RAPID VEGETATIVE PROPAGATION OF EUCHARIS GRANDIFLORA IN VITRO

R.L.M. Pierik, P.A. Sprenkels and J. van Bragt
Department of Horticulture, Publication 504
Agricultural University
P.O.Box 30, 6700 AA Wageningen
The Netherlands

Abstract

To increase the propagation rate and to produce homogeneous plant material for flower production, a simple in vitro system was developed to clone *Eucharis grandiflora*. Excised bulb scale segments produced adventitious bulblets on the basal sides. Factors affecting formation and growth of these bulblets are described.

Nine weeks after excision of the scales the then formed bulblets were subcultured in vitro to study rooting, bulb growth and leaf formation in the following 9 weeks. The complete plants were then transferred to soil to study further growth.

First flowering of the in vitro produced plants was observed 16 months after the start of the in vitro culture. The in vitro progeny was homogeneous and the plants, including the flowers, were not visibly different from the original genotype.

1. Introduction

Eucharis grandiflora (*Amaryllidaceae*) is normally propagated vegetatively by means of daughter (offset) bulbs which arise in the axils of bulb scales. Van Bragt and Sprenkels (1983) showed that the production of daughter bulbs is rather small: after one year of growth a bulb with an original diameter of 20 mm has produced an average of 1.5 side bulbs, whereas a bulb of 40 mm has produced 3.0 side bulbs.

To increase the propagation rate and also to produce homogeneous and relatively cheap material for studies on juvenility and flowering, a system was developed to clone *Eucharis grandiflora* in vitro. The basis of this work was an earlier in vitro study with excised bulb scales of *Nerine*, a genus also belonging to the *Amaryllidaceae* (Pierik and Ippel, 1977). The *Nerine* system (isolation of double scales) was modified and so a simple in vitro propagation method became available, which is described in this paper.

2. Material and methods

Scale explants of *Eucharis grandiflora* Planch. et Lind. were obtained from air-dried bulbs 35 mm in diameter, which had been stored at 22°C for 4 weeks. To reduce the number of infections we used undamaged and healthy bulbs, which were sterilized individually.

Preparation of plant material was as follows. The lower part of the basal plate including the roots, the outer dry and dirty scales and the upper part of the bulb were removed. Care was taken that not too much tissue from the basal plate was removed, since this tissue is of great importance for bulb formation in scale explants of *Amaryllidaceae* (Pierik and Ippel, 1977; Grootaarts et al., 1981).

Subsequently bulbs were longitudinally cut into quarters and then sterilized as follows: immersion in 96% ethanol for a few seconds, rinsing in 10% commercial bleaching liquid (with 10% NaOCl) to which a few drops of Tween had been added (25 minutes) and finally rinsing 3 times in sterilized tap water for 3, 10 and 15 minutes. The scales were then removed from the bulb quarters to be cut into explants.

For each experiment we used at least 3 bulbs. Their scales were randomly divided over the treatments. The explants were rectangular, approximately 2.5 cm long and 0.75 cm wide, and cut out of the basal parts of the scales; they also had a 3 mm thick strip of the basal plate. Explants were wounded at all sides. In most experiments 24 scale explants were placed inverted into the media to a depth of about half of their lengths.

The basic culture medium contained: MS (Murashige and Skoog, 1962) macroelements at half strength, MS microelements (except Fe) at full strength, NaFeEDTA 25 mg/l, saccharose 3%, méso-inositol 100 mg/l, vitamin B_1 0.1 mg/l, Difco Bacto agar 0.7% and pyrex-distilled water. The rooting medium also contained 4.10^{-7} g/ml IBA. The pH was adjusted to 6:0 before autoclaving. Culture tubes were closed with aluminium caps and then covered by Vitafilm. Each tube contained 20 ml of the culture medium.

Test tubes were placed in a culture room at 25°C in continuous fluorescent light (Philips TL 40W/57, 10 W m^{-2}). The effect of temperature was examined in a phytotron, where the light intensity was 20 W m^{-2}.

In vitro propagation can be divided into 3 steps: (1) bulb scale segments were isolated to produce bulblets; (2) bulblets were subcultured to produce roots and the first leaf; (3) complete plantlets were transferred to soil.

Nine weeks after the isolation of the explants, the numbers of regenerated bulblets were counted and the total bulb fresh weight in each treatment determined. The mean number of bulblets per scale explant and their mean fresh weight were calculated over all explants (regenerating or non-regenerating bulblets). Infected cultures were not included. Data on root formation have been ommitted as rooting of bulb scales was erratic.

Rooting experiments with bulblets under the in vitro conditions as described for bulb scales, were discontinued 9 weeks after the isolation of the bulblets from the scale explant culture. In vitro produced plants with roots and one leaf were subsequently transferred to soil. Growth conditions in the glasshouse have been described by van Bragt and Sprenkels (1983).

Details about the treatments are mentioned under results. The principle of our experiments was to vary one environmental or plant factor and keeping all the other factors constant. The values in the table represent the averages of at least 2 experiments.

3. Results

3.1. Introductory remarks

Preliminary experiments were done to eliminate limiting factors for bulb formation, growth and rooting; on the basis of these preliminary results the experimental set-up was chosen as described in material and methods. We found e.g. that twin (double) scaling, as applied for N e r i n e (Pierik and Ippel, 1977) decreased bulblet formation and

particularly bulb growth in E u c h a r i s. Therefore we used single scaling for E u c h a r i s.

3.2. General

Infection percentages at the isolation of scale explants were only about 10%. In contrast to many other A m a r y l l i d a c e a e, regeneration of bulblets in E u c h a r i s started very early, 2 weeks after the isolation. Also the number of bulblets per explant (3.3) in E u c h a r i s was high. Bulb growth started so early that after 9 weeks the experiments with scales were terminated. The bulblets thus obtained were then used for rooting and sprouting experiments.

During the 9 weeks of scale culturing root and leaf formation on the newly formed bulblets were rather poor, except when auxin was present in the culture medium.

3.3. Bulb regeneration on excised scales

Table 1 shows the influence of various plant factors. Bulblets only regenerated at the sites where the scales are implanted on the basal plate. Therefore the effect of the orientation of the explants was studied first. Regeneration was only slightly promoted when the scales were inserted in the medium with their basal ends up. Regeneration was increased when the length and the width of the explants were increased as is specified in table 1.

Data on the influence of various environmental factors on bulb regeneration are given in table 1, which shows that regeneration and bulb weight were not influenced by the concentration of the MS-salts, but were promoted by raising the temperature from 21 to 25°C or 29°C. No effects on regeneration and bulb weight were observed when the daily illumination was extended from 16 to 24 h.

Table 2 shows that the mean number of bulbs is increased by IBA with a maximum of 3.7 at 10^{-6} g/ml; it looks as if IBA also increased the total bulb weight per explant and mean bulb weight. The most remarkable effect of IBA is the promotion of leaf and root growth. In most cases, however, these roots developed from the basal ends of the scales and not from the bases of the newly formed bulbs (fig. 1).

3.4. Rooting and leaf formation on excised bulbs

IBA increased the number of roots per bulblet (fig. 2). Table 3 shows that leaf formation was slightly increased by IBA, with a saturation level at 2.10^{-7}. Mean bulb weight slightly increased, whereas total plant weight strongly increased by raising the IBA conc. We concluded that IBA at 4.10^{-7} g /ml gave the most suitable plantlets for the transfer to soil.

Table 3 shows that rooting, leaf formation and total fresh weight of leaves and roots were increased by increasing the temperature from 17 to 25°C.

3.5. Transfer to soil; growth and flowering

After their transfer to soil, the plants grew on without any stagnation. To evaluate the results of our work on 4 December 1981, we isolated single scales; on 17 February 1982 the bulblets from these scales were then subcultured on the basic culture medium with IBA 4.10^{-7} g/ml for rooting and sprouting. On 4 April 1982 totally 123 bulblets with roots and leaves were transferred to soil. On 23 November 1982 bulb diameters were measured. This experiment with 123 plants yielded the

following result (first number of bulbs; second number between brackets the bulb diameter in mm): 3 (19), 7 (22), 24 (26), 28 (29), 41 (32), 20 (35). Bulbs with a diameter of 35 mm were used for the induction of flowering (van Bragt and Sprenkels, 1983). The plants flowered on 6 April 1983, that is 16 months from 4 December 1981, when the bulb scales have been isolated in vitro. The in vitro progeny was homogeneous and the plants, including the flowers, were not different from the original genotype.

4. Discussion and conclusions

This study demonstrates that with the aid of in vitro culture a very rapid propagation rate can be obtained as compared with the conventional system of propagation by daughter bulbs. Since the price of E u c h a r i s bulbs is rather high, it is worth while to consider whether in vitro propagation is not a more suitable method than in vivo propagation.

It has been shown that the in vitro propagation of E u c h a r i s roughly follows the rules developed for N e r i n e (Pierik and Ippel, 1977), a genus which also belongs to the A m a r y l l i d a c e a e. There are only three exceptions: E u c h a r i s requires a higher temperature (25°C) than N e r i n e, double scaling in vitro as applied for N e r i n e is not a suitable system for E u c h a r i s, whereas rooting and sprouting in excised E u c h a r i s bulbs was strongly promoted by IBA, which is not the case in N e r i n e. Although IBA has a promoting effect on sprouting of bulbs being formed on excised scales, auxin was not added to the media for scale explant culture, because the formation of leaves was of no advantage for the rooting phase.

References

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Table 1 - The influence of various plant and environmental factors on regeneration and weight of bulblets formed on bulb scale explants of E u c h a r i s g r a n d i f l o r a.

Factor	variation	bulb regeneration (%)	mean number of bulbs per explant	total bulb fresh weight (g) per explant	average fresh weight per bulblet (g)
Orientation of the explants	basal end down	80	2.5	0.48	0.19
	basal end up	94	3.0	0.51	0.17
Length of the explants (cm)	1.5	92	2.5	0.25	0.10
	2.5	93	3.5	0.53	0.17
Width of the explants (cm)	0.37	74	1.8	0.20	0.11
	0.75	92	3.3	0.56	0.17
Saccharose conc. (%)	2	89	3.5	0.56	0.16
	3	88	3.6	0.54	0.15
	4	78	3.2	0.51	0.16
	5	85	2.5	0.30	0.12
MS-macro salts	half strength	89	3.1	0.50	0.16
	full strength	88	3.2	0.42	0.13
Temperature (^{o}C)	21^{o}	86	2.6	0.39	0.15
	25^{o}	83	3.0	0.51	0.17
	29^{o}	86	2.8	0.48	0.17
Daylength (hours)	16	86	3.5	0.53	0.15
	24	93	3.6	0.50	0.14

Table 2 - The influence of the IBA concentration on the regeneration and the weight of bulblets formed on bulb scale explants of E u c h a r i s g r a n d i f l o r a.

IBA conc. (g/ml)	bulb regeneration (%)	mean number of bulbs per explant	total bulb fresh weight per explant (g)	average fresh weight per bulblet (g)	mean fresh weight (mg) leaves	roots
0	91	3.1	0.50	0.16	23	61
5.10^{-7}	96	3.4	0.58	0.17	41	59
10^{-6}	95	3.7	0.56	0.15	59	55
5.10^{-6}	95	3.5	0.66	0.19	75	100

Table 3 - The influence of IBA and temperature on rooting, sprouting, bulb weight and total plant weight of excised bulblets of E u c h a r i s g r a n d i f l o r a. Duration of the experiments 9 weeks. The IBA experiment was carried out at 25°C. In the temperature experiment the medium contained 4.10^{-7} g/ml IBA.

Factor	variation	rooting (%)	mean number of roots per bulb	mean root fresh weight per bulb (g)	sprouting (%)	mean number of leaves per bulb	mean leaf fresh weight per bulb (g)	mean bulb fresh weight (g)	mean total fresh weight per plant (g)
IBA	0	100	3.3	0.19	87	0.9	0.12	0.26	0.57
conc.	10^{-7}	100	4.2	0.23	96	1.0	0.17	0.25	0.65
(g/ml)	2.10^{-7}	100	4.7	0.29	100	1.0	0.20	0.27	0.76
	4.10^{-7}	100	5.8	0.36	100	1.1	0.22	0.29	0.87
	6.10^{-7}	100	6.4	0.37	100	1.1	0.21	0.31	0.89
Temp.	17	95	2.7	0.17	0	0.0	0.00	0.38	0.55
(°C)	21	100	3.8	0.33	30	0.3	0.02	0.36	0.71
	25	100	6.0	0.38	100	1.0	0.22	0.38	0.98

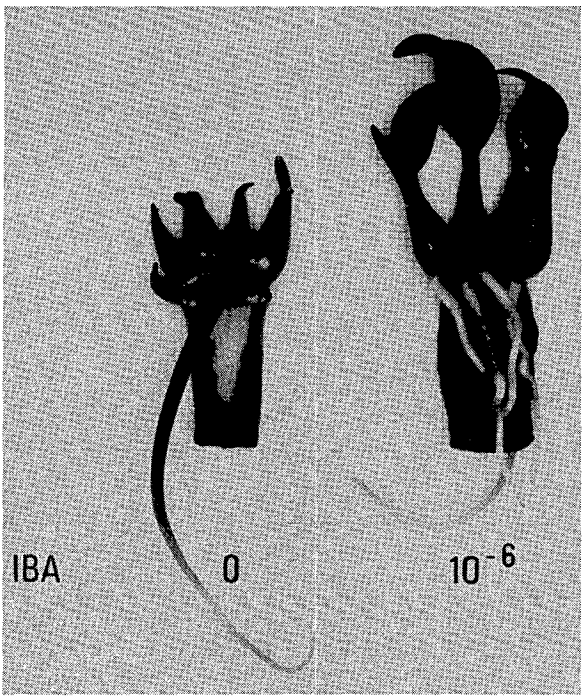

Fig. 1 - Regeneration of bulblets on excised bulb scale explants of E u c h a r i s g r a n d i f l o r a as affected by IBA.

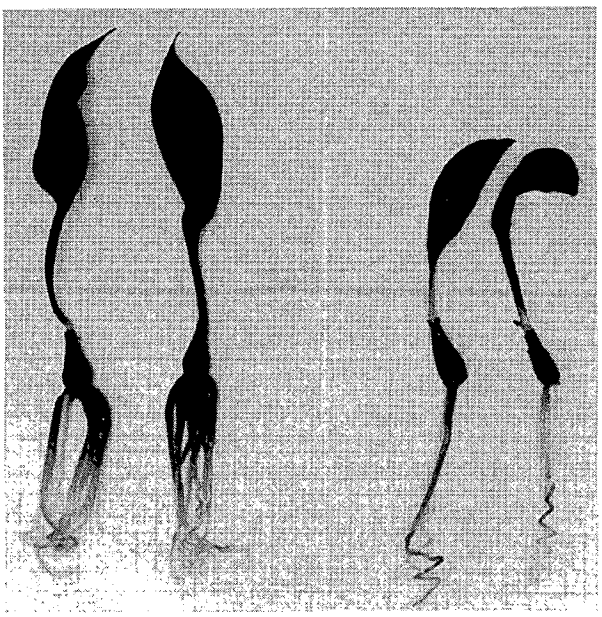

Fig. 2 - Rooting, bulb growth and leaf formation of excised bulblets of E u c h a r i s g r a n d i f l o r a. Left: on a medium with IBA 6.10^{-7} g/ml. Right: on a medium without auxin.