

**CHANGES OF PHOTOSYNTHETIC CHARACTERISTICS IN PERSIMMON
(DIOSPYROS SPP.) ROOTSTOCKS DURING DROUGHT STRESS
SIMULATED BY PEG-6000**

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Keywords: Persimmon rootstocks, PEG-6000 stress, Photosynthetic characteristics

Abstract

Three persimmon rootstocks (*Diospyros lotus*, *Diospyros oleifera* and *Diospyros glaucifolia*) seedlings were selected as research materials, five stress strength treatments (5, 10, 15, 20 and 25%) and four stress duration treatments (1, 2, 3 and 4 days) were set by using PEG-6000 to simulate drought stress environment artificially, and the changes of photosynthesis characteristics of persimmon rootstocks seedlings were studied. The results showed that net photosynthetic rate (Pn), intercellular CO₂ concentration (Ci), stomata conductance (Gs), transpiration rate (Tr) and water utilization efficiency (WUE) of seedling leaves of persimmon rootstocks decreased with the increase of PEG-6000 stress, and the variation of five photosynthesis indexes increased with the extension of PEG-6000 stress duration and the increase of intensity. It may be concluded that, with PEG-6000 stress increase, the Pn, Ci, Gs, Tr, WUE of persimmon rootstocks seedling were restrained which resulted in a significant reduction of photosynthesis.

Drought is one of the most important environmental factors that inhibits the photosynthesis of plants (Liu 1997). Drought stress could cause damage to photosynthetic organs, and thus inhibit photosynthesis (Liu *et al.* 2016, Ma *et al.* 2016). Under light, in the process of carbon assimilation, the ability of chloroplast to utilize CO₂ was restricted by drought stress, then energy consumption was reduced, the proportion of photosynthetic electrons transfer to O²⁻ was relatively increased, and so O²⁻ and H₂O₂ could be formed. When the production of free radicals exceeded the scavenging capacity of the defense system, the accumulation of free radicals would be caused. The balance between the generation and clearance of the free radicals was broken. The accumulation of O²⁻, to a certain degree, causes the destruction of chlorophyll, and the excess of H₂O₂ also inhibits the fixation of CO₂, and accelerate the aging of plants. O²⁻ accumulation to a certain extent causes the destruction of chlorophyll, O²⁻ and H₂O₂, catalyzed by transition metal ions Fe²⁺ or Cu²⁺, could form hydroxyl radical (-OH) by Fenton Haber-weiss reaction, which is more reactive and more aggressive, and these reactive oxygen species could aggravate membrane lipid peroxidation and caused destruction to the whole membrane (Liu *et al.* 1994, Fang *et al.* 2006, Zhao *et al.* 2010, Xie *et al.* 2010).

Recent studies had shown that the destruction of the biofilm structure, caused by the imbalance of active oxygen metabolism in plants, was the main reason for the non-stomatal limitation of photosynthesis (Qi *et al.* 2006, Liu *et al.* 2007, Chu *et al.* 2008, Guo *et al.* 2012, Sun *et al.* 2012, Wang *et al.* 2014, Zheng *et al.* 2015, Zhang *et al.* 2016). Using hydroponic nutrient solution and polyethylene glycol (PEG-6000) to simulate drought stress, the effects of drought stress on water utilization and photosynthetic characteristics of 3 persimmon rootstocks seedlings were studied, so as to provide theoretical basis for the research on drought resistance mechanism of fruit trees.

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Three persimmon (*D. lotus*, *D. oleifera* and *D. glaucifolia*) rootstocks have different drought resistance and their seedlings were used as research materials. The seeds were collected in the national Persimmon germplasm garden of Northwest Agriculture and Forestry University in early November, 2015. In January, 2016 the seeds were placed in the moist sand and placed in the refrigerator at 4°C for 60 days. After the seeds germinated for about 4 days in the 23 - 25 illumination incubator, the peat soil was used as the substrate and the distilled water was used to raise seedlings.

When 5 - 6 true leaves were found to grow in May, 2016 the seedlings with uniform growth were transplanted to the culture bowl and cultured in 1/4 Hoagland nutrient solution. When the 8 - 10 leaves were fully expanded, the nutrient solution was replaced with 1/2 Hoagland nutrient solution and replaced once every 2 days. When the seedlings grew to 10 - 12 leaves, the healthy seedlings with consistent growth were selected and cultured with PEG-6000 of 5, 10, 15, 20 and 25%, and the treatment with only 1/2 Hoagland nutrient solution was used as a blank control. Each treatment was treated with 3 pots and 2 seedlings were cultured per pot. After 24 hrs of treatment, the indexes were measured.

The photosynthetic characteristics were determined by Li-6400 portable photosynthesis analyzer. Under different drought stress, the net photosynthetic rate (Pn), stomatal conductance (Gs), transpiration rate (Tr) and CO₂ concentration (Ci) were measured from 9: 00 a.m. to 11: 00 a.m. everyday and continuously measured for 4 days. Each index was measured and repeated for 3 times, and the water use efficiency (WUE) was calculated by the following formula ($WUE = Pn / Tr, \text{mmol} \cdot \text{mol}^{-1}$). The experimental data were analyzed by SPSS 13, and Microsoft Excel 2003 for plotting.

The net photosynthetic rate (Pn) of persimmon rootstocks decreased with the increase of drought stress and stress time, and the decline degree of different rootstocks was different (Fig. 1). At the first day, compared with the control, treated with PEG-6000 of 5 and 10%, the Pn of *D. lotus* decreased by 25.87 and 34.3%, respectively, and under the stress of PEG-6000 of 15, 20 and 25%, the Pn decreased rapidly by 52.72, 67.5 and 91.77%, respectively. At the second day, compared with the control, the Pn of *D. lotus* slowly decreased under the stress of PEG-6000 of 5 and 10%, and decreased sharply under the stress of PEG-6000 of 15, 20 and 25%. At the third day and the fourth day, compared with the control, under the stress of PEG-6000 of 5 and 10%, the Pn of *D. lotus* decreased down sharply, and under the stress of PEG-6000 of 20 and 25%, the Pn was 0, and the leaves showed withered state. This showed that low concentration (5 and 10%) of PEG-6000 stress had little effect on the Pn of *D. lotus*, but with the increase of the concentration of PEG-6000, the Pn of *D. lotus* gradually reduced. When the concentration of PEG-6000 reached 20%, the Pn of *D. lotus* had decreased by 67.5%, and the seedlings were withered at the third day. These findings are in agreement with the previous research results (Fang *et al.* 2006, Wang *et al.* 2014). At the same time, it was found that the Pn of *D. oleifera* and *D. glaucifolia* showed the same trend as that of *D. lotus*.

With the increase of drought stress and stress time, the Gs of 3 persimmon rootstocks declined, which was in accordance with that of the Pn (Fig. 2). At the first and the second days, compared with the control, under the stress of PEG-6000 of 5, 10 and 15% concentrations, the Gs of *D. lotus* decreased slowly, and under the stress of PEG-6000 of 20 and 25%, the Gs declined sharply. At the third and the fourth day, under the stress of PEG-6000 of 5, 10 and 15% concentrations, the Gs of *D. lotus* decreased rapidly, and under the stress of PEG-6000 of 20 and 25%, the Gs was near to 0, and leaves almost lost their activity. The similar change rule was also observed in that of *D. oleifera* and *D. glaucifolia*. It was found that the Pn and Gs of *D. lotus* and *D. glaucifolia* were higher under normal conditions and decreased greatly under the simulated drought stress.

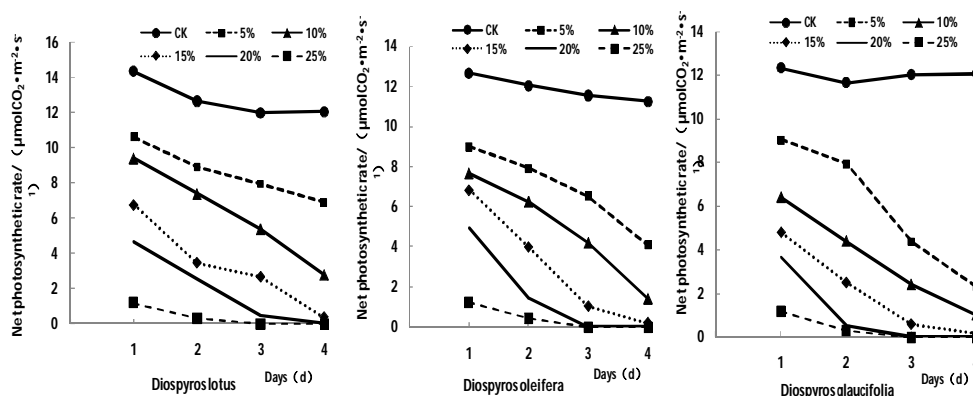


Fig. 1. Effect of PEG-6000 stress on net photosynthetic rate of different persimmon rootstocks.

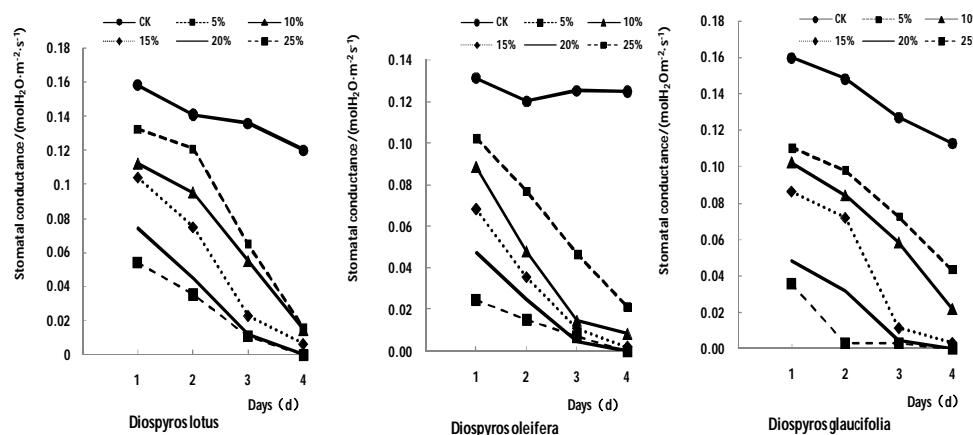


Fig. 2. Effect of PEG-6000 stress on stomatal conductance of different persimmon rootstocks.

The C_i of leaf was reported to be dependent on four factors: CO_2 concentration around the leaves, G_s , mesophyll conductance (GM) and photosynthetic activity of mesophyll cells (Guo *et al.* 2016). It was found that, under different PEG-6000 stress concentrations, with the extension of stress time, the C_i of three persimmon rootstocks was inconsistent in this experiment (Fig. 3). When PEG-6000 stress concentration was 5 and 10%, the C_i of *D. glaucifolia* was significantly higher than that of *D. lotus* and *D. oleifera* at the first and the second day. The C_i of three persimmon rootstocks was significantly lower than that of control, and sometimes even was negative at the third day and the fourth day. When PEG-6000 stress concentration was 15, 20 and 25% respectively, the C_i of three persimmon rootstocks was significantly lower than that of the control at the first and the second day and decreased obviously again at the third and the fourth day. The exchange of CO_2 and water in the leaves was hindered by the reduction of T_r and G_s . This observation is consistent with the previous research results (Cao *et al.* 2004).

With the increase of stress and treatment time, the transpiration rate (T_r) of 3 persimmon rootstocks decreased rapidly and then decreased slowly, which was similar to the changing trend of stomatal conductance (Fig. 4). Compared to the control, all persimmon rootstocks had a downward trend. Under the stress of PEG-6000 of 5, 10 and 15% concentrations, at the first and the second day, the T_r of *D. lotus* decreased compared with that of the control, but not by much.

Under the stress of PEG-6000 of 20 and 25%, the Tr of *D. lotus* decreased slowly. At the third and the fourth day, under the stress of PEG-6000 of 5, 10 and 15%, the Tr of *D. lotus* decreased significantly compared to the control. And under the stress of PEG-6000 of 20 and 25%, the Tr decreased sharply, almost to 0. Under drought stress, the stomatal aperture of plants decreased or partially closed, which resulted in an increase in stomatal resistance and a decrease in transpiration rate, thereby reduced water evaporation in plants (Liu 1994). In this study, from the change of stomatal conductance, it was found that the Tr of rootstocks decreased a lot, the Gs decreased a lot, and there was a great correlation between the size of stomatal opening and Tr. The mechanism of transpiration and stomatal movement of drought-resistant rootstocks need to be further studied.

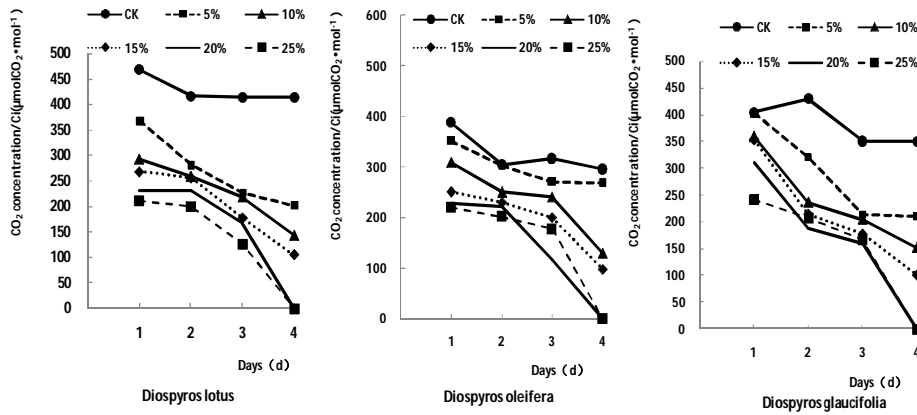


Fig. 3. Effect of PEG-6000 stress on CO₂ concentration of different persimmon rootstocks.

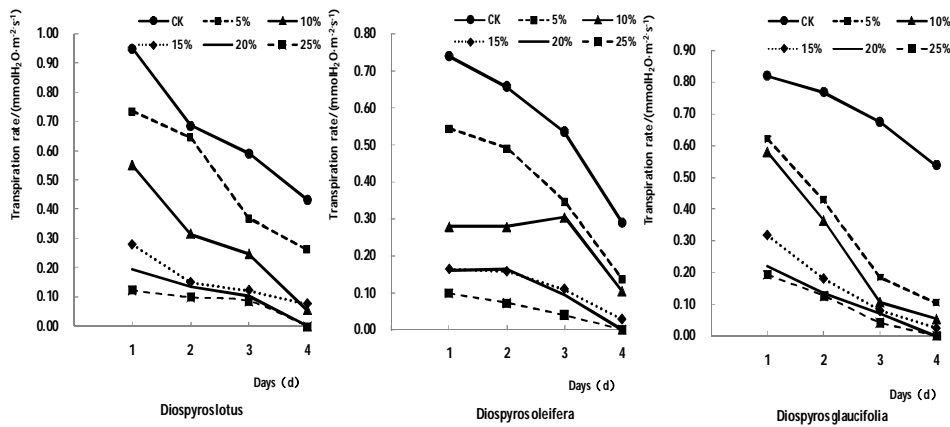


Fig.4. Effect of PEG-6000 stress on transpiration rate of different persimmon rootstocks.

As shown in Fig. 5, under the stress of PEG-6000, the WUE of 3 persimmon rootstocks decreased differently, compared to the control. At the first day of stress, under the stress of PEG-6000 of 5 and 10%, the WUE of 3 persimmon rootstocks declined slowly. *D. lotus* dropped 9.85 to 21.6%, *D. glaucifolia* dropped 23.49 to 29.77%, and *D. oleifera* dropped 18.3 to 30.18%. Under the stress of PEG-6000 of 15%, the WUE decreased further, and there was no significant difference among 3 persimmon rootstocks. With the further increase of drought stress, under the

stress of PEG-6000 of 20 and 25%, the decrease of WUE of each rootstock increased obviously. Compared with the control, *D. lotus* dropped 64.45 to 70.45%, *D. glaucifolia* dropped 68 to 78%, and *D. oleifera* dropped 73.18 to 81.75%, respectively. At second, third and fourth day of stress, under the stress of PEG-6000 of 5, 10, 15, 20 and 25%, the WUE of 3 persimmon rootstocks declined rapidly, especially under the stress of 25% PEG-6000, the WUE decreased to 0.

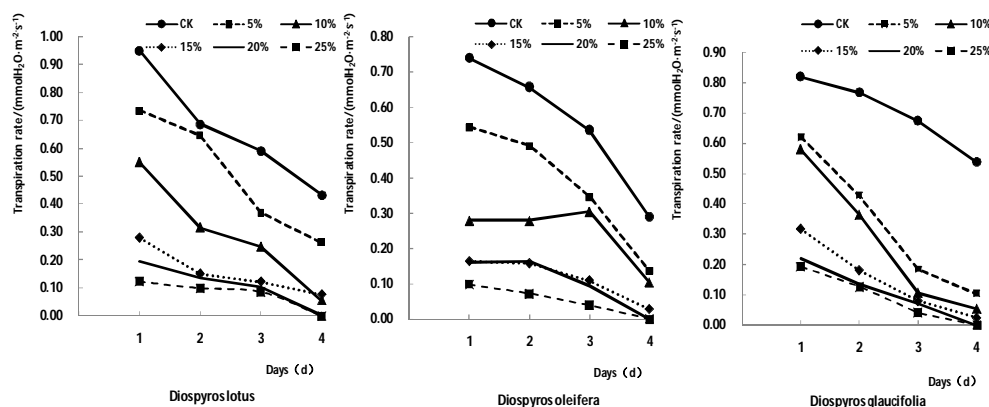


Fig. 5. Effect of PEG-6000 stress on leaf water use efficiency of different persimmon rootstocks.

In the present experiment, both P_n and G_s of the seedling leaves of *D. lotus* decreased with the increase of PEG-6000 stress intensity. When the PEG-6000 concentration was 5 and 10%, the decrease of various indexes in the leaves of *D. lotus* seedlings was not significant at first and second day of stress, and at third and fourth day of stress treatment, all the indexes dropped sharply, which indicated that, under mild stress, the simulated drought stress had little effect on the various photosynthetic indexes of *D. lotus* seedlings. However, with the extension of stress time, the photosynthesis of *D. lotus* seedlings was significantly affected, and the declines of the various photosynthetic indexes were significant. When the PEG-6000 concentration was 15, 20 and 25%, the photosynthetic indexes of *D. lotus* seedlings significantly decreased at first and second day of stress, and at third and fourth day of stress treatment, the leaves of the young *D. lotus* seedlings withered rapidly, and most of the leaves withered away, which showed that the effect of drought stress on photosynthesis of *D. lotus* seedlings increased obviously when the stress intensity increased to a certain extent. Compared to different persimmon rootstocks, the P_n and G_s of *D. lotus* and *D. glaucifolia* were higher under normal conditions, and decreased greatly under the simulated drought stress. The different rootstocks showed different drought resistance ability.

C_i depended on four factors, namely CO_2 concentration around the leaves, G_s , mesophyll conductance (G_m) and photosynthetic activity of mesophyll cells (Guo *et al.* 2016). Under different PEG-6000 stress concentrations, with the extension of stress time, the C_i of three persimmon rootstocks was inconsistent in this experiment. When PEG-6000 stress concentration was 5 and 10%, the C_i of *D. glaucifolia* was significantly higher than that of *D. lotus* and *D. oleifera* at first and second day. The C_i concentration of three persimmon rootstocks was significantly lower than that of control, and sometimes even was negative at third and fourth day. When PEG-6000 stress concentration was 15, 20 and 25% respectively, the C_i of three persimmon rootstocks was significantly lower than that of the control at first days and second days, and decreased obviously again at third and fourth day. The exchange of CO_2 and water in the leaves was hindered by the reduction of T_r and G_s , which was consistent with the previous research results.

Acknowledgements

The authors are grateful to the authorities of the postdoctoral research start-up fund of Henan Institute of Science and Technology, the Henan province postdoctoral research start-up fund, the Natural Science Foundation of China (U1304323), the Science and Technology Research Projects in Henan Province (192102110043), and the Henan Province Engineering Research Center of Horticultural Plant Resource Utilization and Germplasm Enhancement for financial support.

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