7.63bc	0.735
SY Cooky	670a	30.1	1.53bc	0.226	0.343bc	0.05	1.88bc	0.276	4.47abc	0.035	7.3bc	0.208
Drim	613ab	39.5	1.51bc	0.336	0.329bc	0.067	1.84bc	0.404	4.58a	0.114	6.84bc	1.599
SY Mascotte	601ab	52.2	1.44bc	0.838	0.332bc	0.209	1.78bc	1.048	4.41abc	0.192	6.79c	2.242
ES Fortran	618ab	28	1.49bc	0.204	0.327bc	0.047	1.82bc	0.252	4.56ab	0.09	6.27c	0.895
PR 39 K 13	583b	30.3	1.76bc	0.136	0.416bc	0.059	2.18bc	0.195	4.27cd	0.264	8.6ab	1.94
$HSD_{0.05}$	70.2		0.504		0.128		0.632		0.227		1.777	

in columns, means followed by the same letters are not significantly different at 0.05 level.

Table 2 - Estimation of the mean square for of genotypes (MS_g) and mean square for error (MS_e); average values; phenotypic variance (V_p); genotypic variance (V_g) and environmental variance (V_e); phenotypic variation coefficients (*PCV*); genotypic variation coefficients (*GCV*) and environmental variation coefficients (*ECV*); heritability (h^2) and genetic gain (*GG*) for the variables of 13 cultivars of maize

Traits	SPAD	Chlorophyll a	*Chlorophyll b*	*Chlorophyll a+b*	*Chlorophyll a/b*	*Carotenoids*
MS_g	4942	0.265	0.022	0.438	0.097	4.041
MS_e	2412	0.120	0.008	0.189	0.024	1.495
Mean	578	1.601	0.365	1.969	4.422	7.270
V_p	633	0.036	0.003	0.062	0.018	0.637
V_g	1236	0.066	0.005	0.110	0.024	1.010
V_e	2412	0.120	0.008	0.189	0.024	1.495
PCV	4.35	11.87	16.10	12.67	3.041	10.97
GCV	6.08	16.07	20.13	16.81	3.517	13.83
ECV	8.50	21.66	24.17	22.09	3.535	16.82
h^2 (%)	33.9	35.5	41.0	36.7	49.7	40.3
GG	17.5	0.139	0.050	0.189	0.138	0.663

variability, respectively.

Genetic gain was calculated by the formula:

$$GG = c \cdot \Delta p \cdot h^2,$$

where c refers to a constant (equivalent to 2.06 when the intensity selection is 5%), Δp to the standard deviation of the phenotypic variance and h^2 to heritability.

The relationships between observed traits were estimated using Pearson correlation coefficients. Data analysis was performed, using the statistical package GenStat 18.

Results and discussion

General variability of the chlorophyll and carotenoid

Results of analysis of variance indicated that the main effects of cultivars were significant for all the traits of study. The largest value of SPAD was observed for SY Cooky (670), however the smallest for NK Ravello (494) (Table 1). The largest variation of this trait had SY Mascotte (8.69%), however the smallest cultivar Clarica(1.40%). The smallest values of contents of chlorophyll *a*, *b* and *a*+*b* have been observed for Gazele, however the largest for NK Cooler (Table 1). The most stability cultivar regard contents of chlorophyll *a*, *b* and *a*+*b* was ES Paroli, coefficients of variation for these three traits were equal to 1.98, 2.13 and 1.37, respectively. Szulc and Bocianowski (2011) stated that the "stay-green"varieties are more genetically stable compared to the classical hybrids. The "stay-green" type maize varieties, compared to their classic counterparts, are characterized not only by a higher chlorophyll content, but also significantly better yield-forming abilities both for grain cultivation and silage raw material (Szulc *et al.* 2021). Such abilities of this type of maize varieties are determined by the nutrient remobilization factor (Szulc *et al.* 2012). During the period of maize vegetative growth, the flow of nitrogen from the older organs of the plant to the younger ones occurs at a different rate, with soil resources being the dominant source of nitrogen. During the maturation phase, plant nitrogen balance undergoes significant changes. Nitrogen taken up from the soil is only a small portion of the nutrient accumulated in the grain. Most of it is derived from the remobilization of this macronutrient from the storage material previously accumulated in vegetative organs. During plant maturation, the latter source provides 60-92% of the accumulated nitrogen in the grain (Papakosta and Garianas 1991). Hence, the main nitrogen source for grain in cereal plants, including maize, is the one remobilized from vegetative organs of the plant. The amount of nitrogen released in this manner depends on the effectiveness of remobilization processes and the amount of nitrogen available in the soil (Cox *et al.* 1986). The classic model of nitrogen accumulation shows that plants accumulate 85-100% of this nutrient during the vegetative growth period, while the processes of re-mobilization of nitrogen organic compounds from the resources accumulated during vegetative growth occur already during the filling phase, and the uptake of nitrogen from soil resources should be considered as complementary. In the present study, the classical model of nitrogen, phosphorus, potassium and magnesium accumulation was shown in the traditional hybrid. The organic compound re-mobilization

Table 3 - Correlation coefficients between observed traits of maize

Trait	SPAD	Chlorophyll *a*	Chlorophyll *b*	Chlorophyll *a+b*	Chlorophyll *a/b*
Chlorophyll a	-0.159				
Chlorophyll b	-0.191	0.990***			
Chlorophyll a+b	-0.166	1.000***	0.994***		
Chlorophyll a/b	0.308	-0.678***	-0.767***	-0.698***	
Carotenoids	-0.142	0.778***	0.807***	0.785***	-0.711***

*** P<0.001

index was positive in the traditional hybrid, which meant that this variety utilized the components previously collected during vegetative growth in the period of grain filling. In turn, a different accumulation model of nitrogen and other mineral components was observed in plants of the "stay-green" variety. The remobilization index, as well as nitrogen, phosphorus and magnesium translocation indices were negative, and positive only for potassium (Szulc *et al.* 2012). This indicated that soil resources were the main source of nitrogen accumulation during the generative growth phase. The presented model of the "stay-green" plant response should imply a fertilization system of nitrogen and the remaining nutrients. Hence, the expression of the yield potential of a cultivated plant ("stay-green" maize variety) is not possible with a deficiency of even a single mineral component. That is why field studies comparing maize varieties in terms of genetic similarity, as well as its impact on yield are so important. SY Mascotte was the large variation of contents of chlorophylls: *a* (58.19%), *b* (62.95%) and *a+b* (58.88%). Content of chlorophyll *a/b* ranged from 4.06 (for NK Cooler) to 4.61 (for 4.61). However, values of coefficients of variation ranged from 0.76% (for Clarica) to 6.18% (for PR 39 K 13). Values of carotenoids ranged from 6.25 mg g^{-1} (for Clarica) to 9.87 mg g^{-1} (for NK Cooler). Coefficients of variation of content of carotenoids ranged from 2.85% (for SY Cooky) to 33.02% for SY Mascotte).

Environmental, genotypic and phenotypic variability

The largest estimates of genotypic variability coefficients (GCV) were observed for content of chlorophyll *b* (20.13%), however the smallest for the SPAD (6.08%). Large GCV indicates the presence of exploitable genetic variation for these traits. The environmental variation coefficient of variation (ECV) ranged from 3.535% (content of chlorophyll *a+b*) to 24.17% (content of chlorophyll *b*). The polygenic variation may be phenotypic, genotypic or environmental and the relative values of these three types of coefficients give an idea about magnitude of the variability (Nawab *et al.* 2008).

A narrow difference between GCV and PCVwas recorded for content of chlorophyll *a/b* (Table 2) and indicating that this trait is mostly governed by genetic factors with minimal environmental influence on the phenotypic expression of the trait. Hence, selection of this trait on the basis of the phenotypic value may be effective. On the contrary, a wide difference between GCV and PCVwas observed for SPAD, indicating higher influence of environment on the trait thereby reducing possible response to selection on phenotypic basis. GCV values only are not enough to determine the level of genetic variability among cultivars. Genetic variation could further be investigated with the help of heritability and genetic gain estimates. This measures the heritable portion of the total variation.

Heritability and genetic gain

The high heritability (h^2) and low genetic gain (GG) were observed for content of chlorophyllsa/b and b. High heritability and high genetic gain were observed for content of carotenoids. The smallest heritability and the largest genetic gain were observed for SPAD (Table 2). Similar results were found by Sunday *et al.* (2007) in work with rice seeds.

Correlations between observed traits

Table 3 shows a correlation matrix for the traits of maize cultivars. The significant positive correlations were observed between contents of: chlorophylls *a* and *b* (r=0.990), chlorophylls *a* and *a+b* (*r*=1.000), chlorophyll *a* and carotenoids (*r*=0.778), chlorophylls *b* and *a+b* (r=0.994), contents of chlorophyll *b* and carotenoids (r=0.807), contents of chlorophyll *a+b* and carotenoids (r=0.785) (Fig. 1). The significant negative correlations were observed between contents of: chlorophylls *a* and *a/b* (r=-0.678), chlorophylls *b* and *a/b* (r=-0.767), chlorophylls *a+b* and *a/b* (*r*=-0.698) as well as contents of chlorophyll *a/b* and carotenoids (*r*=-0.711) (Fig. 1).

Leaf chlorophyll content is correlated with photosynthetic activity indicators and leaf nitrogen concentra-

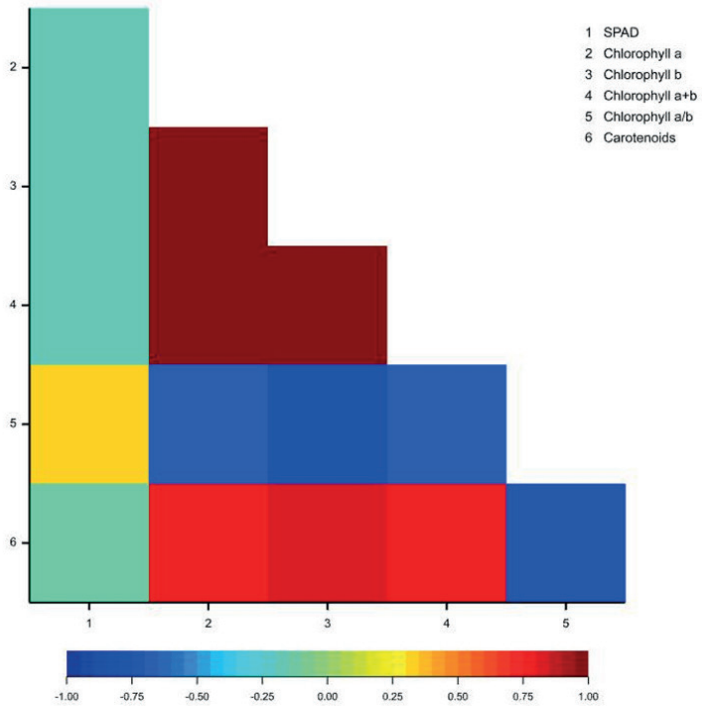

Fig. 1 - Correlation coefficients matrix for observed traits of maize (*Zea mays* L.)

tion, whereas chlorophyll fluorescence has been used successfully as a nondestructive and nonintrusive signal in plant biochemistry, physiology, and ecology (Lichtenthaler and Rinderle 1988; Krause and Weis 1991; Schreiber and Bilger1993; Govindjee1995). There is exists, an internecine record of evidence for a direct relationship between leaf photosynthetic rate and grain yield or biomass production (Sarkar *et al.* 1991). The evaluation of chlorophyll fluorescence, it may be a useful tool to evaluate the energy and metabolic balance of photosynthesis and the level of yields of various plant species under conditions of water deficit (Araus *et al.* 1998). A rapid and non-destructive method of evaluating plant nutritional status in terms of nitrogen is widely applied in agricultural practice (Shah *et al.* 2017). It is based on determining leaf greenness intensity with the use of a SPAD-502 optical apparatus (soil and plant analysis development) or an N-Tester (Hydro) (Uddling *et al.*, 2007). A study reported that maize yield was linearly correlated with the SPAD values and this effect was statistically significant (Rostami *et al.*, 2008). This linear relationship have suggested that the SPAD coefficient may be a tool to evaluate maize biomass yield intended for silage, but also grain yield. The chlorophyll content in the leaves is believed to be a predictor of the size of the yield. It is responsible for more than 98% of the variability in gross primary maize production (Gitelson *et al.* 2008). Chlorophyll content measurements allow to indirectly evaluate the potential absorption of infrared spectrum and the ability of leaves to remain green (Araus *et al.* 2008). The values of the latter index show a positive relationship with maize yield when photosynthetic energy is transported as a result of higher production. The SPAD index can also indicate a negative dependence with performance when energy is re-metabolized from chlorophyll. Ghimire *et al.* (2015) demonstrated that chlorophyll content, presented in the form of the SPAD index, was positively correlated with grain yield.

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