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STUDY OF THE INFLUENCE OF NEGATIVE TEMPERATURES ON BIOMASS ACCUMULATION AND CELL VIABILITY OF CALLUS AND CELL AGGREGATES OF *Rhodiola rosea* L.

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Abstract

As a result of determining the resistance to the action of different negative temperatures of callus cells and cellular aggregates of *Rhodiola rosea*, it was shown that after exposure of callus to -8°C , only 52% of the cells survived. In the case of exposing the experimental variant of *R. rosea* cell aggregates to -8°C , the value of cell viability was 68%. This suggests that the frost tolerance of cell aggregates is higher than that of callus cells, which indicates that the stress factor to be tolerated is higher, the lower the degree of organization of the biological system.

Key words: *Rhodiola rosea* L., callus, cell aggregation, negative temperatures, viability.

Introduction

The culture of plant cells and tissues is an important tool for fundamental research in plant biochemistry. The available methods ensure the regeneration of differentiated cultures (whole plant, plant organs) and dedifferentiated (callus, cell aggregates and suspensions, protoplasts). Dedifferentiated cultures are useful for studying tissue- or organ-specific biosynthetic pathways, which are not always expressed in differentiated cultures. The latter are convenient for large-scale production of secondary metabolites, the investigation of processes at the cellular and molecular level, offering the advantage of a simplified model system for studying the regulation of vital processes in plants [4].

One of the main environmental factors that determine the survival of plants in a given habitat is temperature. Temperature changes cause changes in the metabolic processes in the plant cell, and excessively high or low temperatures cause stress, damage or even death of plants. Depending on the species, the range of favorable temperatures for plant growth and development is between 10 and 35°C . Temperatures above 35°C and below $5 - 4^{\circ}\text{C}$ are considered as temperatures of the critical transition threshold from favorable to unfavorable conditions [9].

It is known from the literature that cells from cell suspension cultures acclimate to cold developing high tolerance to frost [4]. Cold plant acclimation involves various physiological and biochemical changes, such as cessation of growth, reduce in water content, accumulation of abscisic acid, changes in cell membranes, synthesis of sugars or cryoprotective proteins, allow to avoid frost damage [2, 7]. Under natural conditions of high altitude, during the vegetation period *Rhodiola rosea* L. plants are often subject to the influence of low and even negative temperatures. In previous research we have shown [1] that after the application of low positive temperatures, at the stage of transition to the stationary phase the percentage value of the callus growth index of *R. rosea* registers an increase of over 40% compared to that characteristic for the control variant. Considering that in natural habitats *R. rosea* plants grow in harsh and accidental climatic conditions, including extreme temperatures, we aimed to determine the resistance of callus cells and *R. rosea* cell aggregates to the action of various negative temperatures. The general objective of the research is the influence of short-term negative temperatures on the accumulation of biomass and the viability of callus cells and *R. rosea* cell aggregates.

Materials and methods

Callus induction was performed from the leaves of sterile plants of *R. rosea* L. Leaf segments were inoculated on Murashige-Skoog (MS) medium [3], namely 0,6% agar supplemented with 1,5 mg/L BA and 0,5 mg/L NAA, and placed in the culture chamber with a photoperiod of 16 hours and 8 hours in the dark, light intensity of 2000 lx. To maintain the callus, every 40 days of cultivation callus fragments weighing about 2 g are transferred to fresh MS medium with the same composition.

In order to obtain the cell aggregate culture, 2 g of 20-day-old callus fragments were inoculated on the base medium MS - liquid (without agar), supplemented with 1.5 mg/L BA and 0.5 mg/L NAA. The cultivation vessels were placed on a shaker at 75 rpm in the cultivation chamber. To maintain the culture, every 20 days of culture, the cell aggregates were transferred in fresh MS medium.

For testing the short-term action of negative temperatures, the cultivation of callus and cell aggregates was performed at the temperature regime of +26°C (control) and exposure to -16°C, -14°C, -12°C, -8°C, -4°C for 8 hours (experimental variants) on the 20th and 12th day, respectively, after the passage (at the beginning of the exponential growth phase).

The determination of the viability of callus cells and cell aggregates of *R. rosea* was performed by UV-VIS spectrophotometric method at 660 nm [5].

The results obtained using the methods presented above were statistically processed, determining the arithmetic mean, standard deviation, coefficient of variation and degree of correlation R^2 by computerized methods.

Results and discussions

Evaluating the viability of cells, after exposure to negative temperatures (-8°C, -12°C, 14°C and -16°C) for 8 hours, their resistance to shock caused by short-term exposure to these temperatures was determined. Cell viability was expressed in % compared to control (callus cells that were not frozen). Data on the action of negative temperatures on cell viability (%) and accumulation of *R. rosea* callus biomass on solid medium are presented in Fig. 1-3. From the pictures shown in Fig. 3 we can see that exposure to -14°C is critical for the viability of callus cells, the degree of chlorophyll degradation becomes extremely pronounced. The data shown in Fig. 1 clearly demonstrates that once the temperature decrease after exposure to shock at negative temperatures the percentage of dead cells increased practically linearly starting with 24% in the control variant and ending with 89% in the callus previously exposed to shock by -14°C. If 67% of the cells survived after exposure to -4°C, only 52% of cells survived after exposure to -8°C. The decrease of the exposure temperature to -12°C and -14°C caused the increase of the destructive processes and reduce of the percentage of viable cells up to 20% and 11%, respectively. Therefore, the temperature at which the loss of 50% of the total number of cells occurred is about -8°C. The decrease of the percentage of viable cells with the decrease of the negative exposure temperature caused the even more significant diminish of the biomass accumulation (Fig. 1).

Fig. 1. The influence of negative temperatures during 8 hours, applied on the 20th day of cultivation, on the viability of cells and the accumulation of *R. rosea* callus biomass, at the end of the cultivation period (on the 40th day).

Fig. 2. Dependence between biomass values and those of the viability of *R. rosea* callus cells, treated with negative temperatures during 8 hours on the 20th day of cultivation, at the end of the cultivation period (on the 40th day).

Fig. 3. Images of *R. rosea* callus exposed to shock with negative temperatures (-8°C, -12°C and -14°C) during 8 hours on the 20th day of cultivation and subsequently maintained for 20 days at a temperature of +26°C.

Callus biomass decreased from 12,3 g (control) to 10,8 and 7,3 g in the callus variant obtained after exposure to -4°C and -12°C, respectively. The mentioned values constitute the decrease of the callus biomass ratio in the respective variants compared to the control by 12 and 41%.

As mentioned above, the callus of *R. rosea*, subjected to the action of negative temperatures, recorded both a decrease in cell viability and a biomass growth index. The comparison of the legitimacies of

the change of these values demonstrates the existence of a positive correlation between the biomass and the viability index, the correlation coefficient being $R=0.9722$ (Fig. 2). Thus, we can conclude that increasing the percentage of viable cells by one unit leads to an increase in the final biomass of the callus by 0.0799 g. It follows that the division of callus cells under artificially created conditions is limited.

Similar research on the action of negative temperatures on cell viability and biomass accumulation was performed with *R. rosea* cell aggregates cultured in liquid medium. The data obtained are presented in Fig. 4-6. We mention that, as in the case of *R. rosea* callus culture on a solid medium, the negative temperatures caused effects of deterioration. For example, in the experimental variant after exposure to -12°C and -14°C , the mass of the cell aggregates in the liquid nutrient medium was reduced compared to that of the mass in the control variant by 2,8 g and 3,1 g, respectively, which is the decrease by 24% and 27% compared to the control. The viability of the cells after exposure to shock with a temperature of -12°C -14°C decreased from 79% to 19% in the control variant and 16% in the experimental variants.

Fig. 4. The influence of negative temperatures during 8 hours, applied on the 12th day of cultivation, on the viability of the cells and the accumulation of biomass of *R. rosea* cell aggregates, at the end of the cultivation period (on the 20th day).

Fig. 5. Dependence between biomass values and those of cell viability of *R. rosea* cell aggregates, treated with negative temperatures during 8 hours on the 12th day of cultivation, at the end of the cultivation period (on the 20th day).

Fig. 6. Images of *R. rosea* cell aggregates exposed to shock with negative temperatures (-8°C , -12°C , -14°C and -16°C) during 8 hours on the 12th day of cultivation and subsequently maintained for 8 days at a temperature of $+26^{\circ}\text{C}$.

If after exposure of the callus at -8°C only 52% of cells survived, in the case of exposure in the experimental variant of *R. rosea* cell aggregates to -8°C , we noticed that the viability value of the cells is 68%. This suggests that the frost tolerance of cell aggregates is higher than that of callus cells. As in the case of the action of ionizing radiation that the tolerance to the stress factor is the higher, the lower the degree of organization of the biological system (Bergonie-Tribondeau rule) [8]. These results show that lethal temperatures for cell aggregate culture can be considered values lower than -12°C .

The dependence of callus biomass on the percentage of viable cells at the end of the cell aggregate culture period (Fig. 5) shows that the decrease in cell aggregate biomass is directly proportional to the decrease of viable cells, the correlation coefficient being $R=0.9434$. As expected, the angle of inclination of this dependence for cell aggregates is equal to 0.0445 (Fig. 5). It is smaller compared to that characteristic for callus cells 0.0799 (Fig. 2). This further proves that the cells of cell aggregates are more tolerant to frost compared to those of the callus.

It is known that the action on plants with negative temperatures, the dose of which is moderate, can ensure their acclimatization [2]. They are characterized by increased resistance to negative temperatures, which would otherwise be lethal [6]. The formation of mechanisms of adaptation to low temperatures involves various levels of cellular metabolism, the condition of membranes, changes in gene expression, which leads to the accumulation in cells of stress proteins and osmo- and cryoprotective compounds [2]. These changes are meant to ensure that the plant organism not only survives unfavorable conditions, but also returns to normal functioning after the ambient temperature reaches the optimum required. It is also known that the level of tolerance manifested by a particular plant depends on the species, tissue or type of cells subjected to stress [6].

Therefore, the results obtained show that the cells of cell aggregates are more resistant to the action of negative temperatures, tolerating temperatures about 4°C lower compared to callus cells. This suggests a tendency to decrease frost resistance with increasing complexity of the relationships between callus cells compared to those of cell aggregates.

Many researchers have examined the effect of low temperature on the growth of different plant species in both *in vivo* and *in vitro* culture. However, research in this direction with the culture of *R. rosea* has not been performed.

Conclusions

Cellular aggregates survive exposure to temperatures 4°C lower compared to callus cells, which is confirmed by Bergonie and Tribondeau law on decreasing the stress resistance of biological systems with a higher degree of complexity.

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