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RESEARCH ARTICLE

Physiological response of four wolfberry (*Lycium* Linn.) species under drought stress

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Abstract

We studied gas-exchange, chlorophyll pigments, lipid peroxidation, antioxidant enzymes, and biomass partitioning responses in seedlings of four wolfberry species (*Lycium chinense* Mill. var. *potaninii* (Pojark.) A. M. Lu, *Lycium chinense* Mill., *Lycium barbarum* L., and *Lycium yunnanense* Kuang & A. M. Lu) under four water supply regimes. In all four species, drought affected seedlings in terms of chlorophyll content, net photosynthesis rate (P_n), transpiration rate (E), and lipid peroxidation. Drought also increased some antioxidant enzyme activities, such as peroxidase (POD), catalase (CAT), superoxide dismutase (SOD), and ascorbate peroxidase (APX). Significant changes in dry biomass partitioning also occurred in response to water stress. In particular, dry biomass of leaves and fruits decreased significantly. *L. chinense* Mill. and *L. barbarum* L. possessed greater drought tolerance and exhibited superior antioxidant processing ability and other related physiological traits compared to the other two species. *L. chinense* Mill. was the most tolerant to all levels of drought. In contrast, *L. yunnanense* Kuang & A. M. Lu was more affected by water supply and had the lowest resistance to drought stress. These findings would provide some important information regarding genetic resources for future forest tree improvement in relation to drought tolerance.

Keywords: drought, *Lycium* Linn., chlorophyll fluorescence, osmotic adjustment, antioxidant respond, dry matter allocation

1. Introduction

Arid and semiarid zones have traditionally contributed around 40% of the total worldwide production of food grains (Bhatt *et al*. 2011). However, severe water deficits, or droughts, can bring devastating effects on crop productivity in these areas. Drought stress can arrest plant growth and reduce its distributive area (Diego *et al*. 2012). In order to survive under drought stress conditions, plants can reduce leaf water potential, stomatal conductance (G_s), and gas-exchange, and may slow normal growth rates (Reddy *et al*. 2004; Ribas-Carbo *et al*. 2005; Deng *et al*. 2012).

Drought-induced stomatal closure may increase the oxidative load on plant tissues, causing perturbations in biochemical pathways, if the accumulation of excessive reactive oxygen species (ROS) is not stopped. Oxidative stress causes lipid peroxidation and damage to other important biomolecules (Bhatt *et al*. 2011). In addition to morphological adaptations (e.g., extending deep roots to

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tap ground water and closing stomata to reduce water loss), plants have demonstrated a series of physiological and biochemical mechanisms to minimize drought stress. These internal responses range from changes in photosynthetic activity to the development of antioxidant defenses to enhance drought tolerance (Gao *et al*. 2009).

The earliest response to drought seems to be stomatal closure, and both stomatal and mesophyll conductance are the primary limitations to photosynthesis during mild to moderate drought stress. Drought results in the inhibition of photosynthesis, either due to diffusion limitations or metabolic impairment (Ávila *et al*. 2012). Plants protect themselves from drought-induced oxidative damage by producing an array of anti-oxidative enzymes such as superoxide dismutase (SOD), catalase (CAT), guaiacol peroxidase (GPX), and ascorbate peroxidase (APX), which act synergistically to limit levels of ROS (Bhatt *et al*. 2011).

Wolfberry species (*Lycium* Linn.) are perennial, deciduous shrubs that grow in Northwest China and the Mediterranean region. These shrubs possess fast-growth characteristics, a well-developed deep root system, and extensive adaptability to drought and cold (Chen *et al*. 2004; Chang and So 2008). The fruit of *Lycium barbarum* L. (family Solanaceae, also named fructus lycii or wolfberry) has been used for centuries in China as a traditional herbal medicine and as a valuable nourishing tonic (CCP 2010). Recently, medical research has indicated that these fruits have many pharmacological functions, such as improving visual acuity, maintaining liver and kidney function, reducing blood sugar levels, reducing the risk of cancer and cell senescence, and improving immune function (Xu *et al*. 2000; Xie *et al*. 2001; Chang and So 2008).

The depth of existing research on water-stress tolerance in plants varies depending on plant species. Although the general effects of drought on plant growth are fairly well known (Chaves 1991; Wang *et al*. 2011), the essential consequences of drought stress at the physiological level have not been thoroughly studied for wolfberry species (*Lycium* Linn.). This study was aimed to research physiological responses of drought tolerance in four wolfberry species under drought stress. The observations will be helpful for developing high-yield cultivation techniques for wolfberry as well as breeding varieties for drought tolerance.

2. Materials and methods

2.1. Plant materials and growth conditions

The experiment was conducted in a greenhouse at the Wolfberry Germplasm Repository of the Ningxia Academy of Agriculture and Forestry Sciences, China (38°38´N, 106°9´E). The area has a typical temperate continental climate, with an average temperature of 8.5°C, annual average sunshine between 2 800 and 3 000 h (one of the largest amounts of solar radiation and hours of sunshine in China), and an annual average precipitation of 200 mm, most of which falls during the summer.

The four wolfberry species used in the study were *Lycium chinense* Mill. var. *potaninii* (Pojark.) A. M. Lu, *Lycium chinense* Mill., *Lycium barbarum* L., and *Lycium yunnanense* Kuang & A. M. Lu, and the seedlings were collected from one 10-year-old tree, respectively. Semi-lignified branches were collected, and 8-cm hardwood cuttings were taken from the branches with uniform diameters and one or more lateral buds. Cuttings of each clone were collected from stool beds growing at the greenhouse in September 2012. In April 2013, germinating, healthy, and uniform seedlings were transplanted individually to plastic pots (32-cm in diameter and 28-cm in height) containing sand, soil, and vermi-compost (1:3:1, v/v). The mixture weighed about 10 kg per pot and the saturated water content was 24% by mass. All seedlings were grown in a full sunlight greenhouse under a semi-controlled environment (only sheltered from rainfall) during the experiment.

2.2. Experimental design

The experiment was arranged in a completely randomized design for four water supply regimes (75, 55, 35, and 15% field capacity (FC)), of which 75% FC served as the control, and the others served as light, moderate, and severe water drought conditions, respectively. There were 20 pots for each treatment. Watering treatments started on 15 June, 2013. Following natural water loss, all of the water supply regimes reached the required FC by 25 June. After reaching the target FC, the pots received compensatory irrigation by weight at 18:00 everyday during the experiment to maintain a constant soil moisture level. The treatment lasted about 2 months. All measurements were taken 50–60 days after drought.

2.3. Chlorophyll and carotenoid pigments

About 0.5 g of fresh leaf tissue was used for each extraction. Leaf tissue was cut into small pieces, placed in a test tube, extracted in 80% acetone at room temperature, and left on a rotary shaker for 24 h in the dark. The absorbance of the extracts was measured on a spectrophotometer (TU-1900, Beijing, China) at 470, 646, and 663 nm wavelengths, respectively. The concentrations of chlorophylls and carotenoids were calculated according to the method of Inskeep and Bloom (1985) and Arnon (1949), respectively.