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Abiotic Stress and Morphogenic Potential of Wheat *in vitro* in Conditions of Elevated Temperatures

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The influence of the stress effect of an elevated temperature on the morphogenic callus of Tr. durum and Tr, aestivum wheat varieties was studied. The dependence of the intensity of growth processes on the duration of the applied thermal effects schemes is shown. It was established that the presence of endophytic bacterial infection also cardinally changes morphogenetic processes *in vitro*.

Keywords: *Wheat, in vitro, temperature stress, resistance, regenerative potential, endophytic infection*

INTRODUCTION

In recent decades, in crop production, due to significant climate changes and amid unfavorable environmental conditions, there are many problems associated with inadequate plant resistance to abiotic stresses. As a result, obtaining high and stable crop yields is systematically one of the most urgent and important breeding and genetic tasks.

Research on the development of adaptive breeding methods based on modern achievements of genetics and biotechnology, allowing in a short time to improve the effectiveness of traditional breeding approaches based on the creation of new genotypes - donors of resistance to stress factors, are gaining special relevance in realization of aforementioned tasks. Cell selection is a promising method of cell engineering that allows *in vitro* direct selection of genotypes with predetermined properties.

Somaclonal variability, based on genetic mechanisms (chromosomal aberrations, point mutations of DNA (Larkin, 1981)) and epigenetic variability of plants in vitro, which do not affect changes in the nucleotide sequence of DNA and are associated with gene activation or silencing (Kaepleretal, 2000), is the basis of *in vitro* cell selection technologies.

Despite the obvious progress in the cell selection of different cultures, in particular wheat, the development of the theoretical foundations of callus formation, embryogenesis and morphogenesis in cereal culture, the establishment of factors determining the success of non-traditional approaches for increasing useful, inherited *in vitro* variations in selective selection, there are still unsolved problems , associated with a sharp decrease in the proliferation of cell cultures after the stressful effects of abiotic and biotic factors and loss of ability to regenerate (Nikitina et al., 2015; Miguel et al., 2011; Rakoczy-Trojanowska, 2002; Hussain et al., 2001; Abouzied, 2011; Akhtar et al., 2012).

MATERIALS AND METHODS

The purpose of this research was to study the individual schemes for selecting heat-resistant wheat cell lines *in vitro*. The starting material was immature embryos of 3 varieties of *Tr. aestivum* (Gobustan, Azamatli-95, Apsheron) and 3 varieties of *Tr. durum* wheat (Barakatli-95, Garabagh, Saray) of local selection.

Callus tissue was obtained from isolated embryos on days 13-17 after pollination.The embryos were planted scute up on MS medium (Murashiqe et al., 1962) with addition of 2 mg / l of 2,4D.Callus was cultivated at a temperature of 25-260°C.The transplant was performed every 4 weeks, subcultured only with morphogenic callus.Cell selection was carried out after the 2nd passage according to the following schemes:

Scheme I - Callus strains at the end of each passage were subjected to a single exposure at a temperature of 45°C for a different time (20-60 minutes).

Scheme II - the cells were exposed to temperature in the middle of the passage (after 2 weeks of cultivation) and at the end of the passage.

Scheme III - cultivation was carried out at 36-37°C throughout the passage.

The total length of the stress effect was at least 2 passages for all schemes. After stressful action, the induction of morphogenesis was carried out by placing the callus on a medium containing IAA (0.5 mg/l) under illumination conditions and 16/8 photoperiod.

RESULTS AND DISCUSSION

In this paper the results of 60-minute warming of callus masses are discussed (schemes I, II). Visual assessment of the morphogenic potential of the tissue was made on a five-point scale (Nguyen Thi Li an, 1995). Stress effects in all cases reduced the intensity of growth processes, which correlated with the duration of heat exposure, depending on the schemes used (Table 1).

Table 1. The effect of high temperature on the growth of morphogenic callus in different genotypes *Triticum durum* Desf. and *Tríticum aestívum* L. (in % of control)

Variety	Passage	Variants of exposure to high temperature		
		scheme I	schemeII	scheme III
Barakatli-95	1	20.4	19.3	17.2
	2	18.2	17.0	16.1
Garabagh	1	20.6	19.4	40.4
	2	19.1	18.3	43.2
Saray	1	21.3	21.0	35.5
	2	20.1	19.2	35.7
Gobustan	1	30.0	29.2	44.1
	2	28.7	27.1	42.2
Azamatli-95	1	40.0	38.0	30.1
	2	38.4	35.0	32.2
Absheron	1	50.3	46.3	60.7
	2	44.2	41.0	65.6

As follows from the data provided, a single exposure to elevated temperatures had little effect on the growth of calli in solid wheat varieties. Higher temperatures affected growth rates in Tr. aestivum varieties to a greater degree. With the increase in the number of passages, the inhibition of growth activity is slightly reduced under the stresses of schemes I and II. According to the scheme III of exposure, a similar pattern was observed only in varieties Barakatli-95, Gobustan and did not differ in Saray variety.

To a certain extent, the growth of culture under the influence of stress factors can be an integral criterion of its stability. The change in the intensity of growth of callus tissues, depending on the amount of temperature effects, may also indicate the nature of adaptability associated with the selection of cells with increased resistance. Multistage selection was aimed to select cell lines that are resistant to heat stress and a primary evaluation of the genetically determined resistance and adaptive capacity of isolated cells.

Previous research with these genotypes of wheat which studied the effect of elevated temperatures on the induction of callus formation, proliferation, the regeneration capacity of the strains obtained, their ability to maintain the regeneration potential, depending on the contribution of different types of callus cells (individually and in mixed callus types), allowed to establish patterns for selection of genotypes with a certain degree of preservation and expression of signs of resistance, as well as to obtain cell strains possessing increased resistance to high temperatures in comparison with the original forms. However, recent studies with the same genotypes, but grown on a different agrophone,the results of which are presented in this paper, showed a completely different picture of the realization of the morphogenic potential, both in the control and in the experimental variants.

Transplantation of callus strains, subjected to stress on the medium for regeneration, showed that exposure to temperatures in schemes I and II reduced the morphogenic potential by 70-90%, except for Barakatli-95 and Azamatli-95, in which the morphogenic potential and regenerative capacity decreased by 21% and 58% respectively. In the case of varieties of *Tr. aestivum* wheat (Gobustan; Absheron) and Tr. durum wheat (Garabagh; Saray), cultivated on a constant background of high temperatures (Scheme III), there was an even more significant decrease in the morphogenic potential. There was a 30% mortality of strains. Such morphological changes as an increase in the share of rhizogenic callus were observed (Fig. 1). The Gobustan variety showed a change in the morphology and consistency of the callus, a change in its color.

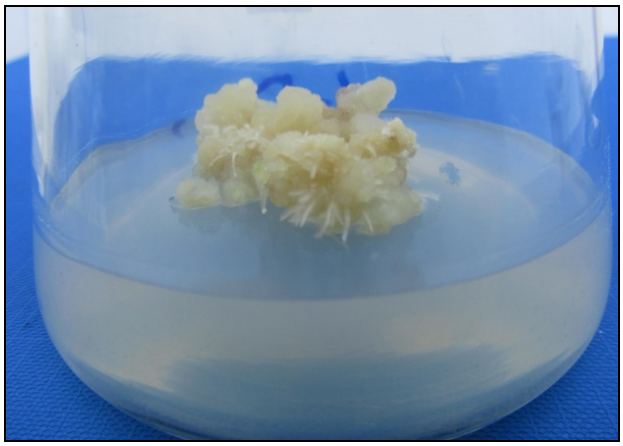

Fig. 1. Risogenesis in callus culture in Gobustan variety

Cellular masses of *Tr. durum* wheat also showed little difference in the degree of suppression of the morphogenic potential. The greatest suppression of the morphogenic potential took place in *Tr. aestivum* wheat varieties. Tr. aestivum wheat also had a varietal relationship with respect to the heat stressor, regardless of the treatment schemes used. With the increase in the cultivation time under the stress scheme II, the suppression of the morphogenic ability in all strains decreased slightly, which can be attributed to the

manifestation of adaptability or the acquisition of a certain resistance to a thermal stressor.

In callus cells, mainly *Tr. durum* wheat, subjected to double treatment with elevated temperatures according to Scheme II, no changes in morphology were observed visually in passage 1. During the 2nd passage the growth of biomass slowed down. In the case of surviving strains of Absheron, Gobustan, Garabagh, Saray, cultured according to Scheme III, in the 4th passage, after the termination of thermal exposure, under normal conditions, the manifestation of endophytic infection was observed. The manifestation of infection was sometimes accompanied by a darkening of the agar medium. Endophytic pathogens were mainly observed on the surface and in the thickness of callus cells (Fig. 2).

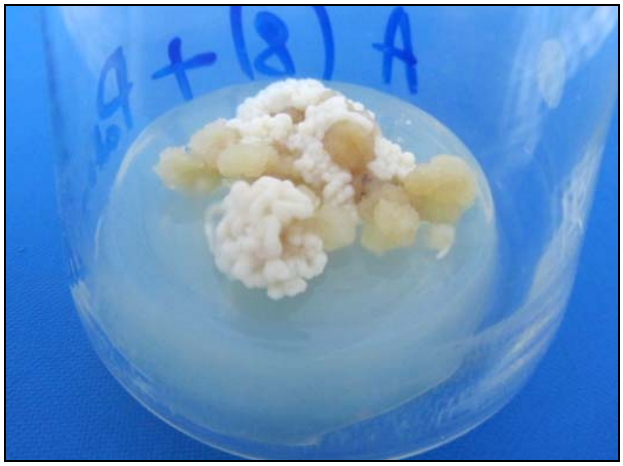

Fig. 2. The manifestation of bacterial infection in variety Absheron.

Transplantation to the hormone-free medium of infected callus cells changed the morphology of pathogens unidentified for this period. Pathogenic microorganisms associated with isolated plant tissues in culture *in vitro* negatively influenced the processes of morphogenesis and this fact should be taken into account in studies on cell selection.

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Abiotik Stress və Yüksək Temperatur Şəraitində Buğdaın *in vitro* Morfogen Potensialı

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Yüksək temperatur stressorının bərk və yumşaq buğda sortlarının morfogen kallusuna təsiri öyrənilmişdir. Böyümə proseslərinin intensivliyinin tətbiq olunan temperaturun təsiri müddəti sxemlərindən asılılığı göstərilmişdir. Müəyyən edilmişdir ki, endofit bakterial infeksiyanın mövcudluğu da *in vitro* morfogenetik prosesləri əsaslı surətdə dəyişdirir.

Açar sözlər: *Buğda, in vitro, temperatur stresi, rezistentlik, regenerasiya potensialı,, endofit infeksiya*

Абиотический Стресс и Морфогенный Потенциал Пшеницы *in vitro* в Условиях Повышенных Температур

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Изучено влияние стрессорного воздействия повышенной температуры на морфогенный каллус у твердых и мягких сортов пшеницы. Показана зависимость интенсивности ростовых процессов от длительности применяемых схем теплового воздействия. Установлено, что наличие эндофитной бактериальной инфекции кардинально изменяет также морфогенетические процессы *in vitro*.

Ключевые слова: *Пшеница, in vitro, температурный стресс, резистентность, регенерационный потенциал, эндофитная инфекция*