World Scientific Research Journal Volume 10 Issue 5, 2024
ISSN: 2472-3703 DOI: 10.6911/WSRJ.202405_10(5).0006

Effects of Air Humidity Difference on Drought Stress State of Leaves on Hydrophilic Side and Hydrophobic Side of Same Lettuce

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Abstract

This experiment investigated the effect of different air humidity on the drought stress state of lettuce. A potted control experiment was used where a separate body of water was placed on one side of the lettuce used to create an air humidity gradient around the lettuce. Air relative humidity, leaf water potential, leaf water content, antioxidant enzyme activities, and malondialdehyde content were determined on the hydrophilic and hydrophobic sides of lettuce. The results showed that significant air humidity differences were formed between the hydrophilic and hydrophobic sides of lettuce, and the relative air humidity, leaf water potential and leaf water content were higher on the hydrophilic side of lettuce than on the hydrophobic side, whereas the SOD activity, CAT activity, POD activity as well as the malondialdehyde content of lettuce leaves on the hydrophilic side were lower than those on the hydrophobic side. The results showed that lettuce under drought stress could absorb water vapor in the air to alleviate its own degree of drought stress, and lettuce leaves on the side with higher relative air humidity could absorb more water vapor to alleviate the degree of drought stress on the hydrophilic side of lettuce.

Keywords

Lettuce; Relative air humidity; Antioxidant enzymes; Drought stress.

1. INTRODUCTION

Water is an important environmental factor in the growth and development of plants, and all normal life activities of plants cannot be separated from the participation of water. Soil water resources are the main source of water for plants, and the main form of water utilization by plants is to absorb soil water through the root system and then be utilized. However, terrestrial water resources are very limited and unevenly distributed spatially and temporally, and plants have evolved a variety of morphological and physiological traits and behavioral strategies to cope with the environmental stresses caused by water stress[1]. Some studies have shown that when soil water is insufficient, plants can improve their overall water status by absorbing water vapor from the air through their leaves, thus sustaining themselves in arid environments[2].

When plants are subjected to environmental stress for a long time, a set of antioxidant enzyme protection system which can maintain normal life activities is produced in order to adapt to survival[3]. Superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT) form an antioxidant enzyme system under the synergistic action in plants, which reduces the damage caused by stress in plants by scavenging reactive oxygen radicals accumulated in plants under adversity and by mitigating the degree of membrane lipid peroxidation in cells[4]. Plants can also measure the magnitude of lipid peroxidation by changes in the amount of the lipid

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peroxidation product malondialdehyde (MDA) produced in response to environmental stress[5]. So the degree of damage to plants by environmental stress can be measured by changes in their antioxidant enzyme protection systems.

In this study, by placing an independent water body on the side of lettuce, an air humidity difference was formed between the hydrophilic side and hydrophobic side of lettuce under a certain drought stress, and the degree of influence of the air humidity difference on the drought stress state of the same lettuce plant was investigated. This experiment provides theoretical support for the drought-resistant and water-saving cultivation of lettuce.

2. MATERIALS AND METHODS

2.1. Experimental materials and experimental environment

Lettuce was chosen as the experimental material, which can be planted all year round and has a developed root system with strong adaptability. The lettuce variety is Green Collar (Nanjing Green Collar Seed Co., Ltd.) This variety is now widely grown in China. Lettuce seeds were soaked for 3 hours and then sown in hole trays for seedlings, and after the lettuce seedlings had grown three leaves, the uniformly growing seedlings were selected for transplanting.

The experiment was conducted from November 2023 to January 2024 in an artificial climate chamber at the College of Agricultural Engineering, Jiangsu University. Each environmental parameter of the artificial climate chamber: light intensity of 400 $\mu mol \cdot m^{-2} \cdot s^{-1}$, temperature 25/20°C, and photoperiod of 10/14h (light/dark).

2.2. Experimental design

The experimental plants were planted in plastic pots (upper diameter 16.5cm, lower diameter 8.9cm, height 11cm). Each pot was filled with one kilogram of soil and the relative moisture content of the soil was set at 36% to 40% to keep the plants under drought stress. A rectangular plastic trough (56cm long, 37cm wide and 8cm high) was chosen for the experiment as a side independent open water body, filled with water and placed on the plant side. The sidewall of the plastic pots near the plastic trough side is called the hydrophilic side, and the sidewall on the opposite side is called the hydrophobic side. The experiment was set up with two treatments: T1 (hydrophilic side) and T2 (hydrophobic side), and each plant included two treatments per pot, with each pot representing one replication, for a total of five pots. No further water was applied to the lettuce during the experiment and the indices were measured after 60 days of treatment.

2.3. Determination index

(1) Leaf water content

Water content of plant leaves was determined by drying method. The measurement site was selected as a fully expanded leaf, and the water content of the leaf on the hydrophilic and hydrophobic sides needed to be determined for each potted plant.

(2) Leaf water potential

Using a dew point water potential meter, plant leaf water potential was measured from 10:00 to 11:00. The leaf selection criteria are the same as above, and the leaf water potential of the hydrophilic and hydrophobic sides of each plant needs to be determined for each pot.

(3) Measurement of antioxidant enzyme activity and malondialdehyde content

Malondialdehyde (MDA) content was determined by thiobarbituric acid method, catalase (CAT) activity was determined by H_2O_2 UV spectrophotometric method, peroxidase (POD) activity was determined by guaiacol method, and superoxide dismutase (SOD) activity was

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determined by NBT (Nitrogen Blue Tetrazolium) photochemical reduction method[6]. Leaf selection criteria were the same as above, and antioxidant enzyme activities and malondialdehyde content were required to be determined on the hydrophilic and hydrophobic sides of each plant in each pot.

(4) Relative air humidity

Use a temperature and humidity sensor to measure the relative humidity of the air. The height of the measurement was kept in line with the top canopy of the plant, and the relative humidity of the air on the hydrophilic and hydrophobic sides needed to be determined for each plant in each pot.

2.4. Data processing

The data collected were analyzed using Excel 2010 software and SPSS 26.0 software. The raw experimental data were organized in Excel and then the independent samples T-test was performed on the experimental data using SPSS.

3. RESULTS AND ANALYSIS

3.1. Effect of air humidity differences on lettuce leaves

Environmental factors are important aspects that affect plant growth and development. This experiment was conducted to analyze the environmental factors affecting the growth of lettuce by measuring the relative humidity of air on the hydrophilic and hydrophobic sides of lettuce. The results of the experiment are shown in Table 1, the air relative humidity on the hydrophilic side of the lettuce is about 64.46% and on the hydrophobic side is about 54.91%, which is 17.39% higher on the hydrophilic side than on the hydrophobic side.

Table 1. Relative humidity of air under different treatments

Treatment	Air relative humidity/%
T1	64.46±0.25
T2	54.91±0.15

The leaf is the organ of the plant that is most exposed to the external environment and is very sensitive to environmental changes. Leaf water potential is the most obvious physiological indicator of water deficit status in plants, which can directly reflect the overall water deficit of plants and show the response of plants to changes in environmental factors[7]. As a general rule, the higher the water potential of a leaf, the greater its internal moisture. The experimental results, as shown in Table 2, showed that the average water potential of leaves on the hydrophilic side of lettuce was about -1.46 MPa and that of leaves on the hydrophobic side was about -1.63 MPa, with the hydrophilic side being 11.64% higher than the hydrophobic side, and the water potential of leaves on the hydrophilic side being significantly higher than that on the hydrophobic side ($P<0.05$).

Table 2. Leaf water potential of lettuce under different treatments

Treatment	Leaf water potential/(MPa)
T1	-1.46±0.04
T2	-1.63±0.04

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Leaf water content can directly reflect the actual situation of crop growth and development, and can be used as an important indicator for detecting crop stress conditions. The results of the experiment are shown in Table 3, the water content of the leaves of the hydrophilic side of the lettuce was about 95.45% and the water content of the leaves of the hydrophobic side was about 93.63%, which was 1.94% higher on the hydrophilic side than on the hydrophobic side.

Table 3. Leaf water content of lettuce under different treatments

Treatment	Leaf water content/(%)
T1	95.45±0.01
T2	93.63±0.01

The results of the above studies showed that due to the presence of separate water bodies on the side, a significant air humidity difference was formed between the hydrophilic and hydrophobic sides of the lettuce, and the leaf water potential and leaf water content of the hydrophilic side of the lettuce were higher than those of the hydrophobic side, indicating that lettuce can absorb water vapor from the air.

3.2. Effect of air humidity differences on antioxidant enzyme systems in lettuce

The levels of antioxidant enzyme activity and malondialdehyde content indicate whether the plant is under environmental stress or the extent to which the plant is under such stress. Reactive oxygen species (ROS) play an important role in plant growth and establishment. The production and elimination of reactive oxygen species (ROS) in plants are in dynamic equilibrium under normal conditions, but this dynamic equilibrium is disrupted when plants are subjected to water stress, resulting in the production and accumulation of ROS. ROS cause oxidative damage to plant cells, at which point the plant scavenges free radicals by increasing the activity of antioxidant enzymes, including superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD), thereby mitigating the damage caused by the stress to the plant. Malondialdehyde (MDA) is a product of the membrane lipid peroxidation process, and its levels are often used as an indicator of oxidative damage[8]. Thus, the levels of SOD, POD, CAT and MDA can indicate the degree of environmental stress suffered by the plant.

As shown in Table 4, the SOD activity, CAT activity, POD activity, and malondialdehyde content of lettuce hydrophilic side were higher than that of hydrophobic side, and all of them had reached the significant level ($P<0.05$), and the SOD activity, CAT activity, POD activity, and malondialdehyde content of lettuce hydrophilic side were 4.77%, 3.48%, 2.77% and 9.93% higher than that of hydrophobic side. The above findings illustrate that by comparing the antioxidant enzyme activities and malondialdehyde contents of leaves on the hydrophilic and hydrophobic sides of lettuce, it was shown that leaves on the side with higher relative air humidity were better able to alleviate the degree of drought stress on the hydrophilic side of lettuce.

Table 4. Effects of different treatment conditions on the activities of superoxide dismutase (SOD), catalase (CAT), peroxidase (POD) and the content of malondialdehyde (MDA) in lettuce

Treatment	SOD/(units/g)	CAT/[U/(g·min)]	POD/[U/(g·min)]	MDA/(umol/l)
T1	150.15±1.85	41.70±0.44	185.61±2.06	1.41±0.02
T2	157.31±2.48	43.15±0.45	191.36±1.81	1.55±0.02

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4. DISCUSSION

In the process of plant development and evolution, environmental changes will have direct or indirect impacts on plants, and plants will also have impacts on their living environment through feedbacks and gradually develop many physiological and morphological adaptive traits to minimize the negative impacts of environmental changes. In this study, by measuring air relative humidity, leaf water potential, leaf water content, antioxidant enzyme activities, and malondialdehyde content between the hydrophilic and hydrophobic sides of lettuce, it was found that lettuce leaves can absorb water vapor in the air to ameliorate their own degree of drought stress, and thus maintain their growth and development.

Leaf water potential refers to the difference in pressure of the water inside the leaf relative to the water in the surrounding air, which is highly susceptible to changes in external environmental factors, and in general, the higher the leaf water potential, the greater its internal water[9]. The levels of leaf water content, antioxidant enzyme activities and malondialdehyde content can characterize the degree of drought stress in plants. In this study, the independent water bodies on the side created air humidity differences between the two sides of lettuce, and the air relative humidity on the hydrophilic side of lettuce was higher than that on the hydrophobic side, which resulted in higher leaf water potential and leaf water content on the hydrophilic side of lettuce than on the hydrophobic side, and also the SOD activity, CAT activity, POD activity, and malondialdehyde content of the leaves on the hydrophilic side of lettuce were lower than that on the hydrophobic side. It was shown that after lettuce sensed the difference in air humidity through the leaves, the hydrophilic side leaves absorbed more water vapor than the hydrophobic side leaves, which to a certain extent better alleviated the degree of drought stress in the hydrophilic side leaves of lettuce.

ACKNOWLEDGMENTS

The research is financed by Major Project of Natural Science Research in Colleges and Universities of Jiangsu Province (15KJA210001).

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