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Evaluation of response of lettuce (*Lactuca sativa* L.) to temperature and light stress

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Abstract

The aim of the study was to assess the effect of irrigation water temperature and shading on the rate of photosynthesis and transpiration in four varieties of lettuce (*Lactuca sativa* L.) – green foliage (‘Salakis’ and ‘Estony’) and red foliage (‘Lollo Rossa’ and ‘Nika’). During the production of seedlings in the greenhouse, two irrigation water temperature regimes (12 and 20°C) were applied. After transplanting in the field plants were grown under three lighting systems (100, 70, and 50% of lighting in the open). The rates of photosynthesis and transpiration were measured at the end of the greenhouse period and 14 days after shading in the field using a Li 6400 infrared gas analyzer. In most varieties, cooling of the irrigation water was not found to have an effect on the rate of photosynthesis. Plants of all the varieties responded to the decrease in irrigation water temperature and to strong shading by reducing transpiration and increasing the water use efficiency. The ‘Salakis’ and ‘Estony’ plants have shown the best adaptability to the changing conditions and therefore they could be used successfully in the practice of extending vegetative growth.

Keywords

*Lactuca sativa* L.; water temperature; shading; photosynthesis; transpiration

Introduction

The establishment of conditions for best productivity of agricultural species is one of the main practical goals. Soil and climatic conditions are the most important environmental factors in the cultivation of vegetables. Adaptation to the temperature and light regimes of the habitat allows crops to more effectively use the absorbed carbon for accumulation of biomass, as they regulate successfully plant water balance [1,2].

Extending the period of vegetative growth and delaying the reproductive phase by lowering the temperature of irrigation water have proved to be an effective approach in agricultural practice [3]. Its validity can be explained by the effects of climate change related to the increasing average summer temperatures. Poor head formation, leaf twisting, early bolting, and reduced yields have occurred when temperate leafy vegetables are grown under hot, high-sunlight conditions [4–6]. Water stress caused by high evapotranspirative demand as well as high air and soil temperatures appear to be the main causes of poor crop productivity of leafy crops in low-latitude regions [4,7]. Yields of semihead lettuce were found to be enhanced with artificial shading during the fall [8]. Lowering of irrigation water temperature is especially effective in leafy vegetables with a short growing season. Combining chilled irrigation water with shading of the crops is an even better approach to prolong vegetative growth.
The application of physiological criteria for the effect of lowered water temperature gives an opportunity to define an optimal irrigation regime for crops. The rates of photosynthesis and transpiration are sensitive characteristics of plant physiological activity. Following their dynamics in different temperature and light conditions allows us to select the most suitable varieties and lines capable of maintaining optimal gas exchange in terms of chilled irrigation water and shading.

Along with drought, low temperature and shading are common abiotic stress factors causing reversible reactions in plants. Plants with a short vegetation period are especially sensitive to their action. A decrease in ambient temperature and illumination can quickly lead to changes in basic physiological processes resulting in increased sustainability [2,9]. Adaptation of leaves to the prevailing light microclimate in crops is an essential component of the overall adaptive capacity of plants [1]. Testing the adaptability of different plant species and varieties to light and temperature stress and creating resistant genotypes are a leading target of selection in agriculture [9]. Higher resistance of plants to abiotic stress can significantly increase their productivity and competitive power [9,10].

The actuality of the problem and the opportunity of wider application of the method in practice give us a reason to focus on some physiological criteria for the best combination of irrigation water temperature and light conditions in several varieties of lettuce.

The aim of this study was to examine the effect of lowered irrigation water temperature during the production of seedlings in a greenhouse and shading in the field on the rate of photosynthesis and transpiration of four varieties of lettuce.

Materials and methods

Plant material and experimental design

The study was carried out with young plants of lettuce (*Lactuca sativa* L.) – varieties ‘Lollo Rossa’, ‘Nika’ (with red foliage), ‘Salakis’, and ‘Estony’ (with green foliage), during two vegetation periods (2013 and 2014) in three regions in Bulgaria. In the first year, the seeds were sown in standard trays (with 228 holes) in a standard peat substrate (70/30), 0.5 mm fraction, pH 5.5–6, EC 0.6–0.8 mmohs. The trays were placed in a chamber at a constant temperature of 18°C until seed germination (2 days). On the 3rd day, the trays with seedlings were placed in a plastic greenhouse. After the 5th day, the plants were irrigated with a water temperature of 12°C and 20°C. The appropriate temperature was achieved by adding ice to the irrigation water. Air temperature and substrate temperature in the greenhouse were recorded with electronic thermometers. The visual condition of the canopy and the general physiological condition of the plants were monitored periodically. Nutrition with Ca(NO₃)₂ was applied once per week in order to form a better root system. Immediately after nutrition, the seedlings were watered with pure water (with the respective temperature). The seedlings were planted in three beds at a spacing of 25 × 25 cm. Previously, the area was milled and beds were formed, then the soil was treated with herbicide against weeds (stomp- pendimethalin 0.350 mL / 1000 m² from BASF). Irrigation was carried out using a drip system. Shadecloth canopies were erected over each of the two plots. The supports consisted of iron beams set at the corners of the plots and anchored 1 m deep in the soil in a grid-shaped pattern. The formed panels of commercial black plastic shadecloth were suspended from the wire rope. Two of the beds were shaded with 70% and 50% levels of lighting in the open, while the third bed was not shaded (100% of full daylight; Fig. 1).

**Fig. 1** Shading system in open field.
Minimum and maximum air temperatures were monitored daily using minimum–
maximum thermometers. After 30 days the seedlings were planted in the field at the
same spacing and with the same levels of shading. On the 14th day after planting, the
gas exchange of the test plants was measured in the three variants of the light mode. In
the 2nd year of the experiment, the same seedling production scheme was used.

Site description

Soil characteristics:
- Rakovski region – the following are soils most prevalent in this area: alluvial,
  meadow luvisolic and podzolic soils. The soils are slightly acidic, neutral to slightly
  alkaline in both horizons: pH 4.9 for 0–20 cm horizon, and pH 5.0 for 20–40 cm
- Sofia region – the soil is alluvial, loose, warm and light, with moderate to good
  water holding capacity and high water permeability.

Climatic characteristics:
- Rakovski region – this area falls within the transitional continental climatic zone,
  located south of the Balkan Mountains. It is characterized by mild winters and dry
  summers. Snow cover lasts on average for 30 days per year. The average daily tem-
  peratures most frequently exceed 10°C. Rainfall during spring has a favorable im-
  pact on the development of agricultural crops. The average duration of sunshine is
  2264 hours per year. The average annual temperature is 12°C. The average monthly
  temperature in July is 23.2°C and in January – 0.4°C. The average annual relative
  humidity is around 70% and the amount of precipitation is 539 mm/year, which is
  below the national average value, due to rainfall shadow of the Balkan Mountains.
- B. Sofia region – the area is located in the center of the Sofia region belonging to
  high fields in western and central Bulgaria of the moderate continental climatic
  zone. The altitude is 500–600 m. The coldest months are December, January, and
  February with average monthly temperatures ranging from −18°C to 0°C. In some
  cases, daily temperatures fall below −20°C. The warmest months are July and
  August.

Environmental conditions in the greenhouse. The average daily temperature during
the period of cultivation of seedlings was 18.1°C and the average relative humidity of
the air was 73.6%. The relatively cool and humid period in the greenhouse was favor-
able for the development of the seedlings and allowed their turgor to be preserved.
The light intensity in the greenhouse ranged from 160 μmol m−2 s−1 (at 9:00 a.m.) to
360 μmol m−2 s−1 (at 12:00 p.m.). Within this range, the illumination was below the
light saturation point of photosynthesis for the test plants [1]. The effect of reduced
light in the greenhouse to some extent was compensated by the favorable combination
of optimal temperature and humidity for cold resistant lettuce.

Gas exchange measurements

The rate of net photosynthesis (An; μmol CO₂ m−2 s−1) and transpiration (E; mmol
H₂O m−2 s−1) was measured with a Li Cor 6400 infrared gas analyzer under controlled
conditions in the leaf chamber and a light intensity of 1000 μmol m−2 s−1. Measure-
ments were carried out between 10:00 a.m. and 12:00 p.m. (at the time of absolute
daily maximum of plant physiological activity) on attached leaves of five plants in
each variant of the experiment. At least 10 records of the respective variables were
logged as the data basis. The water use efficiency (WUE) was determined as a ratio
between the rates of net photosynthesis and transpiration (An/E).
Statistical analysis

The data were analyzed statistically. To determine the statistical significance of the differences between gas exchange parameters at different temperature and light conditions, one-way ANOVA test (Systat 7.0, Systat Software, Inc.; Chicago, USA) was applied. Two-factor analysis evaluated the simultaneous influence of temperature and variety on the rate of photosynthesis and transpiration.

Results

Greenhouse plants

The rate of photosynthesis and transpiration, and the water use efficiency in lettuce under different irrigation water temperature regimes are shown in Fig. 2.

A tendency for a decrease in photosynthesis after watering with cold water was found in the varieties 'Salakis', 'Estony', and 'Lollo Rossa'. However, the trend was statistically significant only for 'Salakis'. In the variety 'Nika', the cooling of the irrigation water did not lead to a change in the rate of photosynthesis. In all of the varieties, the rate of transpiration decreased substantially with a lowering of the water temperature. The significant decrease in transpiration against the background of the almost unchanged photosynthesis as influenced by the chilled irrigation water affected the water use efficiency, as presented by the relationship between the two processes. All the tested varieties responded to lower water temperature with increased water use efficiency. In 'Nika' and 'Estony' plants, the increase in water use efficiency was highly statistically significant.

Plants in open field

In order to establish the simultaneous effect of the abiotic stress factors, the gas exchange of field plants, watered in the greenhouse with water of different temperatures, was monitored in full-day illumination and in two variants of shading.

Rate of photosynthesis

In 'Salakis' plants, shading strongly suppressed the intensity of photosynthesis in both temperature regimes. This trend was expressed more strongly under the temperature regime of 12°C. Therefore, in the variety 'Salakis' irrigation with cold water had a positive effect on the rate of photosynthesis under the conditions of full illumination and heavy shading (Fig. 3).

In 'Lollo Rossa', another type of shading effect on the physiological indicators was determined. Irrigated with cooled water, 'Lollo Rossa' plants showed the highest rate of photosynthesis in slight shading. Under slight shading, the 'Lollo Rossa' plants photosynthesized more intensively in comparison to plants irrigated with uncooled water. The 'Lollo Rossa' plants, derived from seedlings and watered in the greenhouse with cold water, not only managed to maintain, but also to increase their photosynthesis under slight shading in open field. Therefore, in 'Lollo Rossa' photosynthesis was most intense when water cooling is combined with slight shading.
The 'Estony' plants irrigated with cold water and under full light and slight shading revealed more intensive photosynthesis than those irrigated with uncooled water. Under heavy shading, chilled 'Estony' plants had lower intensity of photosynthesis.

In all the light regimes, chilled 'Nika' plants photosynthesized more actively. In 'Nika' plants, the rate of photosynthesis was the lowest under slight shading.

**Rate of transpiration**

The intensity of transpiration of the plants of the tested varieties varies widely under shading (Fig. 4).

The plants with cooled irrigation water had more intensive transpiration than those irrigated with 20°C water at full and 70% of full light.

Chilled 'Lollo Rossa' plants showed the most active transpiration under slight shading.

The explanation is that under slight shading there was the greatest difference between the transpiration rates in plants under both temperature regimes.

Under cooling, 'Estony' and 'Nika' plants showed more intensive transpiration than those irrigated with uncooled water under full light and slight shading.

Under heavy shading, cooled 'Estony' plants had a significantly lower rate of transpiration. Strongly suppressed transpiration in cooled plants at 50% shading was an attempt to save water reserves in order to maintain satisfactory photosynthetic activity, thus allowing them to survive in these conditions. Therefore, under slight shading the cooling of the irrigation water stimulated the transpiration in 'Estony' and 'Nika' lettuce.

**Water use efficiency**

'Salakis' plants responded to strong shading and cooling of the irrigation water with increased water use efficiency (Fig. 5). Therefore, the lower temperature of the irrigation water increased the ability to effectively regulate the water balance, even under...
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**Fig. 4** Rate of transpiration of varieties 'Salakis' (a), 'Lollo Rossa' (b), 'Estony' (c), and 'Nika' (d) lettuce under different light conditions. The data represents mean values ± SE, N = 10 (significance of the differences between the plants under the respective temperature regimes: n.s. – *p* > 0.05; *p* < 0.05; **p** < 0.01; ***p*** < 0.001; ANOVA test).

**Fig. 5** Water use efficiency in 'Salakis' (a), 'Lollo Rossa' (b), 'Estony' (c), and 'Nika' (d) under different light conditions. The data represents mean values ± SE, N = 10 (significance of the differences between the plants under the respective temperature regimes: n.s. – *p* > 0.05; *p* < 0.05; **p** < 0.01; ***p*** < 0.001; ANOVA test).
heavy shading. In full light and under slight shading, chilled 'Salakis' plants exhibited lower water use efficiency.

In 'Lollo Rossa', irrigation with cold water caused inefficient use of water resources under slight shading. When shading was stronger, the effectiveness of water use in cooled plants was higher compared to uncooled plants.

Cooled 'Estony' plants, irrigated with child water, showed the highest water use efficiency under heavy shading. However, the greatest difference between plants in the two irrigation water temperature regimes was found in plants irrigated with chilled water and under heavy shading.

A similar trend was found in 'Nika' plants. Heavy shading led to more efficient water use in cooled plants.

The effect of water temperature ($p < 0.0001$), variety ($p < 0.0001$), and their interaction ($p = 0.006$) was more pronounced on the rate of transpiration than on the rate of photosynthesis ($p = 0.002$, $p = 0.042$, and $p = 0.022$, respectively).

Discussion

Clarifying the dynamics of photosynthesis in changing conditions of the water regime is of practical significance for the cultivation of agricultural plants [12]. The response of net photosynthesis of lettuce to temperature is diminished as a result of morphological plant adaptations, i.e., specific leaf area or top-to-root weight ratio [13]. Therefore, photosynthesis turns to be an unsuitable criterion for temperature control in greenhouses.

Temperature and air humidity are the variables with the greatest effect on stomatal conductance and transpiration rate. Water loss or transpiration is an important physiological process that affects the main quality characteristics of fresh lettuce. The lower intensity of transpiration at the water temperature of $12^\circ$C can be explained by the slow diffusion of water vapor through the stomata and sustained uptake of water from the roots. It has been established that at lower temperatures the incorporation of nitrogen in chlorophyll becomes more effective as a protective response of a plant, which has adaptive importance as this can enhance the photosynthesis [3,10].

An increased contribution of blue light is observed due to shading [14]. Under these conditions, it enhances the synthesis of lipids, which has a protective effect against the decreased temperature. The delayed entry of water from the roots under these conditions probably causes stress, the overcoming of which leads to increased absorption of carbon by plants. It has been found that the enrichment of lettuce plants with blue light as a result of shading improves the efficiency of photosynthesis. This spectral composition of light has a positive effect on the relationship between chlorophylls $a$ and $b$ and increases the specific leaf area. As a result, increased biomass accumulation was shown [3].

Lettuce is one of the main agricultural crops grown in greenhouses where light intensity is low. Such behavior belongs to the long-day characteristics of this species. The best adaptation to a short day is the ability to prolong vegetative growth. Therefore, these plants have great potential to adapt to shading conditions [1,3]. Other authors have concluded that salads absorbed effectively at low light intensity [12]. At lower light intensity, the area covered by plants increased. It was found that the light curve of photosynthesis in lettuce has a smaller slope, which indicates the ability of this species to fully absorb light with a low intensity, showing a good growth rate. Some authors have suggested that in lettuce a lower temperature and short-term shadowing may lead to a more intensive intake of nitrates and their utilization in the synthesis of proteins and the accumulation of biomass [10,15].

More efficient use of water is a successful strategy for survival in reduced access to soil water supply. Such conditions are presented in this experiment, when cooling of the irrigation water slows its entry into the roots and reduces its access to the leaves. To increase the water use efficiency, it is necessary to preserve water resources and ensure their maximum use in biomass [16]. This means to limit transpiration, which can be seen in this experiment in all the varieties tested. Greenhouse plants of the varieties 'Estony' and 'Nika' are most successfully adapted to a lower temperature of the
irrigation water through more efficient water use in growth maintenance. Increased efficiency of water use is a precondition for higher productivity under unfavorable environmental conditions [17,18]. Preservation of water resources provides an opportunity to maintain good physiological activity even in very adverse conditions [14,17,19,20].

In our experiment, plants grown in different irrigation water temperature regimes responded to cooling and shading mainly with water use efficiency.

Conclusions

Most of the lettuce varieties did not show a statistically significant effect of cooling of the irrigation water (in the range of 12°C to 20°C) on the rate of photosynthesis.

Plants responded to the lower temperature of the irrigation water and strong shading by reducing their transpiration and increasing water use effectiveness.

The best adaptability to low temperature and shading was associated with green leaf lettuce (‘Salakis’ and ‘Estony’). Green leaf lettuce can be used in the practice of extending the period of vegetative growth. Plants with red leaves have a higher content of anthocyanins; these pigments can act as antioxidant protectors and directly influence the plant’s adaptability to unfavorable environment. However, further studies are needed to clarify the role of anthocyanins in the adaptability of lettuce to abiotic stress.

References


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Ocena reakcji sałaty (Lactuca sativa L.) na stres termiczny i świetlny

Streszczenie

Celem badań była ocena wpływu temperatury wody użytej do nawadniania oraz cierniowania roślin na tempo fotosyntezy i transpiracji czterech odmian sałaty (Lactuca sativa L.): 'Salkis', 'Lollo Rossa', 'Estony' i 'Nika'. Podczas produkcji rozsady w szklarni do nawadniania stosowano wodę o temperaturze 12 i 20°C. Po przesadzeniu sałaty do gruntu zastosowano 3 systemy oświetlenia (100, 70 i 50% oświetlenia). Tempo fotosyntezy i transpiracji mierzono w końcowym etapie uprawy rozsady w szklarni i po upływie 14 dni uprawy w polu w warunkach zróżnicowanego cierniowania (za pomocą analizatora gazu w podczerwieni Li 6400). U większości odmian sałaty nie stwierdzono wpływu chłodzenia wody stosowanej do nawadniania na tempo fotosyntezy. Odpowiedzią na obniżenie temperatury wody do nawadniania oraz silne cierniowanie wszystkich odmian sałaty była redukcja transpiracji oraz zwiększenie efektywności wykorzystania wody. Odmiany sałaty 'Salkis' i 'Estony' wykazały najlepsze przystosowanie do zmieniających się warunków, dlatego z powodzeniem mogą być stosowane w praktyce z wydłużeniem wzrostu wegetatywnego.