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KLYACHENKO O.L., Cand. Sc. Biology, assistant professor,
KRYLOVSKA S.A., teaching assistant
National University of Life and Environmental Sciences of Ukraine
e-mail: krylovskaya.sv@mail.ru

OBTAINING OF CELL SUSPENSIONS OF DIFFERENT SUGAR BEET (*BETA VULGARIS* L.) GENOTYPES

The influence of the genotype of initial sugar beet (Beta vulgaris L.*) plants on the cell suspension growth was shown. Optimum nutrient media, cultivation conditions of suspension culture were chosen with the aim of its further usage in schemes of cell selection for résistance.*
Key words: *sugar beet, cell suspension, in vitro, Beta vulgaris* L.*, growth dynamics.*

Introduction. Callus tissue is an important material in the work culture of plant cells under conditions in vitro, as well as serve as a source of cell suspensions. The advantage of using suspension cultures of callus is their higher capacity for cell division and the large number of cell generations. [2] The use of sugar beet cell suspensions allows experiments to cells and get them plants with altered morphogenesis and increased adaptive capacity, which may be further used as source material for breeding research [1].

Suspension culture is a convenient object for use in cell selection for resistance to abiotic and biotic factors as cells cultured under conditions in vitro, characterized by physiological, cytological and genetic heterogeneity [6]. Also, from the literature it is known that tissue growth is more intense than the whole plant, which greatly simplifies and accelerates the selective operation. Terms of cells growing in suspension can be controlled by the composition of the culture medium and the cell population, which is a significant advantage when used in different schemes cell selection for resistance [1,5,3].

Therefore, identification of growth parameters growth in sugar beet cell cultures during the cultivation cycle, providing their morphological characteristics are an important task on the initial stages of cell selection [4]. The aim of our work was to obtain cell suspensions of sugar beet and study their growth dynamics.

Materials and methods. The object of the research were varieties of sugar beet Yaltushkivskiy single-seeded 64 and Bilotserkivskiy single-seeded 45, diploid hybrids Yaltushkivskiy MS 72 Ukrayinskiy MS 70, Ukrayinskiy MS 72,Uladovo-Verhnyatskiy MS 37, Uladovo-Veselopodolyanskiy MS 84, Ivanivskiy MS 33, Katyusha, Vorskla, Atamansha and triploid hybrids Alexandria and Bilotserkivskiy MS 57. The aim of our work was to study the dynamics of cell suspensions of sugar beet different genotypes. Researches were conducted in 2012-2013 in plant biotechnology laboratory in National University of Life and Environmental Sciences of Ukraine.

Obtaining of suspension culture. For the growth initiation of cell suspension, proliferating callus tissue with friable consistence of sugar beet different genotypes was used. It was dispersed with a sterile forceps and 2-3 g was placed in Erlenmeyer flasks with liquid nutrient medium with volume 100 ml according to Murashige- Skoog (1962) in various modifications. In our studies, cultivation of cell suspensions was performed according to three different schemes for various MS medium varieties: 1) MS1 with addition of ascorbic acid (2.5 mg/L), 6- BAP (0.4 mg/L) and NAA (2 mg/L); 2) MS2 with addition of 6-BAP (0.25 mg/L) and 2.4-D (0.25 mg/L); 3) MS3 with addition of 6-BAP (0.1 mg /L) and 2.4-D (0.25 mg/L) and cultured at a regulated temperature 25 - 26 ºC in complete darkness with shaking at a speed 120 rpm revolutions per minute. After two weeks of cultivation (first passage) contents of the flask were divided into two parts, each part was placed in fresh culture medium. Also, during the first passage large aggregates were removed by filtration through a nylon sieve. Subcultivation was made every 14 days.

Assessment of cell suspension viability. For the cell viability determination it was stained with 0.1 % Evans blue solution and microscoped. In this case, dead cells were stained in a blue color, but living cells were not stained at all.

Suspension culture density (number of cells per 1 ml of suspension) was determined in the Fuchs Resenthal Counting Chamber, making maceration with the usage of 20% chromic acid at 70 °C for 15 - 20 minutes. The number of cells was calculated by the following formula:

$$X = \frac{1000M}{3,2},$$

M - average number of on-camera with 6 repetitions.

Results and discussion. The composition of culture medium during cell suspensions cultivation influences the growth and development of cell populations. In particular, the quantitative and qualitative indicators of suspension cultures is influenced by the presence and value of growth regulators. Therefore, the main task of the research was to study the dynamics of the impact of the culture media composition on the growth and development of sugar beet cell suspensions of different varieties, di- and triploid hybrids of sugar beet. For this purpose, friable callus tissue was placed in three variants of liquid culture medium. The first variant was the medium containing ascorbic acid, auxin (NAA) and cytokinins (6-BAP) in a ratio 1:5; second and third variants of the medium was supplemented only with auxin (2,4-D) and cytokinin (6-BAP) in concentrations 1:2.5 and 1:1, respectively. Growth characteristics, such as the number of cells in 1 ml of suspension are presented in Table 1.

As it can be seen from the data from 8 passages in all variants of the culture medium suspension culture with pale yellow color and steady growth rate was obtained. The only difference was in the number of cell populations. Phases of the growth cycle in suspension cultures of all genotypes were determined in the first passage, and samples for the quantity and quality of cell populations' determination, as well as for the growth curve tracing, were taken every 2 days.

These data suggest that the addition of auxin, cytokinins and ascorbic acid (MS1) contributed increasing of the cell populations. Cell suspensions of all genotypes that were cultivated in the MS1 medium, distinguished by the presence of a small number of cell lines. Thus, the maximum number of cell populations in this variant of medium was observed in diploid varieties Yaltushkivskiy single-seeded 64, diploid hybrids Katyusha, Vorskla and Atamansha and triploid hybrid Bilotserkivskiy single-seeded 45 and ranged from $25,2\times10^5$ (Bilotserkivskiy single-seeded 45) to $28,6\times10^5$ (Atamansha). During the cultivation of cell populations in media MS2 and MS3 similar trends of growth were observed, but the correlation of auxins: cytokinins (1: 2.5) was more effective than ratio 1: 1 of the same growth regulators. For example, the maximum number of cell colonies on the medium with the addition of vitamin C was observed in genotype Atamansha ($28,6\times10^5$), and the second and third options in the same genotype number of cell colonies was $27,5\times10^5$ and 26.5×10^5 per 1 ml, respectively.

The growth of cells in suspension of studied genotypes for all variants of culture media was visualized with a typical S-shaped curve (Figure 1-4). As a result of research we have found that in all varieties and hybrids phases of the growth cycle began at about the same time. Lag-phase in all genotypes lasted 4 days, log - 6 days, linear - 3 days, then the curve exit to the plateau (phase of slow growth) after which we observed the gradual death of cells in suspension.

The number of cells was maximal at 10 - 14 days of cultivation, depending on the used variant of the culture medium, and after two weeks of cultivation observed decrease in suspension density in all varieties and hybrids irrespective of genotype and growth regulators ratio in media. Difference between suspensions of different genotypes was observed only in active cell division and, consequently, in the number of cells per 1 ml of culture.

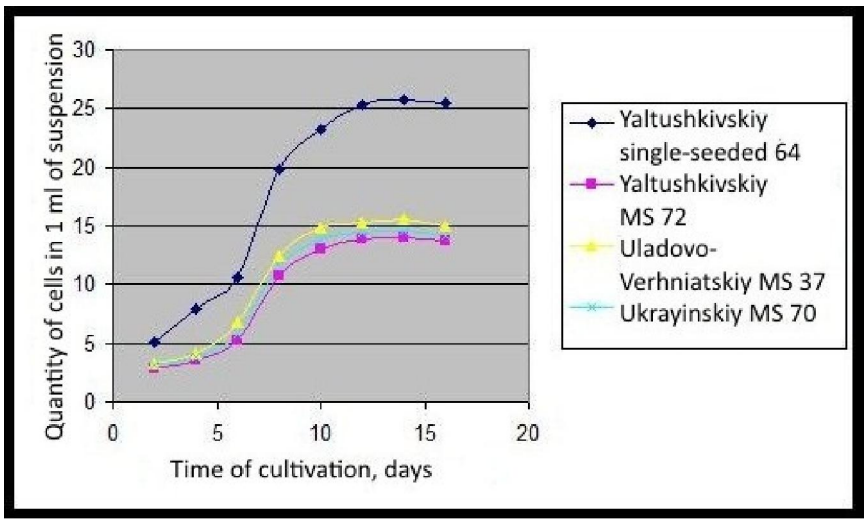
To obtain cellular colonies of sugar beet for the purpose of inducing indirect morphogenesis suspension culture were plated on agarized nutrient medium. After 3 - 4 weeks of cultivation, large sized colonies (up to 2 mm in diameter) were isolated and used for the organogenesis induction.

Table 1

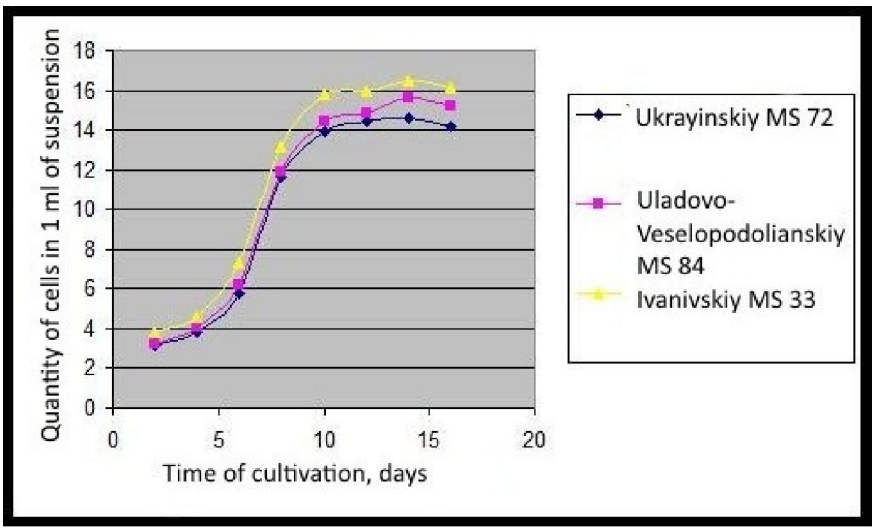
Dynamics of the cell suspensions growth of sugar beet different genotypes in dependence on the culture medium composition

Variety, hybrid	Medium	Number of cells in 1 ml of suspension ($\times 10^5$)							
		Day of cultivation							
		2	4	6	8	10	12	14	16
1	2	3	4	5	6	7	8	9	10
Yaltushkivskiy single-seeded 64	MS1	5,1±0,3	7,9±0,7	10,6±0,3	19,8±1,4	23,2±1,2	25,4±0,4	25,7±0,6	25,5±0,6
	MS2	4,5±0,8	7,5±0,6	10,0±0,8	19,1±0,4	22,5±0,5	24,8±1,0	24,9±1,9	24,0±0,3
	MS3	4,0±0,4	7,1±0,7	9,6±0,7	18,5±1,3	21,9±1,4	24,2±0,7	24,2±0,9	23,5±0,5
Yaltushkivskiy MS 72	MS1	2,8±0,9	3,6±0,2	5,2±0,2	10,8±1,1	13,0±1,3	13,8±0,6	14,0±0,5	13,7±0,4
	MS2	2,3±0,7	3,1±0,4	4,6±0,4	10,1±0,4	12,4±0,5	13,1±0,5	13,3±0,4	12,8±0,2
	MS3	1,9±0,2	2,5±0,1	4,0±0,3	9,5±0,7	11,7±0,3	12,4±0,3	12,5±0,4	11,9±1,1
Uladovo-Verhniatskiy MS 37	MS1	3,2±0,6	4,1±0,3	6,8±0,7	12,5±0,8	14,9±0,3	15,3±0,4	15,6±0,8	15,0±1,1
	MS2	2,8±0,3	3,7±0,6	6,2±0,8	12,0±0,4	14,4±1,1	14,9±1,1	15,0±1,4	14,8±0,3
	MS3	2,3±0,4	3,2±0,8	5,5±0,7	11,3±0,3	13,6±0,8	14,1±0,1	14,5±0,7	14,3±0,8
Ukrayinskiy MS 70	MS1	3,1±0,5	3,8±1,0	5,8±0,3	11,6±1,0	13,9±0,7	14,4±0,4	14,6±0,2	14,2±1,2
	MS2	2,6±0,6	3,2±0,1	5,3±0,1	11,0±0,7	13,2±0,9	13,7±1,0	13,8±1,6	13,7±0,9
	MS3	2,2±0,2	2,6±0,3	4,7±0,8	10,4±0,8	12,6±0,8	13,1±1,8	13,3±0,9	13,1±0,2
Ukrayinskiy MS 72	MS1	3,2±0,5	4,1±0,4	6,2±0,2	11,9±0,2	14,4±0,6	14,9±0,7	15,6±1,9	15,2±0,6
	MS2	2,6±0,4	3,5±0,6	5,5±0,4	11,3±0,7	13,8±0,4	14,2±1,9	15,0±1,0	14,6±0,4
	MS3	2,2±0,1	3,1±0,1	5,0±0,9	10,8±1,0	13,2±0,4	13,6±0,3	14,5±0,2	14,2±1,3
Uladovo-Veselopodolianskiy MS 84	MS1	3,8±0,9	4,6±0,7	7,3±0,1	13,2±0,3	15,8±0,5	16,0±1,6	16,5±1,1	16,1±0,3
	MS2	3,3±1,2	4,1±0,6	7,4±0,2	12,3±0,2	15,0±0,8	15,2±0,7	15,7±0,2	15,5±0,9
	MS3	2,5±0,1	3,3±0,3	6,6±0,7	11,5±0,7	14,2±0,4	14,4±0,8	14,8±0,8	14,6±0,4
Ivanivskiy MS 33	MS1	3,0±0,4	3,8±0,8	5,7±0,2	11,1±0,9	13,9±0,8	15,2±0,8	15,9±1,1	15,6±0,5
	MS2	2,2±0,1	3,1±0,6	5,0±0,1	10,3±0,6	13,0±1,0	14,2±0,9	14,4±0,3	14,1±0,4
	MS3	1,7±0,1	2,7±0,8	4,6±1,1	9,6±0,4	12,3±0,4	13,5±0,6	13,6±0,2	13,4±0,8
Bilotserkivskiy MS 57	MS1	4,0±0,3	5,0±0,7	10,3±0,2	17,7±1,1	21,2±0,2	22,7±1,0	23,2±1,4	22,9±1,1
	MS2	3,1±0,4	4,2±0,3	9,5±0,3	16,9±1,6	20,3±0,4	21,9±0,4	21,8±0,4	21,6±0,1
	MS3	2,4±0,4	3,5±0,9	8,6±0,5	16,0±1,0	19,2±	21,0±0,2	21,2±0,7	21,0±0,5

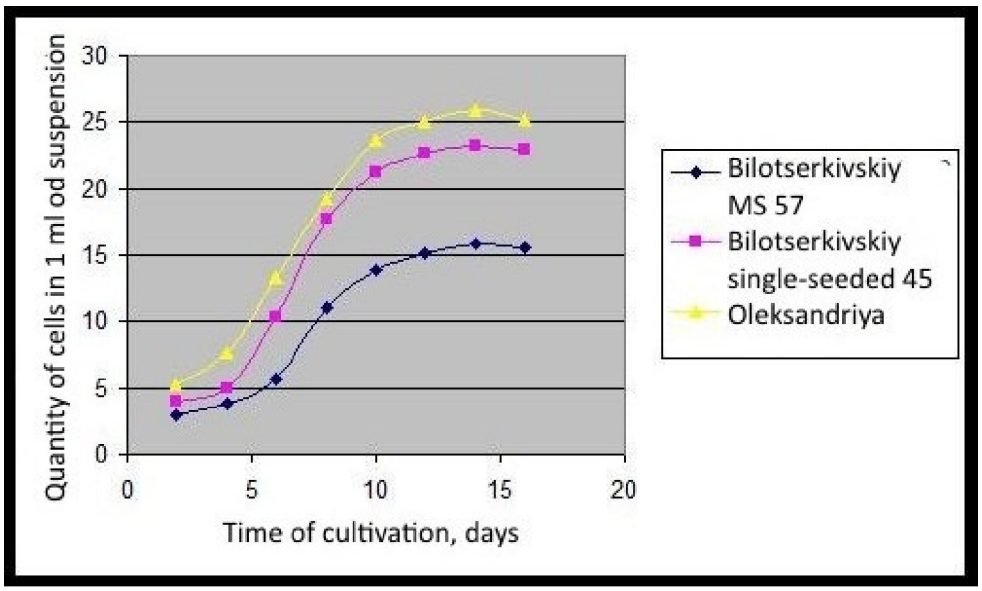
Bilotserkivskiy single-seeded 45	MS1	5,2±0,6	7,6±0,6	13,3±0,4	19,2±0,3	23,6±0,4	25,0±0,1	25,9±0,6	25,2±0,8
	MS2	4,3±0,6	6,8±0,4	12,5±0,3	18,5±0,9	22,2±0,6	23,5±0,3	24,0±1,3	23,7±0,9
	MS3	3,5±0,6	5,9±1,3	11,4±1,2	17,0±0,8	20,7±0,2	22,2±0,6	22,0±0,1	21,8±1,1
Oleksandriya	MS1	4,1±0,4	5,0±0,6	10,3±0,9	16,0±0,1	18,8±0,6	19,6±0,6	21,5±0,2	21,0±1,4
	MS2	3,0±0,1	4,1±0,5	9,0±0,9	15,2±0,3	17,9±1,1	19,0±0,1	20,1±1,1	19,9±0,3
	MS3	2,4±0,8	3,4±0,3	8,3±0,5	14,3±0,9	16,9±0,8	18,8±0,2	18,9±0,4	18,7±0,5
Katyusha	MS1	5,2±1,1	7,8±0,3	13,8±0,8	20,0±0,7	23,6±0,9	25,5±0,9	25,9±1,0	25,4±1,1
	MS2	3,8±0,4	6,2±0,4	12,1±1,1	17,8±0,9	21,2±0,2	21,9±0,8	22,3±0,6	21,9±0,6
	MS3	3,3±0,1	5,7±1,8	11,6±0,2	17,1±0,1	20,6±0,7	21,2±1,7	21,5±0,9	21,3±0,4
Vorskla	MS1	4,3±0,5	6,6±0,8	12,4±0,3	18,8±0,4	22,5±1,0	26,5±1,1	27,3±1,1	27,0±0,3
	MS2	3,8±0,3	6,0±0,6	11,6±0,2	18,0±0,9	21,5±0,3	25,5±0,4	26,0±0,6	25,9±0,9
	MS3	3,4±0,1	5,7±0,5	11,2±0,1	17,5±0,4	21,1±0,6	25,0±0,4	25,7±0,3	25,4±0,9
Atamansha	MS1	4,6±0,1	7,0±0,8	12,6±1,0	19,0±0,4	22,7±0,4	28,3±1,9	28,9±0,4	28,6±0,4
	MS2	4,0±0,6	6,5±1,6	11,8±0,4	18,1±0,7	21,8±1,2	27,0±1,5	27,8±1,0	27,5±1,6
	MS3	3,8±0,4	6,2±0,6	11,5±0,6	17,8±0,8	21,4±1,3	26,0±1,1	26,9±1,0	26,5±1,1



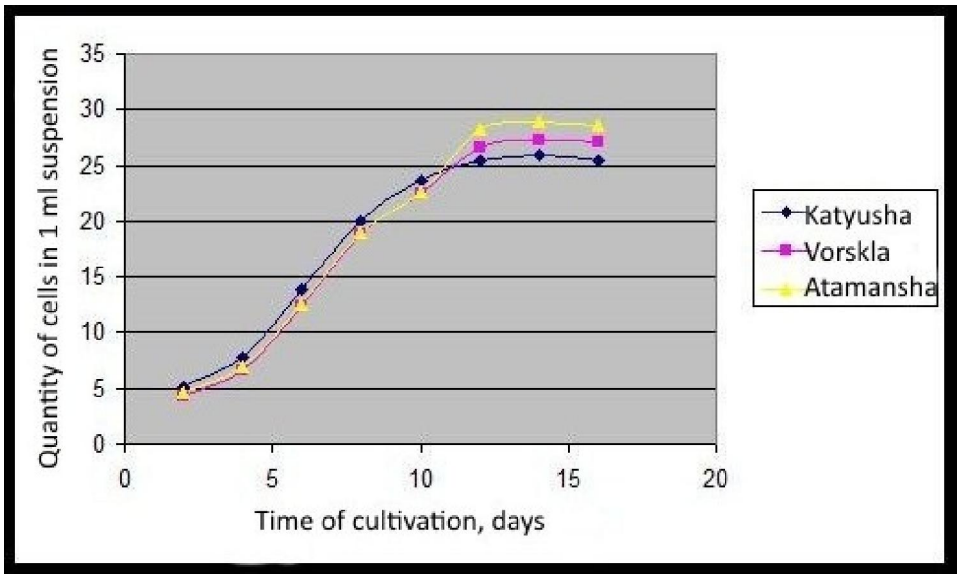
Pic. 1. Growth dynamics of suspension cultures of genotypes Yaltushkivskiysingle-seeded 64,YaltushkivskiyMS 72,Uladovo-Verhnyatskiy MS 37 and Ukrainskiy MS 70 in MS1 medium



Pic. 2. Growth dynamics of suspension cultures of genotypes Ukrainskiy MS 72, Uladovo-Veselopodolyanskiy MS 84 and Ivanivskiy MS 33 in MS1 medium



Pic. 3. Growth dynamics of suspension cultures of genotypes Bilotserkivskiy MS 57, Bilotserkivskiy single-seeded 45 and Alexandria in MS1 medium



Pic. 4. Growth dynamics of suspension cultures of genotypes Katyusha, Vorskla and Atamansha on MS1 medium

Conclusions. Thus, from studies of suspension culture differences in activity of cell division and density of the suspension, which depended on the genotype and the composition of the culture medium, were established. The most intensive growth was observed on the medium with a ratio of 6-BAP and NAA (1: 5) supplemented with 2.5 mg/Lof ascorbic acid regardless of genotype. Growth of cell suspensions in the MS1medium also was marked by the presence of a small number of cell aggregates, which greatly simplified the cultivation contributed to the intensification and growth accelerated further organogenesis and plant regeneration.

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Анотація

Кляченко О.Л., Криловська С.А.

Отримання клітинних суспензій різних генотипів цукрових буряків (Beta vulgaris L.)

Показано вплив генотипу вихідних рослин цукрових буряків (Beta vulgaris L.) на динаміку росту клітинних суспензій. Підібрані оптимальні живильні середовища, умови культивування суспензійних культур з метою їх подальшого використання у схемах клітинної селекції на стійкість.

Ключові слова: *цукрові буряки, клітинні суспензії, in vitro, Beta vulgaris L., динаміка росту*

Аннотация

Кляченко О.Л., Крыловская С.А.

Получение клеточных суспензий разных генотипов сахарной свеклы (Betavulgaris L.)

Показано влияниегенотипаисходныхрастенийсахарной(Betavulgaris L.) на динамику роста клеточныхсуспензий. Подобраны оптимальне питательныесреды, условиякультивированиясуспензионных культур с цельюихдальнейшегоиспользования в схемах клеточнойселекции на стойкость.

Ключевые слова: *сахарнаясвекла, клеточныесуспензии,invitro, Betavulgaris L., динамика роста*