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Micropropagation of *Rudbeckia hirta* L. from seedling explants

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Summary: We conducted experiments for developing an *in vitro* micropropagation protocol starting from meristems of *Rudbeckia hirta* L seedlings. We pre-soaked the seeds in sterile ion-exchanged water for 17 hours, and then achieved surface disinfection in two separate steps. First, we used concentrated household sodium-hypochloride solution for 20 minutes and, also for 20 minutes, we applied hydrogen peroxide of 10%, which was followed by washing with sterile ion-exchanged water three times. For the propagation of seedling meristems, the combination of half-strenght solid Murashige and Skoog (1962) culture medium containing 10 mg/l of kinetin or 2 mg/l of kinetin + 0.1 mg/l of 2iP proved to be the most suitable. The average number of shoot-buds developed from the seedling axillary meristem in the best culture media varied between 5 and 17. Without separating them, we inoculated the shoot-bud clusters on MS culture medium containing 2 mg/l of IAA. After four weeks of incubation we obtained elongated shoots which we separated and inoculated into a new culture medium and we obtained elongated roots. The rooted plants were gradually acclimatised in the cultivation room, potted and carried to a greenhouse, and then planted in open field for subsequent observation. By adopting this method, our laboratory started the micropropagation of the superior and/or elite genotypes of the *Rudbeckia hirta* L. being of special value in respect of breeding.

Key words: *Rudbeckia hirta L., in vitro, tissue culture, seedlings, micropropagation, Hungary*

Introduction

The *Rudbeckia hirta* L. is a wild plant native in the American prairies. Among the open-field annual ornamental plants in our parks in Hungary, which can be propagated traditionally by seed, the *Rudbeckia hirta* L. is a species that has been spreading for over a quarter of a century. *Dr. Zoltán Kovács*, Hungarian plant breeder, produced many internationally recognized varieties of it, in the Horticultural Research Institute (Budatétény, Hungary). It is well known that the Carpathian Basin, thus the Great Hungarian Plain, are exposed, to a great extent, to the warming and sometimes extremely dry conditions, typical of the global change in the climate. Under such ecological conditions – in consequence of the reduction of maintenance costs related to public areas and to the increasing demand for long-flowering decorative species and varieties – the *Rudbeckia hirta* L. species is more and more popular. The production and selection of new varieties suitable for open field plantations require the combined use of traditional plant breeding methods and biotechnological means. It is well known that *in vitro* shoot propagation is an efficient tool in the hands of the plant breeders. On the *in vitro* propagation of this species, a brief publication is available, which was prepared twenty years ago by *Kovács & Ladányi* (1985), and we know four additional publications adopting tissue culture technique (*Łuczkiewicz* et al., 1998, 2001, 2002, *Yuan* et al., 1998). In addition, *Ebringerová* et al. (2002, 2003) and *Kochankov* et al. (1989) published some very interesting results from the tissue culture of other *Rudbeckia* species.

The *in vitro* propagation of the *Rudbeckia hirta* L. is possible in several ways. The most suitable one is the micropropagation of parental genotypes and/or positive individuals, having passed a strict avaliation process and further cultivation under greenhouse condition by adopting a method similar to that used for the breeding of new gerbera cultivars (*Kovács & Ladányi*, 1985).

The purpose of our preliminary investigation has been to learn the micropropagation peculiarities of the *Rudbeckia hirta* L. based on meristem tissues of seedling origin. To our hopes, such *in vitro* cultivation experience can be applicable for developing novel micropropagation technique of the appropriate *Rudbeckia hirta* L. genotypes harboring some special breeding value (i.e., for clonal propagation of positive individuals of seedling-origin combined with progeny testing and *in vitro* germ-plasm preservation, as well as for cellular and molecular genetic interventions).

Material and method

We used three different *Rudbeckia hirta* L. lines obtained by means of traditional breeding process. We pre-soaked the seeds in ion-exchanged water for 17 hours, then treated them in a mixture of sodium-hypochlorite (household Hypo,

Hungary) of 100% and 1% of Tween-20 for 20 minutes. We washed them with ion-exchanged water, then continued disinfecting the surface in the solution of hydrogen peroxide of 10% and 1% of Tween-20 for 20 minutes. Afterwards, we washed them with sterile ion-exchanged water for a short period, and then we repeated it for 2×10 minutes. After dripping, we placed the seeds on Murashige-Skoog (1962) (MS) culture medium solidified with agar-agar containing 3% of sacharose, in VegBox plastic tissue culture boxes. The period of germination was 3 to 4 weeks.

We examined the shoot induction capacity of explants in several kinds of culture media and in several concentration of plant growth regulators. We tested the McCown Woody plant culture medium (MWP) and the MS culture medium with a kinetin content of 2, 5 and 10 mg/l. We conducted the further shoot induction experiments in a culture medium containing six sorts of growth regulators. These were the following: 1/2MS + 2, 5 and 10 mg/l kinetin (KIN), 1/2MS + 2, 5 and 10 mg/l of KIN and 0.1 mg/l of 2-isopenteniladenine (2iP). We removed the roots at the root-neck and placed the seedling cuttings (i. e., shoot tip plus whole hypocotyl part) on different culture media and we kept them for 4 weeks. First, we transferred the small axillary shoot clusters onto hormone-free MWP and MS medium, and onto MS medium containing 2 mg/l of indol-3-acetic acid (IAA). After 4 weeks of incubation we divided the more developed shoot clusters into smaller ones, and then we placed them again onto the same culture medium.

After 2 to 3 weeks of incubation the elongated shoots of the clusters were separated and were placed onto different rooting medium. The following media were tested: MS medium containing 0.5 mg/l of IAA + 1.5 mg/l of α-naphthyl-acetic acid (NAA), MS medium containing 2 mg/l of NAA, Linsmayer-Skoog medium (LS) containing 2 mg/l of IAA, MS medium containing 2 mg/l of indol-3-butyric acid (IBA) and MS medium containing 1 mg/l of Picloram (PIC). Under laminar air flow cabinets the rooted plantlets were potted into glass jars of 800 ml containing sterilised Garri Plus® soil mixture and covered them with transparent thin PVC-foil. Before planting the shoots, the potting soil were wetted with sterile ion-exchanged water of 25 ml. Two days later we drilled 9 holes sized dia. 1 mm and, after an additional period of 3 days, we drilled 12 holes sized dia. 1.5×5 mm in the cover. One day later, we removed the PVC-foil cover. The plants were wetted once in every 2 days, with tapwater. This stage lasted 8 days.

We planted the rooted and acclimatized plants in pots of 9 cm, in the above seedling soil. We kept the plants in cultivating room for 18 days and irrigated them with tapwater of 25 ml once in every 2 to 3 days.

Results

The surface disinfection of seeds proved to be efficient for all three *Rudbeckia hirta* breeding lines. During the treatments, the rate of the seeds' germinating capability –

Figure 1 Shoot induction of *Rudbeckia hirta* L. inbred line on MWP + 10 KIN culture medium, after two weeks of incubation

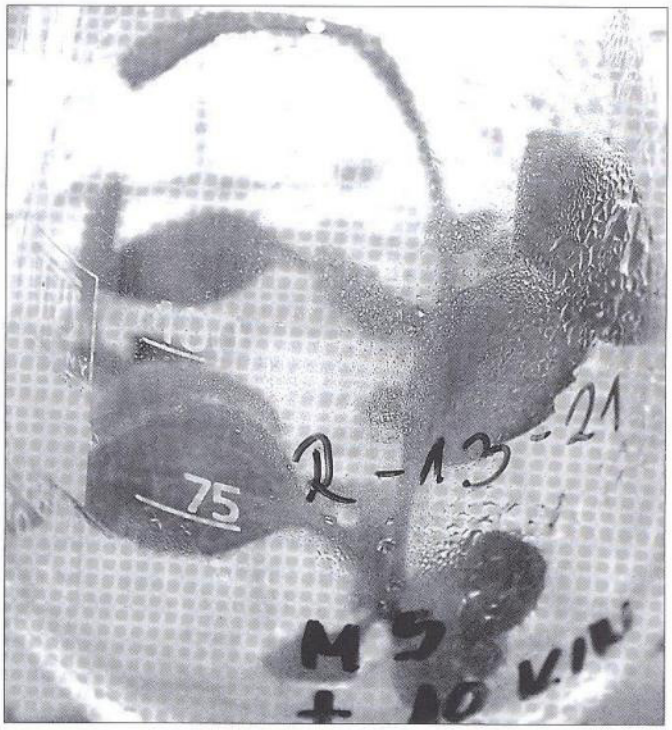


Figure 2 Shoot induction of *Rudbeckia hirta* L. inbred line on MS + 10 KIN culture-medium after two weeks of incubation

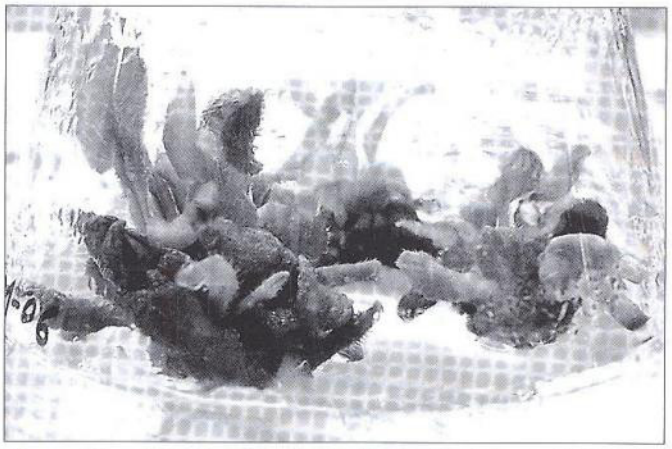


Figure 3 Shoot induction of *Rudbeckia hirta* L. inbred line on MWP + 10 KIN culture-medium after 1 month of incubation

Figure 4 Shoot induction of *Rudbeckia hirta* L. inbred line on 1/2MS culture-medium after 1 month of incubation

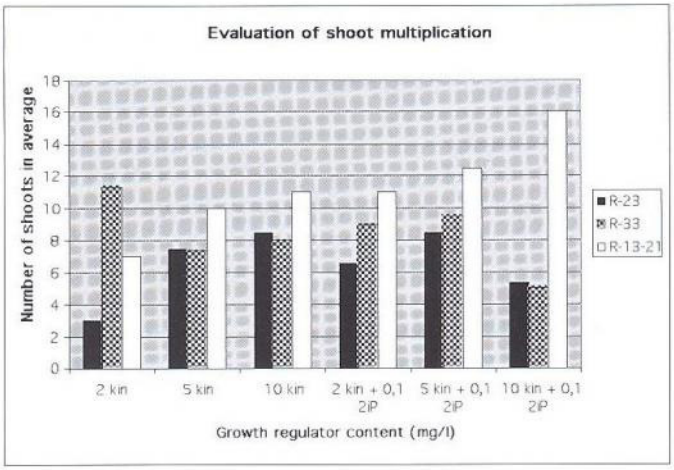


Figure 5 Evaluation of the *Rudbeckia hirta* L. shoot propagation

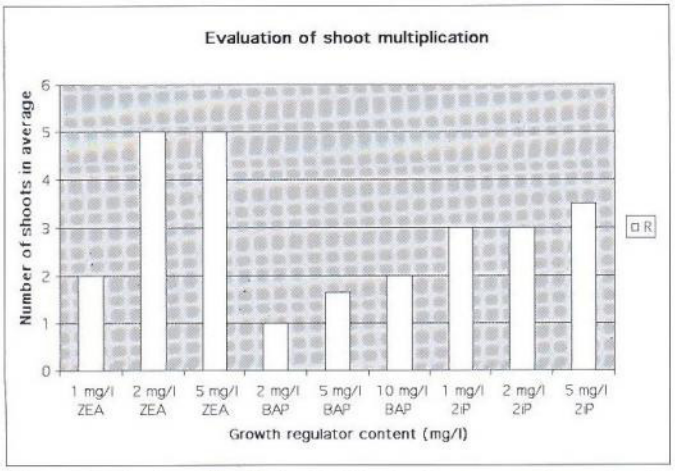
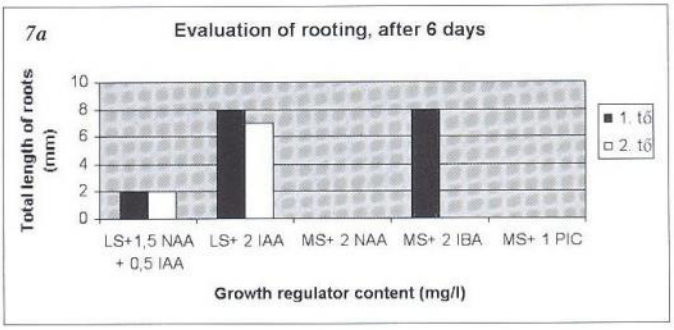
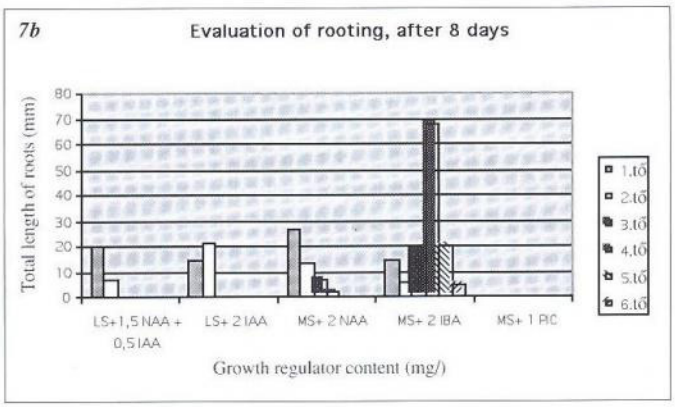


Figure 6 Evaluation of the *Rudbeckia hirta* L. shoot propagation made with the use of cytokinins

depending on the line – diminished to 50 to 67%. For propagating the seedling meristems, the semi-solid MS or full-strenght MWP culture medium combinations containing 10 mg/l of kinetin or 2 mg/l of kinetin plus 0.1 mg/l of 2iP proved to be the most suitable. In these culture media we obtained shoot clusters from the seedling explants containing 5 to 17 mini-shoots of 0.2 to 0.5 cm lenght (*Figures 1–6*).





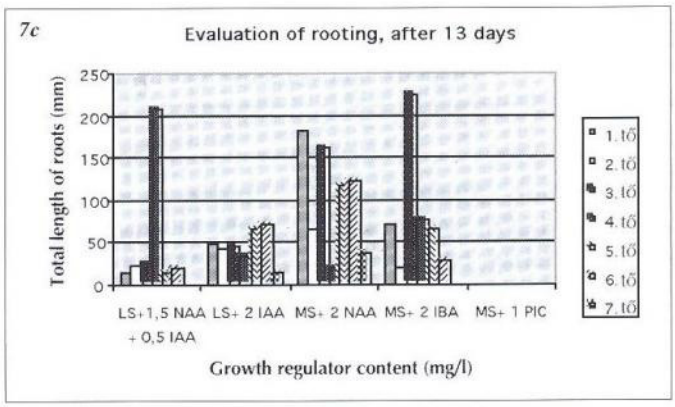


Figure 7a to 7c Evaluation of *Rudbeckia hirta* L rooting

The MS culture-medium containing 2 mg/l of IAA proved to be suitable for further development of the minishoot within the clusters (*Figures 7a–7c*). In this medium, we obtained elongation shoots after four weeks, which we separated, inoculated onto a new culture medium for rooting. We gradually acclimatised the rooted plants in a light-room, potted them and carried them to a greenhouse. Finally, the hardened clones of tissue culture origin were planted to the University's experimental field for further morphological and genetic examination. By adopting the new micro-propagation protocol developed for *Rudbeckia hirta* we started *in vitro* cloning of some elite mother plants selected during the year 2004.

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