

Development and Evaluation of Wheat and Barley Cell Suspension Culture as a Model System of Stress Diagnosis

Terletskaya N.V.

Khailenko N.A.

Abstract – The research objective was the development of suspension culture cells of wheat and barley and assessing the possibility of its use as a model system for physiological studies of cereals stability to abiotic stresses. Experiments in suspension culture showed that stress-resistance of barley cells was greater than wheat cells. The common features of nonspecific action of salt stress on the development suspension cultures of cereals was identified. The results indicate the potential use of the suspension culture cells cereals as a model for physiological studies. Identified under salt stress the possibility of selection of resistant viable cell populations (units) for further regeneration of plants of barley and wheat says about the prospect of work on the cell selection for drought and salt resistant crops.

Keywords – Cell Suspension Culture, Wheat, Barley, Osmotic, Salt Stresses.

I. INTRODUCTION

Genetic diversity of plant cells allows their use for various studies. Thus, the isolated cells are widely used for the study of photosynthesis [1], ion transport [2], [3] Production of secondary metabolites [4], the processes of growth and differentiation [5] and - the programmed cell death [6] Any callus tissue is heterogeneous. Therefore, cells of callus tissue obtained *in vitro*, may be useful in a variety of studies, including - and as a model system for the study of plant resistance to adverse environmental factors [7], [8]

Assessment of the development and stress resistance of wheat and barley in cultured cells as a model system is a multi-step process, which is an important step - is to obtain reliable, viable single cell suspension culture.

Despite the fact that the cell selection work to long and intensively conducted for each species of plants needed to optimize many parameters that cell technology - a selection scheme *in vitro*, selective factor sublethal dose and its duration, the conditions for the regeneration of plants from the resistant lines and other [9], [10]

The purpose of this study was the development of suspension culture cells of wheat and barley and assessing the possibility of its use as a model system for physiological studies of cereals stability to abiotic stresses.

II. MATERIAL AND METHODS

Research material were forms of wheat - Kazakhstanskaya 126 (drought-resistant varieties) and barley - 74/2-2 (salt sensitive line), St-33 (salt tolerant line), which within one month from the start of culture

were obtained good growing loose morphogenic callus tissue.

For suspension cultures of wheat and barley were used callus initiated on a modified B5 medium. Under sterile conditions, cut into small callus pieces weighing about 2 grams wet weight and placed in a 250 ml Erlenmeyer flask with 20 ml B5 liquid medium containing 4 mg/l 2,4-D. Primary cell suspension was cultured on a shaker (140 rpm) at 3000 lux illumination at temperature $26 \pm 20^\circ\text{C}$, relative humidity 60% [11] Subculturing was performed weekly by replacement of one-third volume with fresh medium. After 3 stages of subculture cell suspension containing up to 8×10^4 cells / ml. About 75% of cells were viable.

Stress conditions created by stepwise weekly (three weeks) replacing the nutrient medium at a third adding the medium for the cultivation of NaCl at concentrations of 1.5 and 3%.

To assess the viability of the cell population used the method of vital staining with 0.5% solution of methylene blue [12] Culture viability was determined by the level ratio of the number of live cells to the total number of cells in 1 ml of suspension.

To obtain a homogeneous single embryogenic suspension culture cells and small aggregates of step globule embryoids were separated by filtration through a nylon sieve with a mesh diameter of 200 microns.

All cytological observations in cultured cells and tissues, and in the flowers, anthers and pollen grains were carried out using a microscope «Micros» (Austria), photographed with a video camera YONGXIN OPTICS CAM V200 and computer program YONGXIN OPTICS ScopePhoto version 2.4 for x40 magnification lens.

III. RESULTS AND DISCUSSION

Within three weeks after explanation callus tissues of wheat and barley were counted in a liquid medium the number of living cells (per cent of the total number of cells), in a series of experiments were performed cytological examination. It is noted that after 14 days of culture and suspension cultures of wheat and barley as composed of single cells and cell microcalluses. Up to 90% of the cells were viable. In the process of cultivation for three weeks increased the number of single cells, the density of the suspension of wheat amounted to 85 thousand cells / ml, barley - 80 thousand cells / ml, and the viability of the resulting suspension culture remained stable, which made it possible to conduct further experiments. Thus, good growing slurry consisting

essentially of small actively dividing cells were obtained. (Fig. 1 and 2 about here).

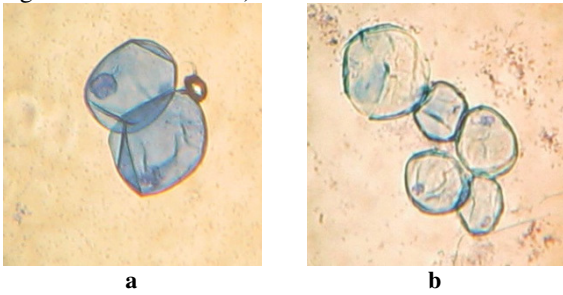


Fig. 1. Suspensions cells: a - cells of barley; b - cells of wheat (magnification x 40)

As shown in Fig. 2, cells in suspension culture cereals are various types of cell division: mitosis addition amitosis observed - "budding" and "overflow" of cellular material formed shoots.

Single cells suspensions of wheat and barley derived from callus cultures were used for studying resistance to abiotic stresses. Experiments were conducted with a suspension of an initial cell population density of 8×10^4 cells / mL and 90% viability.

When creating a cellular background selective breeding for drought tolerance, along with polyethylene glycol, sodium chloride is frequently used as a stressor enables modeled as salt and osmotic stress and receive as salt well as drought resistant forms [8], [13]-[15]



Fig. 2. Dividing of cell suspension barley, line St-33: a - mitosis, b - amitotic division (magnification x 40)

Therefore, after obtaining a relatively homogeneous suspension growing cells in culture medium, we gradually introduce NaCl as osmotic stress agent to achieve the required concentrations of selective - 1.5 and 3%.

It is noted that a single cell suspension cultures of wheat and barley in the absence of stress showed no significant difference in the percentage of live cells for the experiment.

The gradual introduction of the medium for culturing cells of sodium chloride afforded the opportunity to adapt to certain stress factors, however, increasing the concentration and duration of exposure to the stressor leads to a significant reduction in cell viability, as shown in Fig. 3.

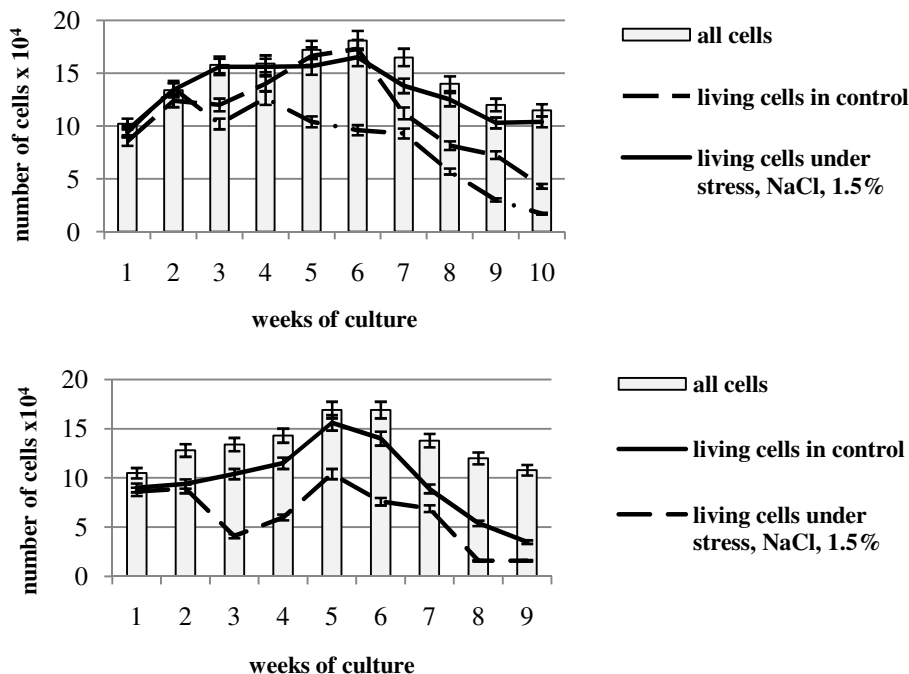


Fig. 3. Effect of NaCl on cell viability (a) barley and (b) wheat suspension culture

This phenomenon refers to a non-specific reaction of the cells to the action of stress factors [16] and shows, first of all, to increase the permeability of cell membranes under the influence of stress, often leading to cell death.

It is noted that in the initial stage of the experiment - from 2 to 5 weeks of culture - in suspension culture dominated meristematic small cell type. However, when

exposed to stress factors at this stage, we observed frequent instances of intense mottled cells shown in Fig. 4.

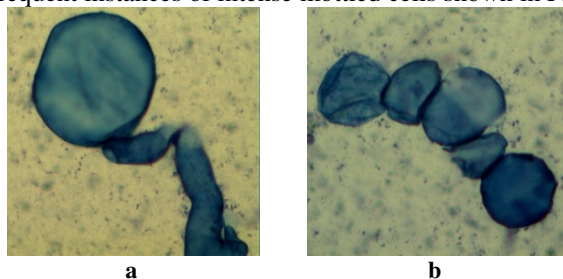


Fig. 4. Coloring cells under salt stress: a) intensive and uneven coloring cells of barley; b) uneven coloring cells of wheat (magnification x 40)

In wheat cells at the sixth week of culture with 1,5% NaCl, and in barley cells at the sixth week of culture with 3% NaCl and at the seventh week of exposure at 1,5% NaCl was noted outbreak enucleated cells, as well as deposition in the lower layers groups suspensions of live cells, cells forming conglomerates or clusters.

In control conditions as barley and wheat, the occurrence of clusters observed at one or two weeks later. This indicates that the acceleration of the completion of all phases of development and destruction of cell cultures under stress. At the eighth week of cultivation under conditions of salt stress concentration when NaCl 1,5% wheat and NaCl at a concentration of 3% was observed in barley pronounced pattern of cell death. The ninth week marked a massive cell death in barley at a concentration of stress factor of 1.5% as shown in Fig. 5.

However, in this case, the studied forms of cereals remain viable cells and cell conglomerates. Therefore, prolonged exposure to salt stress is accelerated completion of all phases of development of the suspension culture and a significant decrease in cell viability of barley and wheat.

But, even with prolonged exposure to salt stress possible selection of resistant viable cell populations (units) for further regeneration of plants of barley and wheat. An important issue when creating selective *in vitro* selection systems is the ability of selected lines resistant to the induction of morphogenesis, since the literature is often marked reduction in their regeneration capacity [9], [17]

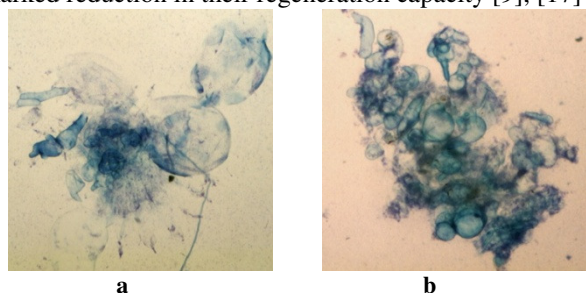


Fig. 5. Cells at ninth week of culture at 1.5% NaCl: a) conglomerate of died wheat cells (magnification x 10); b) conglomerate with living cells of barley (magnification x 10)

Therefore, in parallel, we have considered the possibility of poststress repair and organogenesis in suspension culture cells of callus tissue of barley that have been cultured under conditions of salt stress on the solid medium Gamborg B5.

It is noted that salt sensitive callus lines 74 / 2-2, morphologically characterized as dense, solid, some - with visual signs of necrosis after the transfer of stress conditions in liquid medium without the stressor, the suspension did not form at all. Cytological analysis revealed that almost all of the cells in the callus tissue of these lines were dead. More friable callus tissue salt tolerant line St-33 when administered in suspension culture contained 20% living cells, as shown in Fig. 6.

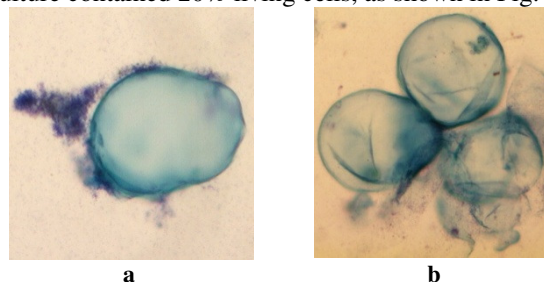


Fig. 6. Dead and living cells of salt-sensitive and salt-tolerant barley a) sensitive lines of barley 74 / 2-2; b) salt-tolerant barley lines St-33 (magnification x 40)

Recovery after vital functions in cells of salt stress lines St-33 occurred eight weeks, after which they are formed in suspension culture perfectly viable cell aggregates - proembryo and embryoids with followed gemmorizogenesis, as shown in Fig. 7.

Consequently, as a result of the experiments demonstrated the ability of salt-tolerant forms of somatic cells to restore vitality after a hard salt stress, which also provides opportunities for cell selection.

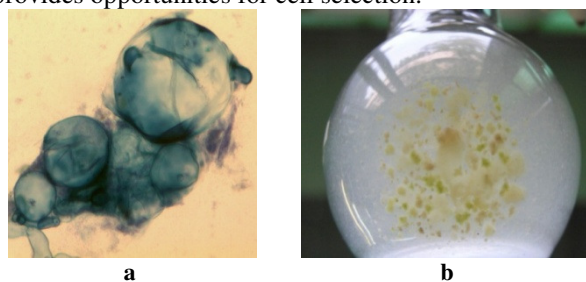


Fig. 7. Viable cells (magnification x 40) (a) and the process of morphogenesis in suspension culture barley (b)

Barley is widely recognized as one of the most salt-tolerant crops and has a large heat-and drought resistance in comparison with wheat. Therefore, and in cell culture shows great osmotolerance of barley than wheat under similar conditions [18]-[20] The fact that cells of wheat and barley in the glass vary in their response to osmotic (saline) stress suggests that plant cells in suspension culture may well be used as a model system in the physiological studies.

IV. CONCLUSION

Presented in this paper are the results of the methodological basis for the development of selective screening system cell lines and regenerated resistant to salt stress and show the availability of such biotechnological methods as suspension culture to create an initial selection of cereals. It should be emphasized that the allocation of intensively growing synchronized capable of regenerating suspension cell lines combined with stressors - is the way to solve the problem of obtaining stress-resistant plants from individual cells and the creation of effective technologies of cell selection. Thus, as a result of the research was obtained suspension culture of barley cells, its parameters are match the requirements of submerged cultivation.

Experiments in suspension culture showed that barley cells was greater resistance to salt and osmotic stresses than the wheat cells. At the same time identified the common features of nonspecific action of salt stress on the development of suspension cultures studied cereals. The results indicate the potential use of the suspension culture cells cereals as a model for physiological studies. Identified under salt stress the possibility of selection of resistant viable cell populations (units) for further regeneration of plants of barley and wheat says about the prospect of work on the cell selection for drought and salt resistant crops.

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AUTHOR'S PROFILE



First A. Author

Nina V. Terletskaia, PhD, Leading Researcher Cell Engineering Laboratory of the Institute of Plant Biology and Biotechnology, Almaty, Kazakhstan, e-mail: teni02@mail.ru



Second B. Author

Nina A. Khailenko, Dr of Science, 1945-2015.