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The Effect of MRET Activated Water on Callus Tissues

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Abstract

This particular article relates to study of the effect of MRET Activated water on the growth and development of callus tissues. Callus tissues are characterized by a nonspecific growth of non-differentiated cells. As a result, an unsystematically growing biological tissue, in which there are no specific and functional attributes, is formed. Such properties of callus tissues are related to the fact that the growth and the cell fission in initial vegetative fragments are accelerated by the influence of special chemical components. In such a mode of the accelerated development, cells do not reproduce the functional organs of a plant. The importance of the study of callus tissues is that they, in a certain sense, are indirect analogs of some diseases characteristic of animal and human origin (for example, psoriasis or cancer). This article provides some evidence that MRET activated water with the modified physical and electrodynamic characteristics may enhance specific molecular mechanisms in living cells of botanical origin. In particular, the discovered significant reduction of activated water viscosity should influence essentially the intracellular/extracellular water exchange in the living cells. The modification of conductivity and dielectric permittivity should render a strong influence on the movement and the characteristics of ions in water (Smirnov, 2007, 2008). The localization of ions is particularly important, since each of the cellular complex structures is expected to have a specific role in the electrical function of the cells and as a result it may affect cells development and division. To verify the validity of the proposed hypothesis the study on callus tissues was conducted at the Institute of Cellular Biology and Gene Engineering of the National Academy of Science, Ukraine.

Keywords: viscosity, dielectric permittivity, Callus tissue, MRET water.

Introduction

The main characteristic of cellular juice is the large content of water which reaches 98% in relative concentration. It implies that water is the main element of a biological system. The introduction of water with any particular characteristics into a cultural medium will definitely influence the basic growing parameters of cells. Callus tissues are characterized by a nonspecific growth of non-differentiated cells. As a result, an unsystematically growing biological tissue, in which there are no specific and functional attributes, is formed. Such properties of callus tissues are related to the fact that the growth and the cell fission in initial vegetative fragments are accelerated by the influence of special chemical components. In such a mode of the accelerated development, cells do not reproduce the functional organs of a plant.
The studies of the influence of MRET activated water on callus tissues were carried out under the supervision of N.A. Matveeva, Ph.D. at the Institute of Cellular Biology and Gene Engineering of the National Academy of Science, Ukraine.

Method and Materials

In order to study the influence of two fractions of MRET activated water (the duration of activation was 1 hour and 0.5 hour) there was prepared the agar-based sterile Murashige–Skoog media with the addition of 2 mg/L of 2,4-dichlorophenoxyacetic acid and 0.5 mg/L of kinetin (Murashige and Skoog, 1962). These biologically active substances are the initiators of accelerated cellular divisions resulting in the

formation of quickly and irregularly growing non-differentiated cells from the ordinary differentiated cells of plants. The experiments on the formation of callus tissues were carried out on segments of a stalk of the plant *Solanum rickii*. Firstly, it was prepared a concentrated solution of the cultural media on the basis of a small amount of the regular distilled water. After the sterilization and the cooling, this solution was diluted with the corresponding fraction of MRET activated water up to the necessary ratio of activated and regular water. The further studies showed that the degree of such a dilution is very important and appreciably determines the efficiency of the prepared solutions.

In the first series of experiments for the preparation of the studied medium there was taken one part of regular distilled water and four parts of water activated for 1 h or 0.5 h. In the control experiment, only initial non-activated distilled water was used for the preparation of the sterile cultural media. For each experiment 20 segments of a stalk of *Solanum rickii* were sowed in every Petri dish that contained the cultural media and the necessary biologically active substances

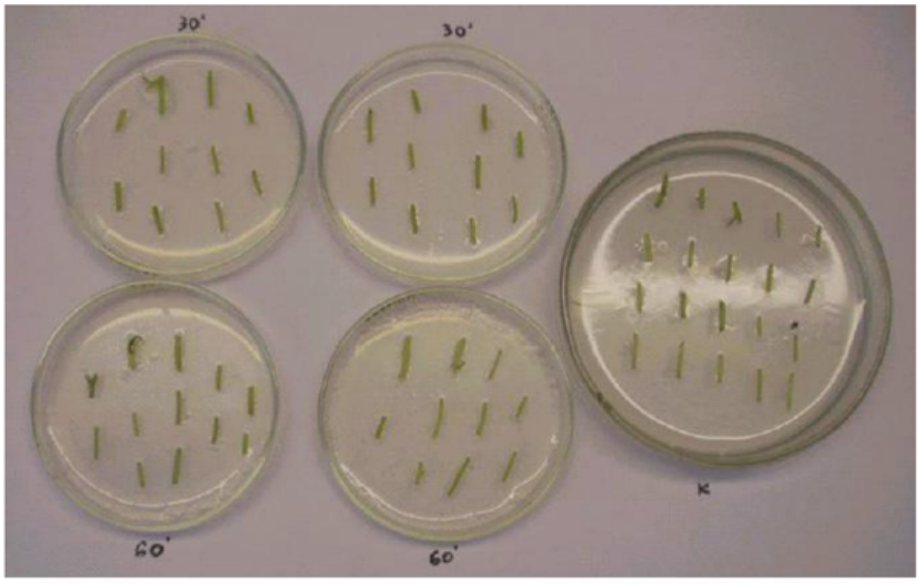
Results

The data of the average weight of initial segments are presented in Table 1. The photos with the general view and the magnified parts of Petri dishes with stalk segments before the beginning of the first experiment of callus tissues cultivation are presented in Figs. 1 and 2. From the data presented in Table 1 and the photos, it is clear that the initial stalk segments were approximately identical.

Table 1. Increment of callus tissue in activated water

Initial average weight of one segment, mg	Fraction of water (the duration of activation)	Final average weight of one segment, mg	Average increment of the weight of one segment, mg	Coefficient of inhibition of the growth of callus tissue
20.2 ± 0.1	1h	20.5 ± 0.1	0.3 ± 0.2	350 - 1800
20.8 ± 0.1	0.5 h	21.2 ± 0.1	0.4 ± 0.2	300 - 900
19.6 ± 0.1	control	193.5 ± 0.1	173.9 ± 0.2	1

First of all, it is worth to note that the survivability of stalk segments in all Petri dishes was identical and equal to 100% in all the experiments without exception. This result has confirmed that MRET activated water is non-toxic and the stalk segments of *Solanum rickii* are normally growing.



t_{act} = 1.0 h t_{act} = 0.5 h Control

Figure 1. The general view of Petri dishes with stalk segments before the beginning of the first series of experiments on the cultivation of callus tissues. The media composition in different Petri dishes: Control is the cultural media on the basis of initial non-activated water; *tact* = 1.0 h means the cultural media containing 20% of non-activated water and 80% of water activated for 1 h; *tact* = 0.5 h means the cultural media containing 20% of non-activated water and 80% of water activated for 0.5 h.

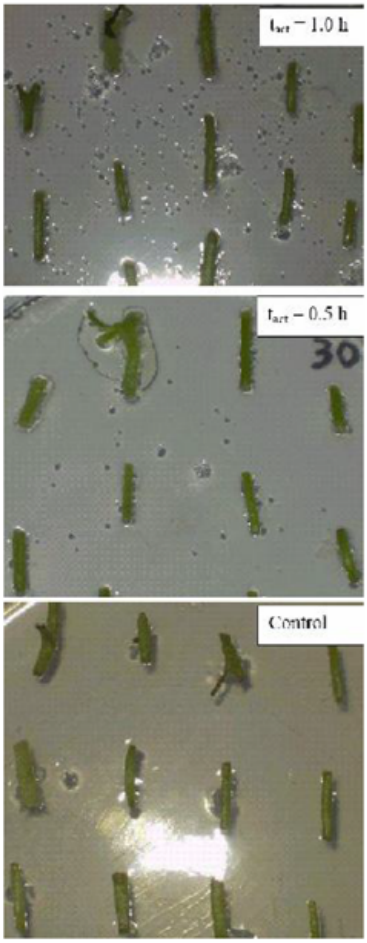


Figure 2. The magnified parts of a working field of Petri dishes containing the initial segments of a stalk before the beginning of the first series of experiments on the cultivation of callus tissues. The cultural media composition in different Petri dishes corresponds to the data presented in Fig.1.

The results of experiments on the cultivation of callus tissues for 14 days at a temperature of 20°C are presented in Table 1 and Fig. 3. The last column in Table 1 characterizes the coefficient of growth inhibition *K* of callus tissues determined as the ratio of the relative increment of the weight of one segment in the control experiment $(\Delta M/M)_{control}$ to the analogous increment of the weight of one segment $(\Delta M/M)_{act}$ of a specific fraction of activated water.

Table 2. Increment of callus tissue in activated water (the repeated series of experiments).

Fraction of water (the duration of activation)	Initial average weight of one segment, mg	Final average weight of one segment, mg	Average increment of the weight of one segment, mg	Coefficient of inhibition of the growth of callus tissues
1 h	22.2 ± 0.1	22.7 ± 0.1	0.4 ± 0.2	470 - 1000
0.5 h	29.8 ± 0.1	30.1 ± 0.1	0.3 ± 0.2	550 - 2500
control	24.6 ± 0.1	257.9 ± 0.1	233.3 ± 0.2	1

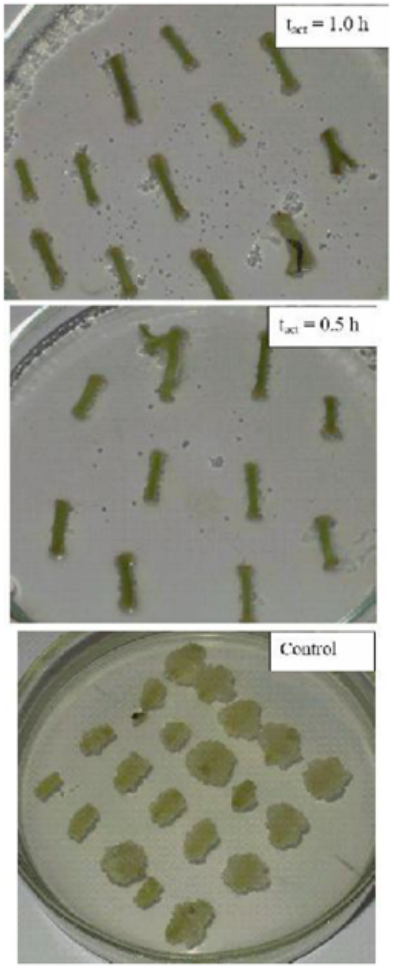


Figure 3. The magnified parts of a working field of Petri dishes containing segments of a stalk in two weeks after the beginning of the first series of experiments on the cultivation of callus tissues. The composition and the arrangement order of Petri dishes are completely identical to those presented in Fig. 2.

It follows from these data that, at a given degree of liquid dilution in the composition of the Murashige–Skoog cultural media (20% of non-activated water and 80% of water subjected to the preliminary activation), both fractions of MRET activated water render extremely strong inhibiting influence on the growth of callus tissues. The great value of the coefficient of inhibition of the development of callus tissues (K = 300 . . . 1000) indicates practically full suppression of the process of cellular division of non-differentiated cells and the termination of the growth of callus tissues in the volume of the activated medium.

t_{act} = 1.0 h t_{act} = 0.5 h Control

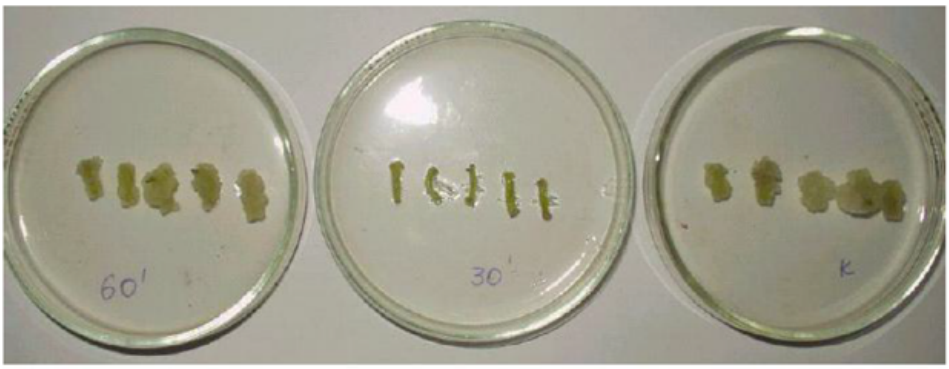
Figure 4. The general view of Petri dishes containing segments of a stalk in two weeks after the beginning of the second (repeated) series of experiments on the cultivation of callus tissues. The cultural media composition and the ratio of non-activated and MRET activated water are completely identical to the first series (see the caption of Fig.1).

To confirm this effect, the second (repeated) series of experiments was carried out under completely identical conditions, with the same media, at the same temperature, and with the same full duration of experiments. The results of these studies are presented in Table 2 and in Fig. 4. It is clear that the results of the second (repeated) series of experiments performed under identical conditions completely confirm (within statistical error) the conclusion about both the extremely strong inhibition of the process of cellular division of non-differentiated cells and practically full termination of the growth of callus tissues on both fractions of the activated media (Vysotskii *at el, 2009*).

The third series of experiments was devoted to the study of the influence of a degree of mixing of ordinary and MRET activated water on the inhibition of the growth of callus tissues. In these experiments, for the preparation of the studied media, it was taken one part of ordinary distilled water and 2.5 parts of MRET activated water (activated for 1 h and 0.5 h respectively). In the control experiment, only initial non-activated distilled water was used. The duration of this series of measurements was also equal to two weeks. The results of these measurements are presented in Table 3 and in Fig. 5.

Table 3. Increment of callus tissue in a diluted mixture of activated and regular water (the third series of experiments)

Fraction of water (the duration of activation)	Initial average weight of one segment, mg	Final average weight of one segment, mg	Average increment of one segment, mg	Coefficient of inhibition of the growth of callus tissue
1 h	22.9 ± 0.1	131.8 ± 0.1	108.9 ± 0.2	1.39
0.5 h	20.8 ± 0.1	26.0 ± 0.1	5.2 ± 0.2	25.5 - 27.5
Control	22.6 ± 0.1	171.8 ± 0.1	149.2 ± 0.2	1



t_{act} = 1.0 h t_{act} = 0.5 h Control

Figure 5. The general view of Petri dishes containing segments of a stalk in two weeks after the beginning of the third series of experiments on the cultivation of callus tissue in the media prepared with a diluted mixture of activated and regular water.

Conclusion

It is possible to make several additional remarks concerning the features and results of our studies. Before realizing the third series of measurements, we would expect *a priori* that a rather small reduction of the relative concentration of MRET activated water from the initial value of 80% (in the first and second series of experiments) up to 71% (in the third series) should result in the same small (within the limits of 10%) predicted reduction of the inhibition effect of the growth of callus tissues for both fractions of activated water. However, such simplified prediction turned out erroneous, and the results turned out to be essentially different. It follows from the obtained results that the dilution of MRET activated water with regular non-activated water does not lead to a monotonous reduction of the effect of inhibition identically for both fractions. The effect turned out much more complex and interesting. From the obtained data, it is clear that the cultural media prepared on the basis of the mixture of regular water and activated water (with the duration of activation of 0.5 h and the ratio of 1: 2.5) is characterized by the essential, though considerably weaker, effect of inhibition. The effect of inhibition for this type of media remains very big ($K \approx 25$), though its value has decreased 20 times in comparison with the media with the mixture of water in the ratio of 1: 4. Contrary to that, for the cultural media prepared on the basis of the mixture of regular water and MRET activated water with the duration of activation of 1.0 h and the ratio of 1: 2.5, the effect of inhibition of the

growth of callus tissues and the influence on the cellular division is manifested slightly and is characterized by a rather small coefficient of inhibition, $K \approx 1.4$. Taking into consideration that MRET activated water has non- toxic property, we may assume that a small impurity of regular non-activated water essentially alters the character of influence of activated water on the cell surface.

Within the framework of such a concept, it is necessary to accept that there is a certain threshold for the surface energy, the overcoming of which makes the cell division disadvantageous. In this case, little changes of the relative concentration of activated water result in similar little changes of the surface energy which can appear lower than the threshold in this case, and the process of division becomes allowed. This prediction may have very important consequences and can promote the development of a real mechanism of the essential influence on biological objects without the presence of side effects, which are undoubtedly manifested on the use of various chemical preparations or ionizing radiation for the same purpose. It is obvious that the determination of the surface energy threshold is one of those perspective tasks of fundamental biology. A special importance of the discovered effect of strong inhibition of irregularly growing non-differentiated cells of botanical origin is related to the fact that cells of the animal origin have similar properties. Thus, hypothetically MRET activated water may inhibit irregular growth of non-differentiated cells of animal origin as well.

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