PLANTLET FORMATION FROM EXCISED BULB SCALE SEGMENTS OF NERINE

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Abstract

Excised bulb scale segments of *Nerine bowdenii* W. Watts. cv. Van Roon produced bulblets which formed roots and leaves after subculture on a fresh medium. Double scales connected by part of the basal plate were grown with basal ends up on a modified Murashige and Skoog medium with 1 mg/l IBA. Cultures were kept at 25°C in continuous fluorescent light.

Special attention was paid to factors affecting the formation and growth of bulblets. The most essential requirement for the formation of bulblets was the presence of part of the basal plate. Bulblet formation was increased by increasing the width and/or the length of the explants. The optimum temperature was 21 to 25°C. The formation and the growth of bulblets were promoted by 0.1 - 1 mg/l IBA.

Rooting, bulbing and leaf development of subcultured bulblets were much better when explant culture took place in darkness instead of in continuous light.

Plantlet formation starting with excised double scales could also be obtained from *Nerine sarniensis* and *Nerine* hybrids.

1. Introduction

Nerine, a genus belonging to the *Amaryllidaceae*, is becoming an important ornamental bulbous plant in the Netherlands. In recent years the cut flower production especially of *N. bowdenii* and to a lesser extent of *N. sarniensis* and other species strongly increased. All *Nerine* species are propagated vegetatively by natural offsets, and sometimes by scaling. Generative propagation is only applied for breeding purposes. Since the propagation rate through offsets is very low, scaling has been introduced which resulted in a much higher propagation rate. Many commercial *Nerine* species are diseased due to infection by several viruses, which decrease flower quality and yield. Because *Nerine* is propagated vegetatively, viruses are continuously transmitted to the newly formed bulbs. Hakkaart et al. (1975) made a successful attempt to eliminate *Nerine* latent virus from *N. bowdenii* bulbs by applying meristem culture.

In the present study *N. bowdenii* was chosen as an experimental plant because in the Netherlands about 90% of the cultivated *Nerines* belong to this species. The purpose of applying in vitro techniques has been to develop methods to increase the multiplication rate of especially virus-free plant material and of new selections. Most attention has been paid to factors affecting the formation of bulblets on excised double scales.

2. Material and methods

Most experiments were done with bulbs of *Nerine bowdenii* W. Watts. cv. Van Roon. After lifting from the field in November 1976, bulbs were stored at 2°C until use. To reduce the number of infections care was taken that only undamaged bulbs were used. The in vitro experiments were carried out from the beginning of February till the end of April 1977. On 7 February a preliminary experiment was done with other *Nerine* material as specified in table 3.

Scale explants from bulbs with a circumference of 12-14 cm were prepared as follows. First of all the roots, the lower brownish part of the basal plate, the outer dry and dirty scales and one third of the upper part of the bulb were removed to reduce the number of infections. Care was taken that not too much tissue from the basal plate was removed, since this tissue is of great importance for bulb formation. Subsequently bulbs were longitudinally cut into quarters. To reduce the spread of microorganisms the old brownish flower stems were removed and the bulbs were sterilized separately. Quarters were sterilized as follows: immersion in 70% ethanol for a few seconds, rinsing in 20% commercial bleaching liquor (containing 10% NaOCl) for 25 minutes and finally rinsing 3 times in sterilized tap water for 30 minutes. In each experiment material from at least 3 bulbs was used and divided at random over the various treatments. Only those scales were used from which standardized 'rectangular' explants, 1.5 cm long and 1.0 cm wide, could be cut. In the experiment with *N. sarniensis* and *Nerine* hybrids the explants were 2.5 cm long and 1.5 cm wide. Each explant consisted out of 2 scales (a so-called double scale) which were always connected by a 3 mm thick strip of the basal plate. Double scale explants (24 per treatment) were placed inverted in the media to a depth of about half their length. Each explant was wounded on 4 sides.

The basic culture medium contained: the MS (Murashige and Skoog, 1962) macroelements at 0.75 strength, MS-microelements at full strength, NaFeEDTA 25 mg/l, saccharose 3%, vitamin B_1 0.4 mg/l, meso-inositol 100 mg/l, the potassium salt of IBA (indolebutyric acid) 1 mg/l, Difco Bacto-agar 0.7% and pyrex distilled water. The pH was adjusted to 6.0 before autoclaving.

Explants were cultured in pyrex glass tubes (height 17 cm, diameter 2.2 cm), each containing 20 ml of culture medium. Cultures were kept at 25°C in continuous fluorescent light (Philips TL 40W/57, 14 W m^{-2}). The effect of temperature was examined in a phytotron with the same light source, but with a light intensity of 28 W m^{-2}.

Twelve weeks after isolation the number of bulblets per explant was counted and the total fresh weight of the bulblets of each treatment determined. The mean number of bulblets per explant and the mean fresh weight of bulbs per explant were calculated over all excised explants except the infected ones. The principle of the experiments reported in this paper was that in each experiment only one plant or one environmental factor was varied, while all the other factors were kept constant.

3. Results

3.1. General observations

When the sterilization procedure was followed carefully, an infection rate of about 10% was found to be normal. Two weeks after isolation a small percentage of explants already showed macroscopically visible bulblet formation, which percentage strongly increased from week 3 to 8. The strongest increase in bulblet weight per explant took place

during week 8 to 12. Without exception bulblet formation occurred on places where the scales were implanted on the basal plate. The size of the bulblets was highly variable. The formation of bulblets was also obtained when the newly formed bulblets were split longitudinally into halves and isolated on a fresh medium as described in the methods. The number of bulblets formed on these explants, however, was very small, possibly due to lack of sufficient scale and basal plate tissue (table 2).

The number of bulblets increased by increasing the number of scales per explant. Whether single scale instead of double scale isolation is the most efficient system of vegetative propagation was not yet examined in detail. Although homogeneous plant material (bulbs from the same clone and size) was chosen, the total number of bulblets which could be obtained from one bulb (size 12-14) varied strongly from bulb to bulb. The lowest number was 44, the highest 77, with an average of 55 per bulb.

3.2. Duration of storage

To answer the question whether the duration of cold storage affects bulblet formation, a group of bulbs was stored at 2°C immediately after harvest in November 1976 until 15 March 1977. Subsequently these bulbs were stored at 5°C and double scale explants were periodically isolated in vitro and grown for 12 weeks as described in the methods. Table 1 shows that the % of bulblet formation was hardly influenced by the date of excision. The mean number of bulblets per explant increased from 16-29 March and then declined. The total bulblet weight per explant was rather stable, but declined strongly after the 20th of April.

3.3. Important factors affecting bulblet formation

Table 2 shows the results of 5 experiments in which the most important factors affecting bulb formation were examined. Each experiment includes one treatment (always the last one) which represents the standard treatment as described in the methods.

The results presented in table 2 can be summarized as follows. Bulblet formation is strongly promoted by raising the temperature, with an optimum at 21-25°C for both number of bulblets and bulblet weight. The number of bulblets per explant is optimum after adding 0.1 mg/l IBA to the medium, whereas the bulblet weight is slightly higher at 1.0 mg/l IBA. The number of bulblets is only slightly influenced by the length of the explants, but the bulblet weight is strongly increased by longer explants. By increasing the width of the explants from 0.3 to 1.0 cm a strong increase in bulb formation and bulb growth was observed. The last experiment from table 2 shows that no bulblet formation occurred without the presence of part of the basal plate, whereas the number of bulblets and the bulblet weight were strongly promoted by increasing the thickness of the basal plate from 1 to 3 mm.

3.4. Bulblet formation on scale explants of *Nerine sarniensis* and *Nerine* hybrids

To examine the ability of other *Nerine* material to form bulblets on excised double scales, three *Nerine* hybrids and five clones of *Nerine sarniensis* 'Corusca Major' were compared. Table 3, which gives the results of this preliminary experiment, shows that all bulbing parameters strongly vary from clone to clone and

from hybrid to hybrid.

3.5. Subculture of bulblets

When after 12 weeks of explant culture the newly formed N e r i n e b o w d e n i i bulblets were subcultured on the same medium as used for the explants, root formation and leaf development rapidly took place. Addition of IBA was not essential to obtain complete plantlets. After 6 weeks of subculturing the plantlets could be transferred to soil without any difficulty and continued their growth in vivo. It should be noted, however, that rooting, bulbing and leaf development from subcultured bulblets were much better when explant culture took place in darkness instead of in continuous light.

When bulblets of N e r i n e s a r n i e n s i s and N. hybrids were subcultured, as specified in table 3, a remarkable variation in reaction within the clones or the hybrids was observed: a few cultures formed only leaves, part of the cultures formed only roots, whereas another part produced both roots and leaves.

4. Discussion

The question whether the formation of bulblets on excised N e r i n e scales is a result of the outgrowth of pre-existing bud initials in the axils of the scales or the result of new formation (regeneration) is difficult to answer. Because no regeneration of bulblets could be observed on scale explants not including part of the basal plate, the outgrowth of bud initials seems to be the most plausible explanation at present. Anatomical and histological research together with the isolation of explants without 'macroscopically visible' bud initials can possibly give a definite answer to this question.

The ability of explants to form bulblets was primarily determined by the plant material, although 2 other factors (temperature and auxin) also played an important role. This paper is the first report on vegetative propagation of N e r i n e in vitro; several other factors have certainly to be examined to optimalize the developed system. However, when only bud initials give rise to bulbs, the in vitro propagation of N e r i n e will not be so very efficient.

For horticultural practice the continuous multiplication of bulblets in vitro starting with virusfree bulblets will be of great importance. For that reason our preliminary experiments dealing with longitudinally splitting of bulblets (chapter 3.1) should be extended. And last but not least more research will be necessary to optimalize rooting, bulbing and shooting of subcultured bulblets as described in chapter 3.5.

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Table 1 - The effect of the date of excision on the formation of bulblets on isolated double scales of *Nerine bowdenii* after 12 weeks.

Date of excision	Bulblet formation (%)	Mean number of bulblets per explant	Total fresh weight of bulblets per explant (g)
16 March	82	2.1	0.11
21 "	79	2.3	0.10
24 "	82	2.7	0.12
29 "	89	2.8	0.11
6 April	96	2.6	0.14
20 "	85	2.6	0.12
28 "	83	2.3	0.09
3 May	74	1.8	0.05

Table 2 - The effect of temperature, IBA concentration, length and width of the explants and thickness of the basal plate on the formation of bulblets on excised double scales of *Nerine bowdenii* after 12 weeks.

Factors	Treatment	Bulblet formation (%)	Mean number of bulblets per explant	Total fresh weight of bulblets per explant (g)
Temperature	13°C	74	1.3	0.05
	17	87	1.7	0.09
	21	78	2.3	0.17
	25	75	2.3	0.16
IBA	0 mg/l	68	2.3	0.11
	0.01	86	2.6	0.14
	0.10	97	3.2	0.18
	1.00	93	2.8	0.20
Length of the explants	0.5 cm	78	2.5	0.02
	1.0	88	2.5	0.07
	1.5	90	3.0	0.21
Width of the explants	0.3 cm	70	1.2	0.04
	0.6	92	2.0	0.08
	1.0	92	2.8	0.16
Thickness of the basal plate	0 mm	0	0.0	0.00
	1	86	2.3	0.09
	3	96	3.2	0.15

Table 3 - Bulblet formation on excised double scales from 5 clones of *Nerine sarniensis* 'Corusca Major' and 3 *Nerine* hybrids after 12 weeks.

Plant material	Bulblet formation (%)	Mean number of bulblets per explant	Total fresh weight of bulblets per explant (g)
Clone		*N. sarniensis*	'Corusca Major'
30	83	1.3	0.46
113	88	1.3	0.33
187	100	1.8	0.55
57/67	45	0.8	0.22
57/40	95	2.9	0.39
Hybrid		*N. sarniensis*	x *N. bowdenii*
55A	52	0.7	0.14
Hera	100	1.4	0.50
16B	87	1.5	0.37