IN VITRO BULB PRODUCTION IN HIPPEASTRUM (HIPPEASTRUM HYBRIDUM)


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ABSTRACT
An in vitro experiment was conducted to find out the optimum hormonal supplement and sucrose level for the bulb production of Hippeastrum. Murashige and Skoog medium supplemented with different hormone concentrations of BAP (0.0, 2.0, 4.0, 6.0 and 8.0 mg/L) and CCC (0.0, 125, 250 and 500 mg/L) and sucrose levels (30, 60, 80, 90 and 110 g/L) were used in this study. Sucrose level at 90 g/L produced the maximum average weight as well as the highest regeneration percentage. The increasing rate of CCC increased the number and average weight of bulb. The maximum bulb formation observed in media supplement with 6.0 mg/L BAP and 500 mg/L CCC fortified with 90 g/L sucrose.

Key words: Hippeastrum (Hippeastrum hybridum), In vitro, bulb production, tissue culture, BAP, CCC, sucrose
SUPPLEMENTAL MATERIALS AND METHODS

Bulbs, generally 30 cm in circumference, were cleaned, and rinsed in tap water for 30 minutes. Two or three outer most scales were removed. The tips of the bulb were removed. The bulbs were cut vertically into four, producing sixteen more identical pieces. Any visible leaf and bud initials were removed at this stage. The pieces of bulb that now remain are then trimmed back so that on each section of root plate and or basal plate there seats two scales. Finally twin scales (adjacent scale pairs jointed by a portion of the basal plate) of different sizes were prepared. The number of twin scales produced depends on the size of the bulb; usually a 30 cm of diameter bulb can be expected to yield 2-3 bulblets in a year of growth [3]. Multiplication of plant from seed will show wide variation in flower colors, plant shape, time of flowering etc. Since the natural multiplication rate of Hippeastrum is slow, twin scaling might be developed to overcome this deficiency. In vitro plantlets production has already been established through twin scaling [13].

RESULTS

Individual effects of sucrose, BAP (Benzyl Amino Purine) and CCC (Chloro Choline Chloride) on in vitro bulb production are presented in Table 1 and the combined effects in Table 2.

Effects of Sucrose
The highest regeneration percentage (93.19%) was found in the medium with 90 g/L sucrose. It was observed that regeneration percentage increased up to 90 g/L of sucrose and then it was decreased. The earliest (36.95 days) bulblets induction was observed at 90 g/L sucrose but it was delayed either increasing or decreasing of sucrose level. The highest (1.79) number and heaviest (2.17 g) weight of bulb was also achieved at 90 g/L sucrose level. Percent of undesirable shooting decreased significantly with increasing sucrose level up to 90 g/L. It appeared that medium supplemented with 90 g/L sucrose was suitable for achieving maximum number of bulbs per twin scales and was used for all further studies in combination with BAP and CCC.

Effects of BAP
The highest percentage (97.48%) of regeneration was achieved in case of 6.0 mg/L BAP while the lowest percentage (81.04%) was observed in absence of BAP. Time required for bulblets induction varied significantly along with different concentrations of BAP. The minimum time (31.17 days) required for bulblets induction was at 6 mg/L BAP and the maximum (37.98 days) was found in absence of BAP. The number of in vitro bulbs per twin scales were increased with increasing the concentration of BAP up to 6 mg/L, and then gradually decreased with further increase of BAP concentration. The maximum number
(2.35) was achieved in case of 6 mg/L. The concentration of BAP at 6 mg/L produced the highest weight (2.77g) of bulb. The undesirable shooting was observed in different concentrations of BAP. The minimum (31.17%) was found in absence of BAP while the shooting percentage was increased with the increasing rate of BAP.

**Effects of CCC**

CCC has significant effect on percent of regeneration. The maximum (93.08%) regeneration percentage was obtained in absence of CCC while minimum (85.33%) percentage was observed at 500 mg/L CCC. CCC concentration significantly influenced the time required for bulblets induction. The shortest time (33.12 days) taken at 500 mg/L CCC while twin scales grown in the medium without CCC took the longest time (38.02 days) for bulblets induction. The number of bulb per twin scales was increased with increasing concentration of CCC. The maximum number (2.28) of bulb was produced with CCC concentration at 500 mg/L. Weight of in vitro bulb was increased with increasing rate of CCC concentrations. The highest bulb weight (2.51g) was recorded in case of 500 mg/L CCC. It was observed that CCC has positive role on average weight of in vitro bulb. Percentage of shooting varied widely (26.71 to 2.99) along with different concentration of CCC. The highest shooting (26.71%) was observed in absence of CCC while the lowest (2.99%) shooting was observed at 250 mg/L CCC. It might be due to the beneficial effect of CCC controlling undesirable shooting, because shooting was undesirable for in vitro bulb production.

**Combined effects of CCC and BAP**

The best regeneration (99.33%) was achieved at 500 mg/L CCC combination with 6.0 mg/L BAP. The time required for bulblets induction was maximum (37.44 days) in absence of CCC and BAP while it was the minimum time (29.54 days) with 500 mg/L CCC and 6.0 mg/L BAP. The combined effects of different concentrations of CCC and BAP were found significant on number of bulb per twin scales. The maximum number (3.33) of bulbs was noticed at combination of 500 mg/L CCC with 6.0 mg/L BAP. The highest average weight (4.34 g) of bulb was found with 6.0 mg/L BAP and 500 mg/L CCC. Average weight of bulb was minimum (1.86 g) in absence of CCC and BAP. It was observed that better average weight of bulb (2.96- 4.34) had found at hormone concentration 6.0 mg/L BAP in combination with different concentration of CCC. CCC at all concentrations produced more than 80 percent small bulb (<2.5 g) in absence of BAP. Benzyl Amino Purine (BAP) at 6.0 mg/L produced the highest (30.99%) percentage of >3.5 g size of in vitro bulb in combination with 500 mg/L CCC which was closely followed by same concentration of BAP with different concentrations of CCC.

**Table 1. Effects of sucrose, BAP and CCC on in vitro bulb production of Hippeastrum**

<table>
<thead>
<tr>
<th>Treatments</th>
<th>% regeneration</th>
<th>Bulblets induction (days)</th>
<th>Bulb per twin scales (no)</th>
<th>Average weight of bulb (g)</th>
<th>% shooting</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sucrose (g/L)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>71.77d</td>
<td>40.35a</td>
<td>0.29e</td>
<td>1.71d</td>
<td>40.41a</td>
</tr>
<tr>
<td>60</td>
<td>73.06d</td>
<td>37.02b</td>
<td>0.49d</td>
<td>1.85c</td>
<td>37.62ab</td>
</tr>
<tr>
<td>80</td>
<td>81.27b</td>
<td>37.05b</td>
<td>1.24b</td>
<td>2.02b</td>
<td>33.27bc</td>
</tr>
<tr>
<td>90</td>
<td>93.19a</td>
<td>36.95b</td>
<td>1.79a</td>
<td>2.17a</td>
<td>30.47c</td>
</tr>
<tr>
<td>110</td>
<td>72.56c</td>
<td>40.49a</td>
<td>1.06c</td>
<td>1.80c</td>
<td>35.60ab</td>
</tr>
<tr>
<td>BAP (mg/L)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.0</td>
<td>81.04d</td>
<td>37.98 a</td>
<td>1.84c</td>
<td>2.11e</td>
<td>31.17c</td>
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<td>2.0</td>
<td>85.28c</td>
<td>33.62c</td>
<td>1.87c</td>
<td>2.26d</td>
<td>42.33a</td>
</tr>
<tr>
<td>4.0</td>
<td>93.68b</td>
<td>31.59d</td>
<td>2.22b</td>
<td>2.61b</td>
<td>42.35a</td>
</tr>
<tr>
<td>6.0</td>
<td>97.48a</td>
<td>31.17d</td>
<td>2.35a</td>
<td>2.77a</td>
<td>34.08b</td>
</tr>
<tr>
<td>8.0</td>
<td>92.18b</td>
<td>36.23b</td>
<td>1.86c</td>
<td>2.39c</td>
<td>42.33a</td>
</tr>
<tr>
<td>CCC (mg/L)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.0</td>
<td>93.08a</td>
<td>38.02a</td>
<td>1.51d</td>
<td>1.88c</td>
<td>26.71a</td>
</tr>
<tr>
<td>125</td>
<td>91.64ab</td>
<td>36.40b</td>
<td>1.70c</td>
<td>2.04e</td>
<td>8.92b</td>
</tr>
<tr>
<td>250</td>
<td>90.90b</td>
<td>35.45c</td>
<td>2.03b</td>
<td>2.22b</td>
<td>6.41b</td>
</tr>
<tr>
<td>500</td>
<td>85.33c</td>
<td>33.12d</td>
<td>2.28a</td>
<td>2.51a</td>
<td>2.99e</td>
</tr>
</tbody>
</table>

Means bearing uncommon letter(s) in a column varied significantly at 5 % level.
Table 2. Combined effects of CCC and BAP on in vitro bulb production of Hippeastrum

<table>
<thead>
<tr>
<th>Treatments* (mg/L)</th>
<th>Percent of regeneration</th>
<th>Bulblets induction (days)</th>
<th>Bulb per twin scales (no)</th>
<th>Avg. weight of bulb (g)</th>
<th>Grading (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>&lt;2.5 g</td>
</tr>
<tr>
<td>C₀ x B₀</td>
<td>82.75j</td>
<td>37.44a</td>
<td>1.443k</td>
<td>1.86m</td>
<td>88.57a</td>
</tr>
<tr>
<td>C₀ x B₁</td>
<td>85.98i</td>
<td>35.78b</td>
<td>1.820ij</td>
<td>2.26jk</td>
<td>61.23c</td>
</tr>
<tr>
<td>C₀ x B₂</td>
<td>93.69h</td>
<td>34.80c</td>
<td>2.127fg</td>
<td>2.57gh</td>
<td>52.71g</td>
</tr>
<tr>
<td>C₀ x B₃</td>
<td>96.83cde</td>
<td>34.95c</td>
<td>2.380de</td>
<td>2.96cd</td>
<td>47.02i</td>
</tr>
<tr>
<td>C₀ x B₄</td>
<td>93.27h</td>
<td>36.36b</td>
<td>1.763j</td>
<td>2.12kl</td>
<td>54.60f</td>
</tr>
<tr>
<td>C₁ x B₀</td>
<td>93.89h</td>
<td>36.36b</td>
<td>1.787j</td>
<td>2.07l</td>
<td>86.89b</td>
</tr>
<tr>
<td>C₁ x B₁</td>
<td>95.33fg</td>
<td>34.46cd</td>
<td>1.907hij</td>
<td>2.76f</td>
<td>51.22h</td>
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<tr>
<td>C₁ x B₂</td>
<td>97.34bc</td>
<td>33.93de</td>
<td>2.320e</td>
<td>2.72fg</td>
<td>40.89k</td>
</tr>
<tr>
<td>C₁ x B₃</td>
<td>97.37bc</td>
<td>32.17gh</td>
<td>2.630bc</td>
<td>3.09c</td>
<td>32.70m</td>
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<tr>
<td>C₁ x B₄</td>
<td>95.18c</td>
<td>34.79e</td>
<td>1.807j</td>
<td>2.28j</td>
<td>47.74i</td>
</tr>
<tr>
<td>C₂ x B₀</td>
<td>94.90g</td>
<td>35.87b</td>
<td>2.013gh</td>
<td>2.32ij</td>
<td>83.45c</td>
</tr>
<tr>
<td>C₂ x B₁</td>
<td>95.81fg</td>
<td>33.26ef</td>
<td>1.983ghi</td>
<td>2.83def</td>
<td>46.82i</td>
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<tr>
<td>C₂ x B₂</td>
<td>97.53bc</td>
<td>31.49hi</td>
<td>2.397de</td>
<td>2.77ef</td>
<td>39.22i</td>
</tr>
<tr>
<td>C₂ x B₃</td>
<td>98.16b</td>
<td>30.94i</td>
<td>2.800b</td>
<td>3.44b</td>
<td>29.53n</td>
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<tr>
<td>C₂ x B₄</td>
<td>96.24def</td>
<td>32.61fg</td>
<td>1.800j</td>
<td>2.27jk</td>
<td>42.36j</td>
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<tr>
<td>C₃ x B₀</td>
<td>96.19ef</td>
<td>33.82de</td>
<td>2.280ef</td>
<td>2.45hi</td>
<td>81.31d</td>
</tr>
<tr>
<td>C₃ x B₁</td>
<td>95.60fg</td>
<td>32.60fg</td>
<td>2.127fg</td>
<td>2.92de</td>
<td>41.62jk</td>
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<tr>
<td>C₃ x B₂</td>
<td>98.18b</td>
<td>31.26i</td>
<td>2.507cd</td>
<td>2.92de</td>
<td>41.18k</td>
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<tr>
<td>C₃ x B₃</td>
<td>99.33a</td>
<td>29.54j</td>
<td>3.330a</td>
<td>4.34a</td>
<td>20.33o</td>
</tr>
<tr>
<td>C₃ x B₄</td>
<td>97.14ed</td>
<td>32.73fg</td>
<td>1.757j</td>
<td>2.69fg</td>
<td>37.89l</td>
</tr>
</tbody>
</table>

Means bearing uncommon letter(s) in a column varied significantly at 5 % level.

* C₀ = Control (without hormone), C₁ = 125 mg/L CCC, C₂ = 250 mg/L CCC, C₃ = 500 mg/L CCC
B₀ = Control (without hormone), B₁ = 2.0 mg/L BAP, B₂ = 4.0 mg/L BAP, B₃ = 6.0 mg/L BAP, B₄ = 8.0 mg/L BAP
DISCUSSION

Regeneration percentage increased up to certain level of sucrose and then it decreased. It was observed from the result that a certain level of sucrose was the prerequisite for bulblets induction within minimum days. The highest regeneration was found at 90g/L sucrose concentration. The earliest bulblets induction was observed from the same concentration of sucrose while it was delayed either increasing or decreasing of sucrose level. Bruyn et al [1] demonstrated that a certain amount of sucrose was needed for regeneration but high sucrose level had a negative effect on the regeneration potential of explants. So it was evident from this study that a certain level of sucrose was the prerequisite for bulblets induction within minimum days. Similar results were reported by Jeoung-Lai et al [6] in potato and Khanam [7] in gladiolus. The highest number and heaviest bulb was also achieved at 90g/L sucrose level. Percent of undesirable shooting decreased significantly with increasing sucrose level up to 90g/L. It was appeared that medium supplemented with 90g/L sucrose was suitable for achieving maximum number of bulbs per twin scales which was more or less similar to that of Zakaria [22] and Jeoung-Lai et al [6] in case of microtuber production in potato.

The time required for bulblets induction varied significantly along with the concentrations of BAP. The minimum time required for bulblets induction was at 6 mg/L BAP. The highest percentage of regeneration was achieved in 6.0 mg/L BAP while the lowest in absence of BAP. It might be due to the positive effect of BAP on regeneration. Dodds et al [2] recommended 5.0 mg/L BAP as optimum to induce tubers in a broad range of potato genotypes. Young et al [21] reported that BAP promoted microtuber initiation. Cytokinins have been considered to be important for in vitro bulb formation due to several reasons. Firstly, cytokinins known to stimulate cell division [14]; secondly, there is indication that it inhibits cell elongation [18] and promote cell expansion [12]. These phenomena are required for bulb formation and development. The number of in vitro bulbs per twin scales was increased with increasing concentration of BAP up to 6 mg/L and then it was gradually decreased with further increase of BAP concentration. The results were similar to the findings of Wang and Hu [20] who reported that the higher concentrations of BAP in the medium decreased the number of microtuber in case of potato. The maximum number and larger bulblets was achieved in the concentration of BAP at 6 mg/L. The shooting percentage was increased with the increasing rate of BAP.

It was evident from this study that CCC has negative role on % regeneration of in vitro bulblets in Hippeastrum. The shortest time for bulblets production taken at 500 mg/L CCC while without CCC took the longest time for bulblets induction. So the time required for bulblets induction was reduced with increasing concentration of CCC. This finding is in agreement with Hossain and Sultana [4] who reported earlier tuberization with 500 mg/L CCC in case of potato. The number of bulb per twin scales was increased with increasing concentration of CCC. Zakaria [22] also found maximum number of microtuber with 500 mg/L CCC in potato. Young et al [20] also reported that CCC increased the number of in vitro microtuber. Weight of in vitro bulb was increased with increasing rate of CCC concentrations. Zakaria [22] disagreed with this finding but Hossain and Sultana [4] reported similar findings in case of potato. Percentage of shooting varied widely with different concentration of CCC. The highest shooting was observed in absence of CCC while the lowest shooting was observed at 250 mg/L CCC. It might be due to the beneficial effect of CCC controlling undesirable shooting, because shooting was undesirable for in vitro bulb production.

The combined effects of different concentrations of CCC and BAP were found significant on regeneration and number of bulb per twin scales in Hippeastrum. The highest regeneration was achieved at 500 mg/L CCC combination with 6.0 mg/L BAP. It might be due to the combined beneficial effect of CCC [4] and BAP [17]. The maximum number (3.33) of bulbs was noticed at combination of 500 mg/L CCC with 6.0 mg/L BAP which is due to the positive response of both BAP [20] and CCC [21]. The highest average weight of bulb was found in 6.0 mg/L BAP with 500 mg/L CCC. CCC at all concentrations produced more than 80 percent small bulb (<2.5g) in absence of BAP. Benzyl Amino Purine (BAP) at 6.0 mg/L produced the highest percentage of >3.5g size of in vitro bulb in combination with 500 mg/L CCC. However, % regeneration, days to bulbles induction, number of bulbs per twin scales, average weight of bulb and grade of bulbs showed positive results with 6.0 mg/L BAP in combination with 500 mg/L CCC.

CONCLUSION

The present experiment was conducted to find out the optimum hormonal supplement and sucrose concentration for the bulb induction in Hippeastrum. Murashige and Skoog (MS) medium was supplemented with different hormone concentrations of BAP and CCC and sucrose levels. Sucrose level at 90 g/L produced the maximum average weight of bulb. The earliest bulblets induction was also observed at 90g/L sucrose and it was delayed either increasing or decreasing of sucrose level. The
increasing rate of BAP and CCC increased the number and average weight of bulb at the sucrose level of 90 g/L. The regeneration percentage was decreased due to increase of hormone concentrations. The maximum bulb formation observed in media supplement with 6.0 mg/L BAP and 500 mg/L CCC fortified with 90 g/L sucrose.

REFERENCES


