Somatic embryogenesis in cultures of the multiflorous chrysanthemum (*Chrysantemum* x *grandiflorum* (Ramat.) Kitam)

Summary

The multiflorous chrysanthemum (*Chrysantemum* x *grandiflorum* (Ramat.) Kitam) is one of the most popular decorative plants. All varieties of chrysanthemums are multiplied vegetatively, by conventional way or with the use of microseedling in *in vitro* cultures. As the demand for seedlings of this plant is enormous, methods to multiply the plant of this species, which would allow to obtain a large number of seedlings in relatively short period of time, are still searched for. Such a method is the somatic embryogenesis, or else the process of embryos initiating from somatic cells in *in vitro* cultures. In spite of its potential, this is a process dependent upon the numerous environmental factors and correlated, to a large degree, with the genotype of plant multiplication. As there is no well worked out method for obtaining somatic embryos of such economically important plant as the chrysanthemum, a trial was undertaken to set forth the maximized number of factors which have an impact on the course of this process.

The aim of the study was to select an optimum plant growth medium to initiate and multiply the callus culture of the multiflorous chrysanthemum, with a particular attention paid to the impact of auxins rarely applied up to now: picloram and dicamba. In the research, a trial was also undertaken to set forth the impact of growth regulators on the intermediate initiations of somatic embryogenesis in callus cultures and direct somatic embryogenesis, with a particular attention paid to the role of polyamines (putrescine, spermine and spermidine) in the course of this process. The callus tissue was induced on growth media solidified with agar, with an addition of auxins: 2,4-D, NAA, IBA, IAA, PIK and DIC, cytokinine BAP as well as polyamines: putrescine, spermine and spermidine. The callus initiated in the first stage of the research was multiplied on solidified growth media, with the addition of the above-mentioned auxins, BAP and polyamines. At the same time, the impact of mineral components content and light on the multiplication of the callus tissue on liquid growth media, in a bioreactor, was set forth.

The callus tissue was multiplied on liquid and solid growth media and fragments of leaves were used as explants in subsequent stages of the research – somatic embryos were induced and then regenerated in one stage on growth media supplemented by a differentiated content of carbohydrates, casein hydrolysate and activated charcoal. The impact of soaked in ABA on embryo conversion was also set forth. The impact of mineral composition of the culture medium, together with organic additives was also determined on the regeneration of somatic embryos. During all stages of research, a particular attention was paid to the impact of the light on the course of callogenesis and somatic embryogenesis.

The purpose of the research was also to determine the scope of variability of regenerants which came into being as a result of somatic embryogenesis, depending on the conditions of regeneration. The DNA was analysed isolated from the callus tissue, multiplied on culture media solidified by agar, for a period ranging from one month to one year, and on liquid culture medium from plants regenerated from embryos by direct or indirect embryogenesis, on the above-mentioned callus tissue. The level of changeability of 50 regenerants obtained in an SE indirect and direct way, pursuant to the record worked out in the paper, was also determined.

Based on the research conducted, it was found out that the picloram and dicamba may be used to induce callus cultures and somatic embryogenesis, however, their efficiency is not significant. An addition of spermidine to the medium has an advantageous impact on the course of callogenesis process and the formation of somatic embryos in additionally lit cultures. The application of this growth regulator at the stage of callus initiation has it that a large amount of callus tissue is created which has an embryogenous nature, and at the stage of somatic embryogenesis induction, it acts favourably on the number of embryos being formed.

The light conditions in which the culture was conducted had a crucial impact on the plants development in *in vitro* cultures. The additional lighting of cultures with red light causes an increase in the mass of the callus tissue and the frequency of the somatic embryos formed. The blue light causes the callus cultures to die and breaks down the formation of somatic embryos. The demand for the light changes, depending upon the stage of research. The callus tissue may be induced and multiplied in the darkness even for six months. It is not possible to form somatic embryos without the access to light, but the darkness has a favourable impact on their sprouting when they have come to an appropriate development stage.

The callus cultures conducted on a liquid MS medium with an addition of 4 $mg \cdot dm^{-3}$ NAA and 3 $mg \cdot dm^{-3}$ BAP in a bioreactor, allow to receive a large mass of callus tissue in a short period of time. However, the tissue which came into being in these conditions is not suitable for inducing somatic embryogenesis.

The suggested record of chrysanthemum multiplication by an indirect somatic embryogenesis covers the initiation of the callus tissue on the MS medium, with an addition 4 $mg \cdot dm^{-3}$ NAA and BAP and 5 $mg \cdot dm^{-3}$ spermidine, its subsequent multiplication on a substrate with an addition of 4 $mg \cdot dm^{-3}$ NAA, 3 $mg \cdot dm^{-3}$ BAP, the initiation of somatic embryogenesis on the medium with an addition of 2 $mg \cdot dm^{-3}$ NAA, 1 $mg \cdot dm^{-3}$ BAP and 5 $mg \cdot dm^{-3}$ spermidine. The conversion of embryos received in this way should be conducted on a substrate which contains ½ of the mineral composition of the medium, according to Murashige and Skoog (1962), with an addition of 45 $g \cdot dm^{-3}$ saccharoze, in the dark.

A large number of somatic embryos may be received when leaf blades are put on the medium, with an addition of 4 $mg \cdot dm^3$ NAA, 2 $mg \cdot dm^3$ BAP and 5 $mg \cdot dm^3$ of spermidine, and then, the embryos are regenerated on a substrate containing ½ of the mineral composition of the medium, according to MS, with an addition of 45 $g \cdot dm^3$ sucrose, in the dark. Based on the evaluation of the genetic uniformity of the regenerats by the ISSR-PCR method, we may claim that the suggested system of regeneration allows to obtain daughter plants with no risk of genetic instability of regenerants.