Plant Omics Journal

POJ

POJ 5(1):1-5 (2012) ISSN:1836-3644

# Effects on physiological characteristics of Honeysuckle (*Lonicera japonica* Thunb) and the role of exogenous calcium under drought stress

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**Abstract**

*Lonicera japonica* Thunb as the traditional Chinese herb is widely planted in southwestern China to harness rocky desertification due to its adaptability to the rock-desertificated environment. However, how the drought stress limits the *Lonicera japonica* Thunb's physiological and photosynthetic characteristics is not clear. Therefore, a series of pot-cultivation experiments were dealt/performed with 30 $mmol.L^{-1}$ and 15 $mmol.L^{-1}$ of exogenous $CaCl_2$ to investigate the chlorophyll content, soluble sugar content, proline content and the photosynthetic rate of *Lonicera japonica* Thunb under drought stress. Moreover, the possible roles of Ca in plant processes, are discussed. Under the drought stress, the content of chlorophyll, soluble sugar, proline, catalase enzyme activity and the photosynthetic characteristics in *Lonicera japonica* Thunb are changed by 1.5 $mg.g^{-1}$, 0.11 $mg.g^{-1}$, 13 $mg.g^{-1}$, 0.5 $u.mg^{-1}$ and 4.3 $umol.m^{-2}.s^{-1}$, respectively. The samples dealt with the additional/higher $Ca^{2+}$ content during drought stress have a higher catalase enzyme activity and chlorophyll content because it can alleviate the cell membrane leakage and the chlorophyll decomposition. Moreover, proline content and soluble sugar content were found decreased/lower compared with the results without the additional $Ca^{2+}$ in the soil.

**Keywords:** *Lonicera japonica* Thunb; Drought stress; Physiological and Photosynthetic Characteristics; Exogenous Calicum; Karst.
**Abbreviations:** CAT-Activity of Catalace, TDR-Time Domain Reflectometer, ICT-Information Communication Technology, NAD-Nicotinamide Adenine Dinucleotide, NADP-Nicotinamide Adenine Dinucleotide Phosphate.

## Introduction

In Southwest China, karst area has the largest exposed limestone area in the world covering about 540,000 $Km^2$ (located) in seven provinces and one municipality. Serious ecological degradation and rocky desertification restricts the local economic and social development (Yuan, 2001). Therefore, comprehensive control of rocky desertification in karstic regions in China has been listed in National Medium and Long-Term Science and Technology Development Plan (2006-2020). Therefore at some karst areas, such as Guangxi, Guizhou province and the municipality of Chongqing, the government explores optimal modes of rocky desertification by establishing *Lonicera japonica* Thunb industry-based methods to restore karst ecological system. *Lonicera japonica* Thunb belongs in the family of *Lonicera* or *Caprifoliaceae*. Its' dry buds or flowers, commonly known as "honeysuckle" are commonly used as a traditional Chinese medicine (TCM) herb. It has been used for centuries in TCM practices for the treatment of sores, carbuncles, furuncles, swelling and affections caused by exopathogenic wind-heat or epidemic febrile diseases at the early stage (Ministry of Public Health of the People's Republic of China, 2004). Moreover, *Lonicera japonica* Thunb is planted to harness rocky desertification due to its strong adaptability to drought-resistance, waterlogging-enduring, cold-resistance, tolerance to barren activities (Peng et al., 2003). In view of its beneficial effects, much attention has been paid to the influences of its quality on the morphological anatomy, drought resistance and water stress in recent years (Xu et al., 2006; Li et al., 2007 and 2008). Calcium is an essential plant nutrient (White and Broadley, 2003; Hepler, 2005). The divalent calcium cation, $Ca^{2+}$, is required for structural roles in the cell wall and membranes (White and Broadley, 2003). In addition to it's role in cell structure, calcium also plays a role as a secondary messenger in coordinating responses to numerous environmental stresses (Gong et al., 1998; Hepler, 2005). When plants are exposed to high temperature, calcium is directly involved in the heat shock signal transduction (Liu et al., 2003). Previous studies showed that pretreatment with $Ca^{2+}$ can also obviously enhance subsequent thermotolerance of tobacco seedlings (Gong et al., 1998). Though numerous studies have been carried out on the physiological mechanism of calcium in plant heat tolerance (Larkindale and Knight, 2002; Liu et al., 2003; Hepler, 2005), no study has been carried out to investigate the involvement of exogenous calcium in osmoregulation and photosynthetic characteristics of *Lonicera japonica* Thunb under karst drought stress. In the present study, we investigated the effects of exogenous calcium on photosynthetic physiological characteristics of *Lonicera japonica* Thunb under drought stress. This investigation provides with important knowledge for irrigation and industrial based usage of *Lonicera japonica* Thunb against the desertificated rocky karst areas.

## Results and discussion

### ***The chlorophyll content variation of Lonicera japonica* Thunb *in the process of gradual soil drought and rehydration***

Chlorophyll is a factor of photosynthesis in higher plants. When plants suffer drought stress, the slice layer structure of chloroplast is destroyed. As a result, the process of chlorophyll biosynthesis is stopped and the decomposition of synthesized chlorophyll is accelerated. Thus, the total amount of chlorophyll and chlorophyll a+b decreases. It is generally believed that the amplitude of content variation of chlorophyll a+b could be used as an index of varietal sensitivity to drought stress and the large amplitude leads to weak drought resistance(Hu et al., 2006; Gao et al., 2002; Zhao et al., 2003). As it is demonstrated in Fig. 1, the content of the chlorophyll in *Lonicera japonica* Thunb is bimodal initialy increasing and subsequently decreasing form April $24^{th}$ to $27^{th}$ in 2007 during the process of soil moisture decline and rewatering. The first rise reflects the adaptability of plants to drought stress and the later depression results in retardation of synthesis and acceleration of degradation simultaneously (Hao et al., 2006). The rise, from $28^{th}$ to $29^{th}$, might be caused by the increasing trend of chlorophyll content that was foiled by the slow decrease of relative water content of leaves when drought stress is prolonged. The chlorophyll content was decreased by rewatering of leaves(Wang et al., 2007). Comparing the chlorophyll content in group K1 with groups K2, K3 and K4, it was found that exogenous calcium, to a certain extent, improved the drought resistance of *Lonicera japonica* Thunb. The higher chlorophyll content of K1 than K2 indicates that imposed exogenous calcium can alleviate the drought stress damage.

### ***The soluble sugar content variation of Lonicera japonica* Thunb *in the process of gradual soil drought and rehydration***

The content of soluble sugar increased significantly when plants is growing under drought stress for the major osmotic adjustment substance of cells (Blackman, 1992). The increasing content of soluble sugar in plant body under drought stress results from the manifestation of plant adaptation to drought stress and the accumulation of osmotic adjustment substance through a certain metabolic activity in cells to maintain the turgor potential (Stewart and Lee, 1974). In addition, due to the destruction of the plant photosynthetic system under adversity stress, the synthesis of carbohydrate macromolecules is prevented. Therefore, it synthesizes substances such as low molecular sucrose (Dure, 1993). The curve of soluble sugar in leaves of *Lonicera j aponica* Thunb was bimodal in the process of drought stress and rewatering (Fig. 2). In the early stages of gradual drought ($24^{th}$-$27^{th}$), with increasing drought stress, *Lonicera japonica* Thunb responded to drought adversity by increasing soluble sugar in body to maintain intracellular osmotic balance. In the late phases, the damage of chloroplast resulted in lower soluble sugar content. Elevated drought lowers the water content of leaves increasing the soluble sugar concentration. This is reversed by rewatering. In order to research the effect of exogenous calcium on soluble sugar variation, different experimental groups are compared in this paper. It was found that soluble sugar content is K1> K2> K3> K4, indicating that exogenous calcium effectively enhanced drought resistance of plants (Fig. 2).

### ***The variation of free proline content in leaves of Lonicera japonica* Thunb *in the process of soil gradual drought and rehydration***

Proline is an aminoacid which exists abundant in plants as components of many plant proteins. The normal proline content in plants is about 0.2-0.7 mg.g-1 of the dry weight, which is just a few percent of total free amino acids. However, free proline in plants is increased by 10-100 times to reach over 40 percent of free amino acids under stress conditions, such as drought, salinity, freezing, etc (Stewart and Lee, 1974). Fig. 3 shows that proline content in leaves of *Lonicera japonica* Thunb is first increased and then decreased from April $25^{th}$ to $27^{th}$ reaching the lowest on $28^{th}$, which reflected to the adaptation of *Lonicera japonica* Thunb under drought stress. The rapid rise, from $28^{th}$ to $29^{th}$, could be caused by the increasing trend of proline content that was foiled by the slow decrease of relative water content of leaves as stress was prolonged. The relative order of proline content in *Lonicera japonica* Thunb under progressive drought and rewatering is K1>K2>K3>K4 which provides with further explanation about the effect of drought tolerance of *Lonicera japonica* Thunb. Moreover, exogenous calcium played an important role in alleviating proline accumulation in *Lonicera japonica* Thunb maintaining the osmotic balance in cells under drought stress.

### ***The variation of free catalase enzyme activity in leaves of Lonicera japonica* Thunb *in the process of progressive soil drought and rewatering***

When plants are subjected to drought stress, negative oxygen ions are produced and damage the plant tissue. CAT plays a vital role in capturing biological free radicals and catalyzing the decomposition of hydrogen peroxide into water and oxygen under the stress. Therefore, hydrogen peroxide does not react with negative oxygen ions which play a defensive role protecting the organ (Gong et al., 1997). During progressive drought, the trend of CAT content in leaves of *Lonicera japonica* Thunb initially increases and later decreases due to the capture of biological free radicals and the promotion of synthesis of protective enzymes at the early stage of drought stress. The content of CAT decline is restricted in the late stage of drought stress when leaves wither. Moreover, the content of CAT in leaves increases to some extent after rewatering of the plant. In order to understand the effect of exogenous calcium concentration on CAT activity of *Lonicera japonica* Thunb, the variation of CAT activity were studied. Addition of 30 $mmol.L^{-1}$ $CaCl_2$ and 15 $mmol.L^{-1}$ $CaCl_2$ to the soil during the process of progressive soil drought and rewatering showed that the exogenous calcium could improve the CAT activity of the plant (Fig. 4).

### ***The characteristics of the photosynthetic rate of Lonicera japonica* Thunb *under progressive drought stress and rewatering***

The photosynthetic rates of *Lonicera japonica* Thunb at 8:00 am were observed continuously in different groups to explore its response of photosynthetic rate. Fig. 5 demonstrates that the photosynthetic rate variation of *Lonicera japonica* Thunb under progressive drought stress ($24^{th}$ -$27^{th}$) first increased and then decreased. With intensifying drought, the photosynthetic rate raised again and

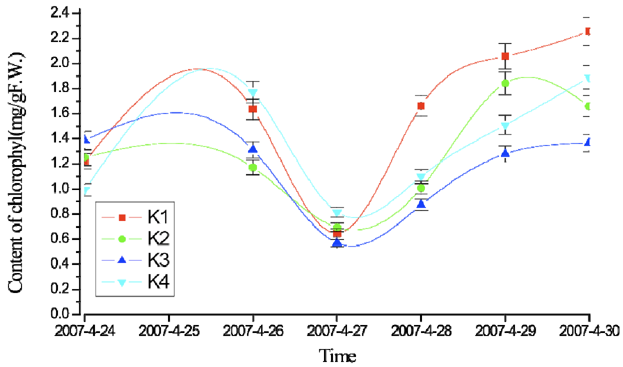


**Fig 1.** The chlorophyll content variation of *Lonicera japonica* Thunb during drought stress and rewatering (K1 and K2 treated with 30 mmol/L and 15 mmol/L of $CaCl_2$; K3 standing for the control group; K4 treated with 150 ml of distilled water).

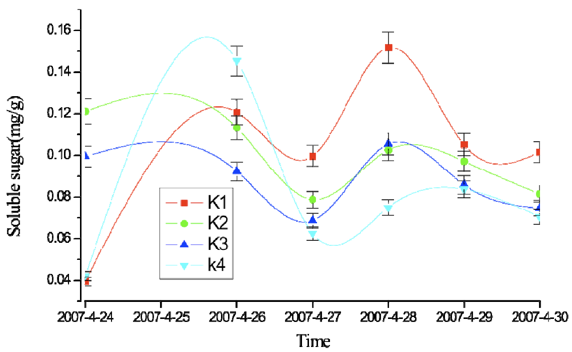


**Fig 2.** The soluble sugar content in *Lonicera japonica* Thunb during drought stress and rewatering (K1 and K2 treated with 30 mmol/L and 15 mmol/L of $CaCl_2$; K3 standing for the control group; K4 treated with 150 ml of distilled water).

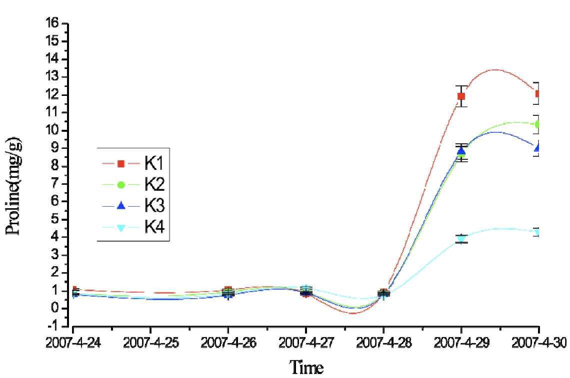


**Fig 3.** The variation of proline in *Lonicera japonica* Thunb during drought stress and rewatering (K1 and K2 treated with 30 mmol/L and 15 mmol/L of $CaCl_2$; K3 standing for the control group; K4 treated with 150 ml of distilled water).

declined after rewatering. Moreover, the addition of 30 mmol.$L^{-1}$ exogenous calcium increased the photosynthetic rate of *Lonicera japonica* Thunb under various treatment conditions. Under drought stress, most stomata are closed to limit gas exchange required for photosynthesis. In addition, plants resist external environment stress by increasing the chloroplast content to ease the damage and intensify the photosynthetic rate (Bogorad and Vasil, 1991; Wu et al., 2001; Xue et al., 2009). The appropriate exogenous calcium treatment increased stomatal resistance and reduced the transportation and respiration rate enhancing photosynthesis in *Lonicera japonica* Thunb. The exogenous calcium can improve phytochrome by over 6-10 mol.$L^{-1}$ and activate NAD kinase for the CaCAM-dependent synthesis of NADP (Jones and Lunt, 1967). In the process of photosynthesis, the light can increase free $Ca^{2+}$ concentration in chloroplast to improve the photosynthetic rate by absorbing exogenous calcium. The photosynthetic rate in experiments treated by various exogenous calcium concentrations, is differnet, which reflected the tolerance ability of plants to exogenous calcium (Fig. 5). Moreover, the experiment also shows that *Lonicera japonica* Thunb treated by exogenous calcium kept a high rate of photosynthesis under drought stress (Farquhar and Sharkey, 1982; Farquhar et al., 1980).

## Materials and methods

### *Materials and treatments*

*Lonicera japonica* Thunb for experiments from Shangqiu city of Henan province where there is no karst were planted in pots with karst soil. The sampling plants were divided into four groups with five pots in each group at random (Deng et al., 2009). Before experiment, the soil was leached by distilled water to remove most of free calcium. The soil for Group K1 and K2 is treated with 30 mmol/L and 15 mmol/L of $CaCl_2$ , solution respectively, under soil progressive drought and rewatering. The soil for group K3 is just under soil progressive drought and rewatering without an addition of $CaCl_2$ solution. The soil for group K4 is treated with 150 ml of distilled water. The relative content of soil moisture is represented by percentage. when leaves are withered with relative content of soil moisture reduced to 30%-35% respectively, the plants are rewatered. At the same time, water content of experimental groups is in kept with each other on the whole.

### *Methods*

Content of chlorophyll is measured by extraction methods (Pan et al., 2007).
Content of soluble sugar is determined by corlorimetry of anthracene and ketone (Zhang et al., 1999). Activity of catalace (CAT) is evaluated by corlorimetry, calculation of CAT activity is according to $(\Delta A240\times V_T)/0.1\times Vt\times t\times Fw$(Zhou, 2000). Content of free proline is measured by chromogenic method of ninhydrin extracted by sulphur radical ortho-hydroxybenzoic acid(Zhi et al., 2005).

### *Measurement of soil moisture*

We obtained degrees of drought stress by the water control method. Before the experiment, soil was irrigated to saturation and hand-held TDR (MP-160) probe of soil moisture produced by ICT company of Austrian was used to

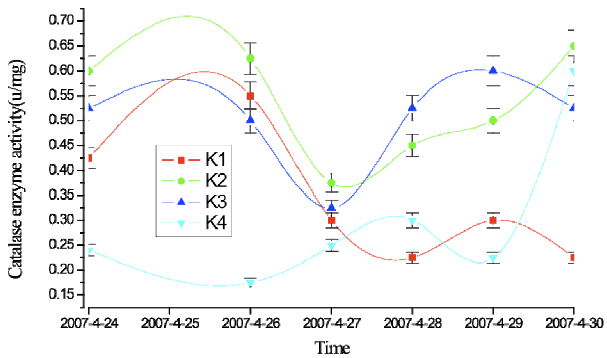


**Fig 4.** The variation of catalase enzyme activity in *Lonicera japonica* Thunb under drought stress and rewatering (K1 and K2 treated with 30 mmol/L and 15 mmol/L of $CaCl_2$; K3 standing for the control group; K4 treated with 150 ml of distilled water).

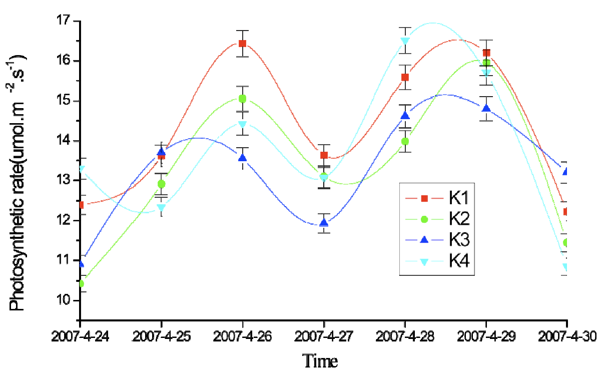


**Fig 5.** The effect of drought stress on the photosynthetic characteristics in *Lonicera japonica* Thunb under drought stress and rewatering (K1 and K2 treated with 30 mmol/L and 15 mmol/L of $CaCl_2$; K3 standing for the control group; K4 treated with 150 ml of distilled water).

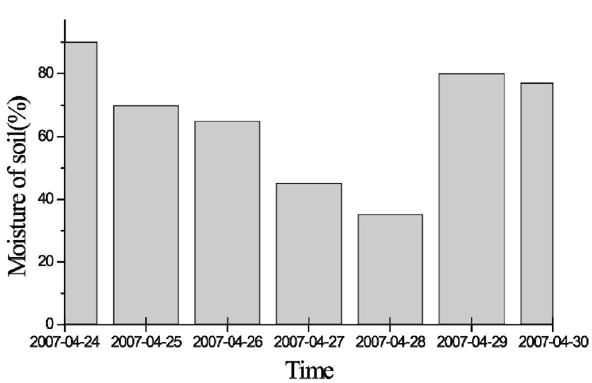


**Fig 6.** The moisture content of soil.

measure volumetric moisture content (W). A day later, gradients of water were acquired, the parameters of photosynthetic characteristics were estimated when the relative content of soil moisture (%) was about 90.8 With water evaporated, a gradient of water was gained to percentage of 70.3%, 65.7%, 45.4%, 35.2%, 80%, 78%, separately (Fig. 6).

### *Measurement of photosynthetic rate*

On a sunny day of April, two pots of plants with similar conditions of mature and healthy leaves above the middle of the plant were selected. Then open system of LI-6400 at 8:00 am was used to estimate effect of exogenous calcium on photosynthetic rate of *Lonicera japonica* Thunb. Three leaves were measured with replicated 6 times to gain average.

### *Statistical analysis*

The combination of Sigmaplot and Origin 6.0 software was used to deal with statistical analysis and drawing. The statistical analysis of the data was performed using the mean values ±S.E.M. Significance was determined by Student's *t*-test for unequal variance or by completely randomized design followed by a least-significant-difference test (Milton and Tsokos, 1983)

## Conclusions

We compared the exogenous calcium effect in various concentrations on chlorophyll, free proline and soluble sugar content, the CAT activity and the daily photosynthetic rate of *Lonicera japonica* Thunb in the process of progressive drought in *Lonicera japonica* Thunb. It found that exogenous calcium improved photosynthetic rate and relieved damage of chloroplast structure from active oxygen by increasing chlorophyll content and activity. Moreover, exogenous calcium reduces accumulation of proline and soluble sugar to ease super oxidation of membrane and fat to maintain relative stability. At last, the appropriate addition of exogenous calcium reduces stomatal aperture for karst drought environment adaption, which is involved in a series of biochemical regulation mechanisms.

## Acknowledgments

This work was supported by the National Science Foundation of China (No.41003038), the National Science Foundation of Guangxi (No.2010GXNSFB013004 and No.2011GXNS-FD018002), the China Postdoctoral Science Foundation (No.20100470802 and No.201104310) and the Special Research Foundation of Institute of Karst Geology, Chinese Academy of Geological Sciences (No.2008002). Special thanks are given to Konstantions Karakostis in Johannes Gutenberg-University Mainz revising the manuscript, the anonymous reviewers and editor for their valuable comments and suggestions, which improved the manuscript a lot.

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