

Induction of embryoid development from apple pollen grains

B. KUBICKI, J. TELEZYŃSKA, E. MILEWSKA-PAWLICZUK

Institute of Genetics and Plant Breeding,
Agricultural Academy of Warsaw, Warszawa, Poland

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Abstract

Globular 32 and 64 celled embryoids were obtained from uninucleate apple microspores (cultivar Jonathan) after 5 weeks of culture on a modified Murashige and Skoog (1962) medium. A similar induction of microspore development was not observed in younger or older stages of anther development. In such anthers only callus was formed from diploid tissues.

INTRODUCTION

Cross pollinating fruit trees are complex heterozygotes, they cannot be reproduced from seeds and constitute difficult material both in genetic studies and in breeding.

The development of a quick method of obtaining genetically pure lines would represent a tremendous advance permitting a precise analysis of the inheritance of characters and a strict programming of the breeding work in these plants. The classical methods depending on self fertilization and selection are difficult to realize in the life span of one breeder due to the long development cycle and the frequently occurring selfsterility and inbreeding depression in these plants.

A great opportunity to overcome these difficulties has been created by the discovery of the possibility of obtaining haploid plants and therefore pure lines from pollen grains.

Even through in herbaceous plants several successful attempts were made to produce haploid plants, in fruit trees there were few attempts in this direction.

The first studies on the culture of apple anthers were reported upon by the Japanese workers Nakayama, Saito, Sato and Tamura (1971), and by Nakayama, Saito and Yamamoto (1972). These

authors have obtained from the anthers a callus that initiated roots. These studies are only preliminary. Of similar nature are the studies of Jordan (1974) who has obtained from *Prunus avium* many celled pollen grains and from *Prunus communis* a callus from the walls of the anthers and rich in strach non-dividing pollen grains.

The present report presents the initial results of attempts to induce the development of haploids from apple pollen grains.

MATERIALS AND METHODS

Apple flower buds (cultivar Jonathan) have been sterilized in 1.5% calcium hypochlorite for 3 min. and washed three times in sterile water. Anthers isolated from sterilized buds have been placed 2—3 per testtube containing 10 ml of a solid medium and held at a temperature of $25^{\circ}\text{C} \pm 1^{\circ}\text{C}$ under continuous luminiscent light of 2000 lux.

In an experiment conducted in 1974, in which embryoids were obtained, use was made of the Murashige and Skoog (1962) medium without vitamins and with the addition of 1 mg/1 of IAA and 1 mg/1 of kinetin. In all the media tested before and in their modifications (media of White 1943; Nitsch 1970, 1972; Blaydes 1966; Gresshoff and Doy 1972) the development of embryoids from pollen grains was not observed.

In the experiments conducted in 1974 the anthers were isolated between February the 26th and April the 19th at the following stages of anther development: Stage I — tetrads and just liberated microspores; Stage II — non vacuolated, uninucleate microspores with a centrally positioned nucleus; stage III — uninucleate microspores with a central vacuole (signet stage); Stage IV — young two-celled pollen grains.

The changes in the inoculated anthers were observed in material fixed at 1—2 week intervals. The anthers were fixed in acetic-alcohol (1 : 3) or in a mixture of 0.2% uranyl nitrate with 20% formalin in the proportion 1 : 1. The first fixative was used for smears in aceto-carmin and the second for the permanent microtome slides which were stained with Ehrlich hematoxylin.

RESULTS AND DISCUSSION

It was observed that in apple similarly as in several other plants (Iyer and Raina 1972; Zenkteler 1971, 1973; Niizeki and Oono 1971; Misiura and Zenkteler 1973) pollen embryoids develop only from anthers set on culture in a defined developmental stage (stage II). In the cultures started in other stages of anther development a callus formed but it did not result in the production of embryoids.

In the anthers inoculated at stage I, one week after planting cell divisions were observed in the walls of the anther, which led after 2—3 weeks to development of a prolific, greening callus, gradually overgrowing the whole of the anther.

In the anthers inoculated at stage III and IV the course of callus development was different. It arose from the tissue of the connective and grew into the pollen sacs. After 3—5 weeks of culturing the pollen sacs were bursting. Through the formed cracks the callus outgrew and formed globular structures, green or stained red with anthocyanins (Figs. 1 and 2).

The callus developing from the anther walls or from the connective did not produce embryoids. They were not observed after 4 months of culturing nor after transfer to a medium of a different composition.

In the anthers placed on culture in stages I, III and IV parallel with the development of the callus a degeneration of the microspores occurred. In these anthers the development of callus from the microspores or from the young pollen grains was not observed.

In the anthers inoculated at stage II various situations were observed. After 4—5 weeks of culturing in the majority of them a callus developed from the connective and the microspores degenerated as in the anthers planted at stage III.

In some anthers, that is in about 1/3 of those studied, there was no callus. The anthers locules were filled with a slime in which the microspores and degenerating protoplasts of the ameboidal tapetum were embedded. There were few degenerating uninucleate microspores. The majority of the microspores were stimulated to development. Among these there were apparently normally developing uninucleate microspores and binucleate pollen grains with a distinct generative cell. However the majority of the microspores in these pollen sacks developed differently. These microspores have had various sizes and were filled with a dense cytoplasm (Fig. 3). The smaller ones were uninucleate and the larger ones were divided into 2 or 4 cells of equal size. Presumably these were the first stages of the development of androgenic embryoids, since in the same anther locules sporadically more advanced stages of the development of embryoids were observed.

The typical globular embryoids in the 32 or 64 cell stage are built from small cells which have a dense cytoplasm and relatively large nuclei, which indicates their meristematic nature. There are no doubts that these are embryoids formed from microspores, since they were enclosed in a wall staining similarly as the walls of pollen grains (Fig. 4).

In the same anthers in which early stages of the development of embryoids was observed, there occurred also very large uninucleate microspores with a large central vacuole and large microspores divided into 2, 4 or 8 cells strongly vacuolated, and appearing devoid of cytoplasm. These are probably embryoids inhibited in development (Fig. 5).

Thus apple embryoids form from uninucleate microspores still undifferentiated into a vegetative and generative cell. In the development of the embryoids a similar participation is observed of the two sister cells formed after the first division of the uninucleate microspore, as in *Nicotiana* (Nitsch 1972). Their development is therefore completely different from that of *Datura metel* (Iyer and Raina 1972) and *Atropa belladonna* (Misiura and Zenkteler 1973) where the embryoids form from the vegetative cell of a pollen grain. Also it is not initiated by the coenocytic stage as described by the latter authors. After each division of the nucleus there occurred a division of the cytoplasm and the formation of a cell wall.

There are hopes that a further development of a haploid plant may prove possible from the obtained embryoids. Further studies are in progress.

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Authors' address

Dr B. Kubicki and co-authors

Institute of Genetics and Plant Breeding

Agricultural Academy of Warsaw;

Nowoursynowska Str. 166; 02-975 Warszawa, Poland

Plate I

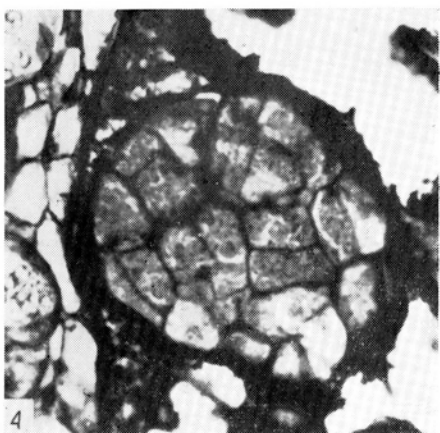
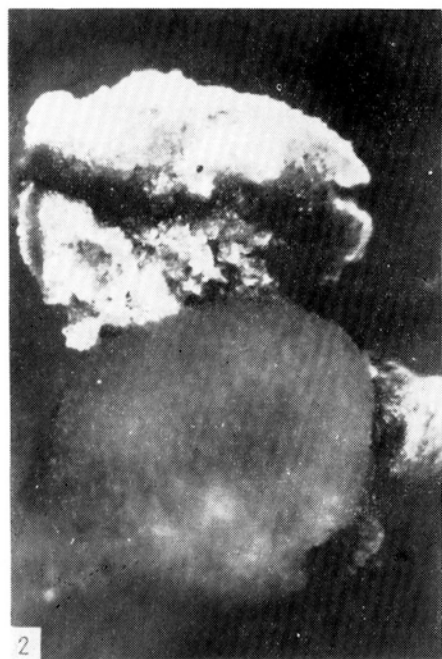
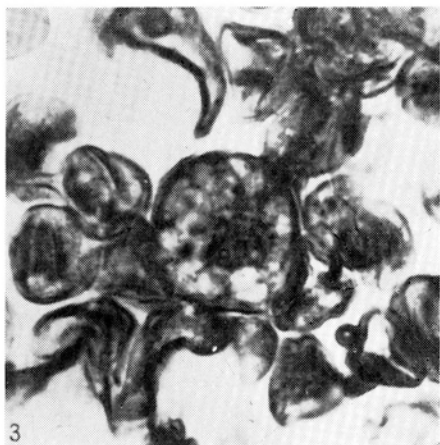
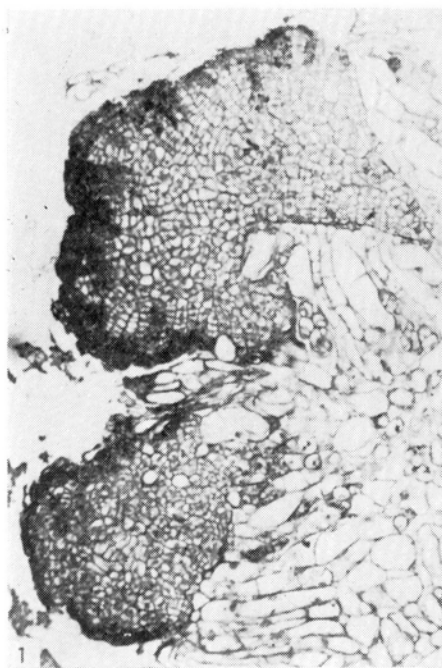


Fig. 1 Callus formation from connective tissues, 80 \times

Fig. 2 Spherical callus emerging from a split anther, 30 \times

Fig. 3 Enlarged uninucleate microspore, 600 \times

Fig. 4 Multicelled globular embryoid, 600 \times

Fig. 5 Inhibition of embryoid development at 4-celled stage, 600 \times

Indukowanie rozwoju embrioidów z ziarn pyłku jabłoni

Streszczenie

Otrzymano globularne 32 i 64 komórkowe embrioidy z jednojądrowych mikrospor jabłoni (odmiana Jonathan) po 5 tygodniach kultury na zmodyfikowanej pożywce Murashige'a i Skoog'a (1962).

Nie stwierdzono podobnej indukcji rozwoju mikrospor w młodszym i starszym stadium rozwoju pylnika. W pylnikach takich tworzył się jedynie kalus z tkanek diploidalnych.