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The effect of shading on nitrate metabolism in stalks and blades of celery leaves (*Apium graveolens* L. var. *dulce*)

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ABSTRACT

The results of two year studies (2003 – 2004) were presented, concerning the effect of various manners of 'Tango' celery shading on NR activity and the contents of NO_3^- and NH_4^+ ions, free amino acids, soluble sugars and dry matter, all of which were determined in the stalks and blades of leaves on three different dates, including the harvest time. Three different shading types were used: shading of leaf stalks only with black film fixed on leaf blade level, whole plant shading and shading by double density planting. Film shading started 16 days before the harvest.

In comparison with the control plants, shading, particularly of whole plants, inhibited NR activity in leaf stalks and blades. Nitrate content in celery stalks decreased in the final two weeks of vegetation regardless of the method of

cultivation. At harvest time, least nitrates were found in leaf stalks of double densed plants. NO_3^- level was significantly higher in the blades of leaves wholly shaded with black film, in which the lowest content of NH_4^+ ions was also found. The significant increase of free amino acids in leaf stalks during vegetation occurred regardless of the method of cultivation, with highest amount of this compound presented in plants with shaded stalks as well as wholly shaded plants. Highest soluble sugar content was found in stalks and blades of the celeries cultivated in double density. Dry matter content in both parts of celery leaves was highest in double density treatment and in the control plants.

INTRODUCTION

Modification of environmental conditions which accompanies horticultural plants cultivation and its effect on the size and quality of the yield is a common knowledge. For example, direct covering of plants and soil mulching significantly improve the growth and yield of several varieties of Chinese cabbage (Kalisz and Cebula 2001). Studies conducted by Siwek (2002) showed that shading can effectively improve the commercial value of some vegetables in respect to their appearance and taste. In the cited study, the author employed an innovative manner of blanching leaf celery stalks using black film, which resulted in obtaining lighter and more delicate stalks, more valued on the market than the unbleached ones.

However, reducing access to light can modify many physiological processes in growing plants, in particular photosynthesis. For instance, celery plants shading was found to have a significant effect on assimilation pigment content in stalks (Siwek et al. 2006b). However, available literature on the subject lacks data on nitrate metabolism in celery stalks depending on the applied shading method. Some works indicate that celery plants may accumulate considerable amounts of nitrates in overground parts (Martignon et al. 1994). The main factors which affect the degree of NO_3^- bioaccumulation in plants, apart from nitrogen fertilization, include the weather conditions which accompany field cultivation of vegetables, such as light, rainfall and temperature (Rożek 2000). Nitrate ion content in plants is closely connected with the rate of their reduction. Nitrate reductase (NR) is responsible for the first stage of this process. Although the induction of this enzyme occurs solely in the presence of the substrate, in this case NO_3^- ions, maximal induction, apart from nitrates, requires light (Lillo et al. 2004). Low light intensity, which is connected with low photosynthesis intensity, has an effect on nitrate content growth in plants, the fact that was proved to occur, for instance, in spinach leaves (Kaiser and Brendle-Benisch 1991).

In the present study, the effect of various methods of limiting light access to stalks of the ‘Tango’ celery on NR activity as well as the contents of NO_3^- and

NH_4^+ ions, free amino acids, soluble sugars and dry matter was determined, separately for leaf stalks and blades, during 16-day vegetation until the harvest date.

MATERIAL AND METHODS

The study was carried out in 2003 and 2004 at the Experimental Station of Agricultural University in Kraków and concerned Tango celery cultivar. Seedlings were prepared in greenhouse conditions from seeds sown to containers on 21.01.2003 and 6.02.2004. At the 4-leaf phase, on 23.04 and 21.04 respectively, seedlings were placed directly in field soil in 30 × 25 cm spacing. Based on the chemical analysis of the soil and on the nutritional needs of celery, the following mineral fertilization was determined: 70 kg N ha^{-1} ($N\text{-}NO_3$), 100 kg P_2O_5 ha^{-1}, and 200 kg K_2O ha^{-1}. Half of the nitrogen dose was introduced before seedling placement into the soil, and the other half as a top-dressing.

The experiment was carried out in a randomized block design in four replications. Each treatment of the experiment included 112 plants. Treatments were made by various manners of shading: 1. without shading (control plants), 2. stalk shading with PP 50 g m^{-2} black film (stalks were shaded by fixing the film which was earlier used as mulch, at the level of leaf blades), 3. whole plant shading with PP 50 g m^{-2} film (on 1 m high tunnel construction), 4. double plant density i.e. sowing plants in 15 × 25 cm spacing. The properties of applied films were presented by Siwek (2002). Shading started on 17.06.2003 and 18.06.2004 and was continued until the harvest time which took place on 3 and 5 July respectively.

The evaluation of the studied indices in celery stalks and blades was carried out in three different times, namely: I – 19 June 2003 and 21 June 2004; II – 26 and 28 June respectively and III (harvest time) – 3 and 5 July respectively. Nitrate reductase activity (abbr. NR) was determined in accordance with the method described by Jaworski with the modifications of Rożek (1982). NO_3^- and NH_4^+ ion content in plant material was determined using ORION ion-selective electrodes working together with UNICAM-9460 ionometer (samples were 0.02M $Al_2(SO_4)_3$ $18H_2O$ extracted). Free amino acid content was determined in accordance with the procedure described by Korenman (1973). Soluble sugar content was determined by the photometric method with antrone reagent, described by Yemm and Wills (1954). In order to determine dry matter content, samples of plant material were dried at 105°C. All the investigations were statistically evaluated using Duncan's test at significance level $p = 0.05$.

RESULTS AND DISCUSSION

The results obtained show that regardless of the applied method of cultivation (Fig. 1) NR activity in stalks was many times lower than in blades. The highest activity of this enzyme was found in the blades of the control plants in the second investigation time, when a significant increase of reductase activity in the blades of leaves harvested from all combinations occurred, except plants which were wholly shaded for the period of 16 days until the harvest date. The latter manifested a drastic nitrate reductase decrease, lasting until the harvest time. Same dependence was shown for leaf stalks of the same plants. The present results confirm the ones obtained by other authors who claim that light is the main external factor which modifies NR activity in leaves on post-translation level, as in result of rapid plant shading the activity of this enzyme quickly decreases (Huber et al. 1992, Lillo 1994). The results obtained by Siwek et al. (2006b) show that in comparison with the control plants shading with each type of film resulted in the decrease of NR activity in celery stalks, with black film having the most inhibitive effect. However, the effect of the used film on nitrate content in celery stalks was not explicitly shown in their work.

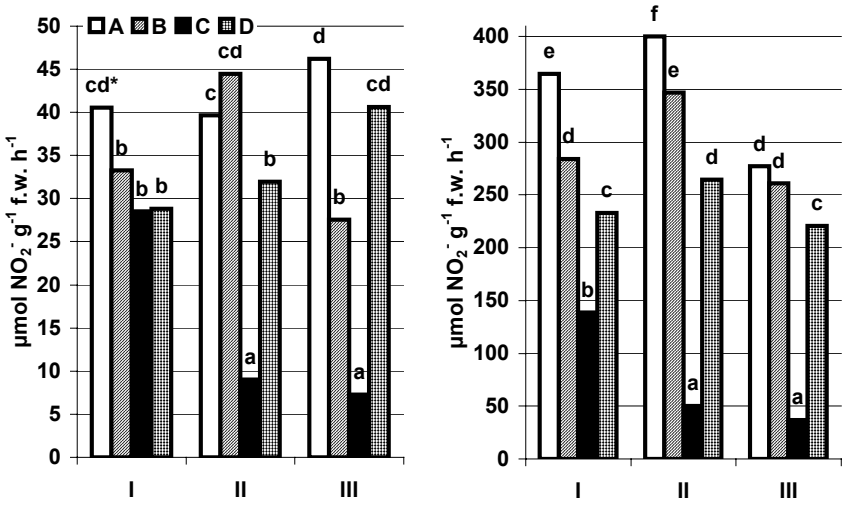

Fig. 1. The effect of shading on nitrate reductase activity in stalks and blades of celery during the vegetation period – means for 2003 – 2004 (I – two weeks before the harvest, II – a week before the harvest, III – harvest time, A – without shading, B – stalk shading, C – whole plant shading, D – double density)

In the present study, in effect of whole plant shading with black film, the decrease of NR activity was accompanied with a significant increase of nitrate level in leaf blades with the simultaneous decrease of NO_3^- in stalks (Fig. 2). The relatively high NR activity in stalks shaded with black film, particularly in the second investigation time (Fig. 1), indicates an interesting co-operation in NO_3^- reduction between the sun-exposed blades and shaded stalks of the same leaves in the initial shading phase. However, in final effect (term III), NR activity in shaded stalks decreased significantly, the fact which was reflected in the nitrate level, the highest in comparison with the other treatments (Fig. 2).

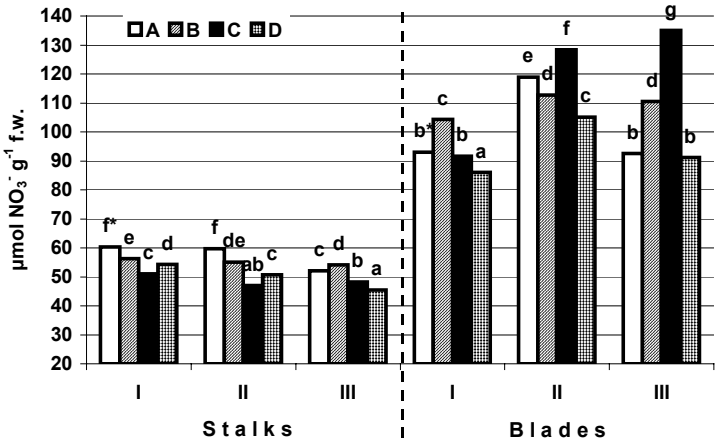

Fig. 2. The effect of shading on nitrate ion content in stalks and blades of celery during the vegetation period – means for 2003 – 2004 (Explanation: as in Fig. 1)

Ammonium ion content in celery leaf stalks significantly increased in the second investigation time, as compared to the results obtained a week before (Fig. 3). Only in case of the stalks harvested from double densed plants, the increase occurred in the third investigation time. As the nitrate content determined in the same objects and on the same dates did not change significantly, the intensifying catabolic changes of amino acids and amides in leaves may have been the probable cause for NH_4^+ level increases in stalks during progressing growth. Lea (1997) reported such processes leading to the increase of nitrate ammonium ion content in plant tissues.

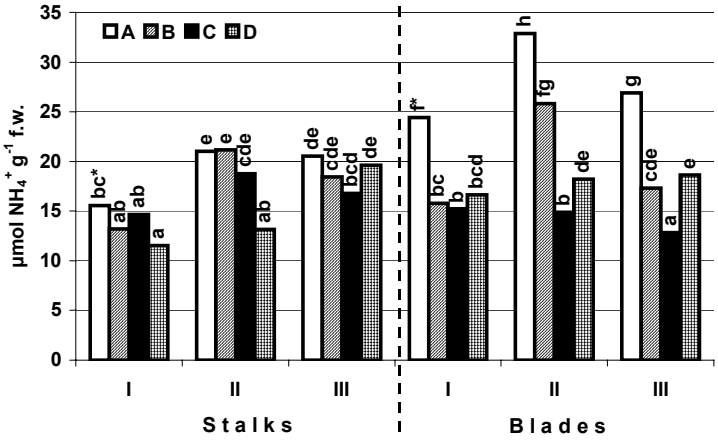

Fig. 3. The effect of shading on ammonium ion content in stalks and blades of celery during the vegetation period – means for 2003 – 2004 (Explanation: as in Fig. 1)

Migge et al. (2000) showed that NR activity may decrease under the influence of certain amino acids, primarily glutamine. The inhibitive effect of large amounts of free amino acids on NR activity in the heads of white cabbage was also shown by Wojciechowska et. al (2000). Comparable results were obtained in the present study (Fig. 4).

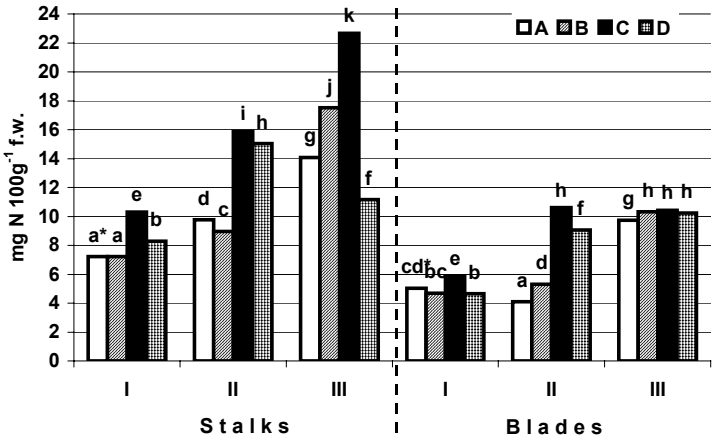

Fig. 4. The effect of shading on free amino acids content in stalks and blades of celery during the vegetation period – means for 2003 – 2004 (Explanation: as in Fig. 1)

Generally, lower NR activity (Fig. 1) was accompanied with a higher content of free amino acids. This regularity was more evident in stalks than in blades of celery leaves. Highest free amino acid content was found following the harvest in stalks of wholly shaded plants, the fact which might have resulted from the advancing, in these conditions, disintegration of ribosomes, leading to the inhibition of protein biosynthesis (Lipton 1987).

Within the two final weeks of vegetation, the significant successive increase of the level of soluble sugars in celery stalks occurred in all treatments except wholly shaded plants (Fig. 5).

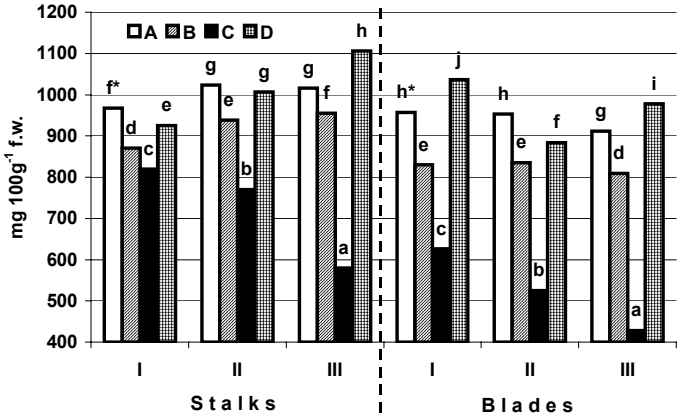

Fig. 5. Effect of shading on soluble sugars content in stalks and blades of celery during the vegetation period – means for 2003 – 2004 (Explanation: as in Fig. 1)

These observations confirm the fact that as celery plants reach harvest maturity, fleshy leaf stalks become a significant sugar acceptor (Starck 2003). The shown decrease of sugar content in blades, which act as main assimilates donor, indicates their successive removal to stalks. It is worth noticing that the increase of sugar content which occurred for stalk shading treatment was linked to the process of photosynthesis in leaf blades exposed to sunlight and acting as the only assimilate donor for shaded stalks. In the former experiment (Siwek 2002), total sugar and dry matter content in bleached leaf celery stalks was even higher as compared to the stalks of the control plants. The fact that stalk shading with black film did not result in the decrease of stalk harvest is also interesting (Siwek et al. 2006a).

Regardless of the investigation time, the effect of particular shading procedures on dry matter content in celery leaves was similar to the one shown for soluble sugars (Fig. 6). However, during vegetation no distinct modifications in dry matter

content in blades were recorded, whereas in stalks the increase was evident. This observation did not concern wholly shaded plants, in case of which the successive decrease of dry matter content in leaves probably resulted from drastic decrease of soluble sugar content, due to the latter being used in intensive breathing processes (Fig. 5). In the no access to light conditions, the energetic balance of a plant gets disturbed (Krug 1997). The decrease of the sugar content may significantly affect the increase of nitrate concentration in plant tissues due to the fact that sugars are deemed to be an internal signal which modulates NR activity (Lillo et al. 2004). The present study has confirmed this finding in respect to wholly shaded leaf blades but not the stalks.

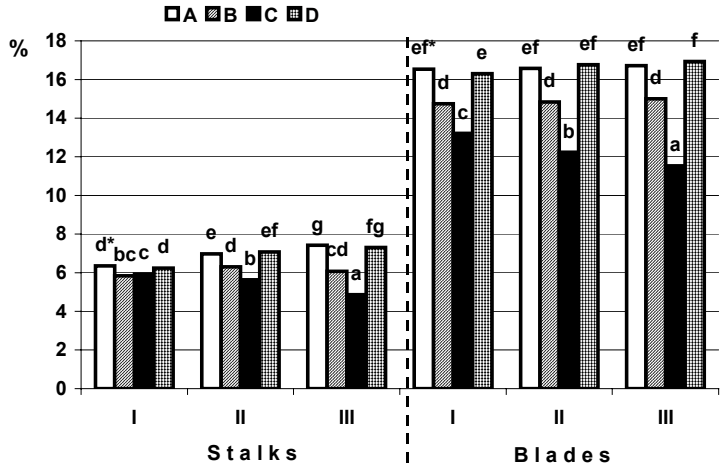

Fig. 6. Effect of shading on dry matter content in stalks and blades of celery during the vegetation period – means for 2003 – 2004 (Explanation: as in Fig. 1)

CONCLUSIONS

1. Nitrate reductase activity was highest in stalks and blades of the control plants (without shading) and lowest in the blades of wholly shaded plants.
2. At the harvest time lowest nitrate content was found in double densed celery stalks.
3. In leaf blades of wholly shaded plants the highest NO_3^- content was shown as compared with other treatments.

4. Highest soluble sugars content was found in leaf stalks and blades of celeries cultivated in double density.

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WPŁYW CIENIOWANIA NA METABOLIZM AZOTANÓW W BLASZKACH I OGONKACH LIŚCIOWYCH SELERA NACIOWEGO (*APIUM GRAVEOLENS* L. VAR. *DULCE*)

Streszczenie: Przedstawiono wyniki dwuletnich badań (2003 – 2004) nad wpływem różnych sposobów cieniowania ogonków liściowych selera naciowego ‘Tango’ na aktywność reduktazy azotanowej, zawartość jonów NO_3^- i NH_4^+, wolnych aminokwasów, cukrów rozpuszczalnych i suchej masy, które oznaczono w ogonkach i w blaszkach liści w trzech terminach. Efekt cieniowania uzyskiwano trzema sposobami: osłaniając same ogonki liściowe czarną włókniną umocowaną na granicy z blaszkami, osłaniając czarną włókniną całe rośliny oraz wysadzając rośliny w podwójnym zagęszczeniu. Cieniowanie włókniną rozpoczynano 16 dni przed zbiorem plonu.

W stosunku do roślin kontrolnych, zabiegi cieniowania wpływały hamująco na aktywność reduktazy azotanowej w ogonkach i blaszkach liściowych, a w największym stopniu – cieniowanie całych roślin. Zawartość azotanów w ogonkach selera zmniejszała się w ciągu ostatnich dwóch tygodni wegetacji niezależnie od metody uprawy. Najmniej azotanów w terminie zbioru plonu zawierały ogonki liściowe roślin podwójnie zagęszczonych. Poziom NO_3^- był wyraźnie najwyższy w blaszkach liści całkowicie cieniowanych czarną włókniną, w których jednocześnie stwierdzono najmniej jonów NH_4^+. Wykazano wyraźny wzrost zawartości

wolnych aminokwasów w ogonkach liściowych podczas wegetacji roślin niezależnie od metody uprawy, przy czym najwięcej tych związków stwierdzono w obiektach z cieniowaniem całych roślin, jak i samych ogonków. Największą zawartość cukrów rozpuszczalnych posiadały ogonki i blaszki liściowe selerów uprawianych w podwójnym zagęszczeniu. Zawartość suchej masy była największa w obu częściach liści selera w kombinacji z podwójnym zagęszczeniem i w obiekcie kontrolnym.

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