DROSERA ROTUNDIFOLIA L. VITROCULTURE ASSOCIATED WITH A SAPROPHYTE FUNGUS

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ABSTRACT. In coculture studies there is an interest in the relationships between phytoinoculs and fungi or bacteria. Apart from the manifestations described by parasitism, a particular importance – both theoretical and practical – is given to the symbiosis and saprophytism relations. Spontaneous infections are detrimental, while artificially produced cocultures represent modern study subjects. The phenomenon of coculture, produced spontaneously in the *Drosera* vitrocultures that we conceived, with a black-grey coloured fungus (which we have not been able to identify yet), led to the formation in the vicinity of the *Drosera minirosettes* colony, of an fungus with a crateriform body fruiting, of larger size than the actual *Drosera* culture. This phenomenon, quite spectacular from a biological point of view, is to be studied in various experimental conditions. We should mention that the culture media was favourable to the phytoinoculs (basis, Murashige – Skoog, 1962), being devoid of growth regulators.

Keywords: *Drosera rotundifolia* L., carnivorous plants, coculture, fungus

INTRODUCTION

The cocultures between phytoinoculs and microorganisms represent a branch of vegetal biotechnology, encompassing more recently procedures of *in vitro* cultivation of plant tissues and cells (Cachiță and Ardelean – in printing).

Studies concerning cocultivation of various types of phytoinoculs with bacteria or fungi referred initially to issues regarding possible situations in laboratories specialised in aseptic procedures, because of the difficulties faced by experimentalists in obtaining the axenic regime, as a result of the production of spontaneous infections generated by various saprophyte or phyto - pathogen agents. Such infections compromise – from the very first week of the inoculation operation – the success of vitrocultures, in all types of cultures intended to be axenic, saprophyte fungi or bacteria 'suffocating' the phytoinoculs.

In vegetal vitrocultures, the presence of sugar in culture media, in 20-30g/l concentrations, often favours the emergence of rather varied fungi infections than bacterial infections. They are spontaneous and are generated either by the improper disinfection of culture media or the use of incorrect aseptic techniques for the vegetal material, or again, the defective sterilisation of culture containers or of the working instruments, or – in the case of subcultures – the negligent execution of the inoculation procedure.

In the case of the description of the situation which makes the object of our current study, it will question of presenting a state of facts, manifested in culture containers and representative for the difficult performance of subcultivating *Drosera rotundifolia* L. (Sundew) propaguls, respectively of the foliar rosettes individualised from the cluster of the numerous differentiated rosettes at the level of an initiating propagul originating in the previous vitroculture.

In the present study we intend to present a particular case of infection, which evolved non invasively over time, paralleled by the *Drosera* culture, and originating eventually in a genuine coculture which lasted for three months, when the experiment was dismantled in order to operate electron microscopic examinations.

MATERIAL AND METHODS

Maintaining a stock of *Drosera* vitroplantlets within the Vegetal Biotechnology Laboratory of the University of Oradea, is accomplished by the procedure of periodically pricking out the vitrocultures through propaguls made up of *Drosera* minirosettes. The operation consists of dismembering and individualising minirosettes, initially regenerated from a similar propagul, and their solitary subcultivation on a fresh media, generally identical (in terms of composition) with their media of origin. This media was prepared according to the recipe described by Murashige – Skoog (1962), which we modified, i.e. without growth regulators, but with an addition of 1mg/l HCl tiamin, HCl pyridoxin and nicotinic acid, 20g/l sugar (instead of 30g/l, as provided in the original recipe for this compound) and with 7g/l agar-agar, instead of 10g/l, as specified by the above-mentioned authors; the pH of the culture media was adjusted to a

5.6 value, and afterwards the culture media were sterilised by 121°C autoclavation, for 30 minutes.

The *Drosera* subculture was operated in uncoloured glass recipients, 10cm high and with a 4.7cm diameter. After the inoculation, the pots were sealed with polyethylene foil and strapped with elastic rings, then, the containers were placed on shelves illuminated by fluorescent white light, in a 16h light/ 24h regime, at a 1700lx lighting, and a temperature of about 23 °C during the period of light and roughly 21°C ±2°C during dark hours.

The vitroculture was kept for *three months* in the growth chamber. When the experiment was dismantled, assays were gathered in order to perform scanning electron microscopy investigations, aiming at visualising details regarding the formation and structure of the fruiting body generated by the fungus, hoping to identify its taxonomic affiliation. Samples were processed using scanning electron microscopy techniques, respectively fragments were fixed according the specific methods, characteristic for the scanning electronic microscopy techniques, and different segments were examined afterwards with this kind of microscope (Cachiţă et al, 2006).

RESULTS AND DISCUSSIONS

As shown in figure 1, after *three months* of vitroculture, both the *Drosera* inocul and the fungus were coexisting in the same culture container, they extended in terms of the occupied surface, the shape of both organism being excellent, proving that they mutually tolerate each other and giving 'birth' to a genuine coculture.

In figure 2 microscopic details are illustrated concerning the composition of the wall of the fungus' fruiting body, as well as aspects regarding the structure of the terminal areas of the fungal hyphae.

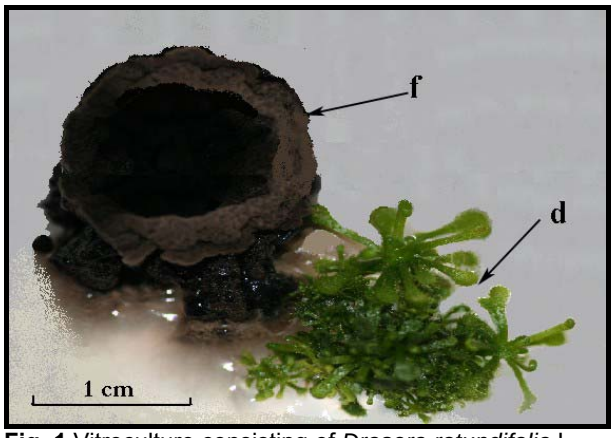
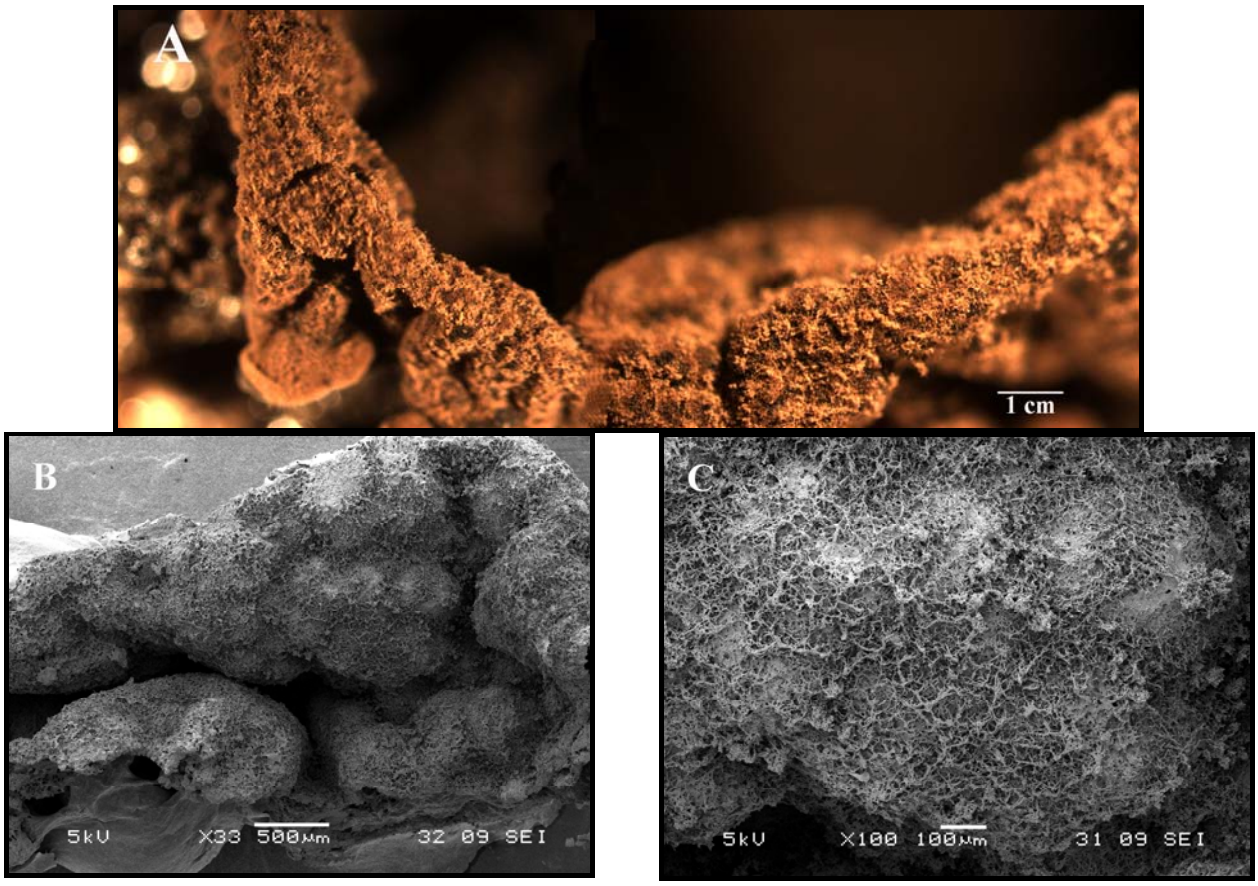


Fig. 1 Vitroculture consisting of *Drosera rotundifolia* L. and a fungus, three months after the operation of the subculture:
at the level of the fungal infection there is evidence of the presence of a crater like fruiting body (d – *Drosera* rosettes; f -fungus fruiting body)



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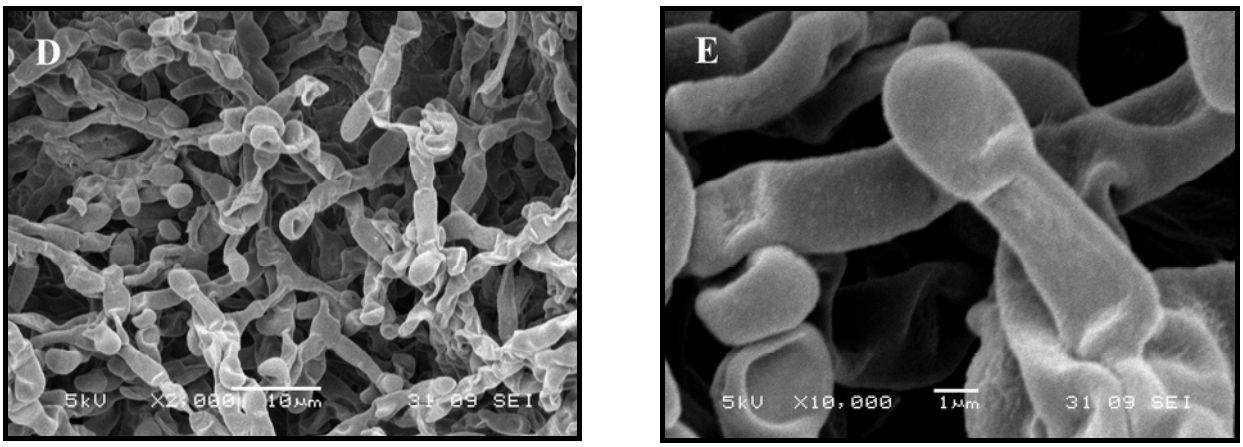

Fig. 2 A-E: Electron microscopic aspects noticed at the level of the basal area of the fungus fruiting body, where: **A**. transversal section through the basal part of the fungus fruiting body; **B-E:** the aspect of the pseudo-plectenchyme generated by the pluricellular fungal hyphae

Starting from the first *ten days* after the operation of *Drosera* subcultures, in only one case – out of the 25 inoculated containers – a small blackish, fungal spot appeared on the surface of the culture media, opposite to the place of inoculation of the *Drosera* propagul (consisting of a minirosette).

Gradually, along with the growth of the *Drosera* inocul, the fungus extended as well.

Approximately *two months* after the inoculation, out of the circular surface of the culture media, in the central area of the fungal mass, extended on a surface of a 30mm diameter, a fruiting body sprang up, having a black-greyish colour, and which at the end of the three months had a 20mm diameter, a 6-7mm height, a circular contour, concave in the central part (resembling a thimble, cavity up) and with about 2mm thick walls.

Very close to this (fig. 1) was the *Drosera* rosettes colony, having intense green colour, which exhibited an intense multiplication.

During the whole experiment there were not any noticeable phenomena of incompatibility, or toxicity, or allelopathic reaction.

In figure 2 A – E pictures of the structure of wall of the fruiting body of the fungus are presented, as seen at the scanning electron microscope. In order to visualise the disposition of the hyphae network of the fungus, the prepared, vitrified through freezing with liquid nitrogen, was fractured, and in the fractured area we could identify a dense network of hyphae, which form a dense pseudo-plectenchyme whose cellular hyphae, unfortunately, have not allowed the taxonomic identification of the fungus yet. Subsequent research is to shed light on this issue.

CONCLUSIONS

This experiment had the advantage of facilitating our seizing of a compatibility phenomenon between a phytoinoculs – with physiological particularities characteristic to carnivorous plants, such as *Drosera* – and a fungus, which developed in the vicinity of the minileaflets colony a vigorous fruiting body that reached up way beyond the level reached by the leaflet rosettes. It is a unique case that which we managed to seize in the 33 years of experiments in the field of vegetal biotechnology, favouring the accomplishment of an enduring coculture between a phytoinoculs and a saprophyte fungus.

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