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RESISTANCE OF CELLULAR VARIANTS OF *NICOTIANA TABACCUM* L. PLANTS TO WATER STRESS

Aim. It has been established that resistance to some heavy metal cations is combined with resistance to osmotic stress. Thus, resistance to barium cations correlates with resistance to water stress. Therefore, the aim of the experiment was to compare the resistance of primary and secondary calli to modelled stresses. ***Methods.*** The object of testing was tobacco, a plant sensitive to water deficit. The level of resistance of the variants was assessed by the relative increase in fresh weight under osmotic stresses: (salinity, 25.0 g/l of sea water salts; sodium sulphate, water stress). Both concentrations are lethal for wild-type tobacco cell cultures. ***Results.*** Ba-resistant tobacco cell cultures were obtained. Tobacco callus were resistant to lethal modelled stresses. The Ba-resistant culture was developed on medium with the addition of sea water salts and sodium sulphate. ***Conclusions.*** The phenomenon of resistance was selected as a result of primary selection on the medium with heavy metal ions. The level of osmotic resistance did not decrease with increasing cultivation time.

Keywords: cell selection, tobacco, water deficit, Ba^{2+} ions, stress, resistance, proline.

The problem of stress/resilience in organisms has been and will continue to be the most challenging of the fundamental problems. Growing environmental degradation and dramatic climate change on a global scale make this problem a priority. Along with solving theoretical issues, there is an urgent need for new forms of plants that have exceptional characteristics that could ensure survival both under conditions of constant stress and changes in environmental parameters. Among abiotic stresses, the most aggressive is osmotic stress, or salinity. In connection with global warming, the task of obtaining plants with an increased level of salt tolerance is becoming a priority [1, 3, 9]. It is obvious that traditional ways of achieving goals are no longer sufficient to meet growing needs. There is a need to come up with new ideas. Alternative methods, such as biotechnological methodologies and cellular selection, are beginning to play a priority role.

Cellular selection has more than a decade of dynamic development and positive results. However, like any technology, this method requires constant improvement. [5, 11, 15]. This method is used to obtain plants with an increased level of resistance to abiotic stress. At the same time, over a long period of use, cellular selection often did not provide tangible benefits. A number of authors have noted its significant drawbacks [5, 10, 11]. Therefore, it became necessary to significantly expand and revise this method in the direction of new directions. However, as in any methodology, it is necessary to take into account the key positions, namely, the choice of the type of selective agent, its initial concentration and the selection marker. If a stressor with a wide range of toxic effects is used as a selective factor, it is possible to select variants with complex stress resistance. Moreover, in this case, this characteristic will be a consequence of genetic changes, but not physiological adaptation.

A number of stress agents release heavy metal ions (HMI), which are particularly toxic in trace amounts [6, 7, 15]. They are characterised by a wide range of pathological effects. Based on these data, we proposed a hypothesis about the targeted use of some HMI to select variants resistant to abiotic stresses. Barium cations (Ba^{2+}) were chosen for the primary selection. Barium ions were chosen because their physiological competition with K^+ ions was established. Barium ions prevented the movement of K^+ ions. On the other hand, it is known that salinity causes a critical loss of potassium. Taking these two facts into account, a hypothesis was proposed to use Ba^{2+} ions to obtain salt-tolerant forms of plants [2, 12, 13].

Since the toxic effect of salinity is largely due to the loss of K^+, it was hypothesised that salt-resistant variants could be selected in in vitro model systems containing Ba^{2+} ions. Subsequently, resistant tobacco cell lines were selected on selective media containing lethal doses of Ba^{2+} ions for cell cultures.

Materials and methods

Ba^{2+} ions were used to create the in vitro model system. The selective concentration was determined in previous experiments. It was considered to be the smallest amount of stressor that stopped the development of the wild-type cell culture. If the vital activity of the culture was restored after returning to normal conditions, the concentration of the stressor was increased. This prevented the selection of adaptants. If the ion used required special modifications of the medium, such changes were made taking into account the preservation of the qualitative and quantitative composition of the culture media [3, 7, 10, 11].

It was necessary to establish the applicability of this approach in crop breeding. The test was carried out using tobacco cell cultures. Since the aim of the experiment was to select comprehensively resistant variants, the cultivation conditions were always changed during each passaging. The alternation of stress 1 – control – stress 1 or stress 1 – control – stress 2 was arbitrary. The number of passages on a single medium (cultivation period) was also arbitrary.

In cell breeding, the phenomenon of a stable cell culture is of great importance. A culture that can only withstand stressful pressure when growth processes are completely inhibited and resume proliferation only under normal conditions is often considered resistant. In our experiments, we selected cultures that maintained growth and development throughout the entire period of cultivation. To determine the stability, the relative biomass growth rate (Δm) was constantly monitored.

$\Delta m = (m_1 - m_0) / m_0$; where m_0 is the mass of cells at the beginning of the passage, m_1 is the mass of cells at the end of the passage.

Results and discussion

Ba-RCL were obtained as a result of primary selection on medium with a lethal concentration of Ba^{2+} ions. After the growth of cell biomass on the selective selection medium, callus was simultaneously passaged on the control medium (n. w.) and on selective media with Ba^{2+}, seawater salts, and sodium sulfate. Figure 1 shows the relative biomass growth (Δm) of different Ba-RCL of tobacco when cultivated under different stress conditions.

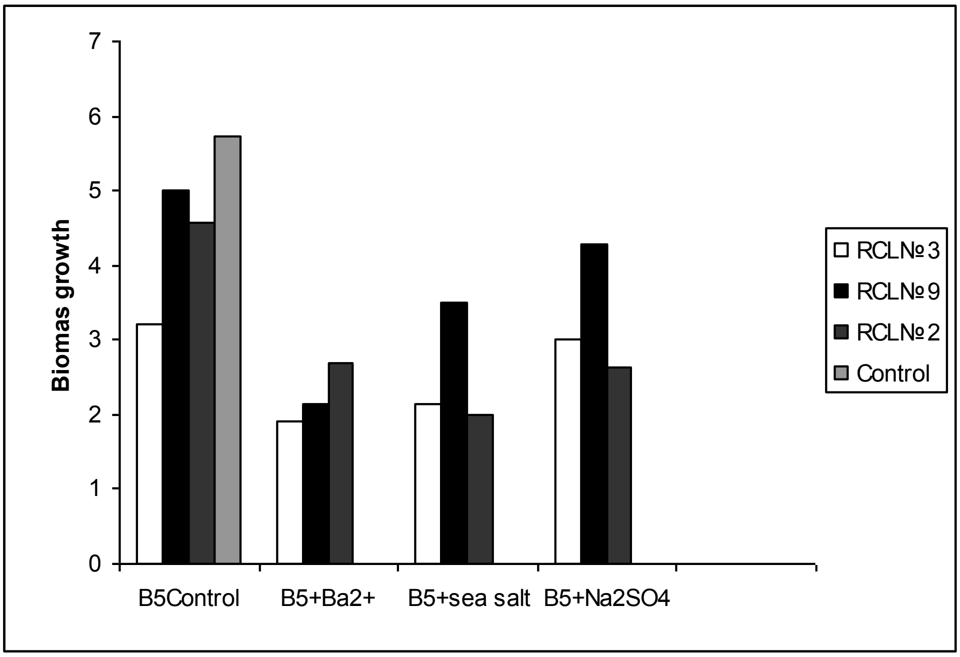

Fig. 1. Relative increase of tobacco Ba-RCL biomass during cultivation under stressful conditions *in vitro*. Concentrations of stress compounds were added in lethal concentrations.

Figure 1 shows that Ba-RCL are characterised by a comprehensive resistance to all modelled stresses. However, the maximum relative increase in cell biomass was observed in the medium with sodium sulfate. It can be assumed that high resistance to sulfate salinity is associated with resistance to barium cations, since the literature shows a higher toxicity of sulfates [4, 8, 11]. It is known that Ba^{2+} ions affect the transfer of K^{+} ions. Ionic interaction/antagonism has been reported for other cations as well [7, 11, 12]. Thus, salinity resistance may be related to the kinetics of toxic cation transport or the structure of the transporters themselves. It is also possible that cross-resistance to Ba^{2+} ions and different types of salinity is associated with changes in membrane viscosity caused by changes in the degree of lipid saturation [13]. One of the established effects that a heavy metal ion can exert in biological systems is the function of a structure stabiliser. This function is also performed in those simple cases when the orientation effect of ions in a complex compound is meant. And in the case when a metal stabilises the structure of proteins in an enzyme without affecting the catalysis process as such. And in the case when a metal ion forms a catalytic structure with qualitatively new characteristics [8, 14]. However, the latter event is only possible in the presence of a metal ion. In our case, the cross-stability of Ba-RCL is confirmed by the proliferation and growth of the culture on any selective medium. Thus, most likely, such an event is due to the compartmentalisation of toxic ions.

This assumption was confirmed by determining the content of free proline in resistant cell lines. Figure 2 illustrates the dynamics of fluctuations of this amino acid during the cultivation of callus on media with different types of salinity.

During one passage of subcultivation, any developing cell culture goes through a number of stages. Among them, the most important are the logarithmic growth stage (7–14 days) and the stationary growth stage (14–21 days). During the logarithmic growth stage, biomass increases due to cell division, and the stage is characterised by a maximum of mitoses. The stationary growth stage is characterised by an increase in individual cell compartments due to the redistribution of intracellular components.

During long-term cultivation under saline conditions, plants and cell cultures accumulate significant amounts of toxic sodium ions [3, 12–14]. This leads to a significant change in the osmotic balance inside the body. To compensate for this disturbance, free proline, one of the possible compatible osmolytes, accumulates in cells. This is obviously the case in the case of cultivation of Ba-RCL in selective medium with the addition of sea water salts (Fig. 2). The maximum absolute value of the proline level was observed on day 14.

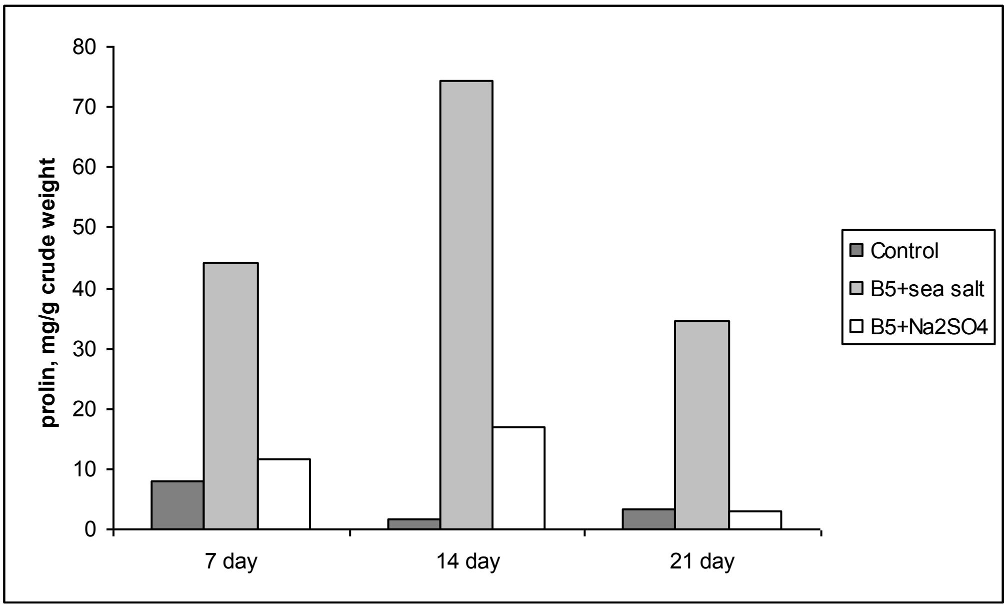

Fig. 2. The content of free proline in Ba-RCL cells during cultivation under salinity conditions.

This is a natural process, since proline has the ability to retain the water necessary for cell division and thus stabilise the required degree of cell colloid viscosity. From 14 to 21 days, the proline content decreases (in absolute terms) but remains high relative to the same value measured under normal conditions. Since the relative increase in biomass of the culture growing under these conditions is the lowest, the protective role of proline becomes even more pronounced.

The expected increase in the free proline content was not observed in the medium with sodium sulfate. This is a particularly interesting phenomenon, since the osmotic pressure of culture media with seawater salts and sodium sulfate is almost the same, and the mechanism of Na^+ ion transfer to the cell is common in both cases. The active growth of the resistant culture in the presence of Na_2SO_4 indicates the absence of stress inhibition. Thus it can be assumed that a different defence mechanism is being implemented in this case: "proline-independent". Such a characteristic phenomenon, combined with salt resistance, was also observed for Ba-resistant tobacco cell lines [3, 6, 12].

Conclusions

Heavy metal ions have a complex effect on living organisms. Resistance to them should cause significant changes that must be genetically determined. When selecting resistant cellular variants, i.e. cells that are characterised by stable growth in the presence of a constant stress factor, it is necessary to investigate the cause of resistance.

The study of cell lines resistant to HMI has shown that cell culture in general and cell selection in particular has not exhausted its potential. As a method of studying the fundamental mechanisms of cell functioning under normal and stress conditions, it is difficult to surpass. As a way to produce resistant plants, it is a promising biotechnological approach. Plant resistance to osmotic stress is a polygenic characteristic. To achieve success, it is necessary to evaluate the maximum number of vital parameters available. This will create an opportunity to actively influence metabolism. The latest biotechnology can be a priority in such experiments.

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СТІЙКІСТЬ КЛІТИННИХ ВАРІАНТІВ РОСЛИН *NICOTIANA TABACCUM* L. ДО ВОДНОГО ДЕФІЦИТУ

Мета. Встановлено, що стійкість до деяких катіонів важких металів поєднується зі стійкістю до осмотичних стресів. Так, стійкість до катіонів барію корелює зі стійкістю до водного стресу. Тому метою експерименту було порівняти стійкість первинного і вторинного калюсів до модельованих стресів. ***Методи.*** Об'єктом тестування обрано тютюн – рослину чутливу до водного дефіциту. Рівень стійкості варіантів оцінювали за показником відносного приросту свіжої маси за дії осмотичних стресів: засолення, 25 г/л солей морської води; сульфат натрію, водний стрес. Обидві концентрації летальні для клітинних культур тютюну дикого типу. ***Результати.*** Отримано Ва-стійкі клітинні культури тютюну. Калюс тютюну відзначався стійкістю до летальних модельованих стресів. Ва-стійка культура розвивалася на середовищі із додаванням солей морської води та сульфату натрію. ***Висновки.*** Явище стійкості відбиралось в результаті первинної селекції на середовищі з іонами важких металів. Рівень осмостійкості не знижувався при збільшенні терміну культивування.
Ключові слова: клітинна селекція, тютюн, водний дефіцит, іони Ba^{2+}, стрес, стійкість, пролін.