Research on the Regulatory Mechanism of Trehalose in Mentha Citrata’s Resistance to Drought

Yingying Feng¹, Lujuan Cai¹
(College of Ecology, Shanghai Institute of Technology, Shanghai 201418, China)

Abstract: Simulation of drought stress by 10% polyethylene glycol (PEG-6000) is conducted to measure the indicators of photosynthetic and chlorophyll fluorescence shown by Mentha citrata seedling foliage at different times and in different conditions. The result suggests that drought stress harms the growth of such seedlings, causing a drastic decline in their photosynthesis and pigment content; exogenous trehalose is effective to mitigate the drought-induced decrease in their \( P_n \), maintain the stability of \( G_s \) and \( T_r \), and meanwhile reduce the content of \( C_i \); such trehalose can mitigate the drought-induced decrease in \( F_m \), \( F_v/F_m \), \( F_v/F_o \), \( q_P \) and \( Y(II) \) to a certain extent, slow the decrease in \( F_o \), and restore \( Y(NPQ) \) to its normal range. Overall, exogenous spraying of trehalose can alleviate the damage that drought stress inflicts on the photosynthesis of Mentha citrata seedlings, maintain a stable photosystem, and enhance such mint’s resistance to drought to some extent.

1. Introduction

People’s increasing demand for water resources accompanied by aggravation of water environment pollution and damage leaves the shortage of water environment one of vital environmental stressors affecting plant growth [1,2]. Drought has a serious effect on plant growth. Mentha citrata, belonging to the family of labiatae, is a perennial herbaceous plant that possesses both appreciation and medical values, having the effect of dispelling wind-heat, mental refreshment and letting out skin rashes [3]. It draws great attention from researchers studying “fragrance” thanks to its aromatic flavor, and furnishes beverages and delicacies with finishing touches, which implies its considerable development potential and promotional value. Nevertheless, insufficient water might kill nepionic Mentha citrata, so a close relationship exists between moisture and the cultivation area that mints are adaptable to.

Trehalose, an exogenous bioregulator that has been frequently discussed over recent years, plays an essential role in the development of foliage, and of plant photosystem including chloroplasts and photosynthetic pigments [4]. A previous study has indicated that there is a close relationship between trehalose, and the metabolism of chloroplasts and photosynthetic pigments [5].

This research discusses the influence that exogenous spraying of trehalose exerts on the photosynthesis and chlorophyll fluorescence properties of mint seedlings under drought stress, in an attempt to reveal the physiological mechanism whereby trehalose enhances such seedlings’ resistance to drought. The purpose is to provide a theoretical foundation for the application of trehalose to mint cultivation so that the planting area mints can adapt to will expand.
2. Materials and Methods

2.1. Experimental materials
Take annual Mentha citrata seedlings as experimental materials and 10% polyethylene glycol (PEG-6000) to simulate arid environment.

2.2. Experimental design
This experiment was conducted in the plant cultivation laboratory equipped with new LED light source in the School of Ecology, Shanghai Institute of Technology in May, 2020.

Select mint seedlings of the same size and similar figure before experimental treatment. Spray clean water on the control group, and 15% PEG and 15% PEG+0.5g/L trehalose respectively on the experimental group. Measure the physiological and biochemical indicators of seedlings in each group on the third, sixth and ninth day, and repeat each treatment three times.

2.3. Items to be measured and methods
The LI-6400XT portable photosynthesis system manufactured by LI-COR Biosciences is adopted to measure net photosynthetic rate ($P_n$), intercellular CO$_2$ concentration ($C_i$), stomatal conductance ($G_s$) and transpiration rate ($T_r$).

The portable PAM chlorophyll fluorometer PAM-2500 manufactured by Walz is used to measure the indicators of original fluorescence ($F_o$), maximum fluorescence ($F_m$), potential photochemical activity ($F_v/F_o$), maximum photochemical activity ($F_v/F_m$), non-photochemical quenching coefficient ($NPQ$), photochemical quenching coefficient ($q_P$) and actual photochemical efficiency [$Y(II)$].

2.4 Statistics
Excel 2016 is used for statistics of experimental data.

3. Results and Analysis

3.1. Exogenous trehalose’s influence on the photosynthetic parameters of Mentha citrata under drought stress
As shown in Figure 1, the $P_n$, $C_i$, $G_s$ and $T_r$ of Mentha citrata leaves treated with 15% PEG are in decline in comparison with those of CK as stress time increases; the $P_n$, $C_i$, $G_s$ and $T_r$ of Mentha citrata leaves treated with 15% PEG+0.5g/L trehalose are also in decline in comparison with those of CK as stress time increases, but the overall downward trend appears lighter compared with that of the group treated with 15% PEG. The difference is subtle between the $C_i$ of Mentha citrata in the 15% PEG+0.5g/L trehalose treatment group and that under drought stress, while the differences of $P_n$, $G_s$, and $T_r$ appear apparent between the 15% PEG treatment group and the 15% PEG+0.5g/L trehalose treatment group when stress time lasts for 9d.
3.2. Exogenous trehalose’s influence on the chlorophyll fluorescence parameters of Mentha citrata under drought stress

As shown in Figure 2, the Mentha citrata leaves treated regardless with 15% PEG or 15% PEG+0.5g/L trehalose see a rising trend in their respective $F_o$ in comparison with that of CK as stress time increases, and a declining trend in their respective $F_m$, $F_v/F_o$ and $F_v/F_m$ in comparison with those of CK, while both the rising and declining trend in the 15% PEG+0.5g/L trehalose treatment group are slighter than those in the 15% PEG treatment group. When stress time lasts for 9d, each indicator of the $F_m$, $F_v/F_o$ and $F_v/F_m$ in the 15% PEG treatment group is smaller than that of the 15% PEG+0.5g/L trehalose group, second to that of the CK.

As shown in Figure 3, the $q_P$ and $q_N$ of the Mentha citrata leaves treated with 15% PEG overall show an opposite trend that the $q_P$ declines while the $q_N$ rises as stress time increases; the same holds true to the 15% PEG+0.5g/L trehalose treatment group, but the trend is slighter compared with that of the 15% PEG treatment group. Compared with that of CK, the degree of change in the 15% PEG+0.5g/L trehalose treatment group is smaller than that of the 15% PEG treatment group.
As shown in Figure 4, the \( Y(II) \) and \( Y(NPQ) \) of the Mentha citrata leaves treated with 15% PEG overall show a declining trend in comparison with those of CK as stress time increases, whereas the \( Y(NO) \) overall shows a rising trend; conversely, in the 15% PEG+0.5g/L trehalose treatment group, the \( Y(NO) \) shows a declining trend along with the \( Y(II) \) and \( Y(NPQ) \), two indicators that remain close to those of CK.

4. Conclusions and Discussions

4.1. The influence of drought stress on the photosynthesis of Mentha citrata seedlings

The net photosynthetic rate \( (Pn) \) can reflect photosynthesis capacity to some extent \(^{[6]}\). The stomatal conductance \( (Gs) \) and transpiration rate \( (Tr) \), essential physiological indicators influencing \( Pn \), affect plant photosynthesis, respiration and transpiration. The shortage of water in plants hinders chlorophyll synthesis and meanwhile accelerates its breakdown \(^{[7,8]}\), which impedes the absorption of light energy...
and eventually engenders the decline in $Pn$ \cite{9}. In this experiment, the constant decrease in the $Pn$, $Ci$, $Gs$, and $Tr$ of Mentha citrata seedlings arising from drought stress as time increases indicates that the arid environment harms the growth of Mentha citrata, resulting in lower $Pn$, $Tr$ normally changes in the same direction with $Gs$, which refers to the degree of stomatal opening, while stomata serve as channels for water evaporation \cite{10}. These two indicators decrease with the extension of stress time, which might be a way that plants protect themselves since the closing of stomata works to minimize water consumption, which is a result similar to that yielded by Zhao Ping et al. \cite{11} in their research on summer Ormosia pinnata. That the $Ci$ decreases in sync with the decline in $Pn$, $Gs$ and $Tr$ as stress time lasts longer shows that the decline in $Pn$ arises from non-stomatal factors\cite{12,13}. In other words, $Pn$ can return from its decline, which results not from light energy utilization, to its normal range under appropriate conditions.

4.2. The influence of drought stress on the chlorophyll fluorescence of Mentha citrata seedlings

Photosystem II (PSII), the photosynthetic apparatus in the thylakoid membranes of chloroplasts, is most susceptible to environmental change \cite{14}. In consequence, further study on chlorophyll fluorescence is conducted on the basis of photosynthesis.

The decline in $Fm$ is regarded as a sign of more non-radiative energy dissipation \cite{15}. The decrease in $Fv/Fm$ indicates that plants are subject to photoinhibition \cite{16}, while such decrease, if accompanied by the increase in $Fo$, signifies that photosystem II has been impaired \cite{17}. This study shows that the treatment of Mentha citrata with PEG causes $Fo$ to increase significantly and $Fm$, $Fv/Fo$, and $Fv/Fm$ to drop, indicating that the non-photochemical energy dissipation of Mentha citrata under drought stress intensifies and the light energy utilization of leaves drops to a lower rate. Stress leaves the potential active center of $PSII$ impaired and inhibits the primary reactions of photosynthesis. The photosynthetic electron transfer is affected and photosynthetic apparatus is damaged, which is unfavorable for Mentha citrata seedlings to convert their light energy captured into chemical energy. This result is consistent with that concluded by Wu Xuexia et al. \cite{18} in their research on tomato seedlings. The quenching coefficient can reflect the utilization of excitation energy in leaves. In this research, the contrary trend of $qN$ and $qP$, which rises and drops respectively, illustrates that more heat of Mentha citrata seedlings dissipates and non-photochemical quenching increases due to drought. $Y(II)$ refers to the actual photochemical efficiency of $PSII$ when plants are in the light. This research shows that the $Y(II)$ of Mentha citrata seedlings under drought stress decreases significantly, which indicates that the actual photochemical conversion efficiency falls in the photochemical structures of internal seedlings. The decrease in $Y(II)$ further triggers the decline in $Pn$.

4.3. Exogenous trehalose’s influence on Mentha citrata seedlings under drought stress

Exogenous trehalose is effective to mitigate the damage caused by drought to photosynthesis of Mentha citrata seedlings. It is demonstrated that, under drought stress, the degree of decline in the $Pn$, $Gs$, and $Tr$ caused by PEG is larger than it is by exogenous trehalose which has a catalytic role in the $Pn$ of such leaves in shortage of water and enhances the seedlings’ resistance to drought. This result is in consistence with that drawn by Pang Chunpeng et al. \cite{19} in their study on tomatoes under stress being sprayed trehalose. The decrease in $Pn$, $Gs$, $Tr$ and $Ci$ concentration of such mints treated with exogenous trehalose under drought stress indicates that the decline in $Pn$ under this circumstance arises from non-stomatal factors, which means trehalose can to some degree protect the reaction center of photosynthesis for Mentha citrata seedlings under drought stress.

The addition of exogenous trehalose improves the $Fm$, $Fv/Fo$, and $Fv/Fm$ significantly and reduces the $Fo$, alleviating the damage that drought stress inflicts on the reaction center of $PSII$, which proves that the potential active center of $PSII$ is possible to suffer less damage caused by drought stress thanks to exogenous trehalose. When Mentha citrata seedlings under drought stress are treated with exogenous trehalose, their $Y(II)$ and actual photochemical utilization efficiency rise, while their $qP$ drops. It turns out that trehalose can to some extent ameliorate the problem that the photochemical efficiency of such seedlings falls because of insufficient water.
In conclusion, Mentha citrata seedlings suffer due to drought stress, while trehalose is a new regulator for plant growth that contributes to protecting plant tissues and structures [20]. This study demonstrates that exogenous trehalose slows the drought-induced decline in $Pn$, $Gs$, $Tr$, $Fm$, $Fv/Fo$, $Fv/Fm$, and $qP$, and lowers the activity of $Fo$ and $qN$ under drought stress. Exogenous trehalose can to some degree protect the photosystem and chlorophyll fluorescence for Mentha citrata seedlings in shortage of water.

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