EFFECTS OF AIR EXCHANGE, SUCROSE, AND PPF ON GROWTH OF *REHMANNIA GLUTINOSA* UNDER ENRICHED CO₂ CONCENTRATION IN VITRO

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Abstract

The explants excised from *Rehmannia glutinosa* plantlets were cultured on Murashige-Skoog (MS) medium with or without sucrose under various environmental conditions such as air exchanges, photosynthetic photon flux (PPF) and CO₂ enrichment. Healthy plantlets, which had normal stomatal activity and high survival rate were obtained by increasing the air exchange rates per hour in culture vessel. More than two times of leaf area and dry weight were observed compared with conventional tissue culture method under increased air exchange rate, high PPF and CO₂ enriched conditions. Although the presence of sucrose led to the maximal growth, the plantlets cultured on sucrose-free medium showed fair growth characteristics. It was clearly shown that plantlets grown under sucrosefree, CO₂ enrichment and high PPF conditions had autotrophic growth characteristics.

1. Introduction

*Rehmannia glutinosa* is a common and important medicinal plant, its root produces some compounds that have the action to clear up the heart, cool the blood, or used as medicine for hematinic and diabetes. The rhizome of the plant was obtained through the process of steaming, slicing, and drying under sunlight of root tubers (Kubo *et al*., 1994). Since Jiang and Mao (1979) first reported that the induction of callus and the regeneration of plants from root, stem, leaf and other organs, various strategies have been followed (Shoyama *et al*., 1983; Wu and Chen, 1986). However, there were no efforts aimed at optimizing the environmental conditions in vitro for higher acclimatization rates of the plants after transferred into the field. Frequently, physiological and morphological disorders such as vitrification could be found caused by high relative humidity, low photosynthetic photon flux and the presence of sugar in vitro (Kozai *et al*., 1992). The objective of our work was to determine the influence of in vitro environmental conditions, especially effects of air exchange, sucrose and light intensity, on plant growth.

2. Materials and Methods

Leafy single stem cuttings of *R. glutinosa* obtained from shoot tip culture were used as explants and cultured on MS medium supplemented with 1.0 mg/l benzyladenine (BA), 0.3 mg/l indole-3-acetic acid (IAA) and 3% sucrose. Four cuttings were inoculated into the polypropylene growth vessels (107 x 107 x 94 mm, Osmotek, Israel) attached with various sizes of membrane filters such as 0.0, 0.8 (Ø10 mm), 2.0 (Ø16 mm), 3.8 (Ø22 mm) and 12.6 (Ø40 mm) cm² containing 100 ml of culture medium. CO₂ concentration
inside and outside the vessels was measured with gas chromatograph (Hewlett Packard 6890, USA). The number of air exchanges per hour were measured by Kozai's method (1986) and it was calculated as 0.0, 0.8, 1.5, 1.9, and 4.4 times, respectively. Different treatments were designed to clarify the effects of air exchange rates and phototrophic conditions on growth of *R. glutinosa* in vitro. Explants were cultured on MS medium in absence or presence of 3% sucrose at 1000μmol mol⁻¹ CO₂, 100μmol m⁻²s⁻¹ PPF to determine the possibility of phototrophic growth in vitro. As control treatment, explants were cultured under conventional culture conditions (with sucrose and non-ventilation). All treatments were conducted in growth chambers, where the environmental conditions were maintained at 70% relative humidity and 16 hr photoperiod and different PPF (70, 140 and 210 μmol m⁻²s⁻¹). The CO₂ concentrations in the vessel under phototrophic conditions were measured at the 4th week. Stomatal diffusive resistance and transpiration (Li-COR 1600, USA), chlorophyll content (SPAD-502, Minolta, Japan), leaf area, shoot length, fresh and dry weight, and survival rates were investigated after transplanting the bulblets into soil 4 weeks later.

3. Results and Discussion

Figure 1 shows the effect of air exchange rates in the vessel on stomatal diffusive resistance and transpiration of the leaves grown under heterotrophic conditions for 4 weeks. The increase of the number of air exchange rate resulted in the increase of stomatal diffusive resistance and the decrease of transpiration, which indicated that the plantlets grown in well ventilated vessels were very sensitive to environmental changes. The stomata closed quickly to inhibit transpiration by the inhibition of K⁺ ions transported from subsidiary cells to guard cells (Salisbury and Ross, 1985). Chlorophyll content per leaf, leaf area and shoot length increased by forced ventilation. Especially, leaf area increased five times more than control when the air exchange rate were 4.4 times. Furthermore, forced ventilation improved the plant survival rates after transplanting them into the field (Table 1). The plantlets grown in poorly ventilated vessel had a lot of small leaves, showing high transpiration rate and numerous roots at the basal node as shown in Fig. 1 and Table 1. Figure 2 shows the effect of air exchange rates on stomatal diffusive resistance and transpiration of leaves grown under autotrophic (without sucrose) and mixotrophic (with 3% sucrose) conditions with 1000μmol mol⁻¹ CO₂ enriched and 40 μmol m⁻²s⁻¹ PPF with 16 hr photoperiod for 4 weeks. Stomatal diffusive resistance increased and transpiration decreased along with the increase of the number of air exchange rates in the mixotrophic conditions. However, few differences were observed in terms of both stomatal diffusive resistance and transpiration at different air exchange rates except for the non-ventilated treatment at autotrophic condition. A remarkable increase of leaf area was observed when the plants were grown in well-ventilated vessels. 4.4 times of air exchange rate gave rise to ten fold increase of leaf area compared with that of non-ventilated vessel (Figure 3). The results suggested that photosynthesis in vitro became vigorous by active CO₂ uptake through stomata under well-ventilated condition (Hayashi et al., 1993; Jeong et al., 1996; Kozai and Sekimoto, 1988). The effects of autotrophic conditions for plant growth are dependent on plants and culture methods. Vigorous shoot growth of potato (Nakayama et al., 1991) and *Cymbidium* (Heo et al., 1996) were observed in autotrophic condition but not significant effect was observed in carnation (Kozai, 1988; 1990), showing higher growth in mixotrophic conditions. Figure 4 represented that the changes of CO₂ concentration in the vessel were strongly affected by different PPF. CO₂ concentration in the vessel decreased rapidly along with the increase of PPF. There were no difference in CO₂ concentration in the vessel between autotrophic and mixotrophic conditions at highest PPF (210μmol
m$^{-2}$s$^{-1}$). Almost half amount of given CO$_2$ remained at lowest PPF condition, while only small amount of given CO$_2$ remained at highest PPF condition, indicating that plants grown in low PPF cannot use CO$_2$ effectively even though high concentration of CO$_2$ was supplied, and considerably high PPF is required for inducing vigorous photosynthetic activity in vitro, accordingly.

Table 2 shows the growth characteristics of *R. glutinosa* under different phototrophic conditions for 4 weeks. Explants at autotrophic and mixotrophic conditions grew very quickly in terms of both leaf area and dry weight. Approximately three folds increase of leaf area in autotrophic and three folds increase of dry weight in mixotrophic condition were observed compared with those under heterotrophic condition. Heterotrophic condition led to the succulent growth, which was caused by inadequate culture condition such as low PPF and low air exchange rates. A remarkable increase of leaf area in autotrophic was the result of photo-dependent growth without sugar, resulting in fully expanded leaves for uptake CO$_2$ needed for the photosynthesis (Desjardins *et al.*, 1995).

The results of this experiment showed that the goal of producing healthier plantlets of *R. glutinosa* could be achieved by appropriate environmental control such as increase of air exchange rate, CO$_2$ supply and high PPF.

4. References


Table 1 - Growth characteristics of *R. glutinosa* cultured at different air exchange rates in heterotrophic culture after 4 weeks in culture

<table>
<thead>
<tr>
<th>No. air exchanges (times/hr)</th>
<th>Chlorophyll content (mg dm⁻²±SE)</th>
<th>Leaf area (cm²±SE)</th>
<th>No. leaves (±SE)</th>
<th>Total fresh wt. (mg/plant±SE)</th>
<th>Survival rate in vivo(±SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>2.9±0.41</td>
<td>1.0 ±0</td>
<td>12.0±0.1</td>
<td>337.8± 9.5</td>
<td>50.2±5.0</td>
</tr>
<tr>
<td>0.8</td>
<td>3.4±0.21</td>
<td>1.83±0.06</td>
<td>8.0±0.1</td>
<td>332.4± 6.0</td>
<td>53.0±7.5</td>
</tr>
<tr>
<td>1.5</td>
<td>3.9±0.42</td>
<td>2.83±0.06</td>
<td>7.0±0.1</td>
<td>407.4±12.6</td>
<td>70.6±6.3</td>
</tr>
<tr>
<td>1.9</td>
<td>4.1±0.57</td>
<td>2.63±0.12</td>
<td>7.0±0.1</td>
<td>366.6±10.6</td>
<td>72.0±6.3</td>
</tr>
<tr>
<td>4.4</td>
<td>4.6±0.72</td>
<td>5.83±0.18</td>
<td>7.0±0.1</td>
<td>423.8±29.3</td>
<td>83.5±1.2</td>
</tr>
</tbody>
</table>

Table 2 - Growth characteristics of *R. glutinosa* grown under different phototrophic conditions with 16 hr photoperiod after 4 weeks in culture

<table>
<thead>
<tr>
<th>Culture Conditions</th>
<th>Plant height (cm±SE)</th>
<th>No. leaves (±SE)</th>
<th>Leaf area (cm²±SE)</th>
<th>Dry weight (mg±SE/plant)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Shoot</td>
</tr>
<tr>
<td>Autotrophic</td>
<td>11.3±0.05</td>
<td>11.3±0.2</td>
<td>75.0±5.0</td>
<td>107.2±3.5</td>
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<td>Mixotrophic</td>
<td>7.3±0.01</td>
<td>11.3±0.1</td>
<td>45.3±0.2</td>
<td>131.3±1.6</td>
</tr>
<tr>
<td>Heterotrophic</td>
<td>12.0±0.4</td>
<td>13.7±0.1</td>
<td>21.2±0.1</td>
<td>n.d</td>
</tr>
</tbody>
</table>
Fig. 1 - Effects of air exchanges per hour of vessel on stomatal diffusive resistance and transpiration of *R. glutinosa* in heterotrophic conditions after 4 weeks in culture. Vertical bars means SE.

Fig. 2 - Effects of air exchanges per hour on stomatal diffusive resistance and transpiration of *R. glutinosa* cultured in autotrophic (without sucrose) and mixotrophic conditions (with 3% sucrose) under 1000 μmol mol⁻¹ CO₂ enriched and 40 μmol m⁻² s⁻¹ PPF with 16 hr photoperiod for 4 weeks. Vertical bars means SE.
Fig. 3 - Effects of air exchanges per hour on leaf area and chlorophyll content of \textit{R. glutinosa} cultured in autotrophic and mixotrophic conditions under 1000 \( \mu \text{mol mol}^{-1} \) CO\(_2\) enriched and 40 \( \mu \text{mol m}^{-2}\text{s}^{-1} \) PPF with 16 hr photoperiod for 4 weeks.

Fig. 4 - Changes of CO\(_2\) concentrations in culture vessels of \textit{R. glutinosa} grown under mixo- and autotrophic conditions at 1000 \( \mu \text{mol mol}^{-1} \) CO\(_2\) enriched after 4 weeks in culture.