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# INFLUENCE OF SOIL SUPPLEMENTATION WITH NITROGEN AND MAGNESIUM ON THE SIZE OF ASSIMILATION AREA OF MAIZE CULTIVARS (*ZEA MAYS* L.) DIFFERING IN GENETIC PROFILE

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## ABSTRACT

Measurements of maize leaves aim at assessing the photosynthetic capacity of a given species and estimating the impact of habitat and agronomic factors on the size of photosynthetic capacity. The paper presents the results of three-year field experiments whose purpose was to analyze the growth of active organs in the process of photosynthesis of two different maize hybrid types, depending on dose of nitrogen and magnesium in the cob flowering stage. The assessment of the leaf area growth was made based on the assimilation area of a single plant and specific leaf area, leaf weight fraction, stem and ear weight fraction, leaf area index. We found that the "stay-green" hybrid developed fewer leaves per plant, while at the same time their weight increased, compared to the traditional variety. The hybrid ES Paroli SG was characterized by a greater total leaf blade area per plant and LAI.

**Key words:** maize, stay-green, nitrogen, magnesium, acid invertase

## ABBREVIATIONS

SLA – specific leaf area, LWF – leaf weight fraction, SWF – stem weight fraction, EWF – ear weight fraction, LAI – leaf area index, AI – acid invertase

## INTRODUCTION

Grzebisz (2008) defines the growth and development of plants as a sequence of genetically controlled processes of division, growth and differentiation of cells, ensuring that every single organism produces tissues and organs necessary to perform basic life functions. Hence, in order to fully exploit the yield potential of maize, carried in its genotype (seed), it is necessary to follow the recommendations of good agricultural practice. According Machul (1995), the normal development of maize plants requires appropriate agricultural technology that ensures a maximum photosynthetic productivity in the period of intensive growth, helping to achieve a high yield of good quality.

Because of its origin, maize belongs to the thermophilic plants. Hence, during growth and development, in addition to proper agricultural technology, it requires more heat than other plants (Kim et al., 2007; Szulc et al., 2011).

Plant productivity is dependent on the so-called photosynthetic production which is the amount of assimilates produced by photosynthesis. It determines to a large extent the biological crop of plants, i.e. the potential to produce fresh or dry weight per unit area of soil (Kopcewicz and Lewaka, 2002). In addition to the normal photosynthesis, characteristic of most species in our climatic zone, at higher air temperatures (above 25°C) and high light intensity, maize starts to assimilate $C_4$, doubling the efficiency of photosynthesis. In one series, it joins two molecules of carbon dioxide (Pengelly et al., 2011). The $C_4$ photosynthesis is an evolutionarily developed adaptive mechanism, occurring mainly in plants inhabiting areas of high temperatures and water scarcity, when $CO_2$ limits the process of photosynthesis. For this purpose, the plant is equipped with PEP carboxylase which has a 10-fold higher affinity for $CO_2$. Owing to that, $C_4$ plants are observed to intensely collect $CO_2$ as soon as the content of this component in the atmosphere is about 0.001% (Kączkowski, 1992). $C_4$ plants, including maize, have therefore an adaptive advantage over $C_3$ plants, as the loss resulting from photorespiration in $C_3$ plants raises with increasing temperature (Matsuoka et al., 1998; Tausta et al., 2002). As a result, maize uses light radiation very efficiently and thus high air temperature, which translates into large increases in leaf area, the main photosynthetic apparatus (Gołębiewska and Sekutowski, 2007). Although photosynthesis changes dramatically under the influence of environmental conditions, it is genetically determined and is characteristic of plant species and varieties (Wojcieska-Wyskupajtys, 1996). Cultivars characterized by very intense photosynthesis very often produce lower yields than varieties showing a lower intensity of the process. This follows from the fact that in biomass production, it is not only the intensity of photosynthesis that plays an important role, but also the size of assimilation area (of leaves), in particular life expectancy and the period of assimilation organs' capacity for efficient photosynthesis (Wojcieska-Wyskupajtys, 1996).

The experiment hypothesis assumed that agronomic factors such as nitrogen and magnesium fertilization or a hybrid type (hybrids of different rates of leaf aging, Fig. 1) can significantly shape the growth of the photosynthetic apparatus and consequently the size of the resulting biological yield. Therefore, the purpose of the field studies was to evaluate the analysis of active organ growth in the process of photosynthesis of two different maize variety types depending on the dose of nitrogen and magnesium.

ES Palazzo ES Paroli SG

Fig. 1. The difference in the appearance of two types of maize in adolescence (modified by Szulc, 2013)

## MATERIAL AND METHODS

### Experimental field

The field experiment was performed at the Department of Agronomy of the Poznań University of Life Sciences, in the fields of the Experimental and Didactic Station in Swadzim (52°26'N; 16°45'E) in 2009–2011. It was conducted in a split-plot×split-block design with three research factors in four field replications. The experiment studied the effect of four doses of urea $CO(NH_2)_2$ – ($N_1$-0, $N_2$-50, $N_3$-100, $N_4$-150 kg $N \cdot ha^{-1}$) and two doses of magnesium ($Mg_1$-0, $Mg_2$-25 kg MgO $ha^{-1}$ in the form of kieserite [25% MgO, 50% $SO_3$ – 20% S, sulfur and sulfate]) on active organ growth in the process of photosynthesis of two types of maize varieties – (ES Palazzo [FAO 230-240] and ES Paroli [FAO 250] of the "stay-green" type – SG). Phosphorus at a dose of 80 kg $P_2O_5 \cdot ha^{-1}$ was used in the form of granular triple superphosphate 46% $P_2O_5$, and potassium at a dose of 120 kg $K_2O \cdot ha^{-1}$ as potassium salt 60% $K_2O$. Nitrogen was applied according to the scheme of the experiment. Nitrogen, phosphorus and potassium fertilizers as well as kieserite were applied before sowing maize. Tillage and other elements of agricultural technology were implemented according to maize cultivation recommendations in seed technology. Soil under study according to the FAO classification was an Albic Luvisol, originated from loam sands, lying on sandy loam. Accordingly to the Polish agronomic evaluation, this soil represents rye good complex.

The macronutrient content and pH of the soil was determined each year before the experiment (Table 1) in accordance with the research procedure/norm of the Regional Agrochemical Station in Poznań: $P_2O_5$ – PB.64 ed. 6 of 10.17.2008; $K_2O$ – PB.64 ed. 6 of 17.10.2008; Mg – PB.65 ed. 6 of 17.10.2008; pH – PB.63 ed. 6 of 17.10. 2008.

Table 1. Soil conditions in Swadzim

| Specification | Year | | |
|---|---|---|---|
| | 2009 | 2010 | 2011 |
| P, mg $P \cdot kg^{-1}$ of soil | 36.1 | 37.8 | 61.2 |
| K, mg $K \cdot kg^{-1}$ of soil | 121.2 | 97.1 | 54.8 |
| Mg, mg $Mg \cdot kg^{-1}$ of soil | 44.0 | 40.0 | 81.0 |
| pH in 1 $mol \cdot dm^{-3}$ KCl | 5.2 | 5.5 | 5.1 |

### Thermal and humidity conditions

Thermal and humidity conditions during the growing season of maize in the period from sowing to the cob flowering phase (BBCH 67) are presented in Table 2. The influence of the thermal and humidity factor is best described in a comprehensive manner by Sielianinov's hydrothermal coefficient in water security [K] (Molga, 1986):

$$K = \frac{10 \cdot \text{monthly total precipitation [mm]}}{\text{number of days} \cdot \text{average daily air temperature in a month [°C]}}$$

Table 2. Meteorological conditions during the research

| Months | Years | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 2009 | | | 2010 | | | 2011 | | |
| | T | O | S | T | O | S | T | O | S |
| IV | 12.9 | 19.2 | 0.49 | 9.3 | 26.8 | 0.96 | 12.4 | 9.8 | 0.26 |
| V | 14.0 | 109.9 | 2.53 | 12.2 | 110.5 | 2.92 | 15.5 | 22.5 | 0.46 |
| VI | 16.0 | 113.8 | 2.37 | 18.4 | 43.4 | 0.78 | 19.9 | 66.5 | 1.11 |
| VII | 20.3 | 75.4 | 1.19 | 22.6 | 97.5 | 1.39 | 18.5 | 218.7 | 3.81 |

T – average monthly air temperature (°C), O – total monthly precipitation (mm),
S – Sielianinov's hydrothermal coefficient (Molga, 1986)

The optimal value of this coefficient is 1. Values below 1 show a drought period, while over 1 – a period of relative humidity. The worst conditions for the emergence of maize occurred in April 2011 (K = 0.26), while close to optimal – in April 2010 (K = 0.96). The value of coefficient K < 1 occurred in June 2010 and May 2011. In the cob flowering phase in 2011, the value of hydrothermal coefficient in water security (K) was 3.81. In addition, it should be noted that in April 2011 ground frost occurred, causing the emerging maize plants to freeze.

The plant material

Samples for analysis were collected each year at the cob flowering stage (BBCH 67). Each experimental plot consisted of four rows. Samples for analysis were taken from two middle rows of each plot, treating the outer rows as the so called sowings (isolation).

Assimilation surface area of a single plant was calculated on the basis of the formula given in the work of Borowiecki and Filipiak (1992):

$$PL = -3.550 + 3.774 \cdot x,$$

where:
PL means assimilation surface area of a single plant, x – sum of the surface areas of the fifth and sixth leaf.

The surface area of the 5$^{th}$ and 6$^{th}$ leaf was determined using Montgomery's (quoted after Borowiecki and Filipiak, 1992): leaf length along the main nerve multiplied by the width determined in the widest place and the result multiplied by 0.75 coefficient.

$$SLA = LA / LW,$$

where:
SLA – specific leaf area ($cm^2 \cdot g^{-1}$), LA – leaf area ($cm^2$), LW – leaf blade weight (g).

$$WF = (W/TW) \cdot 100\ [\%],$$

where:
WF – weight fraction of leaves (LWF), stems (SWF), ears (EWF) – [in %], W – weight of leaves, or stalks, or cobs (g), TW – total plant weight (g).

$$LAI = (LA \cdot LR) / 10000,$$

where:
LAI – leaf area index, LA – leaf area of a single plant ($cm^2$), LR – plant density per 1 $m^2$ (plants$\cdot m^{-2}$).

**Procedure of determination of acid invertase (AI) activity**: the modified method by Copeland and Lea (1990) was used. The sample (400 g) was homogenized in 1.6 $cm^3$ of 50 mM sodium phosphate buffer, pH 7.4, containing 10 µl of mercaptoethanol. The obtained homogenate was than spin-dried for 20 minutes at 15.000 rpm. Next, the AI activity was determined in the obtained supernatant. The reaction mixture contained 0.18 $cm^3$ of supernatant and 0.57 $cm^3$ of acetate buffer, pH 5.0, with sucrose. The tubes with the reaction mixture were incubated in water bath at a temperature of 30°C for 45 minutes; then Tricine buffer was added to each tube and the mixture was boiled at 100°C for 3 minutes. Absorbance of the obtained solution was measured on a spectrophotometer at 560 nm. The results were expressed as absorbance per 1 g of tissue and protein.

**Statistical analysis**

Firstly, the normality of distributions for studied traits were tested using the Shapiro-Wilk normality test (Shapiro and Wilk, 1965). A four-way analysis of variance (ANOVA) was performed to verify the hypothesis of lack of effects of years, nitrogen doses, magnesium doses and a variety type and hypotheses about a lack of interaction between: years × nitrogen doses, years × magnesium doses, years × hybrid, nitrogen doses × magnesium doses, nitrogen doses × hybrid, magnesium doses × hybrid, years × nitrogen doses × magnesium doses, years × nitrogen doses × hybrid, years × magnesium doses × hybrid, nitrogen doses × magnesium doses × hybrid, years × nitrogen doses × magnesium doses × hybrid per number of leaves from a plant, fresh weight of leaf blades from a plant, assimilation area of a single plant, SLA, LWF, SWF, EWF, LAI and acid invertase activity (absorbance per gram of fresh weight and absorbance per milligram of protein). Mean values and standard deviations of individual characteristics were calculated. Least significant difference values were calculated for individual characteristics and on this basis homogeneous groups were determined. All calculations for data analysis were performed using a statistical package GenStat v. 7.1 (Payne et al., 2003).

## RESULTS

All studied traits have a normal distribution. The results indicate the impact of weather conditions varied between years on the number of leaves developed by a single maize plant ($P < 0.001$) (Table 3). On average for the years, the number of leaves developed by a single plant was the lowest in 2010 (10.05 pcs.), while the largest – in 2009 (11.26 pcs.) (Table 4). This is due to a statistically significant effect of interaction between years × variety type ($P = 0.041$) on the value of this characteristic. A maize hybrid type significantly determined the value of this feature in 2009 and 2011 ($P < 0.001$) (Table 3). In both years, ES Paroli SG was found to develop significantly fewer leaves than the hybrid ES Palazzo (Table 4). In 2010, it was found (the value of the feature was statistically at the same level) that the "stay-green" type tended to develop smaller leaf blades, compared to the other variety tested. Furthermore, it should be noted that the demonstrated effect of the variety factor, although modified in the period, was so strong that its influence in the synthetic statement was statistically proven ($P < 0.001$). On average, in the years, a single plant of the hybrid ES Paroli SG developed significantly fewer leaf blades (by 0.32 pcs.) than the cultivar ES Palazzo (Table 4).

Table 3. Mean squares from four-way analysis of variance for observed traits of maize (*Zea mays* L.)

| Source of variation | d.f. | Number of leaves | Leaf weight | ASA | SLA | LWF | SWF | EWF | LAI |
|---|---|---|---|---|---|---|---|---|---|
| Blocks | 3 | 0.02 | 1275.6 | 4947745 | 454.36 | 3.34 | 4.65 | 4.51 | 2.412 |
| N | 3 | 0.33 | 3764.3** | 1353987*** | 78.13 | 0.08 | 5.78 | 5.16 | 0.255* |
| Residual 1 | 9 | 0.18 | 394.7 | 95204 | 35.3 | 1.95 | 13.58 | 19.33 | 0.057 |
| MgO | 1 | 0.01 | 19.9 | 30517 | 7.71 | 0.5 | 1.37 | 0.22 | 0.002 |
| N × MgO | 3 | 0.32 | 202.3 | 29333 | 27.99 | 0.42 | 8.33 | 6.46 | 0.021 |
| Residual 2 | 12 | 0.16 | 111.1 | 56748 | 17.06 | 0.85 | 12.36 | 12.15 | 0.033 |
| Hybrid | 1 | 5.01*** | 1696.7*** | 3793578*** | 30.34 | 179.71*** | 8.75 | 109.14*** | 6.486*** |
| N × Hybrid | 3 | 0.07 | 243.9* | 50948 | 4.81 | 0.04 | 12.96 | 13.69 | 0.011 |
| MgO × Hybrid | 1 | 0.02 | 13.8 | 40 | 3.85 | 4.05 | 2.48 | 12.85 | 0.025 |
| N × MgO × Hybrid | 3 | 0.22 | 121.8 | 33947 | 11.78 | 0.99 | 6.8 | 12.87 | 0.009 |
| Residual 3 | 24 | 0.22 | 68.8 | 85857 | 11.65 | 0.95 | 12.21 | 14.32 | 0.048 |
| Year | 2 | 24.48*** | 3087.3*** | 15160227*** | 1428.94*** | 100.68*** | 2642.78*** | 3064.4*** | 14.335*** |
| Year × N | 6 | 0.29 | 1165.2*** | 634952** | 13.89 | 2.82 | 14.7 | 13.01 | 0.510*** |
| Year × MgO | 2 | 0.08 | 261.1 | 40132 | 11.62 | 0.49 | 12.75 | 18.05 | 0.018 |
| Year × Hybrid | 2 | 0.61* | 337.3* | 89946 | 126.98* | 5.57* | 0.03 | 190.73** | 0.29 |
| Year × N × MgO | 6 | 0.15 | 111.3 | 108421 | 14.14 | 0.59 | 12.49 | 11.37 | 0.032 |
| Year × N × Hybrid | 6 | 0.34 | 12.7 | 24001 | 2.15 | 0.9 | 11.25 | 14.41 | 0.02 |
| Year × MgO × Hybrid | 2 | 0.2 | 5 | 12072 | 3.18 | 0.09 | 0.67 | 0.31 | 0.007 |
| Year × N × MgO × Hybrid | 6 | 0.28 | 224.1 | 28004 | 36.06 | 1.03 | 11.18 | 12.76 | 0.007 |
| Residual 4 | 96 | 0.17 | 124.8 | 160489 | 37.57 | 2.06 | 14.46 | 16.82 | 0.098 |
| * P<0.05, ** P<0.01, *** P<0.001 | | | | | | | | | |
| d.f. - degrees of freedom | | | | | | | | | |

Table 4. Number of leaves (± standard deviation) and their fresh weight at the cob flowering stage (BBCH 67)

| Experimental factor / Factor level | | Number of leaves (pcs.) | | | | Leaf weight (g) | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Years | | | Mean | Years | | | Mean |
| | | 2009 | 2010 | 2011 | | 2009 | 2010 | 2011 | |
| Nitrogen dose kg $N \cdot ha^{-1}$ | 0 | 11.12 ±0.41 | 10.17 ±0.44 | 10.31 ±0.48 | 10.53 ±0.61 | 82.70 ±12.50 | 89.93 ±10.21 | 100.51 ±10.26 | 91.05 ±13.09 |
| | 50 | 11.32 ±0.58 | 10.21 ±0.34 | 10.53 ±0.44 | 10.69 ±0.65 | 108.23 ±10.12 | 92.99 ±10.87 | 103.60 ±10.04 | 101.61 ±12.01 |
| | 100 | 11.18 ±0.50 | 9.93 ±0.41 | 10.39 ±0.56 | 10.50 ±0.71 | 117.79 ±16.34 | 97.77 ±14.63 | 106.19 ±13.59 | 107.25 ±16.77 |
| | 150 | 11.40 ±0.47 | 9.87 ±0.38 | 10.51 ±0.47 | 10.59 ±0.77 | 125.50 ±11.87 | 99.79 ±13.09 | 109.26 ±15.13 | 111.52 ±16.97 |
| $NIR_{0.05}$ | | n.s. | n.s. | n.s. | n.s. | 12.450 | n.s. | n.s. | 6.225 |
| Magnesium dose kg $MgO \cdot ha^{-1}$ | 0 | 11.23 ±0.52 | 10.09 ±0.39 | 10.43 ±0.48 | 10.58 ±0.67 | 110.94 ±20.55 | 95.36 ±11.97 | 103.24 ±11.91 | 103.18 ±16.48 |
| | 25 | 11.28 ±0.47 | 10.00 ±0.43 | 10.44 ±0.50 | 10.57 ±0.71 | 106.17 ±20.64 | 94.88 ±13.49 | 106.55 ±13.19 | 102.53 ±16.87 |
| $NIR_{0.05}$ | | n.s. | n.s. | n.s. | n.s. | n.s. | n.s. | n.s. | n.s. |
| Hybrid type | ES Palazzo | 11.46 ±0.46 | 10.10 ±0.41 | 10.65 ±0.46 | 10.74 ±0.71 | 103.37 ±18.98 | 92.20 ±12.70 | 104.08 ±12.63 | 99.88 ±15.88 |
| | ES Paroli SG | 11.05 ±0.45 | 9.99 ±0.41 | 10.21 ±0.41 | 10.42 ±0.62 | 113.75 ±21.07 | 98.04 ±12.10 | 105.70 ±12.66 | 105.83 ±16.93 |
| $NIR_{0.05}$ | | 0,191 | n.s. | 0.237 | 0.116 | 4.337 | 4.851 | n.s. | 2.488 |
| Mean | | 11,26 ±0.49 | 10.05 ±0.41 | 10.43 ±0.48 | - | 108.56 ±20.57 | 95.12 ±12.65 | 104.89 ±12.57 | - |
| n.s. – no significant difference | | | | | | | | | |

Statistical analysis of the obtained results indicated the importance of the interaction between years × nitrogen doses ($P < 0.001$) (Table 3) and the interaction between years × maize hybrid type ($P = 0.039$) in shaping the fresh weight of maize leaf blades. The results indicate a highly focused impact of these factors and allow for generalization of inference (Table 4). On average, in the years, the fresh weight of leaves developed by a single plant increased at doses ranging from $N_1$-0 kg $N \cdot ha^{-1}$ (91.05 g) to $N_4$-150 kg $N \cdot ha^{-1}$ (111.52 g). It should be noted, however, that the value of this feature for a dose of $N_3$-100 kg $N \cdot ha^{-1}$ and $N_4$-150 kg $N \cdot ha^{-1}$ was statistically at the same level. Synthetically, ES Paroli SG was observed to have a significantly greater fresh weight of leaf blades on a single plant, compared to the variety ES Palazzo. The difference amounted to 5.95 g. The above dependence of the impact of a maize variety type on the value of the discussed feature was demonstrated in two of the three years of the field tests (2009 and 2010). On average, in the research years (regardless of the experiment factors examined), the heaviest leaf blades of a single plant amounting to 108.56 g were found in 2009, while the lightest – in 2010 (95.12 g) (Table 4). The impact of the interaction between a nitrogen dose and hybrid type ($P = 0.045$) on the weight of leaf blades per plant was observed (Fig. 2). This dependence is described by equation 1° (rising curve), while for the variety ES Paroli SG, this relationship runs at a higher level than for the variety ES Palazzo. As for the hybrid ES Paroli SG, the increasing nitrogen dose of 1 kg $N \cdot ha^{-1}$ raised the leaf weight by 0.14 g, while for the hybrid ES Palazzo – by 0.12 g (Fig. 2).

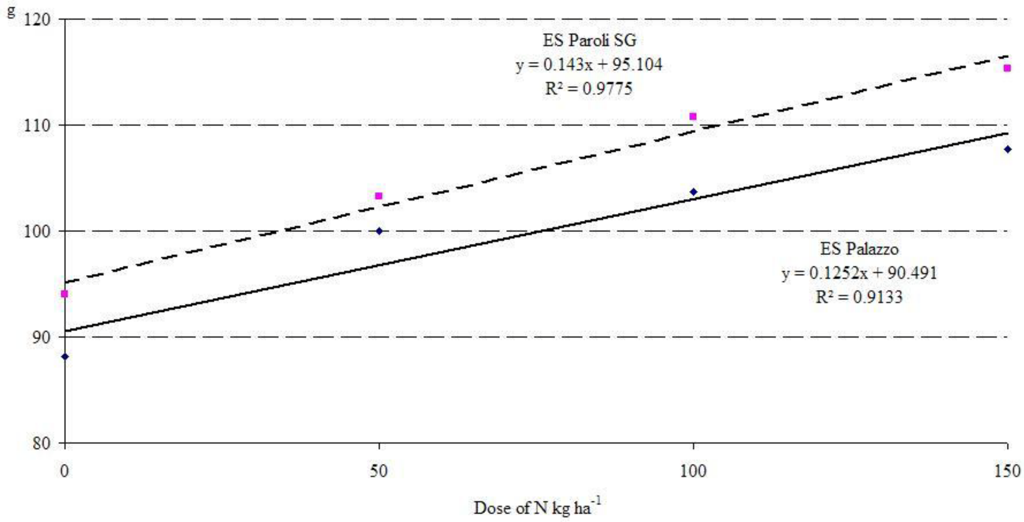


Fig. 2. The effect of a nitrogen dose and hybrid type on leaf weight in the cob flowering stage (BBCH 67) - (2009-2011) (regression coefficients significant at 0.05 level)

The results indicate the importance of weather conditions, varied between the research years, on the size of a single plant assimilation area ($P<0.001$) and LAI ($P<0.001$) (Table 3). On average, in the research years, the values of these features were the lowest in 2009 (respectively: 3456.67 $cm^2$ and 2.39, while the largest in 2011 (respectively: 4315.92 $cm^2$ and 3.24) (Table 5). Synthetically, the assimilation area per plant and LAI were significantly determined by the level of nitrogen application and a maize hybrid type (Table 5). The smallest significant total leaf blade area per plant and LAI was found for a dose of $N_1$-0 kg $N\cdot ha^{-1}$ (respectively: 3520.74 $cm^2$ and 2.61). In turn, the greatest value of the assimilation area per plant was observed for doses of $N_4$-150 kg $N\cdot ha^{-1}$ (3891.01 $cm^2$), and of LAI for a dose of $N_3$-100 kg $N\cdot ha^{-1}$ (2.77). Moreover, in our study, the assimilation area per plant was determined by the interaction between a nitrogen dose and a maize hybrid type ($P=0.004$) (Fig. 3). The curves describing the dependence for both cultivars are shown in equation 1° (rising curves). For the hybrid ES Paroli SG, rising doses of nitrogen by 1 kg $N\cdot ha^{-1}$ resulted in an increase of the assimilation area by 2.48 $cm^2$, and for the variety ES Palazzo – by 2.41 $cm^2$.

Table 5. Assimilation area of a single plant and LAI in the flowering cob stage (BBCH 67) (mean values ± standard deviation)

| Experimental factor | Factor level | Assimilation area of a single plant[#] ($cm^2$) 2009 | 2010 | 2011 | Mean | LAI 2009 | 2010 | 2011 | Mean |
|---|---|---|---|---|---|---|---|---|---|
| Nitrogen dose kg N·ha$^{-1}$ | 0 | 2887 ±300 | 3405 ±328 | 4270 ±600 | 3520.74 ±714.5 | 2.02 ±0.24 | 2.59 ±0.28 | 3.22 ±0.49 | 2.61 ±0.60 |
| | 50 | 3504 ±426 | 3444 ±302 | 4286 ±630 | 3744.71 ±603.2 | 2.46 ±0.33 | 2.50 ±0.33 | 3.25 ±0.51 | 2.73 ±0.53 |
| | 100 | 3725 ±524 | 3509 ±310 | 4348 ±648 | 3860.58 ±617.7 | 2.59 ±0.39 | 2.43 ±0.33 | 3.31 ±0.44 | 2.77 ±0.54 |
| | 150 | 3711 ±387 | 3602 ±308 | 4360 ±596 | 3891.01 ±552.8 | 2.49 ±0.25 | 2.31 ±0.40 | 3.19 ±0.48 | 2.67 ±0.54 |
| $NIR_{0.05}$ | | 338 | n.s. | n.s. | 124.617 | 0.223 | n.s. | 0.115 | 0.097 |
| Magnesium dose kg MgO·ha$^{-1}$ | 0 | 3468 ±483 | 3479 ±290 | 4277 ±590 | 3741.65 ±6002.0 | 2.40 ±0.33 | 2.46 ±0.33 | 3.22 ±0.46 | 2.69 ±0.53 |
| | 25 | 3445 ±585 | 3501 ±340 | 4354 ±627 | 3766.87 ±672.8 | 2.39 ±0.42 | 2.45 ±0.36 | 3.26 ±0.49 | 2.70 ±0.58 |
| $NIR_{0.05}$ | | n.s. | n.s. | n.s. | n.s. | n.s. | n.s. | n.s. | n.s. |
| Hybrid type | ES Palazzo | 3333 ±580 | 3307 ±286 | 4202 ±689 | 3613.69 ±682.8 | 2.24 ±0.38 | 2.20 ±0.26 | 3.10 ±0.51 | 2.51 ±0.57 |
| | ES Paroli SG | 3581 ±456 | 3674 ±221 | 4430 ±493 | 3894.82 ±556.1 | 2.54 ±0.31 | 2.72 ±0.17 | 3.38 ±0.38 | 2.88 ±0.47 |
| $NIR_{0.05}$ | | 160 | 102 | 97 | 68.725 | 0.124 | 0.095 | 0.089 | 0.058 |
| Mean | | 3457 ±533 | 3490 ±314 | 4316 ±605 | - | 2.39 ±0.37 | 2.46 ±0.34 | 3.24 ±0.47 | - |

[#] - according to the method of Borowiecki and Filipiak (1992)

n.s. – no significant difference

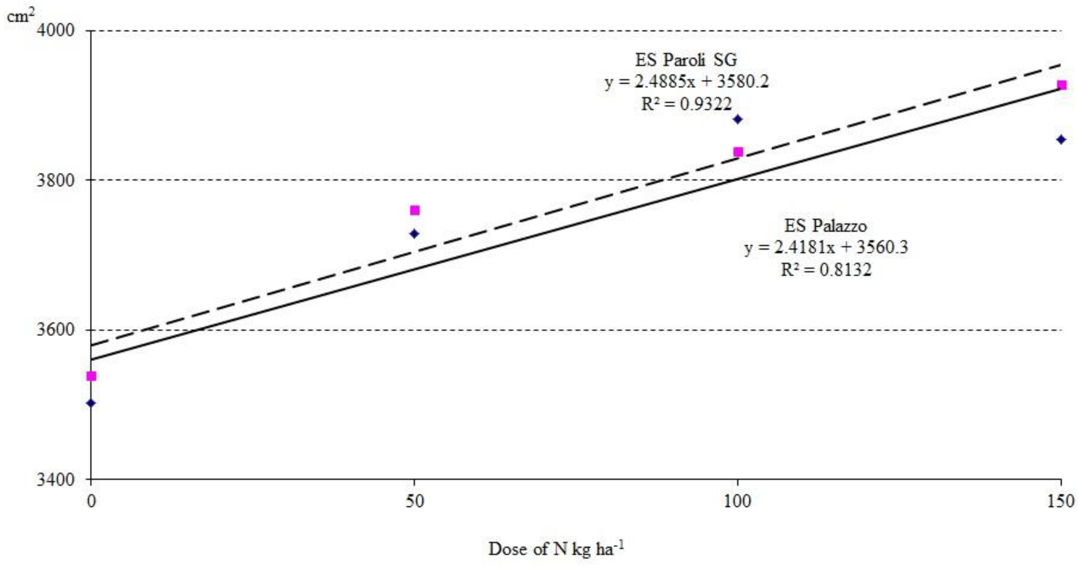


Fig. 3. The effect of a nitrogen dose and hybrid type on a single plant assimilation area at the cob flowering stage (BBCH 67) - (2009-2011) (regression coefficients significant at 0.05 level)

As for leaf weight fraction (LWF) and ear weight fraction (EWF), the statistical analysis showed an impact of the interaction between years and a hybrid type on the value of these characteristics (P=0.043 and P=0.008 for, respectively, LWF and EWF). The direction of these changes in all the years was similar and the statistically confirmed interaction resulted only from differences in the strength of the factor's impact in particular years of the experiment. Thus, seeking to present the dependencies more clearly and formulate generalizations, the paper shows the effect of a hybrid type on these characteristics by using the mean values from the research years (Table 6). On average, in the research years, LWF was found to be significantly higher for the hybrid ES Paroli SG, compared to the variety ES Palazzo (Table 6). As for ear weight fraction (EWF), the hybrid ES Palazzo was characterized by a significantly higher value for this parameter, in relation to the hybrid ES Paroli SG ($P<0.001$) (Table 3). On average, in the research years, none of the factors studied in the experiment significantly differentiated stem weight fraction (SWF) (Table 6).

Table. 6. The fraction weight of leaves (LWF), stems (SWF) and ears (EWF) in the cob flowering stage (BBCH 67) expresses the mass of the plants involved in (2009-2011) (mean values ± standard deviation)

| Experimental factor | Factor level | LWF | SWF | EWF |
|---|---|---|---|---|
| Nitrogen dose kg N·ha$^{-1}$ | 0 | 15.92 ±1.69 | 50.68 ±6.13 | 33.38 ±6.38 |
| | 50 | 15.93 ±1.69 | 49.91 ±6.46 | 34.15 ±6.92 |
| | 100 | 15.98 ±2.04 | 50.09 ±5.88 | 33.91 ±6.64 |
| | 150 | 15.89 ±2.16 | 50.43 ±7.03 | 33.67 ±7.65 |
| $NIR_{0.05}$ | | n.s. | n.s. | n.s. |
| Magnesium dose kg MgO·ha$^{-1}$ | 0 | 15.98 ±1.95 | 50.19 ±6.42 | 33.81 ±6.01 |
| | 25 | 15.88 ±1.84 | 50.36 ±6.31 | 33.74 ±6.86 |
| $NIR_{0.05}$ | | n.s. | n.s. | n.s. |
| Hybrid type | ES Palazzo | 14.96 ±1.64 | 50.49 ±6.64 | 34.53 ±7.06 |
| | ES Paroli SG | 16.90 ±1.61 | 50.07 ±6.07 | 33.02 ±6.62 |
| $NIR_{0.05}$ | | 0.334 | n.s. | 1.056 |
| n.s. – no nsignificant difference | | | | |

While considering specific leaf area (SLA), it was found that the value of this feature was significantly shaped by the changing weather conditions in particular research years ($P < 0.001$) (Table 3). A nitrogen dose significantly changed the value of this feature only in 2009 and 2010 (Table 7). On average, in the years of the research, the value of SLA significantly decreased with increasing levels of nitrogen from $N_1$-0 kg N·ha$^{-1}$ (38.78 cm$^2$·g$^{-1}$) to $N_4$-150 kg N·ha$^{-1}$ (35.74 cm$^2$·g$^{-1}$). The analysis of variance confirmed no significant effect of a hybrid type on the value of SLA ($P=0.120$) (Table 3). However, the interaction between years × a hybrid type was found to have a significant impact on the value of SLA ($P=0.040$). Indeed, the largest specific leaf area of about 0.4 cm·g$^{-1}$ was found for the variety ES Paroli SG, in relation to the hybrid ES Palazzo (Table 7). Specific leaf area in our study was also determined by a nitrogen dose and hybrid type (Fig. 4). The curves describing the dependence for both cultivars are presented as curve 1° (decreasing curve). In the case of both cultivars, the increasing level of nitrogen caused a decrease in this indicator.

Table 7. Specific surface leaf area (SLA) in $cm^2 \cdot g^{-1}$ in the cob flowering stage (BBCH 67) (mean values ± standard deviation)

| Experimental factor<br>Factor level | | Years 2009 | 2010 | 2011 | Mean |
|---|---|---|---|---|---|
| Nitrogen dose<br>kg $N \cdot ha^{-1}$ | 0 | 35.32 ±4.32 | 38.12 ±4.17 | 42.90 ±7.40 | 38.78 ±6.24 |
| | 50 | 32.48 ±3.84 | 37.47 ±5.35 | 41.67 ±7.02 | 37.21 ±6.63 |
| | 100 | 31.91 ±4.43 | 36.37 ±4.41 | 41.70 ±8.89 | 36.66 ±7.35 |
| | 150 | 29.74 ±3.62 | 36.51 ±4.46 | 40.98 ±9.40 | 35.74 ±7.78 |
| $NIR_{0.05}$ | | 3.502 | 1.030 | n.s. | 1.884 |
| Magnesium dose<br>kg $MgO \cdot ha^{-1}$ | 0 | 31.77 ±4.20 | 36.85 ±4.10 | 42.07 ±8.07 | 36.90 ±7.09 |
| | 25 | 32.95 ±4.68 | 37.38 ±5.04 | 41.56 ±8.19 | 37.30 ±7.06 |
| $NIR_{0.05}$ | | n.s. | n.s. | n.s. | n.s. |
| Hybrid type | ES Palazzo | 32.71 ±4.99 | 36.25 ±3.99 | 41.14 ±9.12 | 36.90 ±7.25 |
| | ES Paroli SG | 32.02 ±3.88 | 37.98 ±4.99 | 52.48 ±6.96 | 37.30 ±6.88 |
| $NIR_{0.05}$ | | n.s. | n.s. | n.s. | 0.327 |
| Mean | | 32.36 ±4.45 | 37.12 ±4.56 | 41.81 ±8.07 | - |
| n.s. – no nsignificant difference | | | | | |

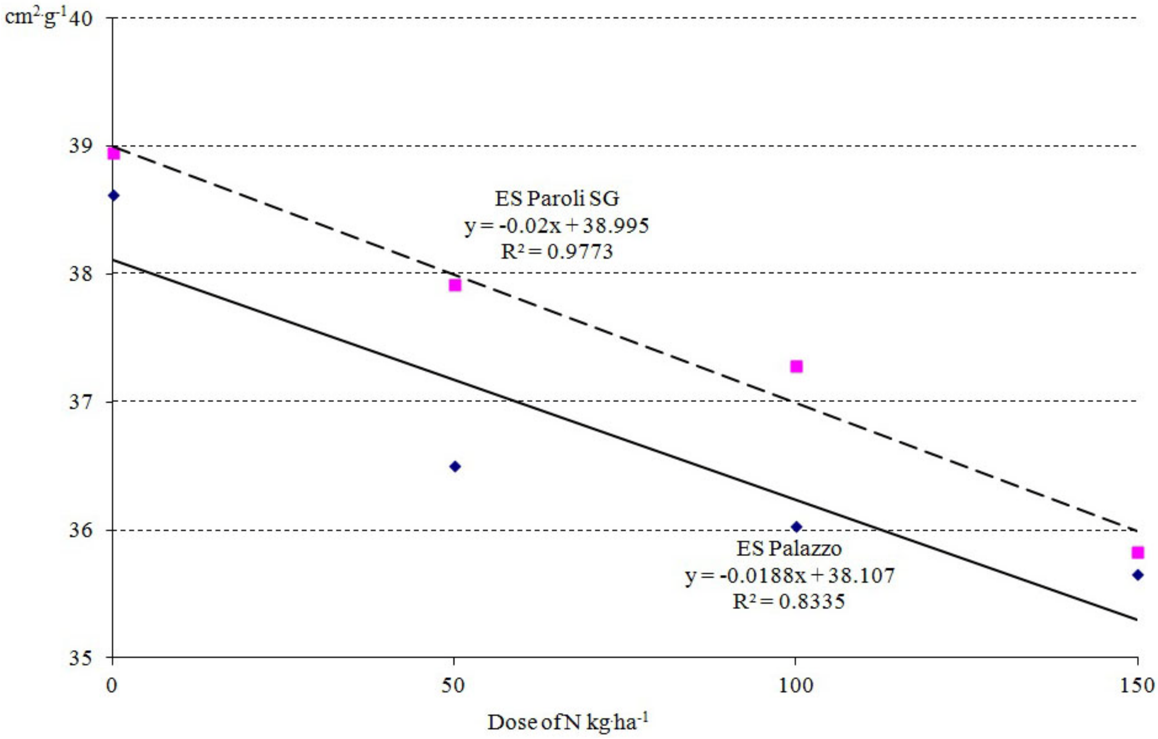


Fig. 4. The effect of a nitrogen dose and hybrid type on the value of SLA in the cob flowering stage (BBCH 67) - (2009-2011) (regression coefficients significant at 0.05 level)

As for acid invertase content in maize leaf blades at the cob flowering stage (BBCH 67), the direction of changes under the influence of the factor levels studied in all the research years was similar ($P = 0.954$) and the statistically confirmed interaction ($P = 0.019$) was due only to differences in the strength of the factor's impact in the individual years of the experiment. Therefore, in order to present the dependencies more clearly and formulate generalizations, the paper shows the effect of the experiment factors studied using the mean values from the research years (Table 8). The research proved no significant effect of the nitrogen dose ($P = 0.754$) and magnesium dose ($P = 0.378$) and the interaction between the nitrogen dose × magnesium dose ($P = 0.551$) on the content of acid invertase in this phase of development, expressed both with the absorbance per gram of fresh weight and per milligram of protein. The content of acid invertase was modified only by a hybrid type (P = 0.031). A significantly higher content of acid invertase expressed in both the absorbance per gram of fresh weight and per milligram of protein was observed for the hybrid ES Paroli SG, compared to ES Palazzo. This difference was, respectively: 0.21 $A \cdot g^{-1}$ of fresh weight and 4.56 $A \cdot mg^{-1}$ of protein.

Table 8. Acid invertase activity in leaf blades of maize cobs in the flowering stage BBCH 67 (2009-2011) (mean values ± standard deviation)

| Experimental factor<br>Factor level | | The absorbance per gram of fresh weight ($A \cdot g^{-1}$ of f.w.) | The absorbance per milligram of protein ($A \cdot mg^{-1}$ of protein) |
|---|---|---|---|
| Nitrogen dose kg $N \cdot ha^{-1}$ | 0 | 0.51 ±0.27 | 14.20 ±5.01 |
| | 50 | 0.61 ±0.15 | 14.47 ±3.86 |
| | 100 | 0.53 ±0.09 | 15.34 ±2.97 |
| | 150 | 0.49 ±0.14 | 14.30 ±3.21 |
| $NIR_{0.05}$ | | n.s. | n.s. |
| Magnesium dose kg $MgO \cdot ha^{-1}$ | 0 | 0.54 ±0.22 | 14.52 ±4.07 |
| | 25 | 0.53 ±0.13 | 14.63 ±3.53 |
| $NIR_{0.05}$ | | n.s. | n.s. |
| Hybrid type | ES Palazzo | 0.43 ±0.11 | 12.30 ±2.43 |
| | ES Paroli SG | 0.64 ±0.17 | 16.86 ±3.52 |
| $NIR_{0.05}$ | | 0.045 | 1.343 |
| n.s. – no nsignificant difference | | | |

## DISCUSSION

The index analysis of plant growth is a method that makes it possible to observe the components of the plant growth process, and to assess the impact of research factors on this process. It consists in measuring the weight gain and plant assimilation area during ontogeny, and then calculating, on the basis of obtained data, the values of indices which are a combination of measured parameters.

Leaf area, and their number per plant are important elements in the assessment of photosynthesis (Boote et al., 1996). In turn, Pandey et al. (2000) claim that various genotypes of maize differ in the number of developed leaves, growth rate and biomass production under conditions of varying amounts of water and nitrogen doses. Keating and Wafula (1992) also indicate that there are significant differences in the number of leaves in maize varieties. In our study, the number of leaf blades developed by a single plant was determined only by the variety factor. ES Paroli SG developed significantly fewer leaf blades, compared to the traditional variety ES Palazzo. Subedi and Ma (2004) obtained different effect of a variety type on the number of developed leaf blades. According to these authors, the SG-type hybrids have a greater number of leaves and their total area is bigger, compared to conventional varieties. In addition, Andrews et al. (2000) and Costa et al. (2002) report that due to a heavier foliage and higher biomass production, the "stay-green" varieties demand more nitrogen than their traditional counterparts.

Measurements of maize leaves aim at assessing the photosynthetic capacity of a given species and assessing the impact of habitat and agronomic factors (e.g. variety fertilization) on the size of the leaf area. Different methods are applied to determine the area, in our study we used the method proposed by Borowiecki and Filipiak (1992). This method represents a major simplification in the experimental technique, because to determine the leaf area of the whole maize plant, it is necessary to measure only two leaves. The assimilation area of a single maize plant is big. One hectare of maize (*Zea mays* L.) can consist of approximately 45.000 $m^2$ (4.5 ha of leaves to 1 ha of soil) of sunlight absorbing surface (Szulc, 2009). In turn, LAI characterizes the size of the plant organ area that is capable of absorbing radiation which affects photosynthesis, and thus indirectly also increases biomass and grain yield (Nieróbca and Faber, 1996). Field and Avissar (1988) indicate, however, that LAI is the main area of gas exchange between the atmosphere and biosphere. It can be measured and analyzed at different spatial scales from individual plants to entire regions or continents. The optimal value of this index for maximum production is between 3 to 5. With such a plant density, their growth rate is maximum, since all the leaf blades are involved in the process of photosynthesis. Furthermore, the productivity of maize hybrids depends on the content of chloroplast pigments in leaf blades. Szulc and Rybus-Zając (2009) claim that the chlorophyll content in the cob flowering stage (BBCH 67) is strictly dependent on the assimilation area of a single plant, as indicated by the straight-line relationship between the chlorophyll content *a*+*b* expressed in SPAD units and the assimilation area of a single plant and LAI. In the presented results, the size of the assimilation area of a single plant and the value of LAI were significantly determined by a nitrogen dose and hybrid type. The values of these characteristics soared with increasing dose of nitrogen. The result obtained in our study confirms the research carried out by Nieróbca and Faber (1996). According to them, nitrogen deficiency decreases LAI as a result of leaf growth reduction, and accelerates their aging. The result obtained in our study also corresponds to the results of Oscar and Tollennar (2006). They report that the size of leaves and LAI soar as a result of increased nitrogen fertilization. Moreover, the macroelement strongly activates growth processes, influencing the development and morphology of plants. Considering the influence of the variety factor on the area absorbing the sun's rays, it was shown that the hybrid ES Paroli SG developed a significantly higher value of the characteristic than the traditional hybrid ES Palazzo. As nitrogen increases the assimilation area, especially of leaves, and extends its physiological performance, intensifying its photosynthesis per unit area or weight, the photosynthetic activity of whole shoots rises (Wojcieska-Wyskupajtys, 1996). This therefore provides potentially greater yielding opportunities of the "stay-green" varieties, in relation to traditional varieties. This can also explain the possibility of obtaining higher grain yield of the "stay-green" hybrids, compared to traditional varieties (Szulc et al., 2008; Szulc and Bocianowski, 2011).

In the process of photosynthesis, in addition to leaves, stems and cob cover leaves may play a significant role. Therefore, our study identified the weight fraction of leaves (LWF), stems (SWF) and ears (EWF). The values of LWF and EWF were substantially modified only by a variety type, while the SWF value was not determined by the rate of nitrogen, magnesium and maize variety type. The total weight of ES Paroli SG plant had a larger share of leaf blades, and a smaller share of cobs in comparison to the variety ES Palazzo. The share of the stems (SWF), regardless of a hybrid type, amounted to about 50% of the plant weight.

Leaf growth rate determines the amount of light energy that can be converted into a new plant weight. The relationship between the size of the leaf area and their weight indicates the intensity of the growth processes of assimilation organs. The basic parameter – relative leaf thickness, known as specific leaf area (SLA) expresses its ratio to the weight (Grzebisz, 2008). In our study, an increase in a nitrogen dose significantly reduced the value of this parameter. In addition, it was demonstrated that the variety ES Paroli SG developed thinner leaf blades, compared to the traditional variety ES Palazzo. Assimilates can accumulate in the form of starch in chloroplasts themselves. When the accumulation of starch in chloroplasts exceeds the daily export of sugars from chloroplasts, and then their export from a leaf blade, leaves become the buffer acceptors of assimilates. They become fatter as their biomass increases (Kopcewicz and Lewaka, 2002). In extreme cases, starch grains can occupy up to 90% of the chloroplast volume, but this makes it difficult to conduct photosynthesis due to reduced light penetration to the photosystem (Kozłowska, 2007). When the demand for organic compounds exceeds the capabilities of current production of assimilates, these reserves of nutrients are released, thus reducing the leaf blade thickness. Hence, from the physiological point of view, thinner leaf blades are more favorable for plants, because the production and transport of assimilates run smoothly. All assimilates produced in the leaves are immediately transported to the acceptor organs. When the sizes of the leaf blades of the studied cultivars were compared, they turned out to be bigger in the “stay-green” variety. On this basis, it can be assumed that the accumulation of plant biomass (in accordance with Figure 5) is far more efficient in the "stay-green" variety than in the ES Palazzo hybrid. It can be also concluded that the transport of assimilates is very efficient in the “stay-green" variety, as evidenced by the lower leaf thickness (SLA).

Acid invertase is the enzyme that indirectly indicates the current leaf carbohydrate balance. It hydrolyzes sucrose, flowing to the young leaf, to monosaccharides. Thus, leaf growth rate is positively correlated with the enzyme content (Grzebisz, 2008). With age, the acid invertase content in the leaf decreases rapidly, thereby reducing the growth rate of the assimilation apparatus of the plant. Roitsch and Gonzales (2004) also report that in mature tissues, the level of invertase is rather low, but is increases under the influence of thermal shock or mechanical damage. In our study, acid invertase activity, expressed both per gram of fresh weight and per milligram of protein was significantly higher in the hybrid ES Paroli SG, compared to the variety ES Palazzo. Therefore, in our study the "stay-green" hybrid was characterized by greater parameters defining the leaf assimilation area, as compared to the traditional variety.

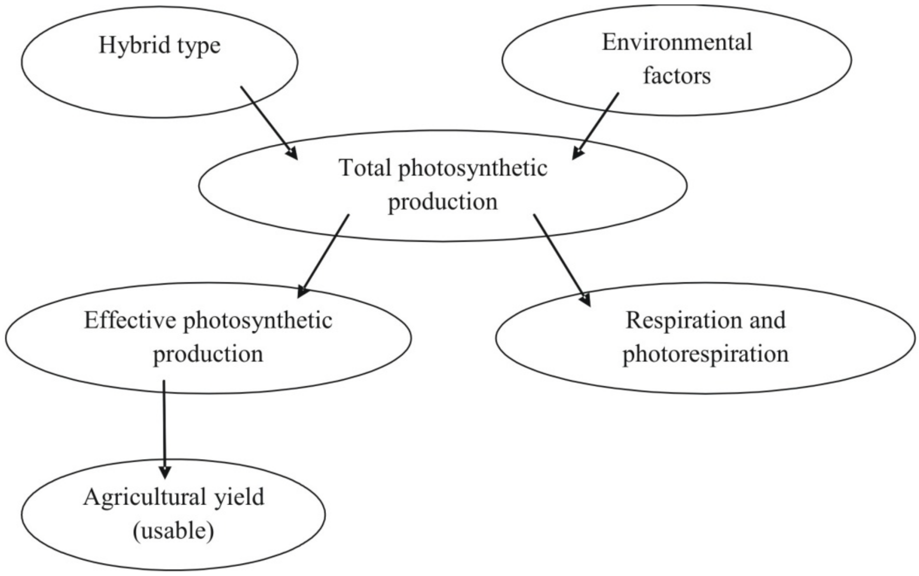

Fig. 5. Influencing the quantity of plant biomass (modified by Kozłowska, 2007)

## CONCLUSIONS

The "stay-green" hybrid developed fewer leaves per plant, while at the same time their weight was bigger, compared to the traditional variety. The increase in the level of nitrogen application raised the weight of leaf blades per plant. The "stay-green" variety was characterized by a larger share of leaf blades in plant weight, compared to the traditional hybrid. As for the share of cobs, the relationship was opposite. Indeed, the smallest values of the assimilation area per plant and LAI were observed for doses of 0 $kg \cdot ha^{-1}$, compared to other tested levels of nitrogen. The value of these features for doses of 100 $kg \cdot ha^{-1}$ and 150 $kg \cdot ha^{-1}$ was statistically at the same level. The total area of leaf blades per plant, and the value of LAI were significantly higher in the hybrid ES Paroli SG, compared to the variety ES Palazzo. The specific leaf area (SLA) significantly decreased with increasing dose of nitrogen. The "stay-green" hybrid was characterized by a lower leaf blade thickness (more efficient transport of assimilates), compared to the traditional variety. Acid invertase activity, expressed both per gram of fresh weight and per milligram of protein, was significantly higher in the hybrid ES Paroli SG, compared to the variety ES Palazzo. In our study, no significant effect of nitroden and magnesium on any of the studied traits was observed.

## REFERENCES


1. Andrews, C. J., Dwyer, L. M., Stewart, J. A., Dugas, J. A, and P. Bonn. 2000. Distribution of carbohydrate during grain-fill in leafy and normal maize hybrids. *Canadian Journal of Plant Science* 80: 87-95.
2. Boote, K. B., Jones, J. W., and N. B. Pickering. 1996. Potential uses and limitations of crop models. *Agronomy Journal* 88: 704-716.
3. Borowiecki, J., and K. Filipiak. 1992. Selection of indicator leaves for the determination of maize leaf surface area (In Polish). *Pamiętnik Puławski* 100: 77-85.
4. Copeland, L., and P. J. Lea. 1990. *Enzymes of sucrose metabolism*. Methods Plant Biochemistry. Acad. Press London 3, p. 73-86.
5. Costa, C. L., Dwyer, L. M., Stewart, D. W., and D. L. Smith. 2002. Nitrogen effect on kernel yield and yield components of leafy and non-leafy maize genotypes. *Crop Science* 42: 1556-1563.
6. Field, C. B., and R. Avissar. 1988. Bidirectional interactions between the biosphere and the atmosphere. *Global Change Biology* 4: 459-460.
7. Gołębiowska, H., and T. Sekutowski. 2007. Effectiveness of herbicides selectivity applied in maize monoculture with of leaf area index (LAI) and mean tip angle (MTA) (In Polish). *Inżynieria Rolnicza* 3(91): 51-58.
8. Grzebisz, W. 2008. *Fertilisation of crops*. PWRiL. Part I. The basics of fertilization.
9. Kączkowski, J. 1992. *Plant biochemistry. Vol. 1. Typical transformations*. Wydawnictwo Naukowe PWN, Warszawa.
10. Keating, B. A., and B. M. Wafula. 1992. Modelling the fully expanded leaf area of maize leaves. *Field Crop Research* 29: 169-176.
11. Kim, S. H., Gitz, D. C., Sicher, R. C., Baker, J. T., Timlin, D. J., and V. R. Reddy. 2007. Temperature dependence of growth, development, and photosynthesis in maize under elevated $CO_2$. *Environmental and Experimental Botany* 61: 224-236.
12. Kopcewicz, J., and S. Lewaka. 2002. *Plant Physiology*. (In Polish). Wydawnictwo Naukowe PWN, Warszawa.
13. Kozłowska, M. 2007. *The physiology of plants. From theory to practice* (In Polish). PWRiL, Poznań Branch.
14. Machul, M. 1995. The influence of pre-sowing field preparation on maize yield grown in five-year monoculture (In Polish). *Pamiętnik Puławski* 102: 191-199.

15. Matsuoka, M., Nomura, M., Agarie, S., Miyao-Tokutomi, M., and M. S. B. Ku. 1998. Evolution of C4 photosynthetic genes and overexpression of maize C4 genes in rice. *Journal of Plant Research* 111: 333-337.
16. Molga, M. 1986. *Agrometeorology* (In Polish). PWRiL Warszawa.
17. Nieróbca, A., and A. Faber, A. 1996. Leaf area index as an indicator of growth and grain yield of winter wheat (In Polish). *Fragmenta Agronomica* 3(51): 54-66.
18. Oscar, R. V., and M. Tollennar, M. 2006. Effect of genotype, nitrogen, plant density and row sparing on the area-per-leaf profile in maize. *Agronomy Journal* 98: 94-99.
19. Pandey, R. K., Maranville, J. W., and M. M. Chetima. 2000. Deficit irrigation and nitrogen effects on maize in a Sahelian environment. II. Shoot growth, nitrogen uptake and water extraction. *Agriculture and Water Management* 46: 15-27.
20. Payne, R., Murrey, D., Harding, S., Baird, D., Soutou, D., and P. Lane. 2003. *GenStat for Windows (7th ed.) – Introduction*. VSN International, Oxford, England.
21. Pengelly, J. L., Kwasny, S., Bala, S., Evans, J. R., Voznesenskaya, E. V., Koteyeva, N. K., Edwards, G. E., Furbanak, R. T., and S. Caemmerer. 2011. Functional analysis of corn husk photosynthesis. *American Society of Plant Biologists* 156(2): 503-513.
22. Roitsch, T., and M.-C. Gonzales. 2011. Function and regulation of plant invertases: sweet sensations. *Trends in Plant Science* 9: 606-613.
23. Shapiro, S. S., and M. B. Wilk. 1965. An analysis of variance test for normality (complete samples). *Biometrika* 52: 591-611.
24. Subedi, K. D., and L. B. Ma. 2004. *Leaf area, ear position and contribution of individual leaf to grain yield in conventional and leafy maize hybrids***.** In the proceedings of the 9th Interregional Corn Improvement Meeting. St Louis, MO. 9-10 February.
25. Szulc, P. 2009. Effect of nitrogen fertilization and methods of magnesium application on chlorophyll content, accumulation of mineral components, and morphology of two maize hybrid types in the initial growth period. Part III. Morphological features of plants. *Acta Scientiarum Polonorum Agricultura* 8(2): 63-75.
26. Szulc, P. 2013. Effects of soil supplementation with urea and magnesium on nitrogen uptake, and utilization by two different forms of maize (*Zea mays* L.) differing in senescence rates. *Polish Journal of Environmental Studies* 22(1): 239-248.
27. Szulc, P., and J. Bocianowski. 2011. Variability and correlation of components of grain yield structure of two different types of maize cultivars (*Zea mays* L.). *Fresenius Environmental Bulletin* 20(10a): 2684-2692.
28. Szulc, P., and M. Rybus-Zając. 2009. Content of chloroplast pigments in the phase of maize blooming depending on nitrogen and magnesium fertilization. *Acta Scientiarum Polonorum Agricultura* 8(3): 43-54.
29. Szulc, P., Bocianowski, J., and M. Rybus-Zając. 2011. Correlation between traits and their influence on grain yield of "stay-green" maize hybrid (*Zea mays* L.). *Fresenius Environmental Bulletin* 20(8a): 2150-2155.
30. Szulc, P., Skrzypczak, W., and H. Waligóra. 2008. Improvement of the effectiveness of maize (Z*ea mays* L.) fertilization with nitrogen by the application of magnesium. Part I. Grain yield and its structure. *Acta Scientiarum Polonorum Agricultura* 7(4): 125-135.
31. Tausta, S. L., Coyle, H. M., Rothermel, B., Stiefel, V., and T. Nelson. 2002. Maize C4 and non-C4 NADP-dependence malic enzymes are encoded by distinct genes derived from a plastid-localized ancestor. *Plant Molecular Biology* 50(4-5): 635-652.
32. Wojcieska-Wyskupajtys, U. 1996. Physiological role of nitrogen in affecting plant yield. Part III. Plant nutrition with nitrogen as affected by photosynthesis productivity. (in Polish). *Postępy Nauk Rolniczych* 3: 29-43.

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