СРАВНИТЕЛНО ПРОУЧВАНЕ НА ЕФЕКТА ОТ АЗОТНИЯ ДЕФИЦИТ ВЪРХУ РАСТЕЖА, ФОТОСИНТЕТИЧНИТЕ ПАРАМЕТРИ И ЕНЗИМНАТА АКТИВНОСТ ПРИ МЛАДИ РАСТЕНИЯ ОТ СЛЪНЧОГЛЕД (*HELIANTHUS ANNUUS* L.) И ОТ ЦАРЕВИЦА (*ZEA MAYS* L.)

COMPARATIVE STUDY OF THE EFFECT OF NITROGEN DEFICIENCY ON GROWTH, PHOTOSYNTHETIC PARAMETERS AND ENZYME ACTIVITY OF YOUNG SUNFLOWER (*HELIANTHUS ANNUUS* L.) AND CORN (*ZEA MAYS* L.) PLANTS

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Резюме

Беше направено сравнително проучване на ефекта от азотния дефицит върху физиологичния статус и растежа на млади растения от слънчоглед (*Helianthus annuus* L. cv. Biser) и от царевица (*Zea mays* L. cv. Knezha 613). Експериментът беше проведен във фитостатен бокс при контролирани условия. Растенията бяха отглеждани като пясъчни култури в два варианта: контролна група от растения, растящи с пълен хранителен разтвор на Кноп, и експериментална група от растения, растящи с хранителен разтвор на Кноп без азот. Растежните и физиологичните параметри бяха отчетени при царевицата след две седмици, а при слънчогледа – след три седмици от поставянето на растенията в условия на азотен дефицит. Значително понижаване на всички измерени параметри беше отчетено при растенията, подложени на азотен дефицит.

Abstract

A comparative study of the effect of nitrogen deficiency on the physiological status and growth of young sunflower plants (*Helianthus annuus* L. cv. Biser) and corn (*Zea mays* L. cv. Knezha 613) was done. The experiment was carried out in a climatic room under controlled conditions. The plants were grown in pots as sand cultures in two variants: control plants grown in full-strength *Knop* nutrient solution and experimental group plants grown in *Knop* without nitrogen supply. The growth and physiological parameters were measured for corn and sunflower respectively two and three weeks after growing the plants under nitrogen deficiency conditions. A significant decrease in all measured parameters was recorded for the plants grown in nitrogen deprivation.

Ключови думи: слънчоглед, царевица, азотен дефицит, растеж, фотосинтеза.
Key words: sunflower, corn, nitrogen deficiency, growth, photosynthesis.

INTRODUCTION

Plants need the right combination of nutrients to live, grow and reproduce. When plants suffer from malnutrition, they show symptoms of being unhealthy. Plant nutrients fall into two categories: macronutrients and micronutrients, and both of them are naturally obtained by roots from the soil. Macronutrients are those elements that are needed in relatively large amounts. They include nitrogen, potassium, sulfur, calcium, magnesium and phosphorus.

Nitrogen plays a significant role in cell division and differentiation, growth and somatic embryogenesis, chlorophyll content, rubisco activity, electron transport rate, photosynthesis, anthocyanin production and is an important component of proteins required for the metabolic processes that take place during plant growth (Chaplin and Westwood, 1980; Mordhorst and Lorz, 1993; Guidi et al., 1998; Jain et al., 1999).

A major consequence of nitrogen deficiency in plants is a decreased growth rate. But nitrogen deficiency limits growth primarily by limiting the rate of leaf area increase, rather than the rate of dry matter accumulation per unit leaf area (Radin and Boyer, 1982). Cechin and Fumis (2004) concluded that metabolic processes, based on protein, leading to increases in vegetative and reproductive growth and yield are totally dependent upon the adequate supply of nitrogen. They found in experiments with sunflower plants that nitrogen deprivation reduces the leaf production, individual leaf area and total leaf area, resulting in a reduced photosynthesis (Cechin and Fumis, 2004).

Up to 75% of leaf nitrogen is found in the chloroplasts, consequently, lower rates of photosynthesis under conditions of nitrogen limitation are often attributed to reduction in chlorophyll content and Rubisco activity (Cechin and Fumis, 2004).

The aim of this study was to investigate the effect of nitrogen deficiency on growth, photosynthetic parameters and enzyme activity of young sunflower and corn plants.

MATERIALS AND METHODS

The seeds of sunflower (*Helianthus annuus* L. cv. Biser) and corn (*Zea mays* L. cv. Knezha 613) were germinated in a Petri dish. After germination the seedlings were planted in pots containing 900 grams sand and were grown in controlled condition: 250 µmol m^{-2} s^{-1} light intensity, 16/8 h photoperiod, 28/22°C day/night temperature and constant relative humidity 65%. All plants were separated in 2 groups – control plants watered with full strength Knop nutrient solution containing micronutrients, and plants watered with full strength Knop nutrient solution containing micronutrients but nitrogen free. The nutrient solution was changed every week. Corn plants were grown two weeks and sunflower plants three weeks in nitrogen deficiency condition. At the end of this period plants of each variant (control and N-deficient) were selected for different measurements.

The selected plants were measured for photosynthetic activity, photosynthetic pigments content, nitrate reductase activity and growth parameters. The parameters of leaf gas exchange were measured with an infrared analyzer LCA-4 (ADC, Hoddesdon, England). The content of photosynthetic pigments (chlorophyll *a*, chlorophyll *b* and carotenoids) was determined spectrophotometrically in fresh leaf tissue through extraction in 85% acetone and calculated according Lichtenthaler (1983). The activity of nitrate reductase was determined as described in Manual of Plant Physiology (Berova et al.,2004).

Statistical analysis of the data obtained was performed using one-way ANOVA (for P<0,05). Based on ANOVA results Tukey's test for the main comparison at 95% confidential level was applied.

RESULTS

Plant growth and dry matter accumulation

The first visible symptoms of nitrogen deprivation were stunted growth and leaf chlorosis (Photos 1a, 1b). All plants from experimental group were smaller in size and developed less number of leaves compared to the control group plants. As it is shown in Table 1, nitrogen deficiency strongly suppressed plant growth and dry matter accumulation. The reduction of dry mass was more severe in sunflower plants where the inhibition was 38% and for corn 17% compared to the control plants. Among plant components of dry weights, leaf dry weight had the greatest decrease under nitrogen deficiency. Sunflower plants shown higher decrease of dry leaf biomass 82% compared to the control, and 48% inhibition for corn plants. Decreased plant biomass was associated with reductions in both leaf area (LA) which was 79% for sunflower and 55% for corn, and photosynthetic capacity.

Chlorophyll content and photosynthesis

Presented in Table 2, results shown significant decrease of all photosynthetic pigments in the plants exposed to nitrogen deficiency. The content of chlorophyll *a* was mostly affected and was 27% from the control value for sunflower plants and 40,5% for corn. Similar results were observed for chlorophyll *b* and carotenoids (for sunflower - 31 and 33% respectively, and for corn 56,2 and 56,6% respectively). The photosynthetic CO_2 assimilation (A) was

Таблица 1. Сравняване на сухата маса и листната площ на растения от слънчоглед и царевица, изложени на азотен дефицит (DW – суха маса на цяло растение, LDW – суха маса на листата от едно растение, LA – листна площ)

Table 1. Comparison of dry weight and leaf area of sunflower and corn plants exposed to nitrogen deficiency (DW – dry weight of whole plant, LDW – leaf dry weight, LA – leaf area)

Варианти Variants	Суха маса Dry weight DW (g/pl)	Листна суха маса Leaf dry weight LDW (g/pl)	Листна площ Leaf area LA (cm^2)
corn (царевица)			
Control (контрола)	0,54a	0,19a	156a
N def. (N дефицит)	0,45b	0,10b	71,7b
sunflower (слънчоглед)			
Control (контрола)	0,45a	0,22a	89,9a
N def. (N дефицит)	0,28b	0,04b	19,42b

Within the same column values followed by the same letter (a, b) are not different for P < 0.05.

Таблица 2. Съдържание на фотосинтетични пигменти (mg g^{-1} свежо тегло) в растения от слънчоглед и царевица, подложени на азотен дефицит

Table 2. Content of photosynthetic pigments (mg g^{-1} fresh weight) in sunflower and corn plants exposed to nitrogen deficiency

Варианти Variants	Хлорофил a Chlorophyll a	Хлорофил b Chlorophyll b	Каротеноиди Carotenoids	Хлорофил a/b Chlorophyll a/b
corn (царевица)				
Control (контрола)	1,9a	0,48a	0,6a	3,99
N def. (N дефицит)	0,77b	0,27b	0,34b	2,89
sunflower (слънчоглед)				
Control (контрола)	2,83a	0,86a	0,94a	3,29
N def. (N дефицит)	0,77b	0,27b	0,31b	2,87

Within the same column values followed by the same letter (a, b) are not different for P < 0.05.

significantly lowered in N deficient plants compared to the control plants, but corn plants were more sensitive and had decrease with 69%, whereas sunflower plants had decrease with 49% (Table 3). Nitrogen supply change transpiration rate (E) in corn deficient plants in higher degree (with 61%) comparing with sunflower plants were transpiration rate was inhibited only with 7% (Table 3). The opposite effect was observed about stomatal conductance where sunflower deficient plants were more sensitive compared to the corn plants (Table 3).

Nitrate reductase activity

The results presented in Figure 1 shown significant decrease in the activity of nitrate reductase enzyme in deficient sunflower plants and the value was only 21% from the control. Different result was observed for corn deficient plants which shown higher enzyme activity compared to the control plants.

In this study we found that nitrogen deficiency may led to growth inhibition of sunflower and corn plants. Since the nitrogen is one of the most important elements its deficiency delayed all developmental phases, reduced plant height and leaf area and also leaf number (Hocking et al., 1987), which was observed in presented experiments (Photos 1, Table 1). Leaf area can be viewed as the result of the rate and duration of leaf expansion (Trapani et al., 1999), and this required structural components as proteins and carbohydrates and water. As the main photosynthetic organ the leaf appeared directly associated with plants dry matter production. But decreased biomass production under nitrogen deficiency was mainly attributed to smaller leaf area (Table 1) rather than the leaf photosynthetic rate (Table 3). However, both leaf area expansion and photosynthesis decreased significantly and led to significantly lower biomass production in exposed to nitrogen deficiency plants (Table 1).

The decreased CO_2 assimilation (Table 3) observed in nitrogen deficient plants could be explained with the very low content of photosynthetic pigments (Table 2) due to disturbed processes of their synthesis, and with inhibited stomatal conductance (Table 3). Toth et al. (2002) reported that leaf chlorophyll concentration of field-grown plants decreased, whereas carotenoids and chlorophyll *a:b* ratio increased. In contrast, our results indicated that carotenoid content and chlorophyll *a:b* ratio decreased in deficient plants compared to the control plants (Table 2).

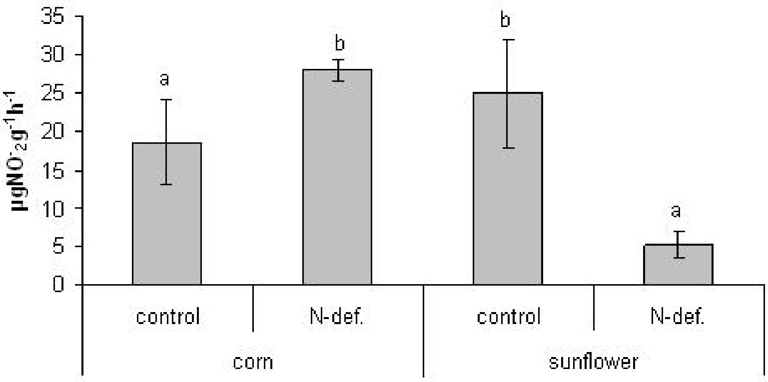
Plants obtain their nitrogen requirements mainly from the soil as nitrates. Most of this nitrate is utilized in the synthesis of proteins and nucleic acids. Nitrate reductase (NR) is the enzyme which is involved in nitrogen

Таблица 3. Листен газообмен при растения от слънчоглед и царевица, подложени на азотен глад; A – интензивност на фотосинтезата (μmol CO_2 m^{-2} s^{-1}); E – интензивност на транспирацията (mmol m^{-2} s^{-1}); g_s - устична проводимост

Table 3. Leaf gas exchange in sunflower and corn plants exposed to nitrogen deficiency; A – intensity of photosynthesis (μmol CO_2 m^{-2} s^{-1}); E – transpiration (mmol m^{-2} s^{-1}); g_s - stomatal conductance

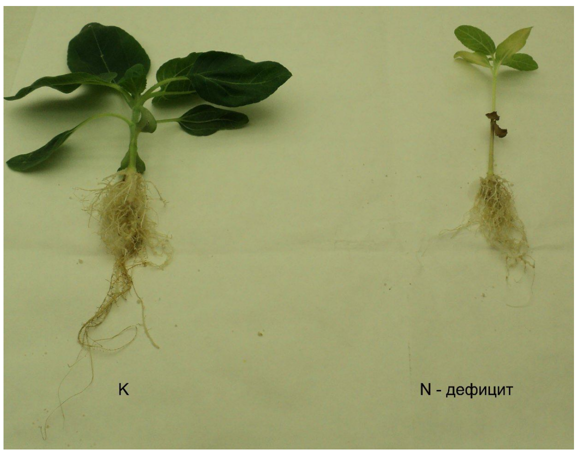
Варианти Variants	Транспирация Transpiration E	Устична проводимост Stomatal conductance g_s	Фотосинтеза Photosynthesis A
corn (царевица)			
Control (контрола)	1,20a	0,04a	7,94a
N def. (N дефицит)	0,47b	0,02b	2,49b
sunflower (слънчоглед)			
Control (контрола)	1,63a	0,7a	10,43a
N def. (N дефицит)	1,52b	0,05b	5,35b

Within the same column values followed by the same letter (a, b) are not different for P < 0.05.



Фиг. 1. *Активност на нитратната редуктаза в листа от растения слънчоглед и царевица, изложени на азотен дефицит. Стълбчетата с еднакви букви за всяка култура (a, b) не са различни при P < 0.05*

Fig. 1. *Leaf nitrate reductase activity in sunflower plants exposed to nitrogen deficiency and after application of foliar fertilizers. The bars with the same letter (a, b) are not different for P < 0.05*



Снимка. 1 а. *Общ вид на контролни и отглеждани при азотен дефицит растения (а) от слънчоглед. Растенията са на еднаква възраст*

Photos 1 a. *Common form of control and grown under nitrogen deficiency sunflower plants (a). The plants had the same age (days after sowing)*

Снимка 1 б. *Общ вид на контролни и отглеждани при азотен дефицит растения (б) от царевица. Растенията са на еднаква възраст*

Photos 1 b. *Common form of control and grown under nitrogen deficiency plants (b) corn. The plants had the same age (days after sowing)* metabolism, play important role in amino acids biosynthesis, and regulates the protein synthesis (Harris et al., 2000). Nitrate is assimilated through a pathway involving nitrate uptake steps and by two reductive steps catalyzed by the enzymes NR and nitrite reductase (NiR). Of these two enzymes, NR is considered to catalyze the rate-limiting step in NO_3^- assimilation because it initiates the reaction when NO_3^- is available (Ahmad et al., 2010). This observation corresponds with our data (Fig. 1) where the very low activity of NR in deficient plants was measured but only for sunflower plants. The higher NR activity in corn deficient plants (Fig. 1) could be explained with their possibilities for translocation and utilization of nitrogen and their better nitrogen metabolism (Radin, 1983).

This study presented a part of our bigger investigation about the effect of nitrogen deficiency in different crops and the possibility to manage this problem after application of different foliar fertilizers. These results will be published latter.

CONCLUSIONS

1. Nitrogen deficiency significantly reduced leaf area, chlorophyll content and photosynthetic activity in both plants species, resulting in lower biomass production.
2. Sunflower plants were more sensitive to nitrogen deficiency than corn plants in respect to reduced leaf area, photosynthetic content, NR activity and dry biomass accumulation.
3. Corn plants shown better possibility for translocation and utilization of nitrogen and thus the deficient plants had higher NR activity.

REFERENCES

Berova, M., V. Kerin, N. Stoeva, A. Vasilev, Zl. Zlatev, 2004. Rakovodstvo za uprazhnenia po fiziologia na rasteniyata. Akademichno izdatelstvo na Agrarnia universitet, 144-146.

Ahmad, S., I.S. Fazili, R. Haque, S. N. Khan, M. Z. Abdin, 2010. Standardization and estimation of nitrate reductase activity in the leaves of *Ammi majus* L. (Bishops weed) in relation to sulphur deficiency and seed yield. – Australian Journal of Crop Science, 4(7): 515-522.

Cechin, I.,T. de Fatima Fumis, 2004. Effect of nitrogen supply on growth and photosynthesis of sunflower plants growth in the greenhouse. - Plant Science, 166, 1379-1385.

Chaplin, M., M. Westwood, 1980. Relationship of nutritional factors to fruit set. - J. Plant Nutri., 4(2): 477-505.

Guidi, L., G. Lorefice, A. Pardossi, F. Malorgio, F. Tognoni, G. Soldatini, 1998. Growth and photosynthesis of *Lycopersicon esculentum* (L.) plants as affected by nitrogen deficiency. - Biol. Plant, 40:235-244.

Hocking, P.J., P.J. Randall, A. Pinkerton, 1987. Sulfur nutrition of sunflower (*Heliantus annuus*) as affected by nitrogen supply: Effects on vegetative growth, the development of yield components, and seed yield and quality. – Field Crops Research, 16 (2), 157-175.

Jain, V., M. Pal, K. Lakkineni, Y. Abrol, 1999. Photosynthetic characteristics in two wheat genotypes as affected by nitrogen nutrition. - Biol. Plant, 42:217-222.

Lichtenthaler, H., A. Wellburn, 1983. Determination of total carotenoids and chlorophylls ***a*** and ***b*** of leaf extracts in different solvents. – Biochem. Soc. Trans, 603: 591-592.

Mordhorst, A., H. Lorz, 1993. Embryogenesis and development of isolated barley (*Hordeum vulgare* L.) microspores are influenced by the amount and composition of nitrogen sources in culture media. - Plant Physiol., 142:485-492.

Radin, J.W. and J.S. Boyer, 1982. Control of leaf expansion by nitrogen nutrition in sunflower plants. - Plant Physiology, 69, 771-775.

Radin, J.W., 1983. Control of plants growth by nitrogen: differences between cereals and broadleaf species. – Plant, Cell and Environment, 6, 65-68.

Toth, V.R., I. Meszaros, S. Veres and J. Nagy, 2002. Effects of the available nitrogen on photosynthetic activity and xanthophylls cycle pool of maize in field. – Journal of Plant Physiology,159, 627-634.

Trapani, N., A.J. Hall, M. Weber, 1999. Effects of constant and variable nitrogen supply on sunflower (*Heliantus annuus* L.) leaf cell number and size. – Annals of Botany, 84: 599-606.

Статията е приета на 11.09.2012 г.
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