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Optimization of potassium for proper growth and physiological response of *Houttuynia cordata* Thunb.

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**A B S T R A C T**

*Houttuynia cordata* Thunb. is an edible herb with a variety of pharmacological activities, but only limited information is available about its response towards potassium supplementation. Sterile plantlets were cultured in media with different potassium levels, and parameters related to growth, foliar potassium, water and chlorophyll contents, photosynthesis, transpiration, *H*₂*O*₂ contents and antioxidative enzyme activities were determined after a month. Results showed that 1.28 mM potassium was the optimum for *H. cordata* as highest values of dry weight, shoot height, root length and number were obtained at this concentration. The optimum potassium concentration resulted in the maximum net photosynthetic rate which could be associated with the highest chlorophyll content rather than limited stomatal conductance. The supply of surplus potassium resulted in higher content of foliar potassium, but negatively correlated with the biomass. Both potassium starvation (0 mM) and high potassium (>1.28 mM) could lead to water loss through high transpiration rate and low water absorption, respectively, and resulted in *H*₂*O*₂ accumulation and increased activities of catalase and peroxidase, which suggested induction of oxidative stress. Moreover, *H. cordata* showed the minimum of *H*₂*O*₂ content and the maximum of superoxide dismutase activity on 1.28 mM potassium, implying its role in inducing tolerance against oxidative stress.

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1. **Introduction**

Potassium (K) as quality element is one of the major nutrients for plant growth and development (Besford and Maw, 1975). It is the most abundant cation in plant cells and plays important roles in metabolisms like enzyme activities, water and assimilate transport, and protein synthesis (Yin and Vyn, 2002, 2003; Véry and Sentenac, 2003; Pettigrew, 2008). Different potassium supplement concentrations significantly affect physiological and biochemical characters of plants (Chartzoulakis et al., 2006). Appropriate potassium concentrations effectively improve plant productivity (Yurtseven et al., 2005), while its deficiency leads to a decrease in chlorophyll content and photosynthetic rate (Zhao et al., 2001; Gerardeaux et al., 2010), and inhibits root and shoot elongation (Drew, 1975; Bednarz et al., 1998; Shin et al., 2005; Kanai et al., 2007). Inappropriate potassium levels may also induce stress responses, and many stresses can result in the accumulation of reactive oxygen species (ROS), such as superoxide radicals (*O*₂⁻) and hydrogen peroxide (*H*₂*O*₂)(Shin and Schachtman, 2004). Plants have evolved antioxidant enzyme system including superoxide dismutase (SOD), catalase (CAT) and peroxidase (POD) to prevent damage from ROS (Bowler et al., 1992; Nayyar and Chander, 2004). SOD scavenges *O*₂⁻, resulting in *H*₂*O*₂ and O₂ formation (Amor et al., 2006). CAT and POD are the main enzymes to eliminate *H*₂*O*₂ (León et al., 2002; Passardi et al., 2005).

*Houttuynia cordata* Thunb. is a pungent, heart-like leafed perennial herb and constitutes a single species of the genus *Houttuynia* in the ancient Saururaceae and in Chinese is known as ‘Yuxing-cao’, which means ‘producing unique fishy smell’. It belongs to thermophilic and hygrophilous species, native to Eastern Asia, especially distributed in middle, southeastern and southwestern provinces and regions in China. It can often be found in ravines, streambeds, forests, wet meadows, slopes, thicket and field margins, trailsides, roadsides or ditch banks in these regions (Wu et al., 2005a).

*H. cordata* has been identified as one of the most potential medical and edible wild plant resources (Wu et al., 2005a). Its young plants are popularly used as wild vegetable, while its mature plants are commonly used as a traditional medical herb in some Asian countries, such as China (Wu et al., 2005b), Korea (Kim et al., 2001), India (Chakrabarti et al., 2006), Vietnam (Ogle et al., 1998; Shin et al., 2005; Kanai et al., 2007).
2. Materials and methods

2.1. Plant materials and growth conditions

H. cordata new line w01-100 with desirable traits like disease resistance, high-quality and yield, was selected out from a collection of more than one hundred H. cordata accessions present in China. The line belongs to chemotype myrcene (Chen et al., 2008) and has chromosome number 90 (Wu et al., 2003). It has been planted for commercial purposes for years in Good Agricultural Practices (GAP) base of 999 Pharmaceutical Group (China).

Sterile plantlets with three leaves were used in this experiment. The uniform plantlets were selected and cultured on MS (Murashige and Skoog, 1962) media, having five different potassium levels (0, 1.28, 2.56, 5.31 and 10.26 mM). The treatment with 0 mM K was negative control. Each treatment consisted of three replicates with at least 30 plants. The control culture conditions were maintained at 24 ± 2 °C under 12 h photoperiod with light intensity of 30 μmol m⁻² s⁻¹ provided by cool-white fluorescent lamps (Philips, China). Plants were harvested after a month to determine water contents, growth parameters, foliar K concentrations, chlorophyll contents, photosynthesis, transpiration, H₂O₂ contents and antioxidative enzyme activities.

2.2. Growth parameters and water contents

Water content and growth parameters including dry weight, shoot height, root length and number were measured. Shoot height and root length were determined using a vernier caliper. The shoot height indicates the value between the top of plantlet and stem base. The length of taproot of individual plantlet represents the root length.

2.3. K concentrations

The second fully expanded leaves were used for determination of K concentrations. Samples were dried as following: firstly, fresh leaves were fixed quickly at 105 °C for 30 min, and then oven-dried at 70 °C for 48 h. The dried material was milled to pass a 0.5 mm sieve. The powder (0.01 g) soaked in 1 mM hydrochloric acid for 5 h, vibrated for 30 min, and then settled to the constant volume of 10 mL using 1 mM hydrochloric acid. The extraction solution was used to determine K concentration by atomic emission spectrophotometry. Foliar K content was calculated in terms of U g⁻¹ dry weight (DW).

2.4. Chlorophyll contents

Chlorophyll contents were estimated by a portable chlorophyll meter (SPAD-502, Konica Minolta, Tokyo, Japan) from the second fully expanded leaves from the top of individual plants. SPAD value was an indicator of chlorophyll content. The average SPAD value of three points per leaf at upper, middle and lower positions was used.

2.5. Photosynthesis and transpiration parameters

The second fully expanded leaves were used for determination of net photosynthetic rate (Pn), stomatal conductance (Cond), transpiration rate (Tr) and intercellular CO₂ concentration (Ci). These parameters were measured by a portable photosynthesis system (LI-6400, Li-Cor, Lincoln, NE, USA) with the air temperature, relative humidity, CO₂ concentration and light intensity inside the leaf chamber controlled at 25 °C, 55%, 450 μmol CO₂ mol⁻¹, 1000 μmol m⁻² s⁻¹, respectively.

2.6. Antioxidative enzyme assays

The second fully expanded leaves were used for determination of antioxidative enzyme assays. Leaves (0.5 g) were homogenized with mortar and pestle in 10 mL 50 mM sodium phosphate buffer (pH 7.8 for SOD, pH 6.0 for POD and 7.0 for CAT) containing 1% polyvinylpyrrolidone (w/v). The homogenate was centrifuged at 15,000 × g for 10 min and the supernatant as enzyme extract was used for antioxidative enzyme assays. The whole extraction procedure was carried out at 4 °C.

SOD activity was determined by monitoring its ability to inhibit photochemical reduction of nitroblue tetrazolium (NBT) at 560 nm (Beauchamp and Fridovich, 1971). Absorbance values were recorded on an ultraviolet and visible (UV-Vis) spectrophotometer (UV-2450, Shimazu Co., Kyoto, Japan). One unit (U) of SOD was defined as the amount of enzyme necessary to inhibit the reduction of NBT by 50%. Enzyme activity was calculated in terms of U g⁻¹ fresh weight (FW). The reaction mixture contained of 50 mM sodium phosphate buffer (pH 7.8), 13 mM L-methionine, 75 μM NBT, 10 μM ethylene diamine tetraacetic acid (EDTA) −Na₂, 2 μM riboflavin, 0.1 mL enzyme extract.

POD activity was determined based on guaiacol oxidation (Hassan et al., 2005). Increase in the absorbance due to guaiacol oxidation was measured at 470 nm. Absorbance values were recorded on the UV-Vis spectrophotometer. One unit of activity was determined by the variety of 0.01 min⁻¹. Enzyme activity was expressed as U g⁻¹ FW. The reaction mixture contained of 50 mM sodium phosphate buffer (pH 6.0), 5 mM guaiacol, 10 mM H₂O₂, and 0.05 mL enzyme extract.

CAT activity was determined by monitoring the destruction of H₂O₂ (Rout and Shaw, 2001). Decrease in the absorbance due to decomposition of H₂O₂ was measured at 240 nm. Absorbance values were recorded on the UV-Vis spectrophotometer. One unit of activity was determined by the variety of 0.01 min⁻¹. Enzyme activity was calculated in terms of U g⁻¹ FW. The reaction mixture consisted of 200 mM sodium phosphate buffer (pH 7.0), 10 mM H₂O₂, 0.1 mL enzyme extract.
2.7. \( \text{H}_2\text{O}_2 \) contents

\( \text{H}_2\text{O}_2 \) content was determined as titanium complex (Brennan and Frenkel, 1977). Leaf samples (0.5 g) were homogenized in 10 mL cold (4 °C) acetone. The homogenate was centrifuged (15,000 \( \times \) G) at 4 °C for 5 min. Subsequently, the supernatant (1 mL) was mixed with 0.1 mL titanium reagent (20% titanic tetrachloride in concentrated hydrochloric acid, v/v), followed by the addition of 0.2 mL concentrated ammonia to precipitate the peroxide–titanium complex. The mixture was then centrifuged at 15,000 \( \times \) G for 5 min. The precipitate was washed with acetone repeatedly and then solubilized in 5 mL of 2 M sulphuric acid. The intensity of yellow color of supernatant was measured at 415 nm by UV-Vis spectrophotometer. The concentration of \( \text{H}_2\text{O}_2 \) in the supernatant was calculated by comparing its absorbance to a standard calibration curve representing \( \text{H}_2\text{O}_2 \)–titanium complex from 0 to 1 mM and expressed as \( \mu \text{mol g}^{-1} \) FW.

2.8. Statistical analysis

Values were presented as means ± standard errors (SE) from three independent treatments. These data were subjected to analysis of variance, correlation and Duncan’s multiple range test \( (P < 0.05) \) using SAS version 9.1 (SAS Institute Inc., Cary, NC, USA).

3. Results

3.1. Growth parameters

\( \text{H. cordata} \) growth was significantly influenced by potassium concentrations (Fig. 1). The four growth parameters including dry weight, shoot height, root length and number showed significant differences with treatments (Table 1). Treatment of 1.28 mM K represented the highest values of these four growth parameters, while potassium starvation (0 mM) severely inhibited the growth of \( \text{H. cordata} \). Similarly high potassium concentrations (>1.28 mM) also caused severe reduction in these parameters. The shortest root and least root number were both found in the treatment of the highest potassium level (10.26 mM).

3.2. Leaf K, water and chlorophyll contents

Foliar K contents were significantly affected by the supplemental levels of potassium (Table 1). The maximum of leaf K concentrations was recorded in the treatment of 2.56 mM K, while higher potassium supplementation led to a decrease of potassium absorption as compared to 2.56 mM K. However, the maximum of potassium absorption did not represent the maximum of dry matter (Table 1).

Plant water contents were also significantly different between treatments (Table 1). The water content was maximum on 1.28 mM K while both no potassium and high potassium resulted in water loss in plant. Coarse small shrinkage of blade was observed in the treatments of potassium starvation and high potassium (Fig. 1).

Maximum chlorophyll content was recorded on 1.28 mM K (Table 1), and represented the darker hue in green leaves (Fig. 1), while the least was observed on no potassium treatment. Surprisingly the chlorophyll content dropped to the next minimum at 2.56 mM and then again showed a slight increase with higher potassium levels (>2.56 mM).
3.3. Photosynthesis and transpiration parameters

The net photosynthetic rate was maximum on 1.28 mM K, while it dropped dramatically on all other treatments, which were almost at par with each other (Table 2). The plants on this treatment had the minimum intercellular CO₂ concentration (Table 2), thereby representing the maximum of carbon assimilation too, which could be partly due to similar correlation between the net photosynthetic rates and chlorophyll contents, and the Pearson correlation coefficient was 0.75.

Transpiration rates were significantly different between potassium starvation and the other four treatments with potassium, which were found at par with each other (Table 2). Similar trend was observed for stomatal conductance, which is usually associated with transpiration (Table 2). This suggested that potassium starvation strongly stimulated stomatal opening and then transpiration, and stomatal conductance was limited in the treatments with potassium.

3.4. H₂O₂ contents and antioxidative enzyme activities

H₂O₂ productions were significantly affected by potassium treatments (Fig. 2a). The minimum of H₂O₂ contents was recorded in the treatment of 1.28 mM K. H₂O₂ contents showed a continuous increment in the treatments of high potassium levels (>1.28 mM). It suggested that the absence of potassium and high potassium stimulated H₂O₂ accumulation, leading to oxidative stress.

The activities of antioxidative enzymes got significantly affected by potassium concentrations. Both CAT and POD activities were low on 1.28 mM K, and both absence of potassium as well as high potassium stimulated H₂O₂ accumulation, leading to oxidative stress.

4. Discussion

Potassium, at optimum concentration, is essential for H. cordata growth. Many studies have demonstrated an obvious increment in plant stature and yield with proper supply of potassium (Mullins et al., 1994; Heckman and Kamprath, 1995; Pettigrew and Meredith, 1997; Buah et al., 2000; Vyn and Janovec, 2001). In the present study, H. cordata plants acquired highest values of dry weight, shoot height, root length and number on 1.28 mM K, and as such represented the most favourable treatment for its growth and development. Potassium starvation, as well as its surplus, caused severe reduction in its growth and development. The length and surface area of root affected the range of nutrient absorption, having direct negative consequences on plant productivity (Catmak, 2005; Hermans et al., 2006; Pettigrew, 2008). Therefore, the decreases in dry weight and shoot height of H. cordata were partly due to similar changes of root length and number. Moreover, although the supply of surplus potassium resulted in higher contents of foliar potassium as compared to 1.28 mM K, the higher leaf K did not improve the biomass. This might contribute to a hypothesis that plant probably needs critical cytoplasmic concentration of K in a certain range (Leigh and Wynn Jones, 1984).

Photosynthesis was significantly affected by potassium concentrations, determining yield. It is well known that photosynthesis is related to carbon assimilation and dry matter production (Gifford and Evans, 1981; Kramer, 1981; Zelitch, 1982) and that chlorophyll content, chloroplast ultrastructure and stomatal conductance are the major factors in photosynthetic rate (Farquhar and Sharkey, 1982; Zhao et al., 2001). However, the optimum concentration of potassium in the present study did not show an increase in the stomatal conductance, and wrinkled leaves as observed in the treatments of potassium starvation and high potassium might be due to water loss and therefore represent poor chloroplast ultrastructure (not measured in this study). Many studies have shown that appropriate potassium concentration can enhance chlorophyll content and photophosphorylase activity and maintain chloroplast inner membrane and proton gradient of thylakoid membranes, promoting photosynthetic phosphorylation (Véry and Sentenac, 2003; Yurtseven et al., 2005; Chartzoulakis et al., 2006; Hermans et al., 2006). Therefore, the maximum of net photosynthetic rate on 1.28 mM K may be mainly associated with high chlorophyll content and stable chloroplast ultrastructure, rather than limited stomata conductance.

Appropriate potassium concentration could maintain a critical water content in H. cordata. It is well known that potassium plays an important role in controlling stomatal aperture because concentration gradient of potassium between inside and outside of

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Table 1
Effects of different potassium supplies on foliar K, water and chlorophyll contents and growth parameters of H. cordata.

|                | K1          | K2          | K3          | K4          | K5          |
|----------------|-------------|-------------|-------------|-------------|-------------|
| Dry weight (mg plant⁻¹) | 4.87 ± 0.39 (c) | 7.45 ± 0.50 (a) | 6.13 ± 0.11 (b) | 5.73 ± 0.19 (bc) | 5.56 ± 0.44 (bc) |
| Shoot height (cm)       | 0.50 ± 0.03 (d) | 0.95 ± 0.01 (a) | 0.75 ± 0.04 (b) | 0.64 ± 0.07 (c) | 0.67 ± 0.09 (bc) |
| Root length (cm)        | 0.51 ± 0.01 (c) | 1.16 ± 0.11 (a) | 0.70 ± 0.07 (b) | 0.53 ± 0.08 (c) | 0.19 ± 0.01 (d) |
| Root number             | 2.13 ± 0.13 (c) | 4.13 ± 0.30 (a) | 2.82 ± 0.07 (b) | 2.95 ± 0.25 (b) | 1.72 ± 0.25 (d) |
| Foliar K (mg g⁻¹ DW)    | 13.50 ± 0.71 (d) | 20.00 ± 1.00 (c) | 34.00 ± 3.64 (a) | 26.33 ± 0.58 (b) | 22.00 ± 1.73 (bc) |
| Water content (g 100 g⁻¹) | 92.08 ± 0.18 (c) | 94.09 ± 0.16 (a) | 93.45 ± 0.25 (b) | 93.42 ± 0.15 (b) | 92.52 ± 0.31 (c) |
| Chlorophyll content     | 23.80 ± 0.60 (e) | 33.01 ± 0.78 (a) | 25.13 ± 0.35 (d) | 27.25 ± 0.84 (c) | 31.10 ± 0.70 (b) |

Note: Each value is mean ± SE (n = 3). Values followed by the different letter in the same lines are significantly different according to Duncan’s multiple range test (P < 0.05). K1, K2, K3, K4 and K5 indicate 0, 1.28, 2.56, 5.13 and 10.26 mM K, respectively.
stomatal guard cells affects solute potential (Fischer, 1968; Fischer and Hsiao, 1968). In the present study the optimum concentration of potassium showed lower stomatal conductance and transpiration rate than potassium starvation, resulting in higher water content, which is important for cell functions. However, although the stomata conductance and transpiration rate of the four treatments with potassium were found at par with each other, high potassium resulted in lower water content as compared to 1.28 mM K. This suggested that high potassium reduced water absorption. Oxidative stress could be induced by potassium starvation and high potassium according to the increase in H$_2$O$_2$ content (Fig. 2a). This result is well in accordance with some other studies (Cakmak, 1994, 2005; Shin and Schachtman, 2004). Excess H$_2$O$_2$ is harmful primarily due to reaction with lipids, proteins, and nucleic acids thus resulting in lipid peroxidation, membrane leakage, enzyme inactivation, and DNA breaks or mutations (Romero-Puertas et al., 2007), chlorophyll destruction (Cakmak, 1994) and growth reduction (Molassiotis et al., 2006). The increased activity of CAT and POD as observed in the present study might be to protect biomolecules from being attacked by H$_2$O$_2$. However, SOD activity did not show an increase with potassium starvation and high potassium. Previous studies have demonstrated that oxygen-dependence photosynthesis may be “leaking” energy to molecular oxygen, forming ROS such as *O$_2^-$ (Wise and Naylor, 1987; Ort and Baker, 2002). It therefore was suggested that accumulation of *O$_2^-$ might be partially suppressed by the low photosynthesis rates in these treatments. All of these implied that potassium starvation and high potassium might not result in an increase of *O$_2^-$ and excess H$_2$O$_2$ should have accumulated from other sources rather than from the conversion of *O$_2^-$ to H$_2$O$_2$. Moreover, the minimum of H$_2$O$_2$ content and the maximum of SOD activity at 1.28 mM K suggested that the optimal level of potassium resulted in lower oxidative stress and endowed plants with the resistance against oxidative stress, respectively.

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