Influence of drought stress on photosynthetic characteristics and protective enzymes of potato at seedling stage

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Abstract The influences of drought stress on the photosynthesis rate, the chlorophyll fluorescence parameters, the activity of protective enzymes of potato, and the biomass fresh weights of potato leaves at seedling stage were investigated, using two different varieties of potato with significant difference in drought resistance to carry out potted plant experiment with water control. Under mild and medium drought stresses, the two potato varieties stabilized the photosynthetic organ functions by light capture reduction, heat dissipation and the regulation of enzyme activity. The damage of photosystem II and antioxidant enzyme system was the non-stomatal limitation factors for the decrease of the photosynthesis rate under serious drought stress. The influence on the physiological parameters of potato Kexin No. 1 was weaker than that of potato Kexin No. 12 under drought stresses. The higher photosynthesis rate and stronger activity of protective enzymes were the important physiological reasons for the drought resistance of Kexin No. 1.

1. Introduction

Water deficiency is a common adverse factor for the growth of plants in the field conditions. It has substantial influence on the growth condition, morphological structure and physiology and biochemistry of plants (Bosabalidis and Kofidis, 2002; Jill et al., 2012; Wu et al., 2008). Under the drought stress, plants usually respond via stomata regulation, osmotic adjustment and anti-oxidative defense, in order to relieve the damage caused by the drought stress. However, long period of high intensity of drought stress could retard the growth of plants, causing the changes of the morphological structure and the distribution pattern of biomass, or even death (Christina and Gisela, 2013; Dias et al., 2007). Therefore, the response of crops to the drought stress and the drought resistance mechanism has drawn an increasing attention. Studies (Efegolu et al., 2009) indicated that drought is an important factor responsible for the inhibited growth of plants and reduced photosynthesis. Drought could prevent the entering of CO2 into the leaves, influence the absorption of CO2 by the carboxylation center and result in the decrease of net photosynthetic rate ($P_n$) (Zhang, 1999). In recent years, there have been studies on...
the relationship between the fluorescence characteristics of leaves and the membrane lipid peroxidation during the photo-oxygenation process. The results indicate that the PS II light energy conversion and the metabolism of reactive oxygen species are closely related to stress (Massacci et al., 1990; Wang et al., 2006). Therefore, the comprehensive study on photosynthesis, activity of protective enzymes and the relationship between the two can help explain the mechanism of the light energy conversion and utilization as well as the mechanism of drought resistance and yield increase.

Potato is one of the four major cereal crops in the world. It is planted in 156 countries and regions worldwide (Breezy, 2013). Potato is also an important agricultural and economic crop in northern China, with a large planting area. Potato is a typical crop of temperate climate, sensitive to water deficiency (Xu et al., 2008). However, most of the potato planting area in China is in shortage of water resources and the water deficiency influences greatly the growth and yield of potato (Lin et al., 2010). But up till now, there have been no systematic and profound studies on the drought resistance physiology and regulation in potato. This research selected different varieties of potato with significant difference in drought resistance to carry out potted plant experiment with water control. The purpose was to study the influence of drought stress on the photosynthesis rate, the chlorophyll fluorescence parameters and the activity of protective enzymes. The work will deepen the understanding of the mechanism of light energy utilization and dissipation in potatoes under drought stress. A theoretical reference is provided for water saving in the planting of potatoes.

2. Materials and methods

2.1. Experiment location and materials

The potted plant experiment was conducted in the mobile canopy in the science park of Farm 858 in Heilongjiang Province in 2012. The experiment location is situated in the eastern part of the Yunchan depression zone of Hulin Basin, with cold temperate continental monsoon climate. The annual average precipitation is 566.2 mm and the frost free period is 141 days. The test soil is the 0–20 cm surface layer of alibic bleached meadow soil. The measurement results of the traits of soil are as follows: Organic matter, 19.82 g kg⁻¹, alkali-hydrolysable nitrogen, 103.41 mg kg⁻¹, available phosphorus (Olsen-P), 23.83 mg kg⁻¹, available potassium (1 mol L⁻¹ NH₄OAC-AAS method), 121.61 mg kg⁻¹, pH (electrode method), 7.21. The varieties of potato used for the experiment were Kexin No. 1, with strong drought resistance and Kexin No. 12, with weak drought resistance. Kexin No. 1 has erect form, with medium number of shoots and sturdy stems. The growth season is approximately 95 days, and the stress resistance is strong. Kexin No. 12 has erect form, with medium number of shoots and sturdy stems. The growth season is approximately 95 days, with resistance to virus diseases and susceptibility to late blight.

2.2. Experiment design

The air dried soil of 15 kg was placed into a plastic barrel with the mouth 36 cm in diameter, the bottom diameter of 28 cm and a height of 38 cm. On the bottom of the barrel were drilled 5–6 holes with the diameter of 1 cm each. The rigid plastic tube inserted into the bottom of the barrel was used for the watering. The completely randomized design was adopted for the potted plant experiment. The degree of drought stress was determined according to the soil moisture content. The watering treatment had 4 levels, i.e. normal watering for each variety (CK), with soil moisture content being the maximum water holding capacity in field; mild drought (T1), with the soil moisture content being 60–70% of the maximum water holding capacity in field; medium drought (T2), with the soil moisture content being 50–60% of the maximum water holding capacity in field; serious drought (T3), with the soil moisture content being 35–45% of the maximum water holding capacity in field. The stress treatment was applied 10 days after the seedling. Every treatment had 10 pots and 3 duplicates. There were 120 pots of each species in total. During the growth of potato, the canopy was shut down in the rainfall, and it would be opened in other times to let potato grow in the open.

Soil moisture content was determined by TZS-IIW soil moisture analyzer equipped with FDR soil moisture sensor (Tuopu Co., China).

On May 10, the seed potato with uniform growth status and one terminal bud was planted in the plastic barrel. Every pot had 1 plant until the natural drought reached the defined range of soil moisture content. Every day at 8:00 am and 18:00 pm, the water was supplemented and controlled with weighing method and the record was made. When the soil relative moisture content indicated drought stress, the third compound leaf under the terminal leaf (fully expanded leaf) of the sample plants was obtained for the measurement of parameters for 7 consecutive days at 9:00.

2.3. Measurement parameters and methods

2.3.1. Measurement of light-response curve of the leaf

The Li-6400 portable photosynthesis system produced by the American company Li-cor was used to measure the Pn-PAR response curve during 9:00–12:00 using Li-6400-02B red and blue light source. The measurement was conducted for 2 consecutive sunny days and 3 duplicates were set. The average value was taken as the measurement value. via the open air channel, the temperature was set at 25 °C, the atmosphere CO₂ concentration (Cao), 400 μmol mol⁻¹, the atmosphere relative humidity, 50–70%, the light intensity scale, 1800, 1600, 1400, 1200, 1000, 800, 600, 400, 350, 300, 200, 150, 100, 50, 20, 0 μmol m⁻² s⁻¹, respectively. The net photosynthetic rate (Pn, μmol m⁻² s⁻¹), stomatal conductance (Gs, mmol m⁻² s⁻¹), intercellular CO₂ concentration (Cic, cmol mol⁻¹) of the leaves at each light intensity were measured. The stoma limit value was calculated according to (Lc) = 1 – Cao/Ci (Larocque, 2002).

2.3.2. Measurement of light-response curves of chlorophyll fluorescence parameters

The PAM-2100 fluorescence analyzer produced by German company WALZ was used to measure the light-response curve of chlorophyll fluorescence parameters at the symmetric points with the leaf vein as the axis. After the dark adaptation for 30 min, the initial fluorescence (F₀) and maximum fluorescence (Fm) in dark adaptation were measured. The 11 light intensities...
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were set up in the range of 0–2000 μmol m$^{-2}$ s$^{-1}$ to measure the maximum fluorescence ($F_{\text{m}}$), the minimum fluorescence ($F_{\text{n}}$) and the stable fluorescence ($F_{\text{s}}$) at light adaptation. The actual quantum yield ($\eta_{\text{PSII}}$), electron transport rate (ETR), photochemical quenching coefficient ($q_{\text{P}}$) and non-photochemical quenching coefficient ($q_{\text{N}}$) were calculated with reference to the method by Demmig-Adams et al. (1996).

2.3.3. Measurement of malondialdehyde content and activity of protective enzymes

The thiobarbituric acid method was used for the measurement of the malondialdehyde content (Zhang, 2010). The following methods were used for the measurement of the protective enzymes (Zhang, 2010): Superoxide dismutase (SOD), nitroblue tetrazolium method; peroxidase (POD), guaiacol coloration method; catalase (CAT), ultraviolet spectrophotometry.

2.4. Data statistics and analysis

The data were processed using Excel 2003 and SAS 9.2.

3. Results and analysis

3.1. Light response of gas exchange parameters of potato leaves under drought stress

Light intensity has substantial influence on the photosynthesis of potatoes. The response of different varieties to light under drought stress is different. Within the range of natural light intensity, $P_n$ of the leaves of the two varieties under different drought stress increased with the increase of PAR. When PAR reached a certain value, i.e. reaching the light saturation point, the curve tended to be steady and then showed a downward trend. Under the same light intensity and different drought stress, $P_n$ of the leaves of the two varieties tended to decrease. In the T3 treatment, $P_n$ decreased significantly and the drop of Kexin No. 12 was greater than that of Kexin No. 1.

The change tendency of the response of $G_s$ to PAR was the same as that of $P_n$. However, under the drought stress treatments, the response of $G_s$ of Kexin No. 12 to the increase of the light intensity was not sensitive, indicating that the stoma of the plants lost the regulation effects, causing insensitivity to light intensity. The change tendency of the light response of $C_l$ and $L_s$ to light was different. Under weak light intensity, $C_l$ of the two varieties decreased as PAR increased. When PAR exceeded a certain limit, $C_l$ increased while $L_s$ tended to drop as the light intensity increased. Under the same light intensity, with the intensification of drought stress, $C_l$ decreased first and then increased; while $L_s$ increased and then decreased. The changing amplitude of Kexin No. 12 was greater than that of Kexin No. 1 (Fig. 1).

3.2. Light response of chlorophyll fluorescence parameters of potato under drought stress

ETR of potato leaves increased and then decreased with the increase of light intensity Under weak light and adapted light intensity, with the intensification of drought stress, ETR of the two varieties showed the downward tendency (Fig. 2). With the increase of light intensity (for example, over 300 μmol m$^{-2}$ s$^{-1}$), the photo inhibition occurred. With the intensification of drought stress, ETR and $\eta_{\text{PSII}}$ of the two varieties decreased in gradient, and the light saturation point was reached in advance. In general, the drop amplitude of Kexin No. 12 was significantly greater than that of Kexin No. 1. Under the CK treatment, ETR and $\eta_{\text{PSII}}$ of the leaves showed no difference between the varieties. Under the T2 treatment, the difference was significant (Fig. 2). The photosynthetic electron transport of Kexin No. 12 basically stopped and $\eta_{\text{PSII}}$ also reached the minimum. At the light intensity over 300 μmol m$^{-2}$ s$^{-1}$, the photo inhibition was observed. The light-response curve under different watering treatments of Kexin No. 1, which has a greater drought resistance, showed a compact distribution. Under the T3 treatment, it had higher ETR and $\eta_{\text{PSII}}$, indicating that the photosynthesis system of the variety with higher drought resistance had a stronger adaptability to drought.

$q_{\text{P}}$ of the potato leaves decreased with the intensification of the drought stress, which is basically consistent with the light response tendency of $\eta_{\text{PSII}}$. With the increase of light intensity and water deficiency, under the T3 treatment for the same light intensity, the drop amplitude of Kexin No. 1 was significantly smaller than that of Kexin No. 12 and the former had higher $q_{\text{P}}$. This indicated that Kexin No. 1 had stronger photosynthetic electron transport capacity under drought stress. $q_{\text{N}}$ of potato leaves under drought stress increased with the increase of light intensity. Under the same intensity, with the intensification of drought stress, $q_{\text{N}}$ showed the increasing tendency. But under the strong light (over 300 μmol m$^{-2}$ s$^{-1}$) in T3 treatment, $q_{\text{N}}$ dropped, indicating that the heat dissipation capacity of $\text{PSII}$ at this moment was damaged. The change amplitude of $q_{\text{N}}$ of Kexin No. 1 under drought stress was smaller than that of Kexin No. 12. It was indicated that the heat dissipation of Kexin No. 1 was lower and the light energy conversion rate was higher (Fig. 2).

3.3. Influence of drought stress on the activity of antioxidant enzymes of potato leaves

With the intensification of drought stress, the change of the activity of SOD, POD and CAT of the leaves of the two varieties both increased and then decreased, reaching the maximum under the T2 treatment. Kexin No. 1 and Kexin No. 12 increased by 29.0%, 17.5%, 18.2% and 13.4%, 8.8%, 9.0%, respectively, in comparison with CK. The result indicated that the medium drought induced the increase of the activity of SOD, POD and CAT, relieving the damage caused by drought. Under the T3 treatment, however, the activity of SOD, POD and CAT decreased significantly, and the drop rate of Kexin No. 1 was slower and the activity was higher than that of Kexin No. 12. The result indicated that the capacity of clearing the active oxygen species in the leaves of Kexin No. 1 was higher than that of Kexin No. 12. The comparison between the 3 types of antioxidant enzymes indicated that the increase amplitude of the activity of SOD under the drought stress was the biggest and that of CAT and POD came next. This result showed that SOD was more sensitive to the drought stress and therefore was the main protective enzyme for the adaptation of potatoes to the drought stress (Fig. 3).
3.4. Influence of drought stress on malondialdehyde content in potato leaves

With the intensification of drought stress, the MDA content of the 2 varieties showed the upward tendency, but the increase amplitude was different. Under the T1 treatment, the difference in MDA content between Kexin No. 1 compared with the CK was not significant; under the T2 and T3 treatments, the MDA content was 34.5% and 84.6% higher than that of CK. In Kexin No. 12, the MDA content was 30.0%, 127.7% and 160.6% higher than that of CK under the T1, T2 and T3 treatments, respectively. Under the T3 treatment, the MDA accumulation of Kexin No. 1 was 1.24 times that of Kexin No. 12, which suffered from a substantial damage in the membrane system (Fig. 3).

3.5. Influence of drought stress on biomass fresh weights of potato leaves at seedling stage

The 2 varieties demonstrated the decrease on the biomass fresh weights of potato leaves with the intensification of drought stress, and particularly Kexin No. 12 presented relatively bigger decrease amplitude, indicating the sensitive response of its biomass fresh weights to the water content condition (Fig. 4). The fresh weights of Kexin No. 12 under the T1, T2 and T3 treatments decreased by 15.5%, 37.4%, and 48.8%, respectively.
respectively, compared with the CK. However, there were no significant differences of the fresh weights of Kexin No. 1 between the T1 and T2 treatments and the CK. For the T3 treatment, the fresh weights of potato leaves of Kexin No. 1 were 20.5% less than those under the CK.

4. Discussion

Under the drought stress, the plants first close the stomata to reduce the transpiration and prevent the entry of CO₂ into the leaves. The photosynthesis of the leaves is influenced by the stomatal and non-stomatal factors (Liu et al., 2005). \( C_i \) and \( L_a \) are the main determining criteria to distinguish the stomatal and non-stomatal factors in the decrease of the photosynthetic rate (Li et al., 2007). In this research, under the mild and medium drought, \( P_n \), \( G_s \), and \( C_i \) of the potato leaves dropped, while \( L_a \) increased. The changing tendency of \( C_i \) and \( L_a \) was just the opposite. The stomatal limitation was the main reason for the drop of \( P_n \). Under serious drought, \( C_i \) increased with the intensification of drought stress, while \( L_a \) showed the downward tendency. The non-stomatal limitation was the
main reason for the drop of $P_n$ in potato leaves. The stomatal and non-stomatal limitations of Kexin No. 1 under drought stress were lower.

The chlorophyll fluorescence dynamics parameters are the ideal method to study and detect the influence of the drought stress on the photosynthesis of plants in a rapid, sensitive and non-invasive way (van Kooten and Snel, 1990). The drought stress mainly damages PSII of the photosynthetic organ of plants (White and Critchley, 1999). PSII can regulate actively the electron transport rate and the photochemical efficiency, as a response to the decrease of the CO$_2$ assimilation capacity. It prevents or relieves the damage caused by excessive light energy to other systems via heat dissipation (Bu et al., 2010).

This research indicated that under mild and medium drought stress, ETR, $\varphi$PSII, $\varphi$P and $\varphi$N of the 2 varieties all decreased, indicating that the PSII system of the leaves suffered from damages to different degrees. $\varphi$PSII is the energy characterization of the CO$_2$ assimilation amount (Leipner et al., 2004). Therefore, it is assumed that the increase in the drop amplitude of $\varphi$PSII could be one of the reasons causing the drop of $P_n$ by non-stomatal limitation. Under the drought stress, the decrease of the activity of PSII of the plants could cause the increase of excitation energy, which results in the excessive energy and produces excessive active oxygen species. If not eliminated in time, it could cause the oxidation stress, resulting in the membrane lipid peroxidation and the damage of the membrane system (Reddy et al., 2004).

A system for clearing the active oxygen species is formed in plants, in which SOD, POD and CAT are the important protective enzymes, which can eliminate the active oxygen species produced in the plants. Under the drought stress, the balance of the production and clearing of the active oxygen species in the plants is disrupted. This leads to the substantial accumulation of active oxygen species and the aggravation of membrane lipid peroxidation. There will be an increase in membrane lipid peroxide (MDA) and the reduction of the photosynthetic capacity of the leaves (Esfandiari et al., 2008).

This research indicated that a mild drought stress could induce the increase of the activity of SOD, POD and CAT in the leaves of the 2 varieties, and relieve the membrane lipid peroxidation. Under serious drought, the activity of the 3 enzymes was significantly inhibited, and the MDA content increased substantially, damaging the structure and functions of the photosynthesis organ. The results of the experiment further proved that the damage of PSII photosystem and antioxidant enzyme system was the non-stomatal limitation factors of the decrease of $P_n$. The comparison between the 3 enzymes indicated that the increase amplitude of the activity of SOD was large under the drought stress and CAT and POD came next. This result indicated that SOD was more sensitive to the drought stress and was the
main protective enzyme against the drought stress. Under the same drought stress, the activity of the 3 enzymes of Kexin No. 1 leaves was significantly higher than that of Kexin No. 12. It was indicated that Kexin No. 1 leaves had a stronger membrane lipid peroxidation resistance capacity under drought conditions and suffered from a milder damage by active oxygen species. Malondialdehyde, the final product of the endomembrane lipid peroxidation, had a significantly lower content in the leaves of Kexin No. 1 than that of Kexin No. 12. This is the main physiological reason for the stronger drought resistance of Kexin No. 1 than Kexin No. 12. Moreover, the research results proved that the PSII light energy conversion and active oxygen metabolism acted in synergy, resulting in the lower capacity of heat dissipation of Kexin No. 1 (lower qN) than that of Kexin No. 12. The actual quantum yield (higher φPSII) was higher than that of Kexin No. 12. The molecular physiological mechanism of the coordinated regulation of PSII light energy utilization and active oxygen metabolism of leaves of different drought-resistant potato varieties requires studies.

5. Conclusion

Under mild and medium drought stress, the 2 potato varieties stabilized the photosynthetic organ functions by light capture reduction, heat dissipation and the regulation of enzyme activity. It is the stomatal limitation factor of the drop of the photosynthesis rate. Under serious drought stress, the damage of photosystem II and antioxidant enzyme system is the non-stomatal limitation factors for the decrease of the photosynthesis rate. The influence on the physiological parameters of Kexin No. 1 was weaker than that of Kexin No. 12 under drought. The higher photosynthesis rate and stronger activity of protective enzymes are the important physiological reasons for the drought resistance of Kexin No. 1.

Conflict of interest

There is no conflict of interest.

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