Activity maintenance of the excised branches and a case study of NO₂ exchange between the atmosphere and P. nigra branches

Chun Chen¹,², Yuzheng Wang¹,², Yuanyuan Zhang¹,²,³, Xiaoxiu Lun⁴, Chengtang Liu¹,², Yujing Mu¹,²,³, Chenglong Zhang¹,²,³, Pengfei Liu¹,², Chaoyang Xue¹,², Min Song¹,², Can Ye¹,², Junfeng Liu¹,²,³,*

¹. Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, Beijing 100085, China
². University of Chinese Academy of Sciences, Beijing 100049, China
³. Center for Excellence in Urban Atmospheric Environment, Institute of Urban Environment, Chinese Academy of Sciences, Xiamen 361021, China
⁴. College of Environmental Science & Engineering, Beijing Forest University, Beijing 100083, China

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ABSTRACT

The efficient maintenance of the activity of excised branches is the powerful guarantee to accurately determine gas exchange flux between the detached branches of tall trees and the atmosphere. In this study, the net photosynthetic rate (NPR) of the excised branches and branches in situ were measured simultaneously by using two photosynthetic instruments to characterize the activity of the excised branches of Phyllostachys nigra. The ratio of normalized NPR of excised branches to NPR in situ was used to assess the photosynthetic activity of detached branches. Based on photosynthetic activity, an optimal hydroponics protocol for maintaining activity of excised P. nigra branches was presented: 1/8 times the concentration of Gamborg B5 vitamin mixture with pH = 6. Under the best cultivation protocol, photosynthetic activity of excised P. nigra branches could be maintained more than 90% within 6 hr in the light intensity range of 200–2000 μmol/(m²·sec) and temperature range of 13.4–28.7°C. The nitrogen dioxide (NO₂) flux differences between in situ and in vitro branches and the atmosphere were compared using double dynamic chambers. Based on the maintenance method of excised branches, the NO₂ exchange flux between the excised P. nigra branches and the atmosphere (from −1.01 to −2.72 nmol/(m²·sec)) was basically consistent with between the branches in situ and the atmosphere (from −1.12 to −3.16 nmol/(m²·sec)) within 6 hr. Therefore, this study provided a feasible protocol for in vitro measurement of gas exchange between tall trees and the atmosphere for a period of time.

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Introduction

Plants have been proved to be an important sink of polluting gases (Hill, 1971; Meetham, 1950). Therefore, the gas exchange between tall trees and the atmosphere has been a concern for biologists, atmospheric scientists and botanists for a long time. There are several methods to research gas exchange, including chamber techniques (Breuninger et al., 2013; Chaparro-Suarez et al., 2011; Hill, 1971; Wang et al., 2004; Zhang et al., 2012), gradient methods (Pryor et al., 2002; Rinne et al., 2000), and eddy covariance (Geddes and Murphy, 2014; Min et al., 2014; Song et al., 2013; Zhu et al., 2015). However, the gradient method and eddy covariance technique are usually limited by funding, because they require large observatories or use tower cranes (Ganguly et al., 2009) to determine tree-atmosphere gas exchange in situ. In addition, these methods are also restricted by terrain, electricity availability, and instrument sensitivity. Therefore, chamber methods, which include static chamber (Fang and Mu, 2006; Geng and Mu, 2006; Jones et al., 2011; Li and Wang, 2007) and dynamic chamber techniques (Breuninger et al., 2013; Chaparro-Suarez et al., 2011), are common methods to study the exchange of gases between vegetation and the atmosphere. Compared with static chamber, one advantage of dynamic chamber is that it can keep the inside and outside ambient of chamber basically consistent. The inside environment of the chamber is close to the external environment, so the measurement value is closer to the real situation (Wang, 2005).

Some studies have determined the gas exchange between trees and the atmosphere using excised branches (Isebrands et al., 1999; Ke et al., 2002; Song et al., 2006; Wu et al., 2006) because it is more convenient. When excised branches were used as research objects to determine the exchange between plants and the atmosphere, the key assumptions are whether the branches activity in vitro and in situ are equivalent or whether the excised branches’ activity can be maintained in a reliable range or not. However, systematic studies of the effective activity maintenance of excised branches are scarce. Koike (1986) discussed the photosynthesis of excised broadleaf trees in Hokkaido. They indicated that a criterion to judge the activity of excised branches could be when the net photosynthetic rate of excised branch returned to 90% of its photosynthetic induction. Tang and Wang (2011) used the same method to research the activity of the main trees of the Northeast China using excised branches. Additionally, there were other ways to define the activity of excised branches. Huang et al. (2009) characterized the activity of the excised branches of Cylindrical (glauca) where the photosynthetic rate in the excised branches was within ±10% of net photosynthetic rate in situ. Both of these previous experiments used one single photosynthesis system to measure one branch from in situ to in vitro. The photosynthesis of the branches in situ was assumed to be constant, and the net photosynthetic rate of the cut branch was used as a reference to judge the difference between net photosynthetic rate of plants in situ and in vitro (Huang et al., 2009; Tang and Wang, 2011). However, the net photosynthetic rate is often affected by meteorological factors such as light and temperature, and the net photosynthetic rate of the branches in situ will also change when the environment changes (Thomas, 1955). As a result, the characterization of activity of the excised branches may be biased. To solve this problem, in our study, two photosynthetic instruments were used to measure the changes in net photosynthetic rate of an excised branch and a branch in situ in order to consider the environmental factors that simultaneously affect the branches in situ and in vitro and to ensure the accurate characterization of the activity of the branches.

The activity maintenance methods of excised branches mainly include wrapping branches with a wet cloth (Wu et al., 2006) or wet soil (Huang et al., 2009) or inserting the excised branches into water (Ke et al., 2002). The main factors that influence the growth activity of plants are light, water and nutrition. At present, as a new research method mainly used for the study of plant ecology (Torabi et al., 2012), hydroponics can provide nutrients and water for plants, and is especially used in plant nutrition studies. The studies focus on the type and concentration of the inorganic salt ions (Lloyd and McCown, 1980) and the acidity of the nutrition solution (Kane et al., 2006). Modern hydroponic technology is mainly based on Hoagland and Arnon solution (Hoagland and Arnon, 1950). Modified Hoagland nutrient solution (Kent and Läuchli, 1985) and Hoagland nutrient solution (Hoagland and Arnon, 1950) are common nutrient solutions for hydroponics. In addition to them, McCown woody plant medium (Lloyd and McCown, 1980), Murashige & Skoog medium (Murashige and Skoog, 1962) and Gamborg B5 (Gamborg et al., 1968) were used to culture oak (Purohit et al., 2002), cowpea tree (Osório et al., 2012), F. cycadis (Demiray et al., 2017), and cherry tree (Bhagwat and Lane, 2004). Generally, Murashige & Skoog medium can be used to culture stem segments, while Gamborg B5 and McCown woody plant medium are mainly used for tissue culture of woody plants. The activity of excised branches could be maintained well by hydroponic culture. Therefore, we cultured excised branches using hydroponics in this study.

In summary, it is of great scientific significance to study how excised branch activity is maintained, and the gas exchange between tall trees and the atmosphere by using excised branches. In this experiment, the activity of common tall tree- P. nigra branches was characterized in vitro through the synchronous determination of net photosynthetic rate of living branches and excised branches. At the same time, the optimal conditions and adaptability of activity maintenance of P. nigra were also studied. Furthermore, the NO2 flux between P. nigra and the atmosphere was measured by using the excised branches and double dynamic chambers.

1. Material and methods

1.1. Reagents and materials

The experiment objects were 3–4 year-old P. nigra deciduous trees, belonging to Salicaceae family, planted in the nursery garden of three-hectare garden at the Beijing Forestry University. P. nigra is growing rapidly and has strong
environmental adaptability. *P. nigra* widely planted and has been used in a wide range of studies. According to the growing habit of plants, we chose branches that were facing the sun, healthy, similar in thickness and canopy position as our experimental objects. One branch was the reference without any changes in the course of the experiment; the other was cut from the tree. The length of excised branch was approximately 50–60 cm.

Five kinds of common culture media were chosen for maintaining the activity of excised branches-modified Hoagland nutrient solution, Hoagland nutrient solution, Murashige & Skoog medium vitamin mixture (MS), Gamborg B5 vitamin mixture (B5), and McCown woody plant vitamin mixture (WPM).

The stoste of Hoagland nutrient solution was liquid, which was a direct result of the preparation method. The other four culture media were powders. These nutrition solutions consist of culture media and distilled water. Specific dosage was in accordance with the prospectus. Modified Hoagland nutrient solution consists of nutrient medium (1.42 g/L) and calcium nitrate tetrahydrate (9.45 g/L). It is worth noting that two kinds of components should be dissolved separately; otherwise, inorganic salt precipitation could occur.

The acidity of the solution was adjusted using 1 mol/L HCl (analytical reagent, Ziyang Chemical Factory) and 1 mol/L NaOH (analytical reagent, Haituo, Sinopharm Chemical Reagent Co., Ltd.) solutions.

1.2. Activity characterization of excised branches

To characterize the activity of excised branches, two poplar leaves that were apart 3–4 leaves positions from the top leaf were chosen as study objects. The leaves were emerald green, intact, without scars, physically healthy and the leaves’ chlorophyll concentrations were between 60 and 70 SPAD. The physical signs of excised leaves and living leaves were synchronously measured by photosynthetic system (leaf area was 5.8 cm², YZQ-100C, Yi Zong Qi Technology (Beijing) Co., Ltd., China). By selecting different leaves, the difference value of initial net photosynthetic rate of living leaves and excised leaves was maintained within 20%.

Comparing the net photosynthesis rate of two leaves, the one had higher net photosynthetic rate was chosen as reference plant, the other was excised. At this experiment, the pruning position was 30–40 cm below the measured leaf. The excised branches were inserted into nutrition solution promptly after pruning and recut immediately at 2–3 cm above the incision to prevent air from entering to block the trachea and absorption of water (Xu, 2006). The net photosynthetic rate of living branches and excised branches was measured synchronously and continuously, then the changes of net photosynthetic rate of excised branches were obtained. To ensure a stable net photosynthetic rate, the experiments were carried out under the condition of clear weather, no rain, with a slight breeze or no wind.

The simultaneous determination of the net photosynthetic rate of living branch and excised branch cultured by MS media changed with time, as shown in Fig. 1, which shows the relationship of the net photosynthetic rate of the branch in situ compared with the net and normalized net photosynthetic rates of excised. It can be seen that the net photosynthetic rate of living branches changes with the environment change. The net photosynthetic rate of the living branches decreased gradually, which was 26.6% lower 60 min after the pruning moment. There is a difference of the original net photosynthetic rate between the excised branches and the living branches of *P. nigra*. It is difficult to assess the changes in net photosynthetic rate after pruning through direct comparison. To more accurately express the change of net photosynthetic rate of living branches, the net photosynthetic rate of excised branches was normalized to keep net photosynthetic rate of two branches consistent with values at pruning time. Generally, after 20 min of stabilization of net photosynthetic rate...
When the activity was less than 1, the activity of the branches in vitro was decreased relative to the living branches, and the activity of the excised branches was greater than the living branches when the activity was greater than 1. \( A_{\text{ex-60}} \) was chosen as a characterization parameter of the activity of the excised P. nigra branches and used for comparison across different experimental conditions.

1.3. Optimum conditions for maintaining activity of excised branches of P. nigra

The effect of light intensity on net photosynthetic rate is significant. The net photosynthetic rate of living P. nigra branches under different light intensity was measured in situ. Fig. 2 shows the relationship between net photosynthetic rate and light intensity. The net photosynthetic rate of P. nigra increases with light intensity until 1200 \( \mu \text{mol/(m}^2\cdot\text{sec}) \), where it reached a plateau. According to the regression line, the saturation light intensity is reached when the light intensity reaches 1200 \( \mu \text{mol/(m}^2\cdot\text{sec}) \). Therefore, in our experiment, the light pattern of YZQ-100C leaf photosynthetic instrument was set as artificial light with 1200 \( \mu \text{mol/(m}^2\cdot\text{sec}) \) light intensity, and the light source is available red-blue light for plants.

The key factors affecting plant growth activity include water, nutrient status, suitable temperature and humidity. The most important concern in hydroponics is the composition, acidity and concentration of the culture solution.

1.3.1. Effects of different kinds of culture media on the activity of excised branches

Different culture media have very different effects on plants. Five common culture solutions were selected: Hoagland nutrient solution, modified Hoagland nutrient solution, MS medium, B5 medium, and WPM medium. Under 1200 \( \mu \text{mol/(m}^2\cdot\text{sec}) \) artificial light intensity, excised branches with leaves were inserted into five different nutrition solutions separately. By comparing the \( A_{\text{ex-60}} \) of P. nigra excised branches cultured in different nutrient solutions, the optimum condition for maintaining the activity of excised branches of P. nigra was determined.

1.3.2. Effect of acidity of culture medium on the activity of excised branches

While the plants grow in the suitable acidity, the acidity of the optimal nutrition culture was adjusted (pH interval was divided into 4 ± 0.1, 5.3 ± 0.1, 6.0 ± 0.1, 6.7 ± 0.1, 7.5 ± 0.1, 8.0 ± 0.1, 9.0 ± 0.1) by adding 1 mol/L HCl or 1 mol/L NaOH, and pH was determined by Mettler pH meter (FE20K, Mettler Toledo, Switzerland). By comparing the \( A_{\text{ex-60}} \) of excised branches of P. nigra cultured in nutrient solution with different pH values, the optimum acidity for maintaining the activity of excised branches of P. nigra was determined.

1.3.3. Determination of optimum nutrient solution concentration

The concentration of nutrient solution also affects the growth of plants. We prepared the best culture medium of different concentrations (1/16, 1/8, 1/4, 1/2, 1, 2 times of original concentration) by adding 1 mol/L HCl or 1 mol/L NaOH to adjust to the optimum acidity. By comparing the \( A_{\text{ex-60}} \) of
excised branches of *P. nigra* cultured in nutrient solution with different concentrations, the optimum condition for maintaining the activity of excised branches of *P. nigra* was determined.

1.4. Adaptability of excised *P. nigra* branches’ activity cultured in the optimal nutrition solution

Plant growth is affected by temperature and light intensity, which vary greatly within a day. After the optimum conditions for maintaining the activity of excised branches of *P. nigra* was determined, the effect of different conditions (temperature and light intensity) on the activity of excised branches of *P. nigra* was investigated.

1.5. The exchange of NO$_2$ between *P. nigra* and the atmosphere

To validate whether the method to maintain the activity of the excised branches plants can also be applied to study gas exchange fluxes, the exchange of NO$_2$ between the atmosphere and *P. nigra* was compared across excised and living branches.

The exchange of NO$_2$ was measured using the dynamic chamber method. Two air inlets are arranged at the symmetrical position at the bottom of the chamber while the air flow was approximately 40 L/min in total through the chamber so that the chamber would have a complete air exchange within 197 s. Six holes with a diameter of 1.5 cm are distributed evenly on the top of the chamber to be used for leaving air. The chamber (inner diameter 52 cm, height 62 cm, volume approximately 131.6 L) was made of stainless steel and coated with a PTFE film. Micro-fans inside the chamber are used to continuously mix the gas to minimize turbulent and boundary layer resistances. The sampling port is positioned on the middle of the box body. To constrain the influence of chamber on the measurement, one chamber acted as sample chamber with vegetation and an identical but empty chamber was used as a reference. The chambers were placed at a height of 1.3 m (above ground) and replenished with ambient air simultaneously. To reduce the measurement error between different instruments, the measurement of blank chamber and sample chamber were switched every 15 min. Temperature, humidity, light intensity and photosynthetic activity radiation (PAR) data were recorded in the blank chamber. To ensure that the living branches and excised branches were less disturbed by environmental factors, two days of stable weather conditions for our experiments were chosen. The living branches were measured on the first day, and the branches were pruned on the second day.

Calculation of NO$_2$ exchange flux is as Eq. (3) (Breuninger et al. (2013)):

$$ F_{ex,NO_2} = - \frac{Q}{A_{leaf}} \left( m_{NO_2,ex} - m_{NO_2,j} + \frac{V}{Q} k m_{NO_2,0} m_{NO_2,0} - \frac{V}{Q} j(m_{NO_2,0}) m_{NO_2,0} \right) $$

where $F_{ex,NO_2}$ is exchange flux density of trace gas NO$_2$ (nmol/(m$^2$·sec)), $Q$ is the purging rate (L/min), $A_{leaf}$ is the leaf area (m$^2$), $m_{NO_2}$ is the volume concentration in ambient air of NO$_2$ (ppb), $m_{NO_2,ex}$ is the volume concentration within the plant chamber of NO$_2$ (ppb), $j(m_{NO_2})$ is the photolysis rate of NO$_2$ ($\lambda \leq 420$ nm) (sec$^{-1}$), $m_{NO_2,0}$ is the volume concentration within the plant chamber of nitrogen oxide (NO, ppb), $m_{NO_2,0}$ is the volume concentration within the plant chamber of ozone (O$_3$, ppb), $k$ is the reaction coefficient of the NO + O$_3$ reaction (cm$^3$/mole·sec) (Atkinson et al., 2003). The concentration of NO$_2$ was measured with a nitrogen oxide analyzer while the concentration of O$_3$ was determined using an ozone analyzer. Temperature, photosynthetically active radiation, and humidity were recorded with an air temperature humidity photosynthetically active radiation light intensity recorder.

2. Results and discussion

2.1. Activity maintenance of excised branches of *P. nigra*

The key factors affecting plant growth activity include water, nutrition, suitable temperature and humidity. The most important factors considered in hydroponics are the composition, pH value and concentration of the culture solution. In our study, the effects of different nutrient solution types, pH value and concentration on the activity of excised branches of *P. nigra* were investigated.

2.1.1. Effect of different kinds of culture media on the activity of excised branches

The activity of the *P. nigra* branches in five kinds of nutrient solutions is shown in Fig. 3. The effects of the five nutrient solutions on the activity of excised branches of *P. nigra* were significantly different and especially notable for the B5 nutrient solution that was better than the other four nutrient solutions. According to the result, B5 is the optimal nutrient solution to maintain branch activity while the A$_{ex-60}$ was 0.754. The WPM nutrition solution ($A_{ex-60} = 0.426$), MS nutrition solution ($A_{ex-60} = 0.345$), modified Hoagland nutrition solution ($A_{ex-60} = 0.323$) and Hoagland nutrition solution ($A_{ex-60} = 0.253$) all had lower $A_{ex-60}$ values. The pH of original five solutions varies from 5.27 to 5.47 (Table 1). Because of similar acidity, the impact of acidity of different nutrient solutions on the activity maintenance of *P. nigra* in vitro could be negligible. Nutrient components in hydroponic cultures have great influence on the activity maintenance, as shown by the significant differences in activity maintenance of excised branches cultivated by five nutrient solutions. In comparison with the MS culture medium, WPM culture medium and B5 culture medium, the modified Hoagland and Hoagland solution did not contain vitamins. When the vitamin content was lower than a suitable level, the metabolic process of plants would be affected (Tang and Yu, 2006). Therefore, the addition of vitamin was beneficial to maintain the activity of excised branches. The experiment result identifies that activity maintenance capacity of the first three nutrition solutions was superior to the other two solutions’ activity maintenance capacity and indicates that the vitamin is good for excised branches to maintain their physiological activity. Comparing the MS culture medium, the WPM culture medium and the B5 culture medium, the WPM culture medium has already been shown to have a
stronger effect than the MS culture medium (Bhagwat and Lane, 2004). The B5 culture medium is the best among these three culture media with vitamins. When the amount of vitamin used is compared, the WPM culture medium is almost the same as the MS culture medium. The only difference is the amount of Thiamine HCl, as it is present in ten times the concentration in the WPM than in the MS. Nevertheless, the B5 nutrient solution has no glycine, in contrast with the former two, and the content of Thiamine HCl in B5 is ten times greater than it is in WPM, and 100 times greater than it is in MS. It can be inferred that the addition of Thiamine HCl was the main reason for the larger effect of B5 nutrient solution on excised branches than two other culture media. In addition, different trace elements in the nutrient solution also play an important role in maintaining the activity of excised branches cultured using hydroponics. For instance, Sagardoy et al. (2009) cultured sugar beet in hydroponic culture, and they found that the increase in Zn ion concentrations could decrease the photosynthetic rate of sugar beet. The Zn ion concentration in B5 nutrient solution used in our study is lower than that of WPM and MS. The experimental results are consistent with those reported in the literature. The results show that the B5 nutrient solution was the optimum nutrient solution to maintain the activity of the excised P. nigra branch in the original formula because of the addition of vitamins, and the differences of nutrient element concentrations. Therefore, the influences of acidity and different concentration of nutrition solution on the

![Fig 3 – $A_{50-60}$ of excised branches cultured in different nutrient solutions under light intensity of 1200 $\mu$mol/(m² sec).](image)

### Table 1 – Composition of the five culture media including major elements, micro elements and vitamin (organic components).

<table>
<thead>
<tr>
<th>Corporation</th>
<th>Hoagland</th>
<th>Modified Hoagland</th>
<th>MS</th>
<th>B5</th>
<th>WPM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Concentration (g/L)</td>
<td>-</td>
<td>2.223</td>
<td>4.405</td>
<td>3.164</td>
<td>2.463</td>
</tr>
<tr>
<td>pH</td>
<td>5.27</td>
<td>5.56</td>
<td>5.43</td>
<td>5.3</td>
<td>5.47</td>
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<tr>
<td>Major elements (mg/L)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NH$_4$NO$_3$</td>
<td>80</td>
<td>1650</td>
<td>400</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mg(NO$_3$)$_2$</td>
<td>493</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(NH$_4$)$_2$SO$_4$</td>
<td>134</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CaCl$_2$</td>
<td>136</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MgSO$_4$·7H$_2$O</td>
<td>180.54</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>K$_2$HPO$_4$</td>
<td>170</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>KH$_2$PO$_4$</td>
<td>170</td>
<td></td>
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<tr>
<td>Na$_2$HPO$_4$</td>
<td>130.44</td>
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<td>NH$_4$H$_2$PO$_4$</td>
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<td>1900</td>
<td>471.26</td>
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<tr>
<td>KNO$_3$</td>
<td>607</td>
<td>2500</td>
<td>990</td>
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<tr>
<td>Ca(NO$_3$)$_2$·4H$_2$O</td>
<td>945</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>K$_2$SO$_4$</td>
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<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Micro elements (mg/L)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>H$_2$BO$_3$</td>
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<td>6.2</td>
<td>6.2</td>
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<td>CoCl$_2$·6H$_2$O</td>
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<tr>
<td>CuSO$_4$·5H$_2$O</td>
<td>0.025</td>
<td>0.025</td>
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<tr>
<td>Fe$_3$O$_4$·EDDHA</td>
<td>30–40</td>
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<td>36.7</td>
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<tr>
<td>FeSO$_4$</td>
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<td>Na$_2$MoO$_4$·2H$_2$O</td>
<td>0.25</td>
<td>0.25</td>
<td>0.25</td>
<td>0.25</td>
<td></td>
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<tr>
<td>(NH$_4$)$_6$Mo$_7$O$_24$·7H$_2$O</td>
<td>0.02</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>KI</td>
<td>0.93</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vitamin (organic fraction) (mg/L)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ZnSO$_4$·7H$_2$O</td>
<td>0.22</td>
<td>8.6</td>
<td>8.6</td>
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<td></td>
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<tr>
<td>Glycine</td>
<td></td>
<td></td>
<td>2</td>
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<td></td>
</tr>
<tr>
<td>Myo-inositol</td>
<td></td>
<td></td>
<td>10</td>
<td>100</td>
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<tr>
<td>Nicotinic acid</td>
<td></td>
<td></td>
<td>0.5</td>
<td>1</td>
<td>0.5</td>
</tr>
<tr>
<td>Pyridoxine HCl</td>
<td></td>
<td>0.5</td>
<td>1</td>
<td>0.5</td>
<td></td>
</tr>
<tr>
<td>Thiamine HCl</td>
<td></td>
<td>0.1</td>
<td>10</td>
<td>1</td>
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</table>

MS: Murashige & Skoog medium vitamin mixture; B5: Gamborg B5 vitamin mixture; WPM: McCown woody plant vitamin mixture.
activity of excised branches of *P. nigra* were studied with B5 nutrient solution.

2.1.2. Effect of acidity on activity maintenance of excised branches

The activity of excised branches of *P. nigra* in different acidities of B5 nutrient solution is shown in Fig. 4. The $A_{\text{ex-60}}$ of excised branches increased first and then decreased with the increase of pH, and the $A_{\text{ex-60}}$ reached the peak of 0.88 when the pH of B5 nutrient solution was 6.0 ± 0.1. Therefore, the pH of 6.0 ± 0.1 is considered the optimal nutrient solution pH in hydroponics.

Previous studies reported that a pH range of 4 to 7 was appropriate for plant growth. Hoagland and Arnon (1950) reported that the optimal pH range for crop in hydroponics was from 5.0 to 6.5. Then, Islam et al. (1980) narrowed the best pH scope for hydroponics from 5.5 to 6.5. The availability of plant nutrients depends on pH. Some nutrient elements’ availability will decrease in slight acidic conditions, such as Mn, Cu, Zn, and especially Fe. Other elements’ availability will decline at a higher pH level, like P, K, Ca and Mg. Hence, Bugbee (2003) reported that in hydroponics a pH of 5.8 was the best for nutrient availability. This conclusion is close to our experiment result.

2.1.3. Effect of nutrient solution concentration on activity of excised branches

Following this determination of the best acidity, the nutrient solution was applied in six concentrations to determine the optimum concentration of nutrient solution. To compare the activity of excised branches of *P. nigra* in different concentrations more clearly, the value of $A_{\text{ex-60}}$ minus 1 ($A_{\text{ex-60}} - 1$) could be regarded as the net variation value. A positive value represents an increase of activity, and a negative value indicates a decrease of activity. As shown in Fig. 5, under the optimum acidity, the variation of the concentration of B5 nutrient solution has an influence on the activity of excised branches of *P. nigra*.
activity maintenance of excised branches of *P. nigra*. The $A_{ex-60}^m$ increased first and then decreased with the increasing concentration of B5 nutrient solution. It can be seen that $A_{ex-60}^m$ of 1/8 times of concentration is significantly different compared to the other concentrations tested ($A_{ex-60}^m$ equal 0.02), as all of the $A_{ex-60}^m$ values for other concentrations were negative. Similarly, the research on the stoma of *A. mamillata* Hance’s leaf by the different hydroponic techniques showed that 1/8 times concentration was

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**Fig. 8** – $A_{ex-60}$ changes over time during the 6 h after pruning. The excised branches were cultivated by 1/8 times concentration B5 nutrition solution with a pH of 6.0 ± 0.1 under light intensity of 1200 μmol/(m² sec).

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**Fig. 9** – The NO₂ exchange between living (9a) and excised poplar (9b) and the atmosphere. While the blue circles stand for the flux of NO₂ between living branch and the atmosphere and red dots are NO₂ exchange between the atmosphere and excised branch. 9c and 9d represent ambient NO₂ of living-branch day (blue) and excised-branch day (red line). In addition, the temperature and light intensity of living-branch day (9e) and excised-branch day (9f), where the green line represents the change of temperature and orange stands for light intensity.
the best culturing solution compared with 1, 1/2, 1/4 times concentration of Hoagland solution (Bai et al., 2010). When the concentration of nutrient solution is high, nutritional stress will enhance the cell wall elastic coefficient, and decrease the water conductivity (Liu and Kuang, 1998). Meanwhile, high concentration nutrient solution also causes osmotic stress and the decrease of transmembrane osmotic pressure of plasma membrane, which will lead to the loss of cell turgor (Apse and Blumwald, 2002; Parida and Das, 2005). When the concentration of nutrient solution is low, the lack of mineral nutrients will limit the synthesis of some important compounds in plants, thus affecting photosynthesis and other metabolic activities (Guo et al., 2010). Therefore, to keep activity of excised branches as long as possible, the 1/8 times concentration of B5 was the best nutrient concentration. The experiments above confirmed that the best condition for maintaining the activity of excised branch of *P. nigra* was 1/8 times B5 nutrient solution with a pH = 6.0 ± 0.1.

### 2.2. Adaptability of culture of branches in vitro

After determining the best conditions for maintaining the activity of excised branches, we studied the adaptability of the excised branch culture. As changes in light intensity, ambient temperature and culture time could affect the activity maintenance of excised branches, the adaptability of excised branches culture was discussed from three aspects: light intensity, temperature and the culture time of branch in vitro.

#### 2.2.1. Adaptation of excised branch culture to light intensity changing

The most appropriate nutrition solution was B5 culture medium with 1/8 concentration, and its pH was 6.0 ± 0.1. Under this solution condition, an artificial light intensity model was adopted. Light intensity was varied across a range of eight values, and excised branches were cultivated under different intensities. The result is shown in Fig. 6. The range of light intensity was from 200 to 2000 μmol/(m²·sec). The *A*_{ex-60} was between 0.97 and 1.05. Hence, we could draw the conclusion that the activity of excised branches is not sensitive to changes in light intensity between 200 and 2000 μmol/(m²·sec). At this range excised branches cultivated in optimal nutrition solution could keep their physiological activity.

#### 2.2.2. Adaptation of excised branch culture to temperature change

During the experiment, the ambient temperature range was 13.4–28.7 °C. In this temperature range, the branches were cultivated in using 1/8 concentration of B5 solution with pH = 6, under the light intensity of 1200 μmol/(m²·sec). The results are shown in Fig. 7. The *A*_{ex-60} was between 1.03 ± 0.10. Wang and Luan (1996) studied on the growth and development features of *P. nigra* and their relationships with temperature and water, and demonstrated that temperature had little influence on the net photosynthetic rate of *P. nigra*. In this experiment, the activity of excised branches of *P. nigra* cultivated in the best nutrient solution was almost the same as that in situ. Additionally, the *P. nigra* is grown in a temperate climate, with its three-basis-point of growth temperatures of approximately 5, 25–30, 35–40°C, so temperature range is suitable for plant growth (Wu et al., 2003). Hence, the optimal temperature conditions to maintain the activity of excised branches are broad. This experiment shows that in this temperature range, the excised branches cultured in this situation can reflect the activity of living branches.

#### 2.2.3. Long-term activity maintenance of excised branches under optimum conditions

Under 1200 μmol/(m²·sec) light intensity, the excised plants were cultured for 6 h under the condition of pH = 6 in a 1/8 concentration B5 nutrient solution. Fig. 8 shows that the *A*_{ex-60} was stable at 1.00 ± 0.09. It means that during the 6 h after pruning, the activity of excised branches can still maintain normal physiological activity.

The time scale of activity maintenance of excised branches under optimum conditions was discussed in previous studies. Huang et al. (2009) wrapped pruned branches in wet soils and found that the reliable time range of the maintenance activity of *C. glauca* was 3–23 min. The deficiency of water resulted in a rapid loss of activity in excised branches. In addition, the excised branches were cut into water twice by Tang and Wang (2011), and the activity time was up to 1 h. In our experiment, the activity of the excised branches of *P. nigra* cultured in the optimum nutrition solution was basically consistent with the living branches for 6 h after pruning, with the activity well maintained across a wide range of temperature and light intensity.

#### 2.3. The exchange of NO₂ between the atmosphere and excised plants and between the atmosphere and living plants

For verifying the feasibility of measuring the exchange of gases between plants and the atmosphere by using cultured branches, the exchange flux of NO₂ between the plant and the atmosphere was measured excised *P. nigra* branches, and compared with living branches.

It can be clearly seen from Fig. 9 that the changing trend of the exchange flux of NO₂ between living branches and the atmosphere is consistent with the exchange flux of NO₂ between the excised branches and the atmosphere. Specifically, the exchange between the atmosphere and excised poplar (from −1.01 to −2.72 nmol/(m²·sec)) with the mean of −1.88 nmol/(m²·sec)) was similar to that between living poplar and the atmosphere (from −1.12 to −3.16 nmol/(m²·sec)) with the mean of −2.11 nmol/(m²·sec)). There is a significant correlation between the two conditions (p < 0.01). Table 2 shows the corresponding parameters of two chambers. There are some slight differences in environmental conditions during the experiment. The uptake

### Table 2 - Data on the exchange of NO₂ between excised and living branches and the atmosphere.

<table>
<thead>
<tr>
<th>Sample chamber</th>
<th>Blank chamber</th>
</tr>
</thead>
<tbody>
<tr>
<td>NO₂ (ppb)</td>
<td>NO₂ (ppb)</td>
</tr>
<tr>
<td>Living</td>
<td>4.10–15.98</td>
</tr>
<tr>
<td>Mean</td>
<td>8.49</td>
</tr>
<tr>
<td>Excised</td>
<td>4.49–8.93</td>
</tr>
<tr>
<td>Mean</td>
<td>5.66</td>
</tr>
</tbody>
</table>

PAR: photosynthetic activity radiation.
of NO₂ was controlled by stomatal conductance and environmental NO₂ concentration (Chaparro-Suarez et al., 2011). There are some differences of environmental NO₂ concentration and stomatal conductance is affected by light intensity and temperature (Wang et al., 2009). Therefore, the differences of NO₂ exchange flux between the atmosphere and living branches and that of excised branches and the atmosphere were due to the different environmental conditions across the two days. The results of this experiment prove that it is feasible to determine the exchange flux of gas through the culture of the excised branches.

3. Conclusions

In this experiment, the net photosynthetic rate of excised branches and living branches of P. nigra was measured simultaneously using two photosynthetic instruments. Additionally, the optimum conditions to maintain the activity of excised P. nigra branches was established. High activity in P. nigra branches in vitro can be maintained across a wide range of light and temperature conditions. The study of the NO₂ fluxes between the atmosphere and the P. nigra was based on this approach, and the NO₂ taken up by excised branches was consistent with that by living branches. The results of this study show that it is feasible to substitute excised branches under specific cultivation conditions for living branches to determine the gas exchange between plants and the atmosphere. It also provides a convenient and reliable method for studying the exchange of gases between tall trees and the atmosphere.

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