Pak. J. Bot., 49(3): 1017-1022, 2017.

THE DYNAMICS OF A DRY MATTER ACCUMULATION IN THE INITIAL PERIOD OF GROWTH OF FOUR VARIETIES OF THE "STAY-GREEN" TYPE OF MAIZE (*ZEA MAYS* L.)

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Abstract

The aim of the study was to determine the impact of weather conditions (temperature, precipitation) on the dynamics of dry matter accumulation and nitrogen nutritional status of maize plants in the type of „stay-green". Four varieties were evaluated: NK Cooler, Delitop, NK Gazelle, NK Ravello. Thermal conditions and humidity in the period from sowing to the phase of 5-6 leaves (BBCH 15/16) shaped the dynamics of dry matter accumulation and nitrogen nutritional status of plants. The differences were found in tested varieties of „stay-green" in terms of the dynamics of initial growth, expressed by the dynamics of dry matter accumulation and their nitrogen nutritional status. In most of the analyzed characteristics, the variety of NK Cooler was characterized by favorable values of these characteristics, as compared to other varieties. The genetic variation of tested varieties is derived from the heterosis cultivation process of F1 hybrids. Currently, cultivated maize varieties (including „stay-green") are F1 hybrids characterized by identical genotype and varietal differences arise from components of parental hybrid genotype (paternal and maternal), as presented in the paper.

Key words: *Zea mays* L., Stay-green, Temperature, Rainfall, Chlorophyll, Nitrates, Nitrate reductase, Initial growth.

Introduction

The basic requirement for acquisition of high yield, simultaneously characterized by high quality, is proper nutrition of the maize throughout the vegetation season (Szulc & Bocianowski, 2011, 2012). In order to get the yield-production potential of this species fully manifested, nutritive components must be applied in such a way that their uptake proceeds corresponding with the rhythm of the plant's growth (Costa *et al*., 2002). Deficiency in any nutrient contributes to bringing about an array of harmful disturbances in the plant's metabolism, which makes the yielding potential impossible to wholly develop.

Of the nutritive components regarded as indispensable for the processes of growth and development to take the proper course, nitrogen is the most important. Deficiency in this particular macroelement is responsible for a decline of the stoma conductivity in leaves, which, consequently, reduces the intensity of photosynthesis. Moreover, a nitrogen deficit lowers the plant's ability to assimilate CO_2 (Zhao *et al*., 2005). Also, Makino & Osmond (1991) maintain that yielding by plants depends on efficient photosynthetic process and distribution of assimilates, among which the key role is that performed by nitrogen.

The dynamics of the developmental processes occurring in a plant is substantially influenced by temperature, water and nitrogen content in the soil. Any disturbance in the composition of these factors results in unsettling of the balance in living activities of a plant, becoming a cause of stress. Under conditions of water deficiency in the soil, functional and structural changes start to occur in the photosynthetic apparatus, the effect of which is hindering of the growth and productivity of plants (Jaing *et al*., 2009). Further, nitrogen undernutrition of the maize at the initial phase of its vegetation period disturbs the processes of formation of leaves, spadices and spadix structure components. These consequences of nitrogen deficiency appear very early, i.e., at the stage of the 8^{th} leaf (Subedi & Ma, 2005). Slowing down of the initial growth due to too low soil temperatures during the maize vegetation season follows a decrease in the uptake of water and nutrients (Kruczek & Szulc, 2006).

The hypothesis of the conducted experiment assumed that maize hybrids falling within the group of "stay-green" cultivars may be characterized by diverse susceptibility to thermal and moisture conditions during the initial phase of development, which, in turn, can decide about varied accumulation of dry matter (thus growth dynamics) and different level of nitrogen nutrition in this period.

The purpose of the field research pursued was to define the dynamics of the initial growth expressed in terms of accumulation of dry matter and juvenile maize plants nutrition with nitrogen in diverse thermal and moisture conditions within a group of cultivars representing the stay-green (SG) type.

Material and Methods

The field experiments were carried out at the Department of Agronomy, Poznań University of Life Sciences, in Swadzim in 2010–2013. The impact of temperature and totalled precipitation on the dynamics of the initial growth expressed as accumulation of dry matter was determined for four maize cultivars of the SG type. The cultivars examined were as follows: NK Ravello (FAO

210), NK Gazelle (FAO 220-230), NK Cooler (FAO 240) and Delitop (FAO 240). The experiment was established in randomized block design, in four repetitions, on typical grey-brown podzolic soil, deriving from shallow coarse sandy soil on light loam. Within the whole experimental field the same level of fertilization with nitrogen, phosphorus and potassium was applied at the amounts of: 100 kg $N \cdot ha^{-1}$ in the form of urea, 80 kg $P_2O_5 \cdot ha^{-1}$ in the form of polifoska 6 and 120 kg $K_2O \cdot ha^{-1}$ in the form of 60% potash salt. Size of the fields was 30.8 m^2 (2.8×1.0 m), while the area of the field under observation was 15.4 m^2 (after excluding outermost seeding rows).

The soil fertility in phosphorus, potassium and magnesium in particular years of study reached the following levels: 2010 – 88 mg $P \cdot kg^{-1}$ of soil, 116 mg $K \cdot kg^{-1}$ of soil, 41 mg $Mg \cdot kg^{-1}$ of soil; 2011–89 mg $P \cdot kg^{-1}$ of soil, 110 mg $K \cdot kg^{-1}$ of soil, 40 mg $Mg \cdot kg^{-1}$ of soil; 2012–129 mg $P \cdot kg^{-1}$ of soil, 64 mg $K \cdot kg^{-1}$ of soil, 79 mg $Mg \cdot kg^{-1}$ of soil; 2013–75 mg $P \cdot kg^{-1}$ of soil, 106 mg $K \cdot kg^{-1}$ of soil, 39 mg $Mg \cdot kg^{-1}$ of soil.

The experimental field in growing seasons was not rainy. The water derived from natural rainfall which actual values are shown in Table 1.

Table 1. Weather conditions in the period from sowing to the 5-6 leaf stage.

	Year			
	2010	**2011**	**2012**	**2013**
Amount of precipitation [mm]	142.3	21.3	89.8	45.9
Mean air temperature [°C]	13.9	14.8	13.5	12.6
Mean soil temperature at a depth of 10 cm [°C]	10.5	11.4	11.8	10.9

At the phase of 5–6 leaves (BBCH 15–16) plant samples (10 plants) were taken from two medial rows of each plot, which was followed by separation of the roots from the above-ground part. After plant drying, dry matter content and dry matter of a single plant were determined.

The nitrogen content in dry matter was assessed using the Kjeldahl method with the device Kjeltec™ 2200 FOSS.

The methodology for estimation of the content of chlorophyll *a*, *b*, *a+b*, of carotenoids and the chlorophyll expressed in SPAD units is available in an earlier (Szulc, 2009).

Determination of the nitrate content and of the nitrate reductase activity (Szulc *et al*., 2012ba) was performed at the phase of 5–6 leaves, exclusively during the first two years of investigations (2010 and 2011).

Procedure of determination of the nitrate content: 250 mg of plant sample (fresh basis) was cut into small pieces, then 20 cm^3 of distilled water was added, and the whole was cooked for 20 minutes. At the second step of the extraction procedure, the cooled extract was spin-dried at 4,000 g for 15 minutes. The concentration of nitrates in the obtained supernatant was determined by the method of Cataldo *et al.* (1975). Next, 0.4 cm^3 of 5% salicylic acid in concentrated H_2SO_4 was added to 0.1 cm^3 of the supernatant and left for 20 minutes, which was followed by addition of 9.5 cm^3 of 2 M NaOH and the samples were cooled at room temperature. Absorbance was measured at 410 nm. The results were expressed as mg of $NO_3^- \, g^{-1}$ of tissue.

Procedure of determination of the nitrate reductase activity (NR): The method described by Jaworski (1971) was employed: plant samples (200 mg) were placed in flasks, then 5 cm^3 of an incubation mixture containing nitrates was added and the whole was left for 1 hour at a temperature of 30°C. At the second step of the procedure, 1 cm^3 of SSA (1% sulphanilamide in 1 M HCl) and 1 cm^3 of NED [0.01% N-(l-naphthyl) ethylenediamine dihydrochloride] were added to 1 cm^3 of the extracted solution and subsequently the mixture was left for 15 minutes. Absorbance of the prepared mixture was measured at 540 nm. The activity of nitrate reductase was expressed as nmole of NO_2^- per 1 g of fresh leaf matter per 1 hour.

Statistical analysis: Two-way ANOVA were carried out to determine the effects of years, cultivars and years × cultivars interaction on the variability of chlorophyll *a*, *b*, *a+b*, carotenoids, SPAD chlorophyll, nitrates, reductase, dry matter from a single plant and nitrogen uptake. One-way ANOVA were performed to determine the effects of cultivars on the variability of nitrogen content. Mean values and least significant differences (LSDs) were calculated. Homogeneous groups were determined on the basis of LSDs. The influence on the studied traits of the total amount of precipitation during the period between sowing and the phase of 5–6 leaves phase, mean air temperature from the sowing season to the 5–6 leaves phase, average soil temperature at a depth of 10 cm in the span from sowing to the 5–6 leaves stage was estimated with the use of regression analysis.Data analysis was performed with the statistical package *GenStat 15*.

Results and Discussion

The knowledge of thermal and moisture requirements of the maize allows, to a certain extent, to execute cultivation of the species irrespective of the fluctuations in weather conditions. All living processes depend to a very substantial degree on the temperature of the soil as well as that of the air, and at low values of these environmental components the particular phases of the maize plants development are decidedly delayed (Szulc *et al*., 2012b).

The greatest amount of total precipitation was characteristic of 2010 (142.3 mm), whereas the lowest was recorded for 2011 (21.3 mm). The average daily air temperature reached the highest value in 2011 (14.8°C), being the lowest in 2013 (12.6°C). Further, an analysis of the soil temperature at the depth of 10 cm reveals that the lowest value of this parameter characterized the first and the last study year (Table 1). In general, the most favourable conditions for the growth and development of the maize during the span from the sowing season until the attainment by the plants of the phase of 5–6 leaves prevailed in the years 2011 and 2012, whereas the worst were in the first and last year of investigations.

The results of ANOVA indicate that the main effects of years were significant for all the traits in the study, except for nitrates (chlorophyll *a*: $F_{3,30}$=8.21, P<0.001; chlorophyll *b*: $F_{3,30}$=7.78, P<0.001; chlorophyll *a+b*: $F_{3,30}$=8.22, P<0.001; carotenoids: $F_{3,30}$=34.75, P<0.001; SPAD: $F_{3,30}$=111.18, P<0.001; nitrates: $F_{1,7}$=2.39, P=0.166; reductase: $F_{1,7}$=15.03, P=0.006; dry matter from a single plant: $F_{3,45}$=6.83, P<0.001; nitrogen uptake: $F_{3,45}$=7.49, P<0.001). The main effects of cultivars were significant for all the traits, except for chlorophyll *a*, nitrates and dry matter from a single plant (chlorophyll *a*: $F_{3,30}$=2.83, P=0.055; chlorophyll *b*: $F_{3,30}$=4.63, P=0.009; chlorophyll *a+b*: $F_{3,30}$=3.22, P=0.037; carotenoids: $F_{3,30}$=9.29, P<0.001; SPAD: $F_{3,30}$=31.38, P<0.001; nitrates: $F_{3,7}$=0.05, P=0.982; reductase: $F_{3,7}$=32.86, P<0.001; dry matter from a single plant: $F_{3,45}$=2.62, P=0.063; nitrogen uptake: $F_{3,45}$=3.27, P=0.030; nitrogen content: $F_{3,12}$=3.65, P=0.044). The year×cultivar interactions were significant for chlorophyll*a* ($F_{9,30}$=2.95, P=0.012), chlorophyll *b* ($F_{9,30}$=3.26, P=0.007), chlorophyll *a+b* ($F_{9,30}$=3.06, P=0.010) and SPAD ($F_{9,30}$=4.68, P<0.001), although they were not significant for carotenoids ($F_{9,30}$=1.26, P=0.296), nitrates ($F_{3,7}$=3.95, P=0.061), reductase ($F_{3,7}$=2.44, P=0.150), dry matter from a single plant ($F_{9,45}$=1.10, P=0.379) or nitrogen uptake ($F_{9,45}$=1.10, P=0.382).

The highest values of the discussed traits were obtained in 2011 (1.74 g, 68.11 mg N plant^{-1}) and 2012 (1.82 g, 80.44 mg N plant^{-1}) (Tables 2 and 3). The lowest values of dry matter from a single plant and nitrogen uptake were characterized the maize in 2010 (respectively 1.38 g and 60.27 mg N plant^{-1}) and 2013 (respectively 1.41 g and mg 59.52 N plant^{-1}). These particular years were distinguishable by the lowest mean daily air temperature and soil temperature at the depth of 10 cm in the span from sowing till the production by the maize of 5–6 leaves (Table 1). Averagely for years, the highest vigour of initial growth was characteristic of hybrid NK Cooler (respectively 1.78 g and 74.56 mg N plant^{-1}), whereas the lowest was typical of cultivar NK Ravello (1.45 g, 59.29 mg N plant^{-1}). In the synthetic approach, the most substantial nitrogen content in dry matter was characteristic of cultivar Delitop (43.54 g kg^{-1}), whereas the lowest – of hybrid NK Ravello (40.58 g kg^{-1}) (Fig. 1).

Unbiased assessment of nitrogen nutrition status is very important due to effectiveness of plant production and quality of obtained raw materials as well as due to growing need of protection of agricultural ecosystems against their eutrophication (Szulc 2013). Each method for assessment of the state of nitrogen nutrition of plants–in order to be widely applied in practice–has to be fast, easy to implement and able to correctly predict the needs of a plant pertaining to a particular nutrient (Szulc & Rybus-Zając, 2009). For this purpose, the contents of chlorophyll *a*, *b*, *a+b*, carotenoids, chlorophyll defined in SPAD units, nitrate content and the activity of nitrate reductase (NR) have been determined in the present study. The chlorophyll content depends on the state of the plants having been provided with nutrients (i.e. nutrition), first of all with nitrogen and sulphurus, and further with magnesium and potassium (Neukirchen & Lammel 2002). The highest values of the contents of chlorophyll *a*, *b*, *a+b*, carotenoids and chlorophyll expressed in SPAD units at the phase of 5–6 leaves have been recorded for the second and third years of research, whereas the lowest for the first and last (Table 4, Figs. 2b, 3b). The varied amount of pigments in particular years was most probably linked with the weather conditions. Year 2010, characterized by the highest level of precipitation and low temperature of the soil and air, contributed to a decline in the synthesis of chloroplast pigments. Averaged for the years, the statistically largest–as compared with the remaining cultivars under study–contents of chlorophyll *a+b* and carotenoids were found for hybrid NK Cooler, whereas cultivars NK Cooler and NK Gazelle were characterized of the highest content of the chlorophyll expressed in SPAD units (Table 4, Figs. 2a, 3a). Maize cultivars exhibit differences in relative chlorophyll content because it is a genetic trait. It explains the result obtained in own research.

Table 2. Mean values (and standard deviations) of dry matter of a single plant in the 5-6 leaf stage [g].

Cultivar	Year				Mean
	2010	2011	2012	2013	
NK Cooler	1.47 (0.35)	2.18 (0.61)	1.94 (0.30)	1.53 (0.26)	1.78 (0.47)
Delitop	1.35 (0.27)	1.73 (0.73)	1.94 (0.38)	1.33 (0.15)	1.59 (0.48)
NK Gazelle	1.33 (0.12)	1.80 (0.37)	1.64 (0.35)	1.39 (0.16)	1.54 (0.31)
NK Ravello	1.38 (0.32)	1.26 (0.23)	1.76 (0.40)	1.40 (0.30)	1.45 (0.34)
$LSD_{0.05}$	n.s.	0.602	n.s.	n.s.	0.213
Mean	1.38 (0.26)	1.74 (0.58)	1.82 (0.35)	1.41 (0.22)	1.59

n.s. – Not significant differences

Table 3. Mean values (and standard deviations) of nitrogen uptake with dry matter of maize in the 5-6 leaf stage (mg plant^{-1}).

Cultivar	Year				Mean
	2010	2011	2012	2013	
NK Cooler	65.46 (15.56)	82.34 (23.20)	85.97 (13.37)	64.49 (10.85)	74.56 (17.76)
Delitop	61.24 (12.43)	69.10 (28.94)	88.40 (17.18)	57.90 (6.55)	69.16 (20.37)
NK Gazelle	55.72 (4.92)	74.67 (15.15)	72.01 (15.21)	58.86 (6.71)	65.31 (13.30)
NK Ravello	58.66 (13.66)	46.33 (8.43)	75.38 (16.94)	56.81 (12.32)	59.29 (15.96)
$LSD_{0.05}$	n.s.	23.471	n.s.	n.s.	8.826
Mean	60.27 (11.62)	68.11 (22.97)	80.44 (15.79)	59.52 (8.99)	67.08

n.s. – Not significant differences

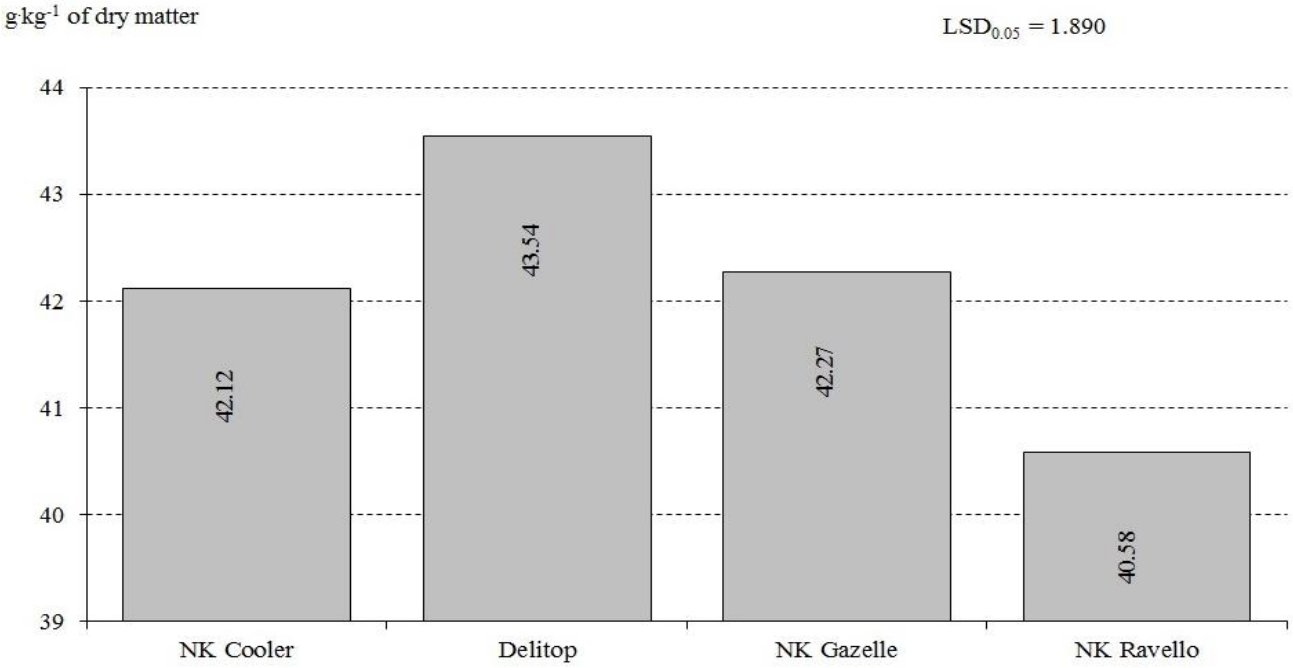

Fig. 1. Content of nitrogen in dry matter of plant in the 5-6 leaf stage – 2010-2013

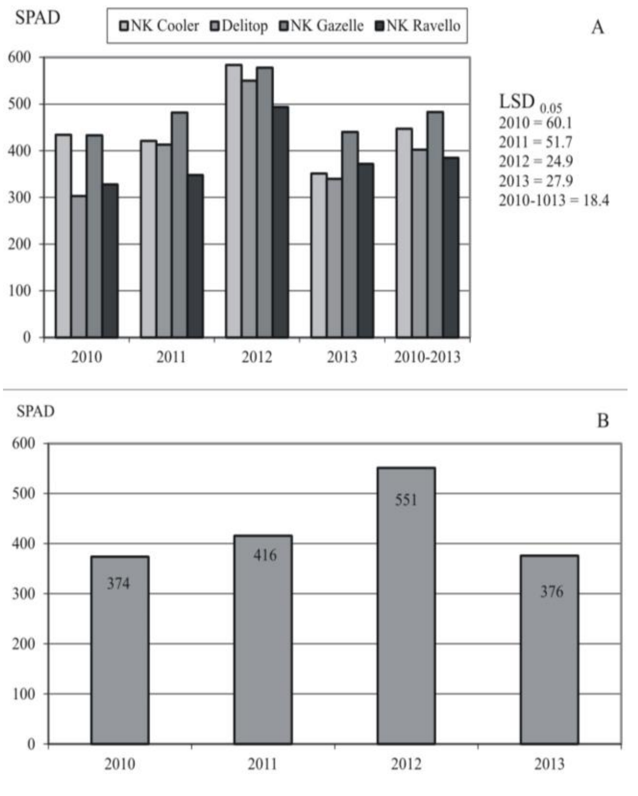

Fig. 2. Content of chlorophyll expressed as SPAD units in the 5-6 leaf stage (A), (B) – mean for years irrespective of cultivar.

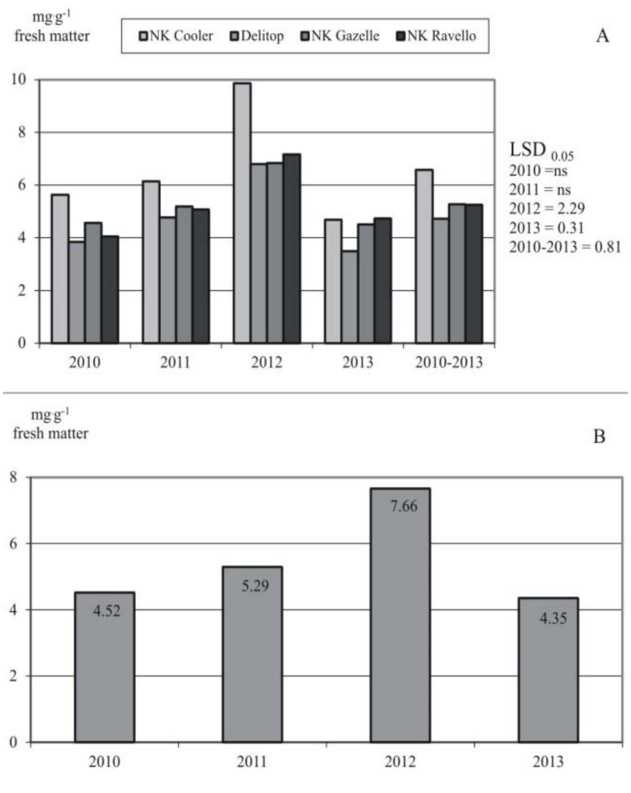

Fig. 3. Content of carotenoids in the 5-6 leaf stage (A), (B) – mean for years irrespective of cultivar.

Table 4. Mean values of chlorophyll *a*, *b* and *a*+*b* content in the 5-6 leaf stage.

Cultivar	Year												Mean		
	2010			2011			2012			2013					
	a	*b*	*a+b*	*a*	*b*	*a+b*	*a*	*b*	*a+b*	*a*	*b*	*a+b*	*a*	*b*	*a+b*
NK Cooler	1.4	0.35	1.79	1.61	0.36	1.98	2.26	0.56	2.83	1.38	0.30	1.68	1.67	0.39	2.07
Delitop	1.16	0.27	1.43	1.48	0.31	1.79	1.88	0.44	2.32	1.01	0.20	1.22	1.38	0.31	1.69
NK Gazelle	1.50	0.34	1.84	1.62	0.34	1.97	1.28	0.28	1.58	1.43	0.31	1.75	1.46	0.32	1.78
NK Ravello	1.45	0.34	1.80	1.77	0.39	2.17	1.55	0.35	1.91	1.32	0.33	1.65	1.52	0.35	1.88
$LSD_{0.05}$	ns	ns	ns	ns	ns	ns	ns	ns	ns	0.21	ns	0.299	ns	ns	0.29
Mean	1.38	0.32	1.72	1.62	0.35	1.98	1.74	0.41	2.16	1.29	0.29	1.58	1.51	0.34	1.86

ns = Not significant differences

Table 5. The influence of amount of precipitation in the period sowing, mean air temperature in the period sowing, mean soil temperature at a depth of 10 cm in the period sowing on the examined traits.

Source of variation	d.f.	Mean squares							
		N uptake	Dry matter of a single plant	Chlorophyll *a*	Chlorophyll *b*	Chlorophyll *a+b*	Carotenoids	SPAD	N content
Model	3	377.34*	0.202*	0.197*	0.011	0.295*	9.325**	27968**	0.213*
Residual	12	83.15	0.044	0.063	0.005	0.102	0.877	2858	0.025
Total	15	141.99	0.075	0.086	0.006	0.136	2.567	7880	0.063
Variate		**Estimate of partial regression coefficients**							
Regression constant		-156.1*	-3.52*	-3.75	-0.932	-4.69	-28.13**	-1337**	4.00**
Amount of precipitation		0.079	0.0003	0.001	0.001	0.002	0.015*	0.815*	0.004**
Mean air temperature		0.50	0.071	0.086	0.015	0.101	-0.085	-10.4	-0.141*
Mean soil temperature at a depth of 10 cm		18.87**	0.370**	0.356*	0.092*	0.450*	3.013***	165.9***	0.165
$R^2 \cdot 100$		41.4	41.9	26.6	18.9	24.8	65.8	63.7	59.8

*, **, *** - p<0.05, 0.01, 0.001

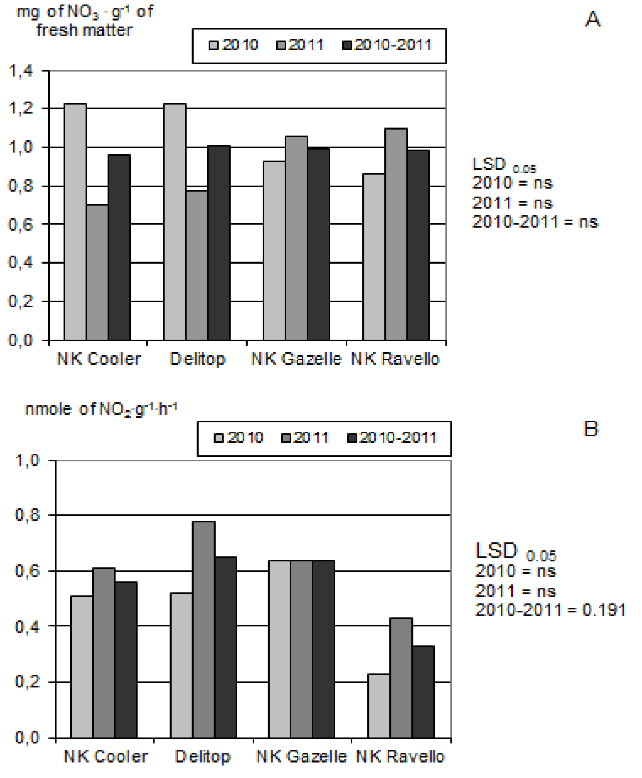

Fig. 4. Content of nitrates (A) and activity of nitrate reductase(NR) (B).

Determination of the content of nitrates is a very useful method for assessment of the level of nutrition of the maize with nitrogen (Geyer & Marschnera, 1990). However, own research has revealed no significant effect of the genotypes or years on the nitrate content in the maize plants at the phase of 5–6 leaves (Fig. 4a). Concerning the nitrate reductase (NR) in the synthetic approach, cultivar NK Ravello has been found to be characterized by the significantly lowest activity of this enzyme, which has also been confirmed by the lowest content of nitrogen in dry matter of its representative in comparison with the other cultivars under analysis (Fig. 1).

The N uptake, dry matter from a single plant, the content of chlorophyll *a*, content of chlorophyll *b* and content of chlorophyll *a+b* were determined directly proportionally by the mean temperature of the soil at a depth of 10 cm in the span from sowing to the phase of 5–6 leaves (Table 5). The carotenoids and SPAD chlorophyll were found to be significantly statistically influenced by the total precipitation within the period from sowing till the 5–6 leaves phase and mean soil temperature at a depth of 10 cm in the spell from sowing to the phase of 5–6 leaves (Table 5). The content of nitrogen was determined directly proportionally by the sum of precipitation in the span from sowing till the 5–6 leaves phase and reversely proportionally by the mean air temperature in the period from sowing to the phase of 5–6 leaves (Table 5). The obtained proportions of the variability accounted for (expressed by R^2) ranged from 18.9% (for the content of chlorophyll *b*) to 65.8% (for carotenoids) and justify the usefulness of the acquired data for programmes of the maize *Zea mays* L. breeding.

Conclusions

1. The cultivars under analysis have been found genetically diversified in respect of the dynamics of initial growth expressed as accumulation of dry matter and the state of plants nutrition with nitrogen.
2. The diversification of the values pertaining to the majority of studied traits (N uptake, dry matter from a single plant, chlorophyll *a* content, chlorophyll *b* content and content of chlorophyll *a+b*) was determined directly proportionally by the mean temperature of the soil at a depth of 10 cm in the sowing season. This proves the great significance of the soil temperature in the shaping of the values of the traits mentioned.
3. A relationship between content of nitrates and activity of nitrate reductase was demonstrated in the examined cultivars. Relatively low activity of the enzyme in NK Ravello cultivar could have resulted from disturbed stability of the enzymatic protein.
4. Genetic diversity of tested varieties in the type of „stay-green" that determine the dynamics of dry matter accumulation and nitrogen nutritional status in the initial period of growth was derived from the cultivation process of heterosis hybrids.

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(Received for publication 11 April 2016)