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# In vitro Haploid Plantlet Induction in *Physalis ixocarpa* Brot. Through Microspore Embryogenesis

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## Abstract

Anthers of Physalis ixocarpa Brot. exised from 2-3 mm long flower buds were treated for 2 d at 3°C, and were cultured on the basal medium of NN (1969), supplemented with plant hormones. They formed embryoids from microspores within 6 weeks. Upon transfer to a regeneration medium, embryoids grew into functional plants with the haploid number of chromosomes (n=12).

## Abbreviations

CM - Coconut-milk, IAA - indole acetic acid, Kn - Kinetin, MS - Murashige and Skoog, NN - Nitsch and Nitsch, ZEA - Zeatin.

## Introduction

Different species of Physalis (Solanaceae) contain steroids of medical importance, some of which have been isolated and characterized (Glotter et al. 1975, Alluri et al. 1976). In the genus Physalis in vitro growth and organogenesis has been successfully achieved in the species Ph. minima Linn. (Bapat and Rao 1977). In further investigations Sipahimalani et al. (1981) demonstrated the biosynthetic potentiality of diploid and triploid callus tissues and regenerants of Ph. minima. Hence, it may be feasible to improve the steroid content of Physalis species by incorporation of a haploid selection step. Such a technique is a valuable tool for increasing the efficiency in the breeding programs of nutritional and medical plants (Nitzsche and Wenzel 1977). The present communication reports the production of haploid plantlets via microspore culture within the anther sac of Ph. ixocarpa. Such haploids or homozygous doubled haploids respectively may be useful also for studies aiming at the elucidation of genetic control for secondary biosynthesis and as a starting material for the isolation, regeneration and manipulation of a protoplast system.

## Material and Methods

Flower buds of 2-10 mm were collected from Ph. ixocarpa plants (2n=24) grown in greenhouses (day temperature 22 ± 4°C). In 2-3 mm long buds, a high proportion of the microspores were in the late uninucleate stage. These buds were harvested and precultured in dry test tubes at 3°C for two d in the dark. After surface sterilization of the buds with 0.1% HgCl for 20 min and subsequent washing with the autoclaved water, the anthers were dissected out and plated on the culture medium of NN (1969) or on MS (1962) basal medium supplemented with plant hormones. Simultaneously anthers were also cultured in the liquid media as float cultures as described by Sunderland and Roberts (1979). For experiments on the isolated pollen culture, anthers were crushed and microspores were separated first by filtration and later by sucrose gradients as described by Wenzel et al. (1975). After washing with the washing medium the microspores were cultured in the liquid media containing different combinations and concentrations of auxins and cytokinins. Differentiated embryoids were transferred to the regeneration medium, a modified MS medium, for plantlet development. Ploidy level of the plantlets was determined by counting chromosomes from young leaves. For each experiment, solid and liquid medium, 15 anthers were cultured per 6 cm petri dishes and each treatment consisted of 5 to 8 replicates. Half the cultures were kept in the dark and half in the 12 h light regime of 2,000 lx at temperature of 26 ± 1°C.

## Results

Of the two basal media tested (NN and MS), embryoid formation from the microspores was achieved only on NN medium, containing 5 $mgl^{-1}$ IAA and 0.2 $mgl^{-1}$ Kn. Fig. 1a demonstrates the first division of microspores on this medium. In contrast to several other reports (Maheshwari et al. 1980), activated charcoal at 0.5% did not enhance the embryoid formation. Illumination was also not essential and there was even a tendency that from anthers kept in the dark about 10% more macroscopic structures developed. After 10 d of anther culture proembryoids could be isolated, which were just bursting through the exine (Fig. 1b). On average 50 - 60% of the plated 2,000 anthers showed proembryoid formation. These structures developed rapidly and reached the typical embryoid structure within 15 d (Fig. 1c). Normally, the embryoids developing from the microspores broke through the anther wall 6 weeks after plating, but sometimes they dropped out much earlier and developed on the medium in isolation from the anther.

This observation together with the fact that usually only one macroscopic structure per anther grew into a plantlet, although many more microspores started showing androgenesis, made it feasible to try isolated microspore culture. However, so far no microspores showed divisions of further development after early separation from the anther tissue. Also anthers cultured in the liquid NN medium as float cultures showed no

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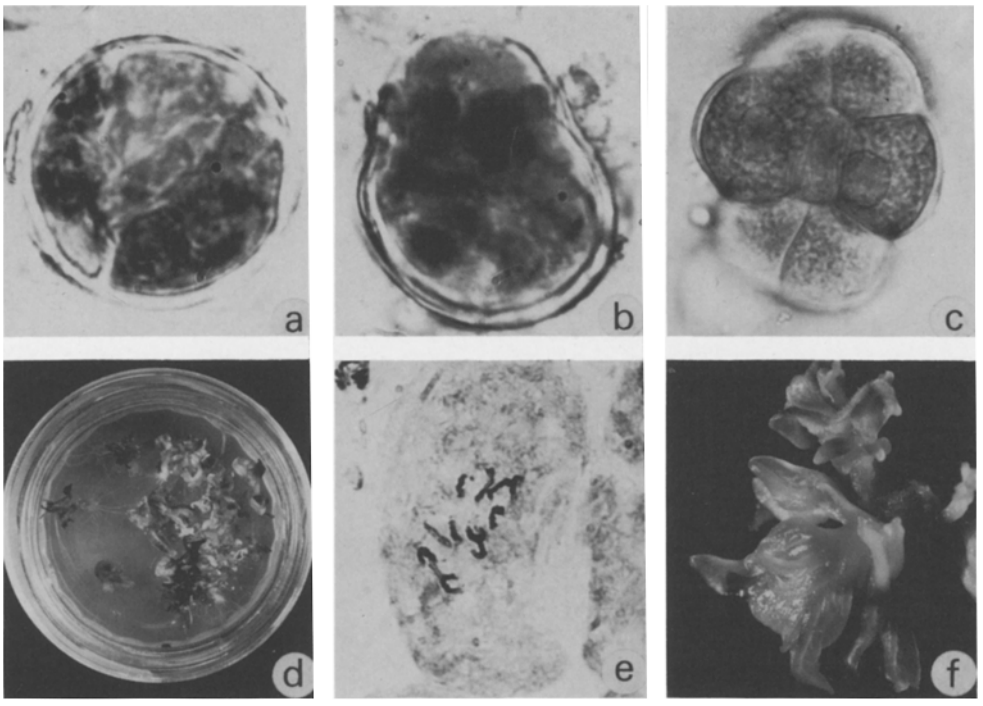


Fig. 1: Development of Physalis ixocarpa microspores into haploid plantlets.

a) Initiation of divisions on a microspore; b) development of an embryoid from a microspore; c) six celled embryoid; d) embryoids growing on MS regeneration medium; e) squash preparation of a young leaf showing haploid number of chromosomes (n=12); f) plantlets developing on Gamborg et al. B5 medium.

embryoid formation. Shedding of the microspores was a slow process. Combinations of growth hormones or the addition of 0.01% of charcoal failed to provoke any positive response from such floating anthers. They remained green for more than 6 weeks, probably demonstrating that the shift from the developmental route of pollen grains to the sporophytic route was not induced successfully.

The embryoids obtained from the microspores cultured within an anther on the agar media were isolated after 6 weeks from the anther tissue and transferred to the regeneration medium, containing macro- and micro-nutrients of MS medium, together with 10% CM and 0.3 $mgl^{-1}$ ZEA. On this medium within 3 weeks multiple bud formation started (Fig. 1 d). From these roots and normal plants were developed (Fig. 1 f) upon transfer to the B5 medium of Gamborg et al. (1968) which grew to full maturity in the greenhouse. Squash preparations from young leaves showed the haploid number of chromosomes (Fig. 1 e).

## Discussion

The present investigation demonstrates that the development of embryoids and haploid plantlets (n=12) from microspores of Ph. ixocarpa (2n=24) is possible; in contrast to the androgenesis of Ph. minima (2n=48) (George and Rao 1979), where all plants showed the triploid number of chromosomes. Probably the formation of 3n plants is more frequent from polyploids as in Ph. minima than from a true diploid species such as Ph. ixocarpa. For the regeneration experiments NN medium was superior over the MS medium, but as in other species, coconut milk was essential for further embryoid development. Comparison of different microspore culture techniques demonstrated that the most frequent regeneration of haploids was obtained from microspore culture within the anther. Even by omitting the agar, and culturing the anthers on identical medium in float culture, no response was observed. Surprising was the clear embryogenic capacity of Ph. ixocarpa microspores. We did not observe callus formation from the microspore or as a secondary effect from an originally formed embryoid. This might be due to the low levels of sucrose in the medium. The relative high concentration of the heat instable IAA was actually in the medium after autoclaving so low that it did not induce callus formation. The direct embryoid formation hastened plantlet formation and, in addition, avoided the possibility for chromosome variations, which are frequent during longer callus stages.

There are no data available yet in the steroid content of the haploid regenerants. Amongst the different regenerants, a wide variability is expected, as the donor material will not be homozygous. It should be possible to increase via polyploidization the secondary product formation, after simple selection at the haploid level. In Ph. minima for example, the callus from triploid plants contained about twice the steroid concentration compared to callus from diploid plants (Sipahimalani et al. 1981). A most favorable approach for haploid genome combination is via protoplast fusion. One prerequisite for such experiments is the regeneration of protoplasts from diploid starting material of Ph. ixocarpa which has been accomplished (Bapat and Schieder 1981).

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