Effect of 5-aminolevulinic Acid on Organogenesis in Protocorn-like Body (PLBs) Culture of Cymbidium Hybrid Cultured In vitro

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Abstract: 5-aminolevulinic acid (5-ALA) has been suggested to be a new natural and environment friendly regulator, which can be widely used in agriculture. This study was undertaken to investigate the effect of 5-aminolevulinic acid (5-ALA) on organogenesis of Cymbidium hybrid in vitro under white fluorescent tube. PLBs of Cymbidium Waltz Idol were cultured on the modified MS supplemented with 5-ALA at various concentrations (control, 0.01, 0.1, 1 and 10 mg/l). The application of 5-ALA in the growth media, showed that 0.01 mg/l of 5-ALA enhanced PLB (100%), shoot (50%). Root formation was found that 0.1 mg/l and 1 mg/l of 5-ALA (20%) to the maximum within 40 days of culture. Whereas the control treatment showed that formation of PLB, shoot and root was the lowest. This result showed that 5-ALA added to culture media act as a plant growth regulator to induce PLB, shoot and root formation of Cymbidium hybrid.

Key words: 5-ALA • in vitro • PLB • Organogenesis

INTRODUCCIÓN

Cymbidium species have been hybridized for over century to produce plants with flowers of rich texture, color and size that have formed the basis of worldwide flower market. Cymbidiums are a large group of grassy-leaved plants. Thus many attempts have been made to develop better methodologies for Cymbidium micropropagation. These hybrids are gaining popularity in many countries, specially Japan [1-6]. To increase the Efficiency of in vitro techniques, plant growth regulators are frequently used for Orchids [7]. Plant growth regulators such as BA improve plant regeneration from PLBs in Cymbidium [2, 8]. Plant growth regulators (PGRs) are organic compounds, other than nutrients, that modify plant physiological processes. PGRs, called biostimulants or bioinhibitors, act inside plant cells to stimulate or inhibit the specific enzymes or enzyme systems and help regulate plant metabolism. Aminolevulinic acid (5-ALA) is a key precursor in the biosynthesis of porphyrins such as chlorophyll and heme. ALA has been suggested to be a new natural and environmental friendly regulator, which can be widely used in agriculture [9]. ALA application increased the yield of garlic, barely, rice and potato plants by significantly enhancing their photosynthetic capacity and plant biomass [10]. Recently, ALA has been shown to be involved in PLB culture of Cymbidium insigne and Cymbidium finlaysonianum [11]. [12] also reported that 5-ALA was the best to enhance the PLBs induction and shoot formation of Dendrobium kingianum. However, the majority of Cymbidium spp. is commercially produced using tissue culture methods. Therefore, in this study the effect of 5-ALA under white fluorescent tube were investigated on the in vitro organogenesis in PLBs of a Cymbidium hybrid.
MATERIALS AND METHODS

Protocorm-like bodies (PLBs) of *Cymbidium* Waltz ‘Idol’ were multiplied in modified MS medium of Shimasaki and Uemoto [2] by transferring the new medium after every two months. Approximately 5 mm long excised PLBs served as explants. Modified MS with quarter strength of ammonium nitrate (412.5 mg/l), half strength of potassium nitrate (950 mg/l), 20 g/l sucrose and 2 g/l Phytagel (Sigma) was used as the culture medium. The pH of media was adjusted using 0.213 g/l 2-(n-morpholino)ethanesulfonic acid sodium salt (MES-Na) before autoclaving at 121°C for 15 min. 5-aminolevulinic acid (5-ALA) at concentrations of 0, 0.01, 0.1, 1.0 and 10 mg/l were added to the culture media before sterilization. Jars (250 ml UM culture bottle, Japan) with plastic caps containing 30 ml of the medium were used as culture vessels. Five explants were placed in each culture vessel and three culture vessels were used for each treatment. All cultures were maintained at 25°C under white fluorescent light tube during 16 hrs photoperiod for 40 days. Experimental data were collected by counting the number of PLBs, shoots, root and fresh weight. The data were statistically analyzed by calculating standard errors of the means (means ± SE, n = 15).

RESULTS AND DISCUSSION

ALA enhanced PLB and shoot formation within 40 days of culture of C. Waltz ‘Idol’ as shown in Table 1. The maximum fresh weight (180 mg) of PLBs, the highest PLB formation rate (100%) and the highest average number of PLBs (3.2 PLBs/explant) were observed in explants cultured in the medium supplemented with 0.01 mg/l ALA, whereas in the control the fresh weight of PLBs was 150 mg, the rate of PLB formation being 80%, with an average of 1.8 PLBs/explant after 40 days of culture (Fig. 1). The highest number of shoots (0.7 shoots/explant) and the highest shoot proliferation rate (50%) were observed in the medium supplemented with 0.01 mg/l ALA compared to the control (0.5 shoots/explant). The highest root formation (20%) and the highest number of roots (0.2 roots/explant) were obtained at 0.1 mg/l and 1 mg/l ALA, compared to the control where there was 13% root formation and the number of roots was 0.2 roots/explant.

Table 1: Effect of aminolevulinic acid (5-ALA) on PLB culture of *Cymbidium* hybrid after 40 days under white fluorescent light

<table>
<thead>
<tr>
<th>Treatments</th>
<th>PLB</th>
<th>Shoot</th>
<th>Root</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-ALA (mg/l)</td>
<td>Average number</td>
<td>Formation rate (%)</td>
<td>Average number</td>
</tr>
<tr>
<td>Control</td>
<td>1.8±0.3</td>
<td>80.0</td>
<td>0.5±0.1</td>
</tr>
<tr>
<td>0.01</td>
<td>3.2±0.5</td>
<td>100</td>
<td>0.7±0.2</td>
</tr>
<tr>
<td>0.1</td>
<td>1.6±0.3</td>
<td>73.3</td>
<td>0.4±0.1</td>
</tr>
<tr>
<td>1</td>
<td>2.6±0.4</td>
<td>86.6</td>
<td>0.5±0.1</td>
</tr>
<tr>
<td>10</td>
<td>2.3±0.5</td>
<td>66.6</td>
<td>0.2±0.1</td>
</tr>
</tbody>
</table>

Value represents means ± S.E. The cultures were examined after 40 days of culture. Each treatment consisted of three replicates and each replicate consisted of five PLBS.

Fig. 1: The Effect of 5-ALA with modified MS on organogenesis on PLB culture of *Cymbidium* hybrid *in vitro*. A: Control, B and C: 0.01mg/l 5-ALA. (Bars=10 mm).
ALA is a linear five-carbon compound thought to act as a plant growth regulator [13, 14]. ALA improves the growth and yield of several plants by enhancing the chlorophyll content and the rate of photosynthesis, including Spirulina (Spirulina platensis), date palm (Phoenix dactylifera) and Arabidopsis (Arabidopsis thaliana) [15-18], rice and horseradish (Armoracia rusticana) [19] and garlic, barley, rice and potato [10]. ALA is known to play a vital role in improving the plant growth. Therefore, the present study confirmed that ALA functions as a plant growth regulator in Cymbidium Waltz Idol. ALA can promote PLBs proliferation of Cymbidium in vitro. Relatively low concentrations of ALA in culture media enhanced the formation of PLBs and shoots of Cymbidium hybrid. In C. Waltz Idol, a 100% PLB formation and a high number of PLBs (3.2 PLBs/explant) were observed in medium containing 0.01 mg/l ALA. The maximum percentage of shoot formation (50%) was obtained with 0.01 mg/l ALA as compared to the control treatment. [20] suggested that ALA at low concentrations could increase the growth of kudzu (Pueraria phaseoloides) by increasing photosynthetic rate. Previous studies have shown that low concentrations of exogenous ALA promote the plant growth, development and responses to environmental stresses, such as crop productivity [21] and stress tolerance [9,22-26]. These previous studies suggest that the effect of ALA on plants depends on the concentration of ALA. ALA at low concentrations is known to promote growth and development of some plants, animals, algae and photosynthetic bacteria [27, 28]. The present study suggests that low concentrations of ALA are also effective in Cymbidium hybrid. In C. hybrid Waltz Idol, the maximum fresh weight (180 mg) was achieved at 0.01 mg/l ALA while the lowest fresh weight (110 mg) was observed at 0.1 mg/l ALA. In agriculture, in addition to growth-promoting effects, ALA can be used as an herbicide and pesticide [29, 30]. ALA is known to regulate several key physiological processes associated with plant growth under saline regimes [20, 18], including seed germination, reduced Na uptake, altered light reactions, improved scavenging of reactive oxygen species (ROS), enhanced photosynthetic assimilation and maintenance of nutrient status [20, 18, 31]. Lower concentrations of ALA were effective in maintaining adequate stability of reactive oxygen synthetis-sis in some plants, such as Komatsuna [32]. Based on these reports, ALA added to culture media acts as a plant growth regulator of Cymbidium micropropagation. ALA is known to have various functions during different stages of plant growth although some of these functions did not apply to our experiments.

Culture media is commonly supplemented with plant growth regulators (e.g. auxins and cytokinins) to control the proliferation of PLBs and formation of shoots in Orchidaceae plants in vitro [2]. In present study, we demonstrated that a low concentration of ALA enhanced the formation of PLBs and shoot in C. Waltz Idol. This result suggests that ALA acts as a plant growth regulator, especially at an optimal auxin and cytokinin ratio, on PLB and shoot formation in a Cymbidium hybrid cultured in vitro. Parmar et al. [33] also obtained similar results in tomato (Lycopersicum esculentum Mill.).

CONCLUSIONS

This growth of Cymbidium hybrid cultured in vitro at lower concentration of ALA likely depend on the stability of reactive oxygen concentrations in cultures, but the mechanism by which ALA facilitates plant growth remains unclear. Thus, further studies required. We showed that low concentration of ALA in culture media act as a plant growth regulator to induce PLB, shoot formation of Cymbidium hybrid.

REFERENCES


