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In Vitro Morphogenesis from Pedicels of *Hippeastrum* x *hybridum*

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In vitro morphogenensis from pedicels of *Hippeastrum* x *hybridum* cv. Hermitage was investigated. Bulblet formation rates and number of bulblets were excellent when the concentrations of naphthyl acetic acid (NAA) and N^6-benzyladenine (BA) were the same. The combination of 5 mgl^{-1} 2,4-dichlorophenoxyacetic acid (2,4-D) with 0.02–2 mgl^{-1} thidiazuron (TDZ) induced somatic embryogenesis with high rate and the combination of 5 mgl^{-1} 2,4-D and 2 mgl^{-1} TDZ gave the greatest number of somatic embryos among the treatments. High concentrations of TDZ (0.2–2 mgl^{-1}) promoted bulblet formation, but reduced root formation. Number of shoot primordia was high with higher 2,4-D concentrations irrespective of TDZ. The highest number of bulblets (6.4) was obtained with 1 mgl^{-1} 2,4-D+0.2 mgl^{-1} TDZ.

INTRODUCTION

We previously reported *in vitro* morphogenesis from ovaries of *Hippeastrum* x *hybridum* (Huang *et al.*, 2005). Peduncle (Seabrook and Cumming, 1977) or flower stem (Hussey 1975, 1976; Seabrook and Cumming 1977) of *Hippeastrum* x *hybridum* was also used for *in vitro* multiplication. Flower stem, pedicel and peduncle were the explant source in many other ornamental bulbous species such as *Amaryllis belladonna* (De Bruyn *et al.*, 1992), *Fritillaria* (Hussey, 1976), *Hyacynthus* (Hussey, 1975; Kim *et al.*, 1981), *Lilium longiflorum* (Liu and Burger, 1986), *Lilium rubellum* (Niimi and Watanabe, 1982; Niimi, 1984), *Muscari* (Hussey, 1975), *Narcissus* (Hussey, 1976, 1977, 1982; Seabrook *et al.*, 1976; Seabrook and Cumming, 1982; Hosoki and Asahira, 1980), *Scilla* (Hussey, 1975) and *Tulipa* (Hussay, 1975; Wright and Anderson, 1980; Alderson *et al.*, 1983, 1986; Rice *et al.*, 1983; Taeb and Alderson, 1987, 1990a, b; Le Nard, 1989; Baker *et al.*, 1990; Alderson and Taeb, 1990a, b).

Plantlets obtained through somatic embryogenesis have been reported in some bulbous plants such as *Scilla* (Chakravarty and Sen, 1987), *Tulipa* (Gude and Dijkema 1997) and *Narcissus* (Sage *et al.*, 2000). We also previously reported the somatic embryo-derived plantlets from ovaries of *Hippeastrum* x *hybridum* (Huang *et al.*, 2005).

In this study, *in vitro* morphogenensis from pedicels of *Hippeastrum* x *hybridum* was investigated.

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MATERIALS AND METHODS

Preparation of plant materials, culture medium and experiments were identical to those reported previously (Huang *et al.*, 2005) except for the explant source. The explant was prepared from horizontally sliced pedicels of *Hippeastrum* x *hybridum* cv. Hermitage with 0.8–1.0 mm thickness.

RESULTS AND DISCUSSION

Effects of NAA and BA

High percentages of protuberances formation were obtained with all the treatments except for the treatments with 0 mgl^{-1} naphthyl acetic acid (NAA) + 0 mgl^{-1} or 5 mgl^{-1} N^6–benzyladenine (BA) (Table 1). Yield of protuberances was high (>8) with 2 mgl^{-1} NAA + 1 mgl^{-1} BA, 1 mgl^{-1} NAA + 0.1 mgl^{-1} BA and 2 mgl^{-1} NAA + 0.1 mgl^{-1} BA (Table 2). Bulblet formation rates and number of bulblets were excellent with the same concentrations of NAA and BA, and with 1 mgl^{-1} NAA + 0.1 mgl^{-1} BA and 5 mgl^{-1} NAA + 2 mgl^{-1} BA. The combinations of the concentrations of NAA and BA that brought the higher yield of protuberances did not always coincide with those gave the higher yield of bulblets. Neither bulblets nor roots were obtained without NAA. Root formation and number of

Table 1. Effects of NAA and BA on morphogenesis from pedicels of *Hippeastrum* x *hybridum* cv. Hermitage.

Treatment (mgl^{-1})		Protuberance	Bulblet	Root formation	Browning
NAA	BA	formation (%)	formation (%)	(%)	(%)
0	0	27	0	0	73
0	0.1	93	0	0	7
0	1	80	0	0	20
0	2	80	0	0	20
0	5	0	0	0	100
0.1	0	93	33	0	0
0.1	0.1	93	100	0	7
0.1	1	93	80	27	7
0.1	2	87	67	13	0
0.1	5	93	67	87	0
1	0	100	80	73	0
1	0.1	93	100	53	0
1	1	100	93	60	0
1	2	100	73	100	0
1	5	87	67	0	7
2	0	73	20	93	7
2	0.1	87	87	80	0
2	1	93	73	93	0
2	2	73	100	80	0
2	5	80	33	67	0
5	0	60	13	100	0
5	0.1	73	60	83	0
5	1	93	67	67	0
5	2	80	100	60	0
5	5	100	100	80	0

Table 2. Effects of NAA and BA on morphogenesis from pedicels of *Hippeastrum* x *hybridum* cv. Hermitage.

Treatment (mgl^{-1})		No. of protuberances / explant	No. of bulblets / explant	No. of leaves / explant	Length of leaves (mm)	No. of roots / explant
NAA	BA					
0	0	4.0 kl*	0	0	0	0
0	0.1	6.6 fgh	0	0	0	0
0	1	6.8 efgh	0	0	0	0
0	2	7.3 def	0	0	0	0
0	5	0	0	0	0	0
0.1	0	7.5cde	0.7 g	0	0	0
0.1	0.1	7.5 cde	5.2 a	3.8 a	2.1 bc	0
0.1	1	7.9 abcd	2.3 d	2.7 b	2.5 a	0.5 j
0.1	2	6.9 efgh	1.4 f	0.7 ef	0.2 efg	0.2 j
0.1	5	6.7 fgh	1.3 f	1.6 d	0.3 efg	6.3 f
1	0	6.7 fgh	2.6 d	1.5 d	2.5 a	6.3 f
1	0.1	8.3 ab	4.2 b	3.2 b	0.9 d	1.3 i
1	1	7.7 bcd	3.7 c	2.9 b	0.8 d	1.6 i
1	2	7.2 defg	1.5 ef	1.6 d	0.4 ef	8.6 d
1	5	6.9 efgh	1.3 f	0.9 e	0.3 efg	0
2	0	4.4 kl	0.2 h	0.2 gh	0.2 fg	13.7 b
2	0.1	8.2 abc	2.5 d	0.3 fgh	0.5 e	7.5 e
2	1	8.6 a	1.9 e	0.3 gh	0.2 efg	7.4 e
2	2	5.2 j	4.1 bc	2.1 c	2.2 b	3.8 g
2	5	5.5 ij	0.5 gh	0.4 fgh	0.1 g	2.6 h
5	0	4.0 l	0.1 h	0.1 h	0.1 g	10.3 c
5	0.1	4.7 jkl	1.4 f	0.7 ef	0.1 g	14.6 a
5	1	6.2 hi	1.8 e	0.7 efg	0.2 fg	13.0 b
5	2	4.8 jk	4.1 bc	0.1 h	0.4 ef	1.6 i
5	5	6.5 gh	5.6 a	2.2 c	2.0 c	4.2 g

* Values followed by different letters are significantly different at 5% level.

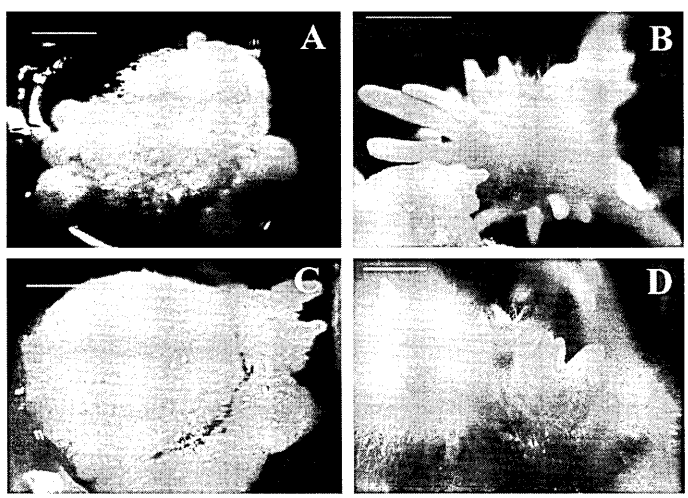

Fig. 1. Protuberance (A), shoot (B), root primordia (C) and root formation from pedicels of *Hippeastrum* x *hybridum* cv. Hermitage. Bars=3 mm for A and B, 2 mm for C and D.

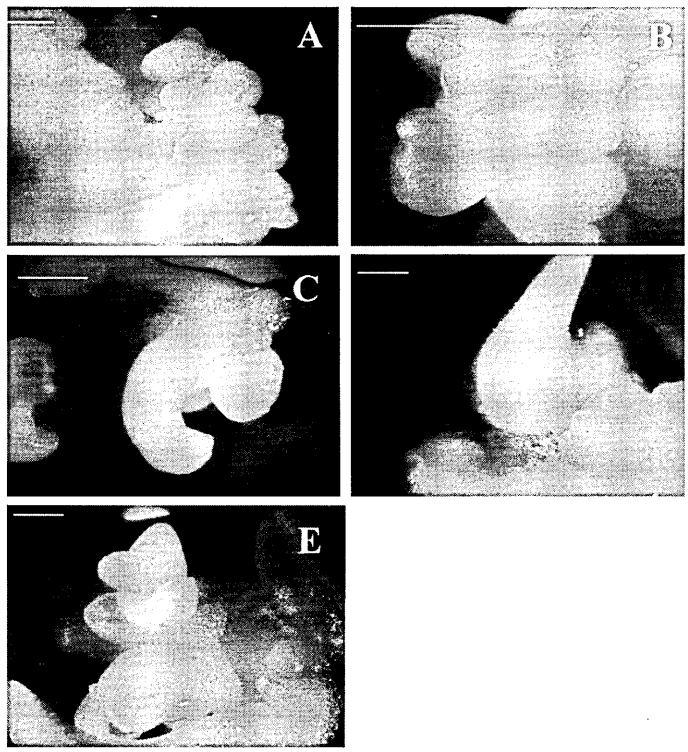

Fig. 2. Process of organ formation from somatic embryos. Organogenesis from somatic embryogenesis (A) to bulblet formation (E) through globular embryos (B), cotyledonous embryos (C) and shoot formation (D). Bars = 1 mm.

roots were greater with higher NAA (2 and 5 mgl^{-1}) and lower BA (0–1 mgl^{-1}) concentrations. Figure 1 shows protuberance (A), shoot (B), root primordia (C) and root formation from the pedicels.

Pedicels seems a little better than ovary for obtaining bulblets in vitro, although not so much difference in morphogenesis with NAA and BA between the explant sources, ovary (Huang *et al.*, 2005) and pedicel (this study), was found. In agreement with our current results, Seabrook and Cumming (1977) described that peduncle or scape tissues of *Hippeastrum* was more productive for shoot induction than other tissues including ovary.

Effects of 2,4–D and TDZ

Somatic embryogensis was confirmed by histological observations as reported previously (Huang *et al.*, 2005) (figures not shown). It was little or not observed in the treatments with 0 mgl^{-1} 2,4–dichlorophenoxyacetic acid (2,4–D) + 0.02 or 0.2 mgl^{-1} thidiazuron (TDZ) and with 2 mgl^{-1} 2,4–D + 0 or 0.02 mgl^{-1} TDZ, whereas shoot primordium formation occurred in all the treatments (Table 3). Among the treatments, the combina-

Table 3. Effects of 2,4–D and TDZ on morphogenesis from pedicels of *Hippeastrum* x *hybridum* cv. Hermitage.

Treatment (mgl^{-1})		Shoot primordium formation (%)	Somatic embryogenesis (%)	Bulblet formation (%)	Root formation (%)	Browning (%)
2,4–D	TDZ					
0	0	80	33	60	0	20
0	0.002	93	20	13	0	7
0	0.02	80	0	0	0	7
0	0.2	73	0	27	0	0
0	2	100	40	20	0	0
0.1	0	83	40	73	33	7
0.1	0.002	100	60	27	40	0
0.1	0.02	93	67	20	7	0
0.1	0.2	100	53	73	0	0
0.1	2	100	80	100	0	0
1	0	100	60	27	93	0
1	0.002	100	93	93	87	0
1	0.02	100	100	87	67	0
1	0.2	100	93	100	13	0
1	2	100	33	40	13	0
2	0	93	0	27	87	0
2	0.002	100	27	0	100	0
2	0.02	87	7	0	80	13
2	0.2	100	67	73	20	0
2	2	100	80	100	0	0
5	0	100	67	27	87	0
5	0.002	100	73	20	67	0
5	0.02	93	93	73	53	0
5	0.2	100	87	93	27	0
5	2	100	100	80	7	0

tion of 5 mgl^{-1} 2,4–D + 0.02–2 mgl^{-1} TDZ induced somatic embryogenesis with high rate and that of 5 mgl^{-1} 2,4–D + 2 mgl^{-1} TDZ gave the greatest number of somatic embryos (10.5) (Tables 3 and 4). High concentrations of TDZ (0.2–2 mgl^{-1}) promoted bulblet formation, but reduced root formation. Number of shoot primordia was high with higher 2,4–D concentrations irrespective of the existence of TDZ. The highest number of bulblets (6.4) was obtained with 1 mgl^{-1} 2,4–D + 0.2 mgl^{-1} TDZ. The process of bulblet formation (Fig. 2E) from somatic embryogenesis (Fig. 2A) through globular embryos (Fig. 2B), cotyledonous embryos (Fig. 2C) and shoot formation (Fig. 2D) is shown.

In vitro vegetative propagation through somatic embryos from pedicels is also possible in *Hippeastrum* as well as from ovaries as previously described (Huang *et al.*, 2005). Advantage of bulblet or plantlet yield through somatic embryogenesis over the yield through direct adventitious shoot formation, however, was not clearly proved with pedicels in this study and ovaries in the previous study (Huang *et al.*, 2005). More improvement of the methods such as liquid culture is necessary.

Ability of somatic embryogenesis and bulblet formation is considered to be a little higher from pedicel (this study) than from ovary (Huang *et al.*, 2005).

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Table 4. Effects of 2,4–D and TDZ on morphogenesis from pedicels of *Hippeastrum* x *hybridum* cv. Hermitage.

Treatment (mgl^{-1})		No. of shoot primordia / explant	No. of somatic embryos / explant	No. of bulblets / explant	No. of leaves / explant	No. of roots / explant
2,4–D	TDZ					
0	0	5.1 defgh*	0.7 fgh	2.0 defg	1.8 cd	0
0	0.002	4.0 ghi	0.3 h	0.3 fg	0.2 ef	0
0	0.02	1.9 i	0	0	0	0
0	0.2	2.7 hi	0	0.9 defg	1.0 cdef	0
0	2	5.2 defgh	0.7 fgh	0.8 efg	0.5 def	0
0.1	0	4.2 fghi	0.6 fgh	1.6 defg	1.0 cdef	0.5 f
0.1	0.002	4.5 fghi	0.7 fgh	0.5 efg	0.4 ef	0.9 ef
0.1	0.02	4.3 fghi	1.0 fgh	0.4 efg	0.4 ef	0.1 f
0.1	0.2	4.3 fghi	0.9 fgh	1.3 defg	0.8 cdef	0
0.1	2	5.7 defg	2.8 ef	4.9 ab	0.5 def	0
1	0	5.7 cdefg	1.0 fgh	0.8 efg	0	5.5 ab
1	0.002	4.8 efgh	2.6 ef	2.8 cd	2.0 bc	5.0 abc
1	0.02	4.2 fghi	7.0 bc	2.3 de	1.4 cdef	3.8 abcd
1	0.2	10.1 ab	4.6 de	6.4 a	3.3 ab	0.1 f
1	2	3.9 ghi	0.6 fgh	0.8 efg	0.6 def	0.2 f
2	0	5.5 defg	0	0.1 g	0	5.3 ab
2	0.002	3.6 ghi	0.6 fgh	0	0	5.8 a
2	0.02	5.2 defgh	0.4 gh	0	0	3.7 abcd
2	0.2	6.9 cde	1.6 fgh	1.8 defg	1.3 cdef	0.4 f
2	2	11.1 a	2.1 fgh	5.6 ab	4.1 a	0
5	0	11.1 a	1.0 fgh	0.4 efg	0.3 ef	3.5 bc
5	0.002	5.3 defg	1.1 fgh	0.3 fg	0.1 f	2.1 def
5	0.02	6.7 cdef	7.9 b	1.3 defg	0.6 def	2.8 cde
5	0.2	8.1 bc	5.6 cd	4.3 bc	3.1 ab	0.1 f
5	2	7.5 cd	10.5 a	2.1 def	1.5 cde	0.4 f

* Values followed by different letters are significantly different at 5% level.

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