EX VITRO ESTABLISHMENT OF *IN VITRO* PRODUCED PLANTLETS AND BULBLETS OF HIPPEASTRUM (*HIPPEASTRUM HYBRIDUM*)

M.N. A. SIDDIQUE[1], J. SULTANA[2], N. SULTANA[3] AND M. M. HOSSAIN[4]

[1]Scientific Officer, Soil Resource Development Institute, District Office, Dinajpur, [2]MS, [3]Associate Professor, [4]Professor, Department of Horticulture, Bangabandhu sheikh Mujibur Rahman Agricultural University (BSMRAU), Gazipur – 1706, Bangladesh

Accepted for publication: 05 May, 2007

ABSTRACT

Siddique, M.N.A., Sultana, J., Sultana, N. and Hossain, M.M. 2007. *Ex Vitro Establishment of In Vitro Produced Plantlets and Bulblets of Hippeastrum (Hippeastrum hybridum)*. Int. J. Sustain. Crop Prod. 2(3): 22-24

This study examined the *ex vitro* establishment performance of *in vitro* produced plantlets and bulblets. For rooting of plantlets different concentration of NAA (0.0, 0.1, 0.2, 0.3 and 0.4 mg/l) were used singly. A well developed root system was achieved in the MS media supplemented by 0.2 mg/l NAA. No separate *in vitro* rooting was required for bulblets. Different substrates constitute of Soil, Soil and Sand (1:1), Soil & Compost (1:1) and Soil, Sand & Compost (1:1:1), to observe the *ex vitro* establishment performance. Plantlets and bulblets shown maximum survival, minimum time for leaf emergence, maximum number of leaf and desired plant height against the substrate contained equal part of Soil, Sand and Compost (1:1:1).

Keywords: Plantlets, bulblets, NAA, substrate

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 and commonly known as Royal Dutch Amaryllis (Jana, 1995). They are native to Central and South America, and are easily grown in the tropical and subtropical regions (Okubo, 1993). 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 (Vijverberg, 1981). Conventional propagation of Hippeastrum hybrids by bulb offsets is slow, seasonal and variable with some hybrids not producing offsets (Smith *et al.*, 1999). In fact, normally a plant produces 2-3 bulblets in a year of growth (Dohare, 1989). Multiplication of plant from seed will show wide variation in flower colour, plant shape, time of flowering etc. Since the natural multiplication rate of Hippeastrum is slow, a standard protocol for *in vitro* plantlets (Siddique *et al.*, 2006) and bulblet (Sultana *et al.*, 2006) production of Hippeastrum is already developed but no well developed method was found for their *ex vitro* establishment. Considering this fact the experiment was undertaken with the following objective: to develop a protocol for *ex vitro* establishment of *in vitro* produced plantlet and bulblet of Hippeastrum.

MATERIALS AND METHODS

In vitro produced plantlets and bulblets were used as experimental materials. The *in vitro* produced plantlets were cultured on MS medium supplemented with different level NAA (0.1, 0.5, 1.0 and 2.0 mg/l). The p^H of the medium was adjusted to 5.8. For rooting of plantlets they were incubated at 24±1°C under 85% relative humidity in a light (16 hrs) and dark (8 hrs) cycle. The well rooted *in vitro* grown plantlets were transferred for hardening to different substrates, Soil, Soil and sand (1:1), Soil and compost (1:1) and Soil, sand and compost (1:1:1), to observe the *ex vitro* performance. The *in vitro* produced bulblets were also transferred to the same substrates, then the rooting and growth performance was observed. The data were analyzed by analysis of variance using MSTAT-C statistical package. Differences among the means were computed for significance following Least Significance Difference Test (LSD) at 5% level.

RESULTS AND DISCUSSION

Plantlet Establishment

***Effect of NAA on rooting*:** NAA at 0.2 mg/l come out with the best (99.61%) response to rooting. Higher concentrations of NAA did not showed good result in case of response to rooting. The treatment NAA at 0.2 mg/l took minimum days (6.21) for root induction. Higher concentration required considerably more time for rooting although maximum days (15.55) took at the control treatment. In case of length of root highest (8.75 cm) root length was measured with treatment 0.2 mg/l NAA. In respect of number of roots per plantlets, NAA at 0.2 mg/l yielded the maximum number with the longest roots (9.33 and 8.75 cm) that was followed by the treatment NAA at 0.1 mg/l (7.14 and 6.45 cm). Both in case of length and number of root in each plantlet up to a certain level, i.e., 0.2 mg/l NAA, positive effect was noticed then increasing the concentration affects the values negatively (Table 1).

Table 1. Effect of different concentrations of NAA on rooting of Hippeastrum plantlets

NAA concentrations (mg/l)	Percent of response	Days to root induction	After 7 weeks	
			Length of root	Number of roots/plantlets
Control	21.16d	15.55a	2.75e	2.36e
0.1	97.81a	7.53d	6.45b	7.14b
0.2	99.61a	6.21e	8.75a	9.33a
0.3	73.79b	10.74c	5.58c	5.51c
0.4	44.36c	13.88b	3.98 d	3.77d

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

***Effect of substrates on establishment*:** Among different substrates, the best survival percent (98.75%) was found with substrate contained equal part of soil, sand and compost. Minimum time (21.87 days) required for fourth leaf emergence in case of soil, sand and compost (1: 1: 1) substrate. The percent of fifth leaf emergence varied widely (12.10-100) for different substrates. However hundred percent plantlets emerge fifth leaf in response to soil, sand and compost (1: 1: 1) substrate. Number of leaf recorded maximum (5.45) in the mixture of soil, sand and compost (1: 1: 1) substrate and the maximum plant height (22.45 cm) measured at the soil, sand and compost (1: 1: 1) substrate (Table 2). The best performance of soil, sand and compost (1 : 1 : 1) substrate for *ex vitro* establishment of plantlets might be due to optimum water holding capacity, good aeration system and nutrient status of the substrate. Similar result documented by Khanam (2000) in case of Gladiolus. Furthermore the findings regarding *ex vitro* establishment of plantlets agree with the protocol developed by Saker (1998) for *in vitro* propagation of *Hemerocallis aurantiaca*, *Hippeastrum vittatum*, *Crinum asiaticum* and *Polianthes tuberosa* and reported that micro propagated plantlets were successfully transferred to soil with a survival rate exceeding 95%.

Table 2. Effect of different substrates on *ex vitro* establishment Hippeastrum plantlets

Type of substrates*	Percent of survival	Days to fourth leaf emergence	Percent of fifth leaf emergence	After 5 weeks	
				Number of leaf/plantlets	Plant height(cm)
S_1	53.33d	43.67a	12.10d	3.10c	12.65d
S_2	68.48c	35.34b	42.15c	3.75c	14.15c
S_3	83.11b	27.33c	74.75b	4.14b	17.33b
S_4	98.75a	21.87d	100a	5.45a	22.45a

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

*S_1 = Soil S_3 = Soil and compost (1:1)
S_2 = Soil and sand (1:1) S_4 = Soil, sand and compost (1:1:1)

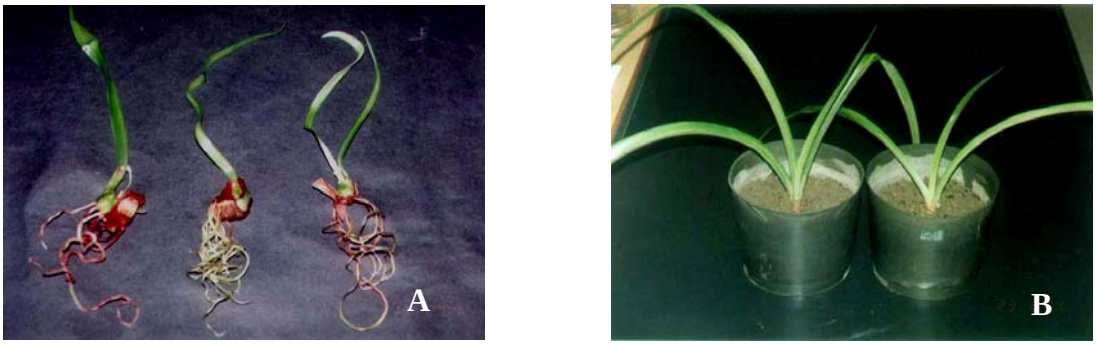

Figure 1. *In vitro* rooting (A) on MS medium supplemented by 0.2 mg/l NAA and established plant (B) of Hippeastrum in the pots.

Bulblet Establishment

Effect of substrates on establishment: The best survival percent (95.25%) was found with substrate contained equal part of soil, sand and compost (1: 1: 1). Minimum time (7.33 days) required for first leaf emergence in case of soil, sand and compost (1: 1: 1) substrate. The percent of third leaf emergence varied widely (55.33– 99.33) for

different substrates. However 99.33% bulbs emerge third leaf in response to soil, sand and compost (1: 1: 1) substrate followed by 78.66% third leaf in the soil and compost (1: 1) substrate. Maximum Number of leaf (4.87) and plant height (24.25 cm) measured at the soil, sand and compost (1: 1: 1) substrate. The best performance of soil, sand and compost (1 : 1 : 1) substrate for *ex vitro* establishment might be due to optimum water holding capacity, good aeration system and nutrient status of the substrate. Similar result documented by Khanam (2000) in case of Gladiolus.

Table 3. Effects of different substrates on *ex vitro* establishment Hippeastrum bulblets

Type of substrates*	Percent of survival	Days to first leaf emergence	Percent of third leaf induction	After 5 weeks	
				Number of leaf/plantlets	Plant height (cm)
S_1	47.33d	14.81a	55.38d	2.25c	12.52d
S_2	62.71c	12.46b	67.29c	2.61c	14.61c
S_3	80.66b	9.75c	78.66b	3.66b	19.33b
S_4	95.25a	7.33d	99.33a	4.87a	24.25a

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

REFERENCES

Dohare, S. R. 1989. Amaryllis and Hippeastrum. *In*: Commercial Flowers. T. K. Bose, R.G. Maiti, R. S. Dhva, (eds.). Naya prokash, Calcutta. pp. 573-593.

Jana, B. K. 1995. Cultural requirements of Hippeastrum. *In*: Advances in Horticulture Vol.12-Ornamental plants (eds.) Chadha, K. L. and S. K. Bhattacharjee. Malhotra publishing House, New Delhi, India.

Khanam, D. 2000. *In vitro* cormel production of Gladiolus (*Gladiolus grandiflorus*). MS in Horticulture thesis submitted to the Department of Horticulture, Bangabandhu Sheikh Mujibur Rahman Agricultural University. pp. 53-55.

Okubo, H. 1993. Hippeastrum (Amaryllis). In: The physiology of flower bulbs. A. DE Hertogh and M. LE Nard (Eds). Elsevier. pp. 321-324.

Siddique, M. N. A, N. Sultana, M. A. Haque, M. M. Hossain and J. U. Ahmed, 2006. Effects of Twin Scale Size and Hormones on *In Vitro* Propagation of Hippeastrum (*Hippeastrum hybridum*). Plant Tissue Cult. & Biotech. 16(2): 105-110.

Sultana J, N. Sultana, M. M. Hossain and M. T. Hossain. 2006. Investigation on phenology, *in vitro* bulb production and vase life of Hippeastrum (*Hippeastrum hybridum*). MS thesis. Bangabandhu Sheikh Mujibur Rahman Agricultural University. Gazipur-1706, Bangladesh.

Saker, M., M. Rady, M. E Bahr. 1998. Towards commercial production of ornamental bulbs *in vitro*. Egyptian J. Hort. 25(1): 113-128.

Smith R. H., J. Burrows and K. Kurten, 1999. Challenges associated with micropropagation of *Zephyranthes* and *Hippeastrum* sp. (Amaryllidaceae0. *In vitro* cellular and develop. Biol. Plant. 35(40: 281-282.

Vijverberg, A. J. 1981. Growing Amaryllis. Grower Book, London. P 57.