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STABILITY OF MORPHOGENIC AND UNMORPHOGENIC CALLUS OF WINTER RAPE (BRASSICA NAPUS L.) AGAINST SALT STRESS AND IT RESEARCH

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The results of research salt stress actions in processes of callus genesis and morphogenesis of isolated tissue culture of winter rape (Brassica napus L.) has presented in this article. Advantages of using morphogenic calluses for breeding processes in vitro has proven. The experimental data and conclusions are the basis for developing method of stepped cell breeding for salt-tolerance to gain valuable breeding material of winter rape.

Keywords: *salt-tolerance, rape, morphogenesis, callus, cell breeding.*

Abiotic stress considered as one of the reasons for losing more than 50% of most crops harvest [7]. One of the stressful environmental factors causes a significant damage to agriculture is soil salinity, which is associated with irrigation and intensive using of fertilizers. High levels of soil salinity is causing ions unbalance, creating toxic levels of cytoplasmic sodium and water stress factors. Salt and water factor stresses are cause violations of basic biosynthetic functions including the process of photosynthesis and carbohydrate metabolism [5]. Comprehensive study of plants regeneration of winter rape (*Brassica napus L.*) to high salt content in environment and in experimental conditions reveals many morphological, physiological and biochemical mechanisms of salt-tolerance [2,3].

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Purpose of research – study characteristic action of NaCl at processes of callus and morphogenesis of winter rape varieties (*Brassica napus L.*) *in vitro* for develop methods of cell breeding for resistance to salinity.

Materials and research methods. The research material served as plants of winter rape varieties like Antariya, Chornyi velenen' and hybrid NK Technik. For callus tissue explants were used a leaf blade and their segments. Callus cultures were cultivated on modified agar nutrient medium by Murashige and Skoog medium (MS) [6]. For initiate callus were used MS culture medium with the addition of Adenine – 10 mg/L, Gibberellic acid (GA) – 0.05 mg/L, 6-Benzylaminopurine (6-BAP) – 0.5 mg/L and Naphthaleneacetic acid (NAA) – 0.5 mg/L. Obtained calluses were passaged on culture medium of the same composition every 24 days of cultivation.

Callus tissue was transferred on callus genesis culture medium with the addition of NaCl at concentrations of 0.2; 0.4; 0.6; 0.8; 1.0; 1.2% for research the effect of salinity.

Control variant is a nutrient medium without NaCl. At the end of growing cycle were determined callus tissue mass, growth index (GI), which is calculating as the ratio of callus tissue weight to the explants initial mass and GI as a percentage to the control variant. In stepped cell breeding callus were cultivated on medium with the addition of 0.4% NaCl (first passage) and 0.6% NaCl (second passage).

For morphogenesis induction, callus tissue was transferring to MS medium with the addition of Kinetin – 0,25 mg/L. After 24 days of cultivation in regeneration medium we defined morphogenesis frequency as the amount of callus with buds and shoots in percentage of the total number of analyzed callus. Callus cultures were growing in the cultural dark room with the controlled temperature +25 – 26 °C and relative humidity 80%. Morphogenic callus tissue

and seedlings were growing in the light cultural room with photoperiod of 16 hour (light intensity: 3000 – 4000 lux). Obtained regenerated plant later we transferred on rhizogenic nutrient medium – ½ MS + 0.5 mg/L NAA + 3 mg/L BAP.

Plants with advanced root systems planted in sterile soil and periodically watered by Macro Salts and Micro Salts solution with MS prescription and addition of 30 g/L saccharose. During analyze the impact of salinity on the development of meristem cultures performed explantation of initial meristem varieties and derived resistant lines of regenerate plants on control medium and medium with the addition of 1% NaCl.

Results of research and discussion. For many plant varieties in modeling of salt stress in the culture medium is injected NaCl, Na_2SO_4 or sea water salt [1, 3, 4]. In our research the creation of breading medium we used chloride salinity. The primary goal in the early stages was evaluate the action of various concentrations of NaCl on growth callus mass. In the end of the cultivation cycle during several passages we defined GI and relative growth control. So concentration of 0,2 NaCl carried through breeding effect for reducing callus growth (Fig. 1). Growth of callus on this medium decreased relative to control an average of 45% during cultivation about four passages. Concentrations of NaCl above 1% were lethal force, it resulting to complete inhibition of callus proliferation, its blackening and necrosis. For breeding resistant to salinity winter rape (*Brassica napus* L.) at this stage of research it was appropriate to using concentration 0.6% of NaCl, and observed a slight increase of growth during two passages – an average of 5% compared to control. At the same time allocated to 15 – 25% callus set, those in selective medium were 8 – 25% of growth relative to the control, and then after deprived a selective charge gradually grow up a lot of mass.

For obtaining resistant set (lines) of winter rape genotypes is quite successful stepped breeding in which gradually increases the level of stress factor on the medium with the addition of 0.2% NaCl (1 passage) – 0.4% NaCl (first passage) – 0.6% (second passage). This variant of breeding allowed to increase the growth of callus tissue in 3 – 4th passages compared with direct breeding and get three resistant sets with GI 1.5 – 2.

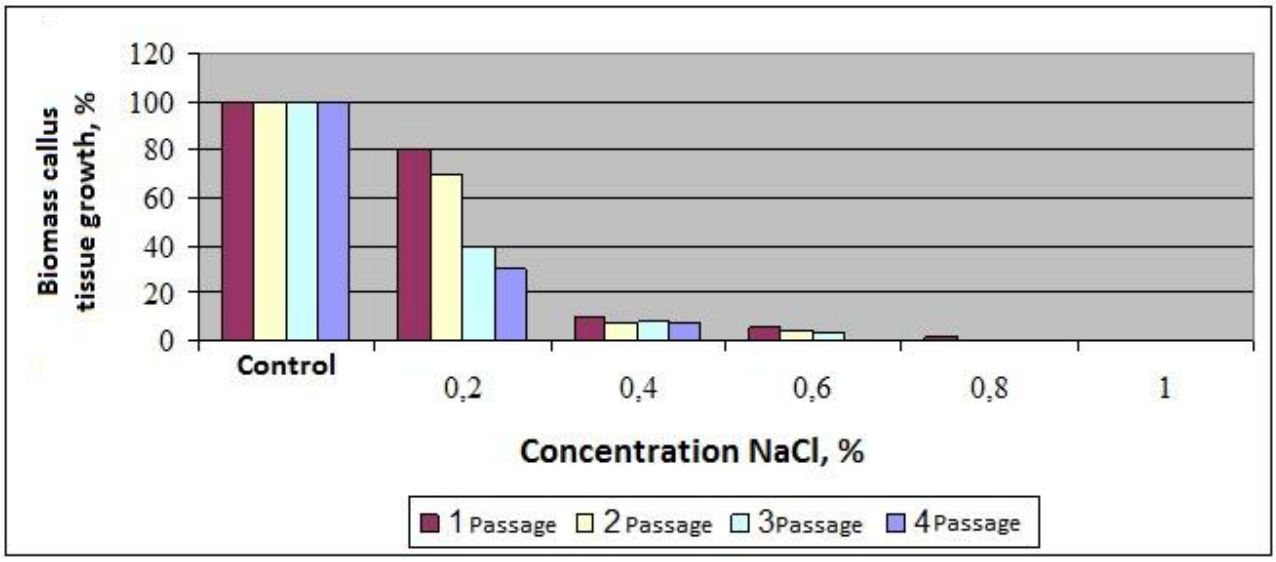

Figure 1. The total dynamics of the growth index of unmorphogenic callus of investigated genotypes depending on the NaCl concentration in the culture medium

During carrying, obtained the resistant sets (lines) on regeneration medium a capacity for morphogenesis maintained. Frequency decreased with increasing concentration of stress factor and duration of cultivation (Fig. 2). However, even for transfer lines (or sets), selected on medium with 0.6% NaCl, at medium to induce morphogenesis observed weight growth and morphogenesis frequency within 1 – 4 passages. It should be in breeding lines morphogenesis passed slowly than in controls and marked developmental delay shoots, formation of a large number of anatomically incorrect seedlings.

Resistant lines which selected in medium with NaCl were transferred to regeneration medium without selective charge. This sublethal concentration of salt was not very high – 0.6% NaCl.

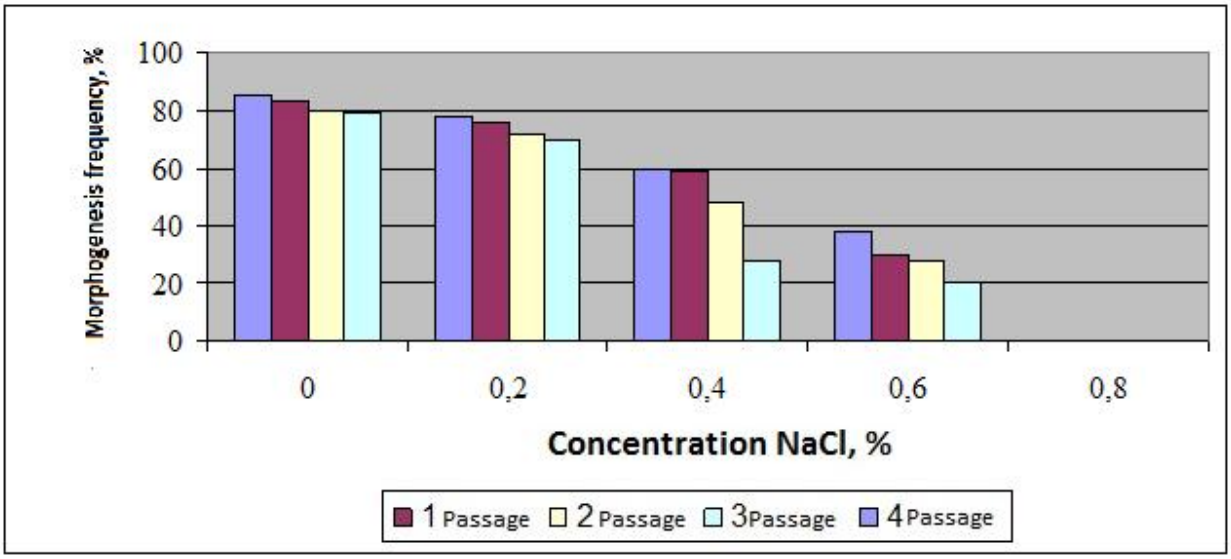

Figure 2. Total dynamics of morphogenesis induction of research genotypes for the transfer of winter rape unmorphogenic callus on regeneration medium under the influence of different concentrations of NaCl in the culture medium for callus genesis

Further investigated the effect of chloride salinity by adding NaCl to the culture medium for induce morphogenesis. Unmorphogenic callus cultivated on medium for callus genesis without selective factor, and then transferred to regeneration medium with the addition of different concentrations of NaCl. The biggest sublethal concentration of salt was 1% and marked by the highest callus tissue proliferation compared with morphogenic callus, during three passages observed 15 – 30% of increase in callus biomass relative to control (Fig. 3).

During the first callus passage on regeneration medium with 0.8% NaCl, observed the active formation of green meristematic centers. Buds and small shoots appear only in the second passage. Resistant plants with well developed

root systems planted in sterile soil and watered periodically by solution of Macro Salts and Micro Salts by prescription MS with the addition of 30 g/L saccharose.

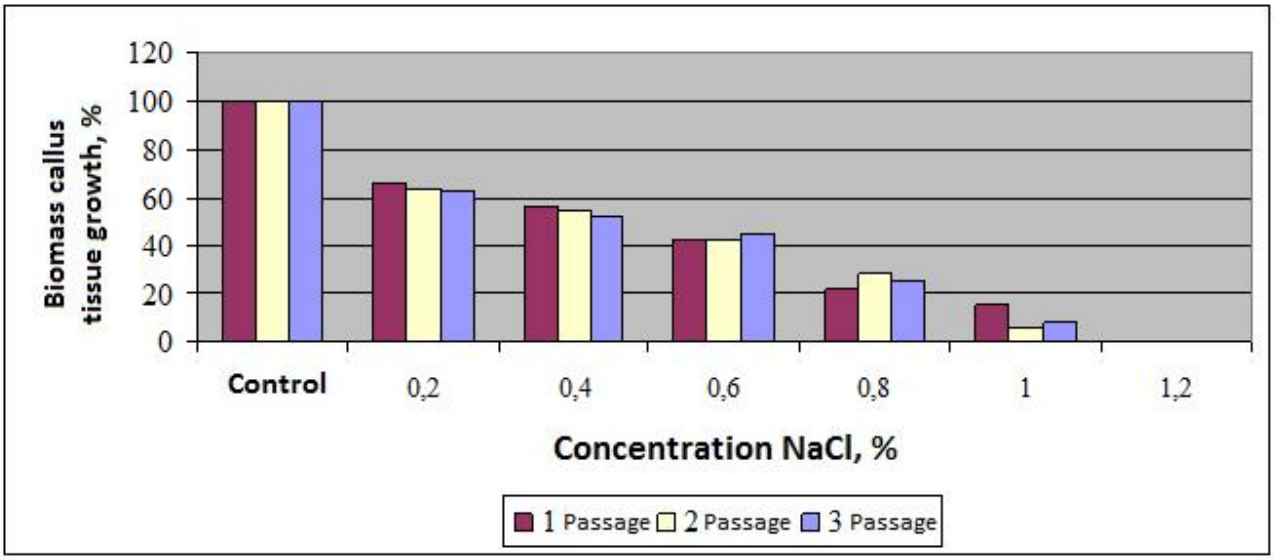

Figure 3. Total change dynamics of callus tissues growth index researched genotypes of winter rape (*Brassica napus* L.), depending on the concentration of NaCl in regeneration medium

Conclusions

1. Performed research of salinity influence on the callus genesis process and morphogenesis of winter rape (*Brassica napus* L.) have identified sublethal concentrations of NaCl for unmorphogenic callus – 0.6%.

2. For research of the chloride salinity action on the process of morphogenesis induction sublethal salt concentration was 1% and marked by the highest callus tissue proliferation compared with morphogenic callus.

3. Experimentaly marked preference for receiving breeding of soil salinity with injected NaCl in the culture medium to induce morphogenesis and recruiting morphogenic callus lines (sets) against the background of stress factor.

4. Obtained the experimental data – the basis for developing a method of stepped cell breeding of soil salinity to gain valuable breeding material of winter rape.

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ДОСЛІДЖЕННЯ СТІЙКОСТІ МОРФОГЕННОГО І НЕМОРФОГЕННОГО КАЛЮСУ ОЗИМОГО РІПАКУ (BRASSICA NAPUS L.) ПРОТИ СОЛЬОВОГО СТРЕСУ

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Представлені результати досліджень дії сольового стресу на процеси калюсо- і морфогенезу в культурі ізольованих тканин озимого ріпаку (Brassica napus L.). Доведена перевага використання морфогенних калюсів для селекційних процесів in vitro. Отримані експериментальні дані і висновки, є основою для розробки методу ступінчатої клітинної селекції на солестійкість для отримання цінного селекційного матеріалу озимого ріпаку.

Ключові слова: *солестійкість, ріпак, морфогенез, калюсогенез, клітинна селекція.*

ИССЛЕДОВАНИЕ УСТОЙЧИВОСТИ МОРФОГЕННОГО И НЕМОРФОГЕННОГО КАЛЛЮСА ОЗИМОГО РАПСА (*BRASSICA NAPUS* L.) К СОЛЕВОМУ СТРЕССУ

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Представлены результаты исследований действия солевого стресса на процессы каллюсо- и морфогенеза в культуре изолированных тканей озимого рапса (*Brassica napus* L.). Доказано преимущество использования морфогенных каллюсов для селекционных процессов *in vitro*. Полученные экспериментальные данные и выводы, являются основой для разработки метода ступенчатой клеточной селекции на солеустойчивость с целью получения ценного селекционного материала озимого рапса.

Ключевые слова: *солеустойчивость, рапс, морфогенез, каллюсогенез, клеточная селекция.*