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The in vitro development of isolated rye proembryos

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Abstract

Isolated 7 day-old rye proembryos, 130–150 μm long, which were inoculated directly onto a medium, degenerated by 100%. Proembryos of the same size, introduced into culture using nurse endosperm, survived by 74%, from which 64% developed normally, giving green and rooted plants.

Key words: *proembryos, nurse endosperm, in vitro culture, Secale cereale*

INTRODUCTION

One of the key factors in the in vitro culture of proembryos of various plants is the adjustment of the medium to the its developmental stages (Raghavan 1976, Batygina and Vasilyeva 1981, Zenkteler 1984). The younger the embryo, the more dependent it is on the external environment. That is why numerous attempts are being made to adjust the composition of the artifical medium to the natural environment of the developing proembryo (Norstog 1965, 1967, Kruse 1974, Monnier 1978, Williams and De Lautour 1980, Tilton and Russell 1984). Results presented in this paper describe the in vitro culture of rye proembryos by application the nurse endosperm technique.

MATERIAL AND METHODS

Isolated proembryos from *Secale cereale* L. cv. Strzekęcińskie, cultivated in the Poznań Botanical Garden, were used in this study. Seven days after controlled pollination, the middle sections of the ears were sterilized for 0.5 min in 70% ethyl alcohol and then rinsed several times in sterile

distilled water. The proembryos were isolated under a stereoscopic microscope in a drop of sterile water with 3% sucrose.

Two types of explants, marked A-1 and A-2 (Fig. 1) were used. A-1 denotes the isolated proembryos inoculated directly onto the medium and A-2 those proembryos which were inoculated onto the medium by applying the nurse endosperm technique. The endosperm used as the "nurse" belonged to the same variety of rye and was at the same developmental stage as the isolated proembryos (at the wax ripness stage).

The medium used in these experiments was according to Gamborg et al. (1968) and it was supplemented with 1 $mg \cdot dm^{-1}$ gibberellic acid (GA_3), 0.5 $mg \cdot dm^{-1}$ kinetin (KIN) and 3% sucrose. The medium was poured into Petri dishes of a diameter of 60 mm and 5 explants were introduced into each dish. For the first 24 hrs of culture, the explants were kept in the dark, after which they were transferred to a photoperiod of light: dark — 18: 6 h (approx. 3000 lux). After 2–3 weeks the proembryos were transferred to a fresh medium of the same composition. The plants obtained from the proembryos were again transferred to test-tubes and only later to hydroponic cultures.

RESULTS

The 7 day-old rye proembryos inoculated onto the medium were oval in shape and from 130 to 150 μm long. They were composed exclusively of meristematic cells and did not have any axial organ primordia discernable by their ultrastructure (Stefaniak 1984).

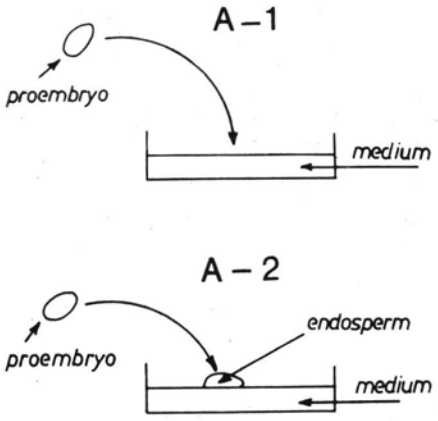
Table 1

The growth and development of isolated preombryos inoculated onto a medium according to Gamborg et al. (1968) supplemented with 1 $mg \cdot dm^{-1}$ GA_3, 0.5 $mg \cdot dm^{-1}$ KIN and 3% sucrose

Type of medium	Number of inoculated proembryos	% of degenerated proembryos	% of deformed proembryos	% of green plants
Medium only (A-1)	2200	100	–	–
Medium + "nurse" (A-2)	2414	26	10	64

PROEMBRYOS IN THE MEDIUM (A-1)

During the first week of culture, the explants did not exhibit any macroscopically visible changes. They started to become brown several days later and after about 10 days of culture they completely degenerated (Table 1).



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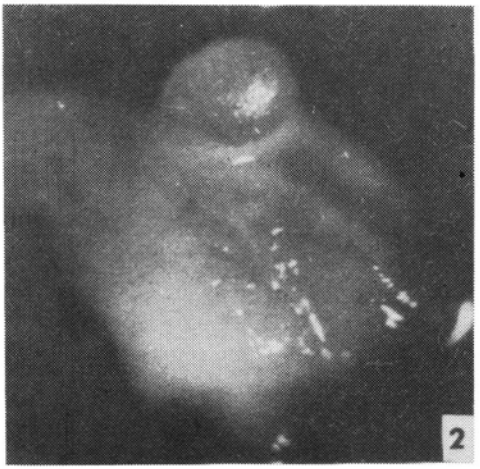

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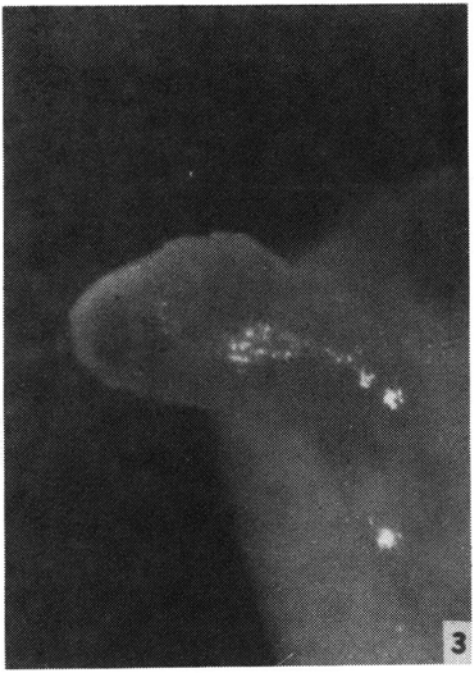

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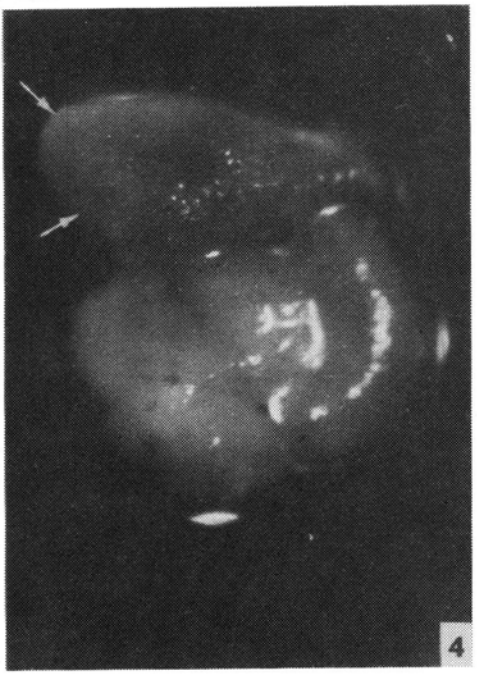

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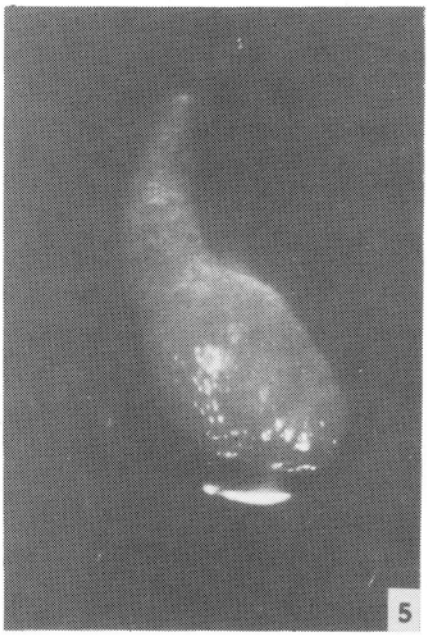
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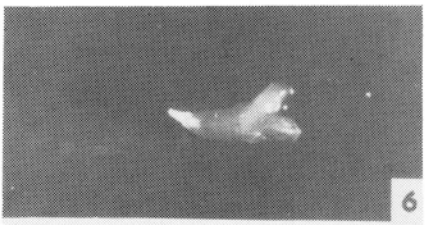
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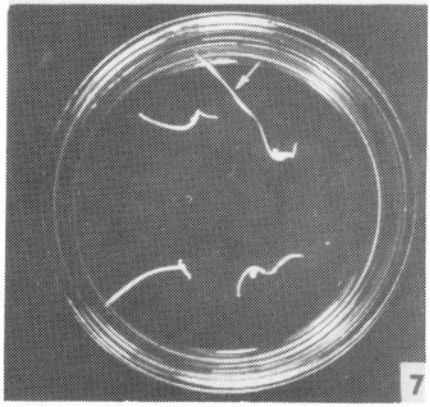
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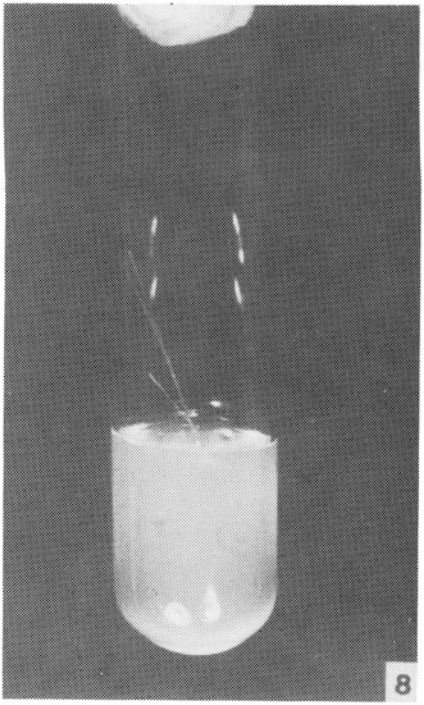
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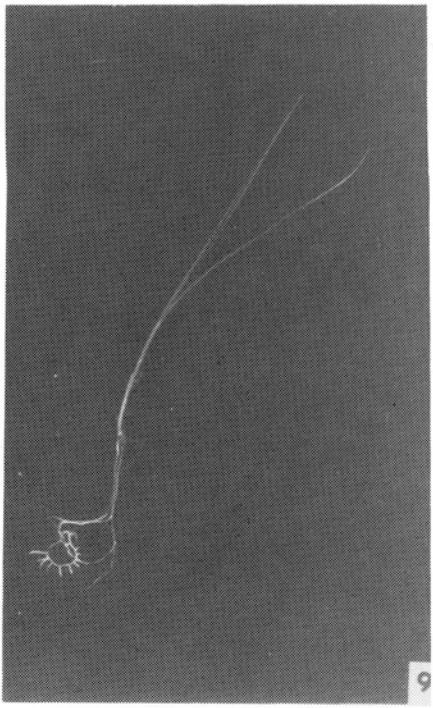
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PROEMBRYOS ON NURSE ENDOSPERM (A-2)

After 10 days of culture, the proembryos which were inoculated on nurse endosperm survived by 74%, from which 64% (Table 1) grew and developed in a similar way to the process of embryogenesis of rye in vivo. Twenty-four hours after inoculation, proembryos retained the same color and firmness as during isolation (Fig. 2). Next, they began to swell slightly and, beginning from the fourth day of culture, to elongate (Fig. 3). It is most probable that at this stage the differentiation processes had begun, since after 12–14 days of culture, the scutellum was becoming visible (Fig. 4). At this stage the embryos were, on average, about 500 μm in length. The nurse endosperm was removed on the 15th day of culture and the developing embryos were transferred to fresh medium (Fig. 5). The transfer and removal of the nurse endosperm did not have a negative effect on the further development of the embryos. The development and elongation of the axial organs continued and 19–20 days after inoculation, completely developed embryos, on average 1500 μm long, were observed. During the next 10 days of culture, the radicles and shoots "germinated" (Fig. 6). After 35 days of culture, the coleoptile began turning green, and the first green leaves appeared after 40 days (Fig. 7). Green and rooted plants were obtained 7 weeks after culturing the isolated rye proembryos using the nurse endosperm technique (Figs. 8 and 9).

The remaining small part of the explants (about 10%, Table 1) grew in an unorganised manner, forming highly deformed axial organ primordia. In spite of further subculturing, these explants did not develop further, did not form callus tissue and all of them soon degenerated.

PLATE I

Fig. 1. Types of inoculated explants. A-1 — directly onto the medium, A-2 — using the nurse endosperm technique

Figs. 2–4. Proembryos inoculated onto an agar medium according to Gamborg et al. (1968), supplemented with 1 mg·dm^{-1} GA_3, 0.5 mg·dm^{-1} KIN and 3% sucrose and by applying the nurse endosperm technique. Fig. 2. An isolated rye proembryo after 24 hrs of in vitro culture. 170×. Fig. 3. An elongated rye proembryo after 10 days of culture. 120×. Fig. 4. A 13 day-old rye proembryo with the contours of the scutellum visible (arrows). 120×

PLATE II

Fig. 5. A 15 day-old rye proembryo after removal of the "nurse" and subculturing on a fresh medium according to Gamborg et al. (1968)+1 mg·dm^{-1} GA_3, +0.5 mg·dm^{-1} KIN+3% sucrose. 100×. Fig. 6. A "germinating" rye embryo 28 days after inoculation. 16×. Fig. 7. 42 days of culture — elongated coleoptiles, first leaf (arrow) and roots are visible. 1.2×. Fig. 8. A five-week-old rye plant. 1:1. Fig. 9. A green and rooted plant obtained about 6 weeks after isolated rye proembryos were inoculated onto the medium using the nurse endosperm technique. 1:1

DISCUSSION

The results presented here indicate that the ingredients of the medium used for culture did not suit the isolated rye proembryos inoculated directly onto the medium (A-1) as all of the explants died soon after the culture. Most of the cultured proembryos as seen in the electron microscope had highly plasmolysed cells (Stefaniak — unpublished results). The reason of this is the lack of osmotic balance between the medium and the proembryo cells (Norstog 1967, Monnier 1978). However, in considering the causes of the high mortality of the rye proembryos inoculated directly onto the medium, such factors as temperature, light and humidity cannot be overlooked.

One way in which the negative influence of an artifical environment on the inoculated proembryos can be reduced by the application of the nurse endosperm (Kruse 1974, Williams and De Lautour 1980, Tilton and Russell 1984). It should be emphasized, however, that the positive results reported by the cited authors, concern mainly the immature embryos and not the proembryos.

In this study, the nurse endosperm technique was used in the culture of isolated rye proembryos (A-2). Both a high survival rate (74%) and a normal course of embryogenesis (64%) were ascertained. In the presence of the nurse endosperm, the proembryos developed axial organs. Only when the scutellum appeared, the "nurse" was removed as embryos at this stage of embryogenesis have already been capable to develope further when in direct contact with the medium.

It is worthwhile, then, to consider the role the nurse endosperm plays in in vitro cultures. It is known from the studies by Wakizuka and Nakajima (1975) and Lagriffol and Monnier (1984) that even a zygote or spherical proembryo is capable of development in a medium, but only inside an ovule containing an endosperm. The lack of an endosperm or the presence of it but not in a viable stage causes the collapse of the proembryo. It seems possible, then, that the main causes of the failures of in in vitro culture of proembryos are to be found not in the composition of the mediums, but in the lack of nutrients in the most suitable form for assimilation by proembryos. A similar opinion express Williams and De Lautour (1980), who suggest that the nurse endosperm is the source of these substances. Therefore, on the basis of the observations of these authors and on the results of this study, it is possible to accept that the role of the nurse endosperm in in vitro cultures is two-fold. First, it provides the proembryos with a microenvironment similar to their natural one, thus lessening the shock evoked by such a radical change in their living conditions, secondly, it constitutes a reservoir of nutrients in a form assimilable by the

proembryos. Unfortunately, at this stage, it is not known whether the nutrients indispensable for the development of isolated proembryos derive exclusively from the cells of the nurse endosperm or whether the "nurse" also participates in the transport of these substances to the explant. This question requires further to be studied in details.

Acknowledgments

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Rozwój izolowanych prazarodków żyta w warunkach hodowli in vitro

Streszczenie

Izolowane 7-dniowe prazarodki żyta o długości od 130 do 150 μm, wyszczepiane bezpośrednio na pożywkę, degenerowały w 100%. Tej samej wielkości prazarodki wyszczepiane przy zastosowaniu bielma-"niańki" przeżywały w 74%, z czego 64% rozwijało się prawidłowo, dając w efekcie zielone i ukorzenione rośliny.