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THE PRELIMINARY ATTEMPTS OF IN *IN VITRO* REGENERATION FROM PETIOLES OF RECALCITRANT SPECIES OF *CEPHALOTUS FOLLICULARIS* LABILL.

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Cephalotus follicularis Labill. is representative of the extraordinary carnivorous group of plants. Carnivorous plants with pitcher traps grow in nutrient poor, sunny and wet habitats, they have adapted themselves to growth in bogs, sandy soils and obtain some nutrients (e.g. nitrogen and phosphate) from insects and other arthropods or protozoa, even from small mammals occasionally (Król *et al.* 2012).

C. follicularis belongs to the monotypic family Cephalotaceae, and it is the endemic plant of south-western Australia. This Australian pitcher plant is heterophyllous with different types of leaves, specialized either for prey capture or photosynthesis. In the spring, non-carnivorous leaves responsible for photosynthesis, while later, in the summer the second type – carnivorous pitcher-shaped leaves occur (Pavlovic 2011).

In this paper we present the first observation of tissue culture of *C. follicularis* for regeneration protocol, taking into account the absence of organic and inorganic nitrogen as well.

We kindly achieved the *in vitro* plants of *C. follicularis* clones: 00001/01 and 0004/03 from collection of Mr. Kamil Pásek (http://www.bestcarnivorousplants.net/) from Czech Republic.

These plants have been maintained on MS (Murashige and Skoog) media, in sterile conditions for several years. Under these conditions the plants are smaller than in the nature, do not develop pitchers properly and do not produce flowers, and intensive multiplication of shoots is observed. Therefore we tried to ensure such conditions, which will enable the proper development of this species in tissue culture. Plants of 00001/01 and 0004/03 clones were transferred to MS solid medium modified by reducing the amount of MS major salts to ½ and ¼ (½ MS and ¼ MS respectively). Plants were placed on these media in two ways: in small groups and individually.

These same rules were applied when using subsequent ½ MS based media supplemented with 8,2 μM 2,4-D with 9,3 μM KIN (first fodder) and 4,9 μM IBA with 18,2 μM TDZ (second fodder). In addition, on the same media we put photosynthetic and pitcher leaves separately. All cultures were kept in light conditions.

It occurred, that in the long term culture (after 47 days) on the ¼ MS medium the plants growing in small groups, started to develop pitcher and non-carnivorous leaves similar to these in the nature. Plants growing individually on the same medium did not change their appearance. The similar situation was observed for 00001/01 genotype, with one difference, bigger quantity of plants created properly shaped pitcher leaves on ½ MS medium. Decreasing the quantity of MS major salts to ½ gave good results in the case of rooting of plants of the *Drosera intermedia* Hayne (Kromer *et al.* 2000) and in *Cephalothus* regeneration system from root mass (Ko *et al.* 2010).

The first symptoms of morphogenesis were noted on 42 day of culture as the direct

organogenesis and somatic embryogenesis. The adventitious shoots and young somatic embryos were observed in 0004/03 on photosynthetic leaves, on the medium supplemented with 2,4-D and KIN. The pitcher leaves did not induce morphogenesis.

Histological analysis confirmed direct morphogenesis. Somatic embryos and adventitious shoots appeared on the petioles without callus formation and originated from epidermal and subepidermal layers of the explants.

Taking into account the specific way of nitrogen uptaking by *Cephalothus* plants, the photosynthetic and pitcher leaves were maintained on the conditions with the lack of organic source of nitrogen and lack of inorganic nitrogen in MS medium. The observations showed that in the absence of inorganic nitrogen in the medium the non-carnivorous leaves become white and slowly degenerate. At the same conditions the pitcher leaves growing in small groups develop well traps, but they are fragile and strong red. In this small group the non-carnivorous leaves are not present.

When the medium is free of organic nitrogen the non-carnivorous leaves become white but still alive, while the pitcher leaves are weak and do not develop well shaped traps. Further investigations are necessary for improving the culture protocols for more efficient plant regeneration and improvement of shoots formation, as well as analysis of the nitrogen influence on leaves of this plant.

Conclusions

1. The time of reaction was long for both genotypes of *C. follicularis* in all applied experimental conditions.

2. These plants prefer the presence of other individuals, and then they develop much better.

3. The callus formation was not observed in tissue culture of *C. follicularis*.

4. The morphogenesis was noted as the somatic embryogenesis and organogenesis on photosynthetic leaves only.

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