ACTA SOCIETATIS
BOTANICORUM POLONIAE
Vol. 50, nr 3: 399—404
1981

Plant regeneration from excised bulb scale segments of *Zephyranthes robusta* Baker

MIROSŁAWA FURMANOWA, HANNA OLĘDZKA

Department of Pharmaceutical Botany, Medical Academy,
Banacha 1, 02-097 Warszawa, Poland

(Received: May 7, 1980)

Abstract

Zephyranthes robusta Baker excised bulb scale segments with the basal plate were grown on Murashige and Skoog (MS) medium in four modifications. The cultures were kept at 25°C in darkness. The best results (bulbing, leaf development and rooting) were obtained on MS medium with 1 mg/l IBA, 2-6 bulblets being developed from one explant. After 6 weeks the plantlets (4-6 cm high) were transferred to pots filled with sterilized soil mixes. After two months the leaves of the plants reached a length of 20 cm.

INTRODUCTION

Zephyranthes robusta Baker *(Amaryllidaceae)* is a South American perennial, cultivated in Poland in botanical gardens. The species has been examined chemically, and three alkaloids: lycorine, haemanthamine and maritidine have been isolated (K r i s h n a R a o 1969, 1979). Lycorine is used therapeutically in the USSR (S a d i k o w and S z a k i r o w 1972). This alkaloid has shown a pronounced antiviral activity (I e v e n et al., 1979). Other biological properties of lycorine and haemanthamine have been described by F u r m a n o w a and O l ę d z k a (1978). The same authors (1980) have studied the growth and alkaloid production of *Zephyranthes robusta* roots in tissue culture.

In recent years many studies on the problem of *in vitro* organogenesis of some members of *Amaryllidaceae* have been carried out. *In vitro* propagation of plants of genus *Nerine* Herb. and *Narcissus* L. *(Amaryllidaceae)* was described by P i e r i k and I p p e l (1977), H u s s e y (1977).

The aim of this investigation was to establish whether rapid vegetative propagation of *Zephyranthes robusta* Baker could be obtained by culturing the excised bulb scale segments. This technique was described by Pierik and Ippel (1977).

MATERIAL AND METHODS

Special care was taken to obtain bulbs free from infection. After many experiments, the culture was initiated from seedlings cultivated in sterile culture in modified liquid Street and McGregor medium (1952). The seeds were obtained from the Botanical Garden of Warsaw University. The bulblets obtained from seedling were the first source of scale explants. They were infection free. Scale explants were prepared in the following way: the roots, brownish part of the basal plate and outer scales were removed from the small bulbs; then the bulbs were longitudinally cut into small parts consisting of 2-3 scales connected by the basal plate. These explants were placed in the medium to a depth of about half their length (Fig. 1).

The nutrient medium contained Murashige and Skoog (1962) salt mixture, kinetin—0.50 mg/l, mesoinositol—100 mg/l, thiamine—0.10 mg/l, pyridoxine HCl—0.10 mg/l, nicotinic acid—0.50 mg/l, glycine—3.0 mg/l, saccharose—2%, Difco-agar—0.8% and distilled water. The pH was adjusted to 6.0 before autoclaving. 2,4-D—0.008 mg/l, 2,4-D—2 mg/l, NAA—0.5 mg/l and IBA—1 mg/l were added separately to the medium, and thus four varieties of the medium were obtained. The explants were cultured in glass tubes, each containing 20 cm^3 of culture medium. The cultures were kept at 25°C.

Six weeks after initiating the culture explants were developed in various ways according to the medium employed.

RESULTS AND DISCUSSION

The results obtained are shown in Figs. 1, 2, 3, 4. The bulblet formation depended on the auxins. On the MS medium with 2,4-D (2 mg/l) the explants were brownish and became dry (Fig. 1), smaller concentration of 2,4-D (0.008 mg/l) caused only slight growth of explants (Fig. 2).

On the MS medium with NAA (0.5 mg/l) only one bulblet was developed from one explant (Fig. 3). MS medium with IBA 1 mg/l had the most favourable effect upon bulblet formation and rooting. Four weeks after isolation of the scales, 2-5 bulblets were observed, two weeks later they developed green leaves and numerous roots (Fig. 4).

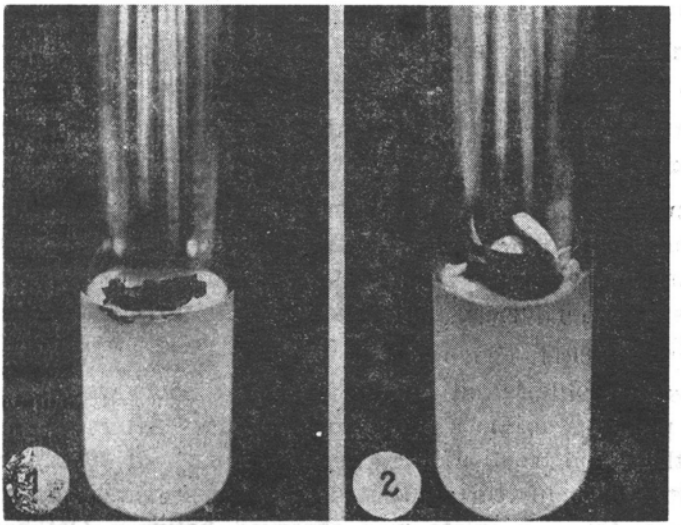

Fig. 1. Scale explant on agar MS medium with 2,4-D — 2 mg/l, six weeks after inoculation

Fig. 2. Scale explant on agar MS medium with 2,4-D — 0.008 mg/l, six weeks after inoculation

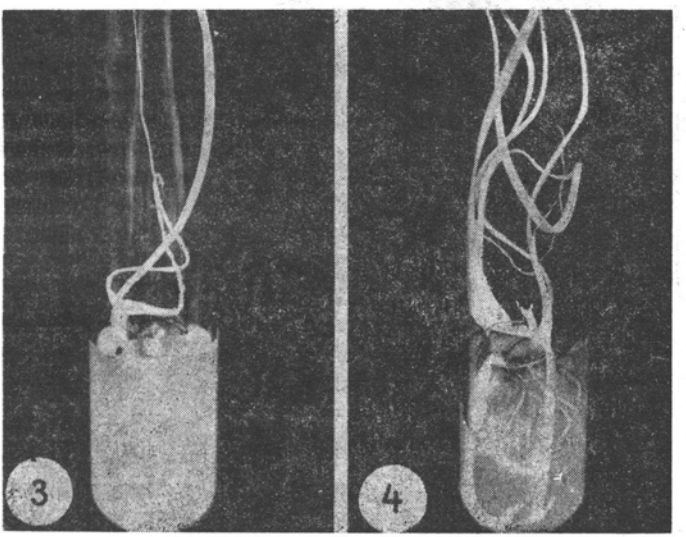

Fig. 3. Plantlet developed on agar MS medium with NAA — 0.5 mg/l, six weeks after inoculation

Fig. 4. Three plantlets developed on agar MS medium with IBA — 1 mg/l, six weeks after inoculation

These bulblets may be either cut longitudinally into four parts and used for initiating of a new culture or they may be transferred to pots filled with sterilized soil mixes (Fig. 5).

For initiation of a new culture the bulblets were cut into quarters. Scales lacking part of the basal plate did not form the bulblets.

The number of bulblets augmented by increasing the number of scales in one explant. After two divisions 144 bulblets from one bulb were obtained (one bulb was cut into four parts and the mean number of bulblets per explant was 3). This rate of multiplication is similar to that described for *Nerine bowdenii* (P i e r i k and I p p e l 1977).

Bulblets with one or two leaves and a few roots were transferred to pots. The plantlets were watered periodically. After two months the leaves further developed and reached the length of 20 cm (Fig. 6). All the experiments were carried out from September to July, the most intensive growth of bulblets was observed between October and February.

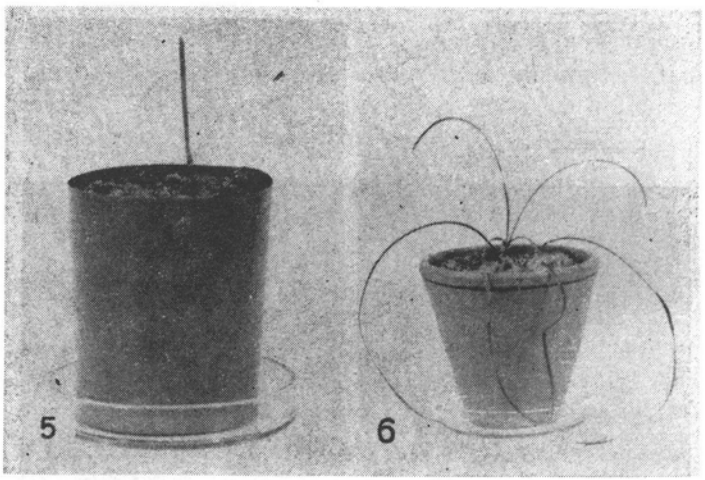

Fig. 5. Plant developed in the *in vitro* culture, one week after transferring it from agar MS medium with IBA (1 mg/l) to the pot
Fig. 6. Fully developed plant transferred from test tube to pot (2 months *in vitro*)

The results presented in this paper indicate the possibilities of vegetative propagation of *Zephyranthes robusta* Baker by tissue culture methods (Fig. 7). Plantlet regeneration from excised bulb scales has demonstrated that the rate of multiplication of *Zephyranthes robusta* by this method is higher than the rate of natural vegetative propagation. It seems to be of great importance for phytochemical investigation, since more plant material can be obtained in a short period of time.

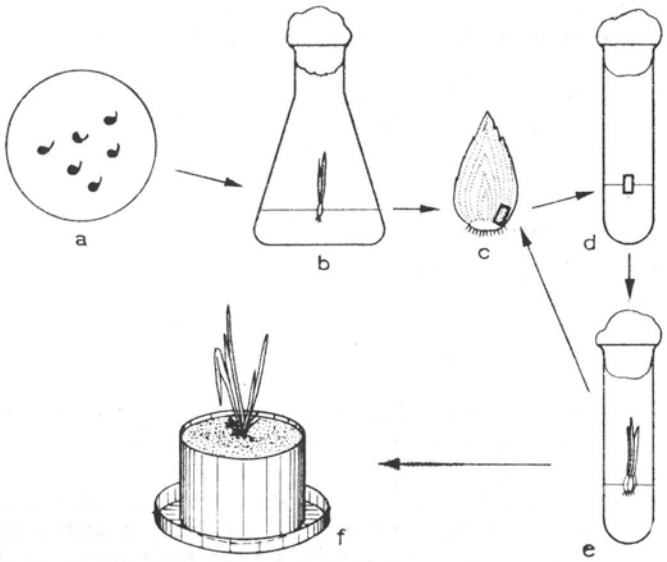

Fig. 7. a — Petri dishes with seedlings of *Zephyranthes robusta* Baker in sterile culture
b — Plant developed from seedling in liquid Street and McGregor medium.
c — Bulb scale segment with a part of the basal plate which was to be removed from the bulb.
d — Explant on solid MS medium.
e — Plantlets developed from the explant.
f — Regenerated plant 2 months after transferring it to the pot

Acknowledgments

The authors wish to thank the Director of Botanical Garden of Warsaw University for seeds.

REFERENCES

Furmanowa M., Olędzka H., 1978. Comparison of the effects of extracts of *Zephyranthes robusta* Baker, haemanthamine and lycorine on mitosis and DNA synthesis in *Allium cepa* roots. Caryologia 31: 449-456.

Furmanowa M., Olędzka H., 1980. *Zephyranthes robusta* Baker roots *in vitro* culture — growth and alkaloid production. Acta Polon. Pharm. 37: 107-112.

Hussey G., 1977. *In vitro* propagation of some members of the *Liliaceae, Iridaceae* and *Amaryllidaceae*. Acta Hort. 78: 303-309.

Ieven M., Vanden Berghe P. A., Vlietinck A. J., 1979. Inhibition of polio virus by lycorine, a plant alkaloid. Planta Medica 36: 254-255.

Krishna Rao R. V., 1969. Alkaloidal components of *Zephyranthes robusta*. Indian J. Pharm. 31: 62-63.

Krishna Rao R. V., 1979. Occurrence of the rare alkaloid maritidine in *Zephyranthes robusta* and Z. *sulphurea*. Current Science 48: 110-111.

Murashige T., Skoog F., 1962. A revised medium for rapid growth and bioassays with tobacco tissue culture. Physiol. Plantarum 15: 473-497.

Pierik R. L. M., Ippel D. J., 1977. Plantlet formation from excised bulb scale segments of *Nerine*. Acta Hort. 78: 197-202.

Sadikow T., Szakirow T. T., 1972. O wydielenii likorina. Chim. Prir. Sojed. 1: 134.

Street H. E., McGregor S. M., 1952. The carbohydrate nutrition of tomato roots. III. The effects of external sucrose concentration on the growth and anatomy of excised roots. Ann. Bot. 16: 185-205.

Regeneracja Zephyranthes robusta Baker z łusek cebulowych

Streszczenie

Tematem pracy jest regeneracja *Zephyranthes robusta* Baker z łusek cebulowych. W tym celu fragmenty soczystych łusek cebulowych z częścią piętki hodowano na pożywkach Murashige'a i Skoога (MS) w różnych modyfikacjach. Najlepsza okazała się pożywka MS z 1 mg/l IBA. Na tej pożywce z jednego wycinka rozwijało się 2-6 cebulek, które ukorzeniały się i wyrastały z nich liście. Po 6 tygodniach wzrostu *in vitro* roślinki wysokości 4-6 cm przenoszono do doniczek. Po dwóch miesiącach wzrostu *in vivo* długość liści osiągnęła 20 cm.