MICROPROPAGATION OF AQUARIUM PLANT, ANUBIAS SP. ‘WHITE’ USING ADENINE SULFATE AND 6-BENZYLAMINOPURINE

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ABSTRACT

Introduction: Anubias sp. ‘White’ is distinguished from other Anubias species by mutation. The leaves are white and green, which is popular and has great market demand. The growth of this aquarium plant is slow.

Method: The combination of adenine sulfate (Ads) and 6-benzylaminopurine (BAP) into Murashige and Skoog (MS) medium. A comparison of three substrate materials, i.e., filter pads (FP), husk ash granules (HAG) and rockwool (RW) were used in ex-vitro acclimatization of the plantlets.

Results: Ads and BAP had combined effects on the plantlets, leaves and roots of the apical bud (p<0.05). The treatment with only BAP at 0.5 mg/L in MS medium obtained the highest number of shoots, which was 7.40 shoots/explant (p<0.05). There is an interaction between 25 mg/L Ads and 1-1.5 mg/L BAP to induce callus. The treatments with HAG were better than the other planting materials in terms of growth performance (p<0.05). HAG yielded the best growth (fresh weight of 0.74 g/plant), which was better than FP and RW, respectively, and showed a significant (p<0.05) difference in terms of root number and plant height except root length.

Conclusion: There was an interaction between 25 mg/L Ads and 1-1.5 mg/L BAP to induce callus. The treatment with only BAP at 0.5 mg/L in MS medium obtained the highest number of shoots, which was 7.40 shoots/explant (p<0.05). The HAG as a planting medium was the optimal ex-vitro acclimatization of Anubias sp. ‘white’ plantlets.

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MICROPROPAGATION OF AQUARIUM PLANT, *ANUBIAS* SP. ‘WHITE’ USING ADENINE SULFATE AND 6-BENZYLAMINOPURINE

**RESUMEN**

Introducción: *Anubias* sp. “White” distingue-se de outras espécies de *Anubias* por mutação. As folhas são brancas e verdes, o que é popular e tem grande procura no mercado. O crescimento desta planta de aquário é lento.

Método: A combinação de sulfato de adenina (Ads) e 6-bencilaminopurina (BAP) em meio Murashige e Skoog (MS). Uma comparação de três materiais de substrato, ou seja, almofadas filtrantes (FP), grânulos de cinza de casca (HAG) e lã de rocha (RW) foram utilizados na aclimatação ex-vitro das plântulas.

Resultados: Ads e BAP tiveram efeitos combinados nas plântulas, folhas e raízes da gema apical (p<0.05). O tratamento apenas com BAP na dose de 0,5 mg/L em meio MS obteve o maior número de brotos, que foi de 7,40 brotos/explante (p<0.05). Há uma interação entre 25 mg/L de Ads e 1-1,5 mg/L de BAP para induzir calosas. Os tratamentos com HAG foram melhores que os demais materiais de plantio em termos de desempenho de crescimento (p<0,05). O HAG apresentou o melhor crescimento (peso fresco de 0,74 g/planta), melhor que FP e RW, respectivamente, e apresentou diferença significativa (p<0,05) em termos de número de raízes e altura de plantas, exceto comprimento de raiz.

Conclusão: Houve interação entre 25 mg/L de Ads e 1-1,5 mg/L de BAP para induzir calo. O tratamento apenas com BAP na dose de 0,5 mg/L em meio MS obteve o maior número de brotos, que foi de 7,40 brotos/explante (p<0,05). O HAG como meio de plantio foi a melhor aclimatação ex-vitro de *Anubias* sp. mudas ‘brancas’.

Palavras-chave: *Anubias* sp. ‘Branco’, planta de aquário, micropropagação, sulfato de adenina, 6-Benzilaminopurina.
mayor número de brotes, el cual fue de 7.40 brotes/explante (p<0.05). Existe una interacción entre 25 mg/L de Ads y 1-1,5 mg/L de BAP para inducir callos. Los tratamientos con HAG fueron mejores que los otros materiales de siembra en términos de comportamiento de crecimiento (p<0.05). HAG produjo el mejor crecimiento (peso fresco de 0,74 g/planta), que fue mejor que FP y RW, respectivamente, y mostró una diferencia significativa (p<0.05) en términos de número de raíces y altura de la planta, excepto la longitud de las raíces.

Conclusión: Hubo una interacción entre 25 mg/L de Ads y 1-1,5 mg/L de BAP para inducir callos. El tratamiento con solo BAP a 0.5 mg/L en medio MS obtuvo el mayor número de brotes, el cual fue de 7.40 brotes/explante (p<0.05). El HAG como medio de siembra fue la óptima aclimatación ex vitro de Anubias sp. Plántulas "blancas".

Palabras clave: Anubias sp. ‘Blanco’, planta de acuario, micropropagación, sulfato de adenina, 6-bencilaminopurina.

1 INTRODUCTION

Anubias spp. is the most traded species of aquatic plants and has the highest export value. They are monocotyledonous angiosperms in the family Araceae. The stem is an underwater rhizome. The leaves are ovate to lanceolate, with a dark green color. Both perfect flowers and imperfect flowers are formed. The flowers are small and apetalous. Anubias spp. grow well at temperatures of 20-25°C at pH 6.5-7.4 (Unnikrishnan, 2002) and the genus is well adapted to growing in the climatic conditions of Thailand. Popular Anubias species include A. nana, A. barteri and some emerging species from mutation or farmed selection include A. barteri, var. nana ‘Petite’, A. barteri var. nana ‘Variegated’, A. barteri var. nana ‘Wrinkle Leaf’ (Han, 2002). Anubias sp. ‘white’ distinctive features of the leaves are white or greenish white heart-shaped broad leaves (Figure 1). The height of the plant is about 5-7 cm due to these outstanding features. As a result, Anubias sp. ‘white’ is popular and has high market demand. But the propagation is quite slow, not enough to meet market demand. Micropropagation is currently applied to aquarium plants as a tool for large-scale multiplication of elite plants. However, information concerning the details of media and growth regulator amendments is still a fundamental requirement of the intense commercial production of Anubias sp. ‘white’. Therefore, plant tissue culture technology can increase the number of plantlets and be disease-free. Adding plant growth regulators to promote optimum growth. It is also very important to increase the number of species. The next step is planting in the surrounding environment, which requires the selection of planting material that is suitable for transplanting Anubias sp. ‘white’ is obtained from tissue culture to enhance its quality for growth in a hydroponic
system. It is enough to get a good-quality Anubias sp. 'white', and there is a standard that is needed by the aquarium plant market.

Several species of the genus Anubias have been propagated via in vitro cultures, such as Anubias congensis (Kломкamhaeng et al., 2021), Anubias sp. ‘Petite’ (Laohavisuti et al., 2019), Anubias heterophylla (Rittirat et al., 2021) and Anubias barteri var. nana (Rittirat et al., 2023). This study investigated the effects of the variable concentrations of adenine sulfate (Ads) and 6-benzyl aminopurine (BAP) in Murashige and Skoog (MS) medium on the shooting formation of Anubias sp. ‘white’, developing sufficient tissues of the Anubias genus as well as providing a standard of quality that meets the export requirements of Anubias sp. ‘white’.

**Figure 1**

Anubias sp. ‘white’

2 MATERIALS AND METHODS

2.1 EFFECT OF COMBINATION ADS AND BAP IN MS MEDIUM ON SHOOTING FORMATION OF ANUBIAS SP. ‘WHITE’

The experimental design was completely randomized in a 4 x 4 factorial scheme (Ads x BAP) with twenty replications, where each replication was composed of one shoot. There are two factors: Ads at 0, 25, 50 and 75 mg/L combined with BAP at 0, 0.5, 1.0 and 1.5 mg/L in MS medium (Table 1).
The sterilized shoots of *Anubias* ‘white’ were obtained from the Sun Aqua Plant Laboratory, Nong Bun Mak Nakhon, Ratchasima province. The sterile shoots were cultured in MS medium (Murashige and Skoog, 1962) for multiplication under a light intensity of 1250 Lux and a photoperiod of 16/8 h light/dark to increase the number of shoots. The sterile shoots were cultured in MS medium for different combinations of Ads and BAP (Table 1). One sterile shoot was in a 6-ounce bottle. All treatments were cultured under light intensity of 1250 Lux and a photoperiod of 16/8 h light/dark for 6 weeks. Aquatic plant growth was counted weekly for their individual number of shoots, roots and leaves, plant height and callus formation.

### 2.2 COMPARISON OF SUBSTRATES FOR THE EX-VITRO ACCLIMATIZATION OF *ANUBIAS* SP. ‘WHITE’ PLANTLETS

The experimental design was designed as a completely randomized design (CRD). Three growing substrates of planting media were used for the investigation of the acclimatization of *Anubias* sp. ‘white’ plantlets. Three substrate materials (Figure 2), i.e., filter pads (FP), husk ash granules (HAG) and rockwool (RW), were used as planting media for the *ex-vitro* acclimatization of *Anubias* sp. ‘white’ plantlets. Each treatment used four replications and each replicate comprised ten pots and one plantlet per pot.

**Table 1**

*Combination of concentration Ads and BAP in MS medium*

<table>
<thead>
<tr>
<th>Ads (mg/L)</th>
<th>BAP (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>0</td>
<td>0.0(</td>
</tr>
<tr>
<td>25</td>
<td>25.0(</td>
</tr>
<tr>
<td>50</td>
<td>50.0(</td>
</tr>
<tr>
<td>75</td>
<td>75.0(</td>
</tr>
</tbody>
</table>
Plantlets of Anubias sp. ‘white’ were pre-planted from tissue culture. They were partially sewn into rockwool cubes (2 cm×2 cm ×2 cm) that were placed in plastic containers in a flexible polyethylene tray then transferred to a plastic tank (10 cm×10 cm×20 cm) and fully covered with a transparent plastic sheet for controlling humidity. Every day, the humidity was reduced by partially uncovering the sheet to mimic the natural condition within 1 week before starting the experiment. The plantlet was grown in a greenhouse with natural light, a temperature of 30-32 °C and a humidity of 70-80%. Fresh weight, leaf number, root number, root length and plant height were measured every week.

2.3 STATISTICAL ANALYSIS

All the data were statistically analyzed by analysis of variance (ANOVA) and Duncan’s new multiple range test (DUNCAN) to determine differences among treatments at 0.05 alpha levels using the SPSS program.
3 RESULTS

3.1 EFFECT OF COMBINATION ADS AND BAP IN MS MEDIUM ON SHOOTING FORMATION OF ANUBIAS SP. ‘WHITE’

3.1.1 To induce shoot formation

After 6 weeks, it was found that Ads and BAP had combined effects on the plantlet of the apical bud (p<0.05). The treatment with only BAP at 0.5 mg/L in MS medium obtained the highest number of shoots, which was 7.40 shoots/explant (p<0.05). The increment of BAP supplemented in the medium tended to increase shoot number (p<0.05). Mean shoots per explant cultured in MS medium with BAP added at 1.5 mg/L (5.27 shoots/explant) 1 mg/L (4.90 shoots/explant) and 0.5 mg/L (4.80 shoots/explant) had no significant difference between them and were higher than those of the treatment without BAP (2.35 shoots/explant) (p<0.05) (Table 2, Figures 3 and 9). Thus, the increment of BAP supplemented in the medium tended to increase shoot number. The mean shoots per explant cultured in MS medium without adding Ads (5.32 shoots/explant) were higher than those of the treatment adding Ads at 25 mg/L (3.80 shoots/explant), 50 mg/L (4.02 shoots/explant) and 75 mg/L (4.17 shoots/explant) (p<0.05). Thus, the increment of Ads supplemented in the medium tended to decrease shoot number.
3.1.2 To induce leaves of *Anubias* sp. ‘white’

After 6 weeks, it was found that the interaction between Ads and BAP could induce the leaves of the apical bud (p<0.05). The treatment with Ads 75 mg/L and BAP 1.5 mg/L in MS medium obtained 4.40 leaves/explant (p<0.05) which gave the highest number of leaves per explant. Table 2, Figures 4, 5 and 9. The mean leaves per explant cultured in MS medium with Ads 75 mg/L (3.22 leaves/explant) were higher than those of the other treatments (p<0.05). The mean leaves per explant cultured in MS medium with BAP at 1.5 mg/L (3.40 leaves/explant) were higher than those of the other treatments (p<0.05). The result indicates that the highest concentration of both Ads (75 mg/L) and BAP (1.5 mg/L) gave the highest leaf numbers.
Figure 4

Shoots number and leaves of Anubias sp. ‘white’ were cultured in MS media adding Ads 0 mg/L and BAP 0.5 mg/L for 6 weeks.

Figure 5

Effects of Ads and BAP on Anubias sp. ‘white’ leaf numbers at the sixth week of culture. The means±SE followed by different letters do differ significantly differences according to the DUNCAN at p < 0.05.
3.1.3 To induce roots of *Anubias* sp. ‘white’

After 6 weeks, it was found that Ads and BAP had combined effects on the roots of the apical bud (p<0.05). The treatment with only Ads at 75 mg/L in MS medium obtained 4.00 roots/explant (p<0.05), which gave the highest number of roots per explant (Table 2, Figures 6 and 9). The mean roots per explant cultured in MS medium with Ads 75 mg/L (2.57 roots/explant) were higher than those of the other treatments (p<0.05). While adding ads at 75 mg/L, there was no significant difference at 25 mg/L of Ads, whereas at 25 mg/L there was no significant difference at Ads 0 and 50 mg/L. Thus, the increment of Ads supplemented in the medium tended to increase root number. The mean roots per explant cultured in MS medium without adding BAP (3.00 roots/explant) were higher than those of the other treatments (p<0.05). The increment of BAP supplemented in the medium tended to decrease root number (p<0.05).

**Figure 6**

*Effects of Ads and BAP on Anubias sp. ‘white’ root numbers at the sixth week of culture. The means±SE followed by different letters do differ significantly differences according to the DUNCAN at p < 0.05*
3.1.4 To induce plant height of *Anubias* sp. ‘White’

After 6 weeks, it was found that Ads and BAP had combined effects on the plant height of the apical bud (p<0.05). The treatment with Ads 75 mg/L and BAP 1.5 mg/L in MS medium obtained 11.67 mm. (p<0.05), which gave the highest height of plant but had no significant difference with the combination between Ads 0 mg/L and BAP 0.5 mg/L (11.31 mm.) (Table 2, Figures 7 and 9). The mean plant height of plants cultured in MS medium with Ads 75 mg/L (8.65 mm.) was higher than that of the other treatments (p>0.05). The mean plant height per explant cultured in MS medium with BAP at 1.5 mg/L (9.29 mm.) was higher than that of the other treatments (p>0.05), except for the culture without adding BAP. The result indicates that the highest concentration of both Ads (75 mg/L) and BAP (1.5 mg/L) gave the highest plant height.

**Figure 7**

*Effects of Ads and BAP on Anubias sp. ‘white’ plant height at the sixth week of culture. The means±SE followed by different letters do differ significantly differences according to the DUNCAN at p<0.05*
3.1.5 To induce callus of *Anubias* sp. ‘White’

After 6 weeks, it was found that Ads and BAP had combined effects on the callus of the apical bud (p<0.05). The treatment with Ads 25 mg/L and BAP 1.0-1.5 mg/L in MS medium induced callus (p<0.05) (Figures 8 and 9).

Figure 8

*Callus formation of Anubias sp. ‘white’* (A) Adding Ads 25 mg/L combined with BAP 1.0 mg/L and (B) Adding Ads 25 mg/L combined with BAP 1.5 mg/L for 6 weeks
Figure 9

Development of Anubias sp. ‘white’ apical bud which adding Ads and BAP at different concentrations for 6 weeks

![Image showing development of Anubias sp. ‘white’ apical bud with different concentrations of Ads and BAP after 6 weeks]
Table 2

Effect of Ads and BAP combination in MS medium on shoot numbers, leaf numbers, root numbers and plant height of Anubias sp. ‘white’ in vitro for 6 weeks

<table>
<thead>
<tr>
<th>Factor</th>
<th>concentration</th>
<th>Shoot number (no/explant)</th>
<th>Leaf number (no/explant)</th>
<th>Root number (no/explant)</th>
<th>Plant height (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ads A</td>
<td>0</td>
<td>5.32±0.84a</td>
<td>2.42±0.42a</td>
<td>1.37±0.28b</td>
<td>8.39±0.87a</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>3.80±0.53b</td>
<td>2.35±0.43b</td>
<td>2.17±0.30b</td>
<td>8.22±0.67a</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>4.02±0.44b</td>
<td>2.12±0.31b</td>
<td>1.42±0.17b</td>
<td>7.86±0.59a</td>
</tr>
<tr>
<td></td>
<td>75</td>
<td>4.17±0.52b</td>
<td>3.22±0.74a</td>
<td>1.71±0.31b</td>
<td>8.65±0.91a</td>
</tr>
<tr>
<td>BAP B</td>
<td>0</td>
<td>2.35±0.23b</td>
<td>1.85±0.10c</td>
<td>3.00±0.34a</td>
<td>6.31±0.29b</td>
</tr>
<tr>
<td></td>
<td>0.5</td>
<td>4.80±0.33a</td>
<td>2.58±0.22b</td>
<td>2.25±0.20b</td>
<td>9.00±0.31a</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>4.90±0.26a</td>
<td>2.30±0.18bc</td>
<td>1.42±0.20c</td>
<td>8.52±0.30a</td>
</tr>
<tr>
<td></td>
<td>1.5</td>
<td>5.27±0.20a</td>
<td>3.40±0.28a</td>
<td>0.90±0.25c</td>
<td>9.29±0.43a</td>
</tr>
</tbody>
</table>

F-test A  | *  | *  | *  | ns
F-test B  | *  | *  | *  | *
A×B       | *  | *  | *  | *
CV%       | 22.66 | 20.93 | 37.97 | 11.00

Mean ± SE values in each column followed by different superscript letters are significantly different (p<0.05) * = significant (p<0.05) ns = non-significant (p>0.05)

3.1.6 Comparison of substrates for the ex-vitro acclimatization of Anubias sp. ‘White’ plantlets

A comparison of three substrate materials, i.e., filter pads (FP), husk ash granules (HAG) and rockwool (RW) were used as planting media for the ex-vitro acclimatization of Anubias sp. ‘white’ plantlets. After 4 weeks, the results showed that the treatments with HAG were better than those with FP and RW, respectively, in terms of growth performance (p<0.05), except root length. HAG yielded the best growth (fresh weight of 0.74 g/plant), which was better than those of FP and RW, respectively, and showed a significant (p<0.05) difference in terms of leaves number, roots number and plant height, whereas root length had no significant difference between the three substrate materials (Table 3 and Figure 10). Thus, HAG provides better growth for Anubias sp. compared to the other substrates.

Table 3

Comparison three substrate materials on Anubias sp. ‘white’ plantlets growth performance for 4 weeks

<table>
<thead>
<tr>
<th>Substrate materials</th>
<th>FP</th>
<th>HAG</th>
<th>RW</th>
<th>F-test</th>
<th>CV %</th>
</tr>
</thead>
<tbody>
<tr>
<td>fresh weight (g/plant)</td>
<td>0.66±0.06ab</td>
<td>0.74±0.07a</td>
<td>0.57±0.07b</td>
<td>*</td>
<td>12.57</td>
</tr>
<tr>
<td>Plant height (cm)</td>
<td>2.5±0.15b</td>
<td>3.69±0.15a</td>
<td>2.60±0.17c</td>
<td>*</td>
<td>19.06</td>
</tr>
<tr>
<td>Leaves number /no./plant</td>
<td>6.45±0.67ab</td>
<td>8.00±0.36c</td>
<td>6.45±0.63b</td>
<td>*</td>
<td>12.85</td>
</tr>
<tr>
<td>Roots number /no./plant</td>
<td>7.55±0.63ab</td>
<td>8.95±0.79a</td>
<td>6.80±0.70b</td>
<td>*</td>
<td>14.05</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Root length (cm)</th>
<th>4.40±0.46</th>
<th>5.32±0.54</th>
<th>4.32±0.57</th>
<th>ns</th>
<th>11.87</th>
</tr>
</thead>
</table>

Mean ± SE values in each row followed by different superscript letters are significantly different (p<0.05) * = significant (p<0.05) ns = non-significant (p>0.05)

Figure 10
Anubias sp. ‘white’ plantlets cultured in (A) FP, (B) HAG, and (C) RW after transparent 4 weeks

4 DISCUSSION

After examining the effect of combination Ads and BAP in MS medium on micropropagation in Anubias sp. ‘white’ for 6 weeks, it was found that there was an interaction between Ads and BAP, which are plant growth regulators in the category of cytokinin. Cytokinins are widely used to promote tissue proliferation and the development of shoots in plant tissue culture (Van Staden & Mooney, 1988).

Regarding the induction of shoot formation of Anubias sp. ‘white’, the result showed that increasing Ads concentration led to a decrease in the shoot number compared to not adding Ads. By contrast, an increase in BAP concentration leads to an increase in shoot number. This result indicates that both Ads and BAP act as cytokinins, which stimulate tissue proliferation and shoot formation (Van Staden & Mooney, 1988).
Interestingly, the treatment with only BAP at 0.5 mg/L in MS medium obtained the highest number of shoots, which was 7.40 shoots/explant (P<0.05). This means that adding only BAP (without adding Ads) was sufficient for cultured and enhanced division of cells in the shoot of Anubias sp. ‘white’. This is because the cytokinin affinity of BAP for the receptors in the Anubias plant is likely to be better than that of Ads (Lomin et al., 2012). This means that BAP molecules are able to bind to the cytokinin receptor better than Ads. The result leads to BAP transporting into the Anubias cells better than Ads. Therefore, the more molecules of BAP transport into the cells, the more shoot induction formation occurs. Adding Ads to the induction of shoot formation, it was observed that increasing the concentration of Ads resulted in a decrease in shoot numbers. This indicates that Ads performed a stimulating effect on shoot development like cytokinins (Van Staden et al., 2008). However, the sensitivity of the cytokinin property of Ads to the induction of shoot formation is unlikely to be as strong as BAP's performance on the induction of shoot formation, as is the reason for the cytokinin receptor affinity of BAP compared to Ads.

The Ads and BAP combinations are like multiplication shoots in other Anubias species. Micropropagation of Anubias sp. ‘Petite’ research showed that using combination treatment with 75 mg/L Ads and 1-2 mg/L BAP induced 4.93 and 5.03 shoots/explant, respectively (Laohavisuti et al., 2019). Moreover, Laohavisuti et al. (2017) used Ads 0, 25, 50 and 75 mg/L combined with BAP 0, 0.5, 1.0 and 1.5 mg/L in micropropagation of the aquatic plant Bucephalandra sp. In the family Araceae for 8 weeks, it was found that treatment supplemented with BAP 0.5 mg/L (2.45 shoots/explant) induced a higher number of shoots than the other treatments. Previous studies have shown that BAP is more effective for shoot induction in aquatic plant species in the family Araceae, such as Anubias barterii var. nana ‘Petite’ (Stanly et al., 2011), A. barteri var. nana (Kanchanapoom et al., 2012), Cryptocoryne lucens (Kane et al., 1990), Cryptocoryne sp. (Herath et al., 2008), Cryptocoryne wendtii and C. becketti (Sheeja et al., 2015).

In the induction of leaves and plant height of the Anubias species, the greatest concentration of both Ads (75 mg/L) and BAP (1.5 mg/L) produced the greatest leaf number and plant height. This result indicated that both plant growth regulators, Ads and BAP, play a role in the performance of cytokinins. According to these results, they are
similar to the induction of shoot formation by *Anubias* sp. ‘white’ which demonstrates that both Ads and BAP function as cytokinins (Van Staden & Mooney, 1988).

Regarding the induction of root formation in *Anubias* sp. ‘white’, the result showed that increasing Ads concentration led to an increase in root number. By contrast, an increase in BAP concentration leads to a decrease in root number. This result indicates that both Ads and BAP act in different ways. The role of BAP showed a cytokinin-like utilization of cytokinins in plant propagation at higher concentrations results in strong inhibition of rooting (Van Staden & Mooney, 1988). While adenine exhibited cytokinin-like activity on shoot development, it did not show the inhibitory effect on root development typical of cytokinins. This compound, particularly at higher concentrations, stimulated both axillary shoot development (Wroblewska (2012) and Jana and Shekhawat (2011)).

After 4 weeks, *Anubias* sp. ‘white’ plantlets grown with different substrates for the ex-vitro acclimatization FP, HAG and RW were significantly different (p<0.05), in terms of root number and plant height whereas, root length had no significant difference between the three substrate materials (p>0.05). This study suggests that the HAG is suitable for the ex-vitro acclimatization plantlet. Ceramics and Construction Materials Research Group (2010) mentioned that HAG properties are porosity 70% and water holding capacity 54.4%. The substrate chosen is the physical medium that supports plants by the stem and keeps them under appropriate growing conditions by providing an aseptic environment with good oxygenation, capillarity, chemical inertia and biological inertia (Velazquez-Gonzalez et al., 2022). Sholichah et al. (2020) reported that sand as a planting medium provides better growth for *Anubias* sp. when compared to the other three growing media (husk charcoal, gravel and rockwool) by hydroponics.

**5 CONCLUSION**

Adding Ads and BAP to MS medium had combined effects on plantlets, leaves and roots of the apical bud (p<0.05). There was an interaction between 25 mg/L Ads and 1-1.5 mg/L BAP to induce callus. The treatment with only BAP at 0.5 mg/L in MS medium obtained the highest number of shoots, which was 7.40 shoots/explant (p<0.05). The HAG as a planting medium was the optimal ex-vitro acclimatization of *Anubias* sp. ‘white’ plantlets.
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