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

INTRODUCTION

Hippeastrum (Hippeastrum hybridum) is an ornamental bulbous flowering plant belongs to the family Amaryllidaceae, it has large and showy flowers with many bright colours [23] and commonly known as Royal Dutch Amaryllis [5]. They are native to Central and South America, and are easily grown in the tropical and subtropical regions [9]. Hippeastrums are often erroneously described as Amaryllis (Amaryllis belladona) although these two plants have distinct difference between them. Propagation can be accomplished by using seed, offset bulblets and twin scaling [19]. Conventional propagation of Hippeastrum hybrids by bulb offsets is slow, seasonal and variable with some hybrids not producing offsets [15]. In fact, normally a plant produces 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]. But in vitro bulb production is more advantageous than raising plantlets regarding time, storage, handing, establishment of planting materials etc. Considering the above facts the experiment was undertaken with the objective to develop a standard protocol for in vitro bulb production of Hippeastrum.

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 apical third of the bulb tips was removed. The bulbs were cut vertically into four segments and each segment cut again 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 60-80 sections of twin scales. Prepared explants were taken in beaker and suspended in Clorox solution (10-12% active chlorine) for 10 minutes then washed 3-4 times by double distilled water. Sterilization was carried out in the aseptic condition under a laminar flow cabinet. Then twin scales were suspended in 70% ethanol for 30 seconds, finally washed 3-4 times by double distilled water. The twin scales were dried superficially between two sheets of sterile filter paper before final use. The twin scales of size 1.0 cm in width × 2.5 cm in height was used as explants. The explants were cultured on MS medium [8] supplemented with different level of sucrose (30, 60, 80, 90 and110 g/L), BAP: Benzyl Amino Purine (0.0, 2.0, 4.0 5.0 and 8.0 mg/L) and CCC: Chioro Choline Chloride (0.0, 125, 250 500 mg/L) singly and the best level of sucrose (90g/L) was used in combination with BAP and CCC. The pH of the medium was adjusted to 5.8. For regeneration and growth of inoculated twin scales were incubated at 24±1°C under 85% relative humidity in dark condition. Subculture was carried out regularly at an interval of 4 -5 weeks. The well-developed bulbs were collected from conical flask after 9 weeks. Analysis of variance was done individually by a statistical package [11] and test of significance was done by F-test [10, 16]. Differences among the means were computed for significance following Least Significance Difference Test (LSD) at 5% level.

RESULTS

Individual effects of sucrose, BAP (Benzyl Amino Purine) and CCC (Chioro 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 90g/L of sucrose and then it was decreased. The earliest (36.95 days) bulblets induction was observed at 90g/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 bulbets 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 appeared 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

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

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

Treatments* (mg/L)	Percent of regeneration	Bulblets induction (days)	Bulb per twin scales (no)	Avg. weight of bulb (g)	Grading (%)		
					<2.5 g	2.5-3.5 g	>3.5 g
C_0 x B_0	82.75j	37.44a	1.443k	1.86m	88.57a	11.43j	0.00f
C_0 x B_1	85.98i	35.78b	1.820ij	2.26jk	61.23e	38.77de	0.00f
C_0 x B_2	93.69h	34.80c	2.127fg	2.57gh	52.71g	45.63bc	0.00f
C_0 x B_3	96.83cde	34.95c	2.380de	2.96cd	47.02i	52.98a	0.00f
C_0 x B_4	93.27h	36.36b	1.763j	2.12kl	54.60f	45.40bc	0.00f
C_1 x B_0	93.89h	36.36b	1.787j	2.07l	86.89b	13.11ij	0.00f
C_1 x B_1	95.33fg	34.46cd	1.907hij	2.76f	51.22h	48.78ab	0.00f
C_1 x B_2	97.34bc	33.93de	2.320e	2.72fg	40.89k	33.04fg	26.08d
C_1 x B_3	97.37bc	32.17gh	2.630bc	3.09c	32.70m	37.97def	29.33ab
C_1 x B_4	95.18g	34.79c	1.807j	2.28j	47.74i	52.26a	0.00f
C_2 x B_0	94.90g	35.87b	2.013gh	2.32ij	83.45c	16.55ij	0.00f
C_2 x B_1	95.81fg	33.26ef	1.983ghi	2.83def	46.82i	39.15de	14.03e
C_2 x B_2	97.53bc	31.49hi	2.397de	2.77ef	39.22l	33.59fg	27.19c
C_2 x B_3	98.16b	30.94i	2.800b	3.44b	29.53n	40.57de	29.90ab
C_2 x B_4	96.24def	32.61fg	1.800j	2.27jk	42.36j	43.01cd	14.63e
C_3 x B_0	96.19ef	33.82de	2.280ef	2.45hi	81.31d	18.69i	0.00f
C_3 x B_1	95.60fg	32.60fg	2.127fg	2.92de	41.62jk	39.56de	18.82e
C_3 x B_2	98.18b	31.26i	2.507cd	2.92de	41.18k	29.65gh	29.17b
C_3 x B_3	99.33a	29.54j	3.330a	4.34a	20.33o	48.70ab	30.97a
C_3 x B_4	97.14cd	32.73fg	1.757j	2.69fg	37.89l	37.48def	18.63e

Means bearing uncommon letter(s) in a column varied significantly at 5 % level.
*C_0 = Control (without hormone), C_1 = 125 mg/L CCC, C_2 = 250 mg/L CCC , C_3 = 500 mg/L CCC
B_0 = Control (without hormone), B_1 = 2.0 mg/L BAP, B_2 = 4.0 mg/L BAP, B_3 = 6.0 mg/L BAP, B_4 = 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. Cytokinin has 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 the 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 bulblets 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.

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