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ANDROGENIC CALLUSES OF SUGAR BEET

The article describes a way to obtain androgenic callus of sugar beet by culturing anthers in conditions in vitro. The basic factors that affect the process of induction of androgenic particular composition of culture media for culturing anthers, proliferation callus. It is shown that the content and growth regulators (auxin - cytokinins) is crucial to get callus and stimulate their morphogenic activity.

Keywords: *sugar beet, culture in vitro, anthers, callus, androgenesis*

Introduction. Creation of new varieties and hybrids of sugar beet, one of the most important, not only technscal crops, but and bioenergy crops in Ukraine, conventional breeding methods and classical genetics is a time consuming process with a huge amount of time. One of modern methods, which allows to create hybrids with a high yield and complex of useful signs, is a method of experimental androgenesis - receipt of haploid in the anther culture and isolated microspores. The ability to quickly create homozygotic lines at diploidisation of haploid lines makes this method very attractive for breeders [1, 2, 4]. In a culture in vitro induction of the formation of embryos can occur in two ways - directly - in the culture of morphogenetic microspores and indirectly through the formation callus induction and formation of somatic embryos. In recent years, rapidly developing methods for homozygous doubled haploid lines with in vitro anther culture and formation embriogenic callus. And as you know, genetic diversity cells of callus can use them also for cell selection for resistance to adverse environmental factors, pathogens, and increased productivity.

Currently, developed methods for obtaining androgenic haploid plants for many cultures, including - vegetables, fruits, cereals and others [5, 6, 7, 8]. However, some species of plants receiving androgenic haploids attempts were unsuccessful. It is to such crops include sugar beet. Thus, the development of methods for obtaining haploid plants of sugar beet by androgenic relevant. In connected with that, the key step is to obtain indirect androgenic morphogenetic callus aim of our research was to develop methods for callus of sugar beet anthers (the basis of the method of indirect androgenesis) in culture in vitro.

Materials and methods. The study was conducted at the Institute of bioenergy crops and sugar beet during 2009-2013 years. In studies using selective material of experimental breeding stations in Belaya serkov and Yaltushki - di-and tetraploid of sugar beet pollinators that are grown in a field environment. During the flowering shoots of the selected seed buds from which explants treated - anthers. Stems and buds of sugar beet seed plants were subjected to cold preprocessing in the refrigerator at a temperature of 6-10 C, with 16 -hour lighting 1.0-2.0 KLK within 7-30 days. For sterilization of explants of sugar beet using sodium hypochlorite and the following profiles: 25 % solution "Белизна" for 20-35 minutes, triple rinsing with sterile distilled water, 3-10 % hydrogen peroxide solution for 10-20 minutes.

Due to the fact that our previous studies have shown that certain schemes and methods developed for different crops [2, 3, 4, 6, 7, 8], can be successfully applied in the anthers and pollen from sugar beet to obtain haploid in culture in vitro, they were used only as a basis for the modification and optimization.

Thus, for the cultivation of anthers developed the series of nourishing environments. Selection and optimization of the medium composition was performed factors: macronutrients, micronutrients, plant hormones, carbohydrates, amino acids, vitamins and other additives. The basis of using mineral medium Murasihe - Skoog [12] with full and reduced by 2 times the number of macronutrients, the addition of vitamins by prescription for Gamborg [10], ascorbic acid - 1.0 mg/l, sucrose - 15-100 g/l regulators of growth 2,4 -D -1,0-2,0 mg/l 6-BAP - 0,2-1,0 mg/l, kinetin - 2.0-3.0 mg/l, IAA - 0.2-0,5 mg/l, NOC - 0.5-1.0 mg /l, CC - 0.2-0.5 mg/l, and mixtures of amino acids, which include glutamine - 12,5-500,0 mg/l, aspartic acid - 30,0-50,0 mg/l, arginine - 2,0-250,0 mg/l, and proline, hydroksyprolin, tyrosine, glycine at a dose of 2,0-5,0 mg/l. In some environments using maltose instead of sucrose. Anthers cultivated after planting on nutrient medium in the dark at a temperature of 26-32 ^{0}C and relative humidity of 50-70% to proliferate of calluses. Formed calluses transferred to a culture room conditions with lighting KLK 1-2 for 18 hours and after a certain period of time (1-3 weeks) were transplanted into a new series of nourishing environments, which differed from the previous content and dosage of growth regulators. In particular, in the first series of environments was used for proliferation calluses content was higher auxin content of cytokinins (2,4- D - 1-2.5 mg/l, BAP - 0.3-0.8 mg/l) in the second, designed to support the growth function callus contrary - cytokinin content was higher auxin content (6- BAP - 1.0 mg/l or 2.0 mg/l, 5.0 mg/l, 2.4 -D - 0.2 mg/l), and in some environments auxins were absent. In the third series, which was used to induce morphogenesis, content and other value growth regulators - 6-BAP -1,0-5,0 mg/l, IAA or NOC 0.2-0.6 mg/l, GA - 0,2-1,0 mg/l kinetin - 0,1-1,0 mg/l. Calluses proposed cultured on medium to induce organogenesis before the appearance of primary roots and buds.

At various stages of cultivation callus determine their number and variety. Callus distinguished : consistency (loose, semi-solid , solid), color (white, green, brown, yellow, multi-colored), surface structure (smooth, grainy, tuberous, nodular), chlorophyll content (chlorophyll, nonchlorophyll), structure (homogeneous or heterogeneous), presence of meristematic centers and potential the formation of morphogenic meristems and organogenesis.

Results and discussion. As you know, the process of androgenic induction in isolated anther culture depends on many interrelated factors, in particular, is the selection and preprocessing explants, components of the culture medium for culturing anthers, microspores, induction callusogenes and modes of cultivation [2]. However, these factors of specific narrow not only different species, but also the type of explants, and even for different stages of culturing explants of one species [13]. Therefore developed specific conditions and methodical bases of indirect androgeneses, which were focused on creating new breeding material in the culture of isolated anthers and microspores in particular sugar beet. It is well known that one of the main factors affecting the androgenic induction in vitro culture is the composition of the culture medium [10]. Therefore, special attention was paid to the development of nourishing environments.

It was established that the formation callus tissues in anthers of sugar beet under conditions in vitro occurs on modified environment of Murasihe-Skoog containing reduced 2-fold dose of macronutrients and micronutrients full dose, addition of growth regulators 2,4 -D - 1,0-2 5 mg/l, 6-BAP - 0.3-0.8 mg/l, vitamins for Gamborg and ascorbic acid at a dose of 1.0 mg/l of amino acids: glutamic - 250.0 -500.0 mg/l, aspartic acid - 30,0-50,0 mg/l, tirozyn - 1,0-10,0 mg/l, arginine - 2,0-10,0 mg/l, hydroxyproline - 2,0-4,0 mg/l. Further growth callus provided second environment – modified environment of Murasihe- Skoog , with the addition of BAP - 0.5-1.5 mg/l , vitamins and amino acids according to the scheme of the first medium and the appearance of primary roots and buds. Third environment - modified environment of Murasihe - Skoog, with the addition of BAP -

1,0-5,0 mg/l, IAA or NOC 0.2-0.6 mg/l, CC - 0,2-1,0 mg/l, and vitamins and amino acids also first circuit protection.

To initiate callusogenesis isolated by culturing anthers and generate more calluses, stimulation of morphogenesis further modification of the composition of the basic medium was performed factors: growth regulators, carbohydrates, amino acids and vitamins.

We know that the most essential is the selection of optimal concentrations of hormonal components of the nourishing environments [9]. Determined that the contents of 2,4 -D (1-2.5 mg/l) as a major regulator of growth in the presence of 6-BAP (0.3-0.8 mg/l) as part of the first series nutrient , promotes proliferation when cultured anthers callus of sugar beet. While increasing the content of cytokinins in the second series of environments and adjustment of the ratio of 2,4-D in a way that their content was higher than the content of 2,4-D contributed to the growth and development of callus. Morphogenetic activity of callus, obtaining a large number of species, stimulation of organogenesis, with the advent of primary roots and buds environment contributed to the third series, with different content and value growth regulators - 6-BAP - 1,0-5,0 mg/l, IEC or NOC , 0.2-0.6 mg /l, CC - 0,2-1,0 mg/l, kinetyn - 0,1-1,0 mg/l.

From the literature it is known that an increase in the nourishing environments of sugar cultivation of sugar beet unripe embryos almost doubles the frequency of seedling establishment [11]. According to our data, the cultivation of sugar beet anthers increase the amount of sugar in the medium did not significantly affect the number of tumors. Replacing sucrose to maltose also had no effect on the number callus formed, but contributed to a significant increase in the size of planted anthers. Thus, the size of anthers on nourishing environments with maltose was almost 2-2.5 times greater than the one that was in anthers cultured on environments with sucrose.

Observations showed that the first tumors appeared 6-7 days from the beginning of cultivation (Fig. 1, 2). Callus developed with different structures: the surface of the anthers from residues stamen s thread due to its elongation and initiating development callus tissue structures at their tips with pollen. They were mostly white or translucent and had a homogeneous structure (Fig. 3, 4)

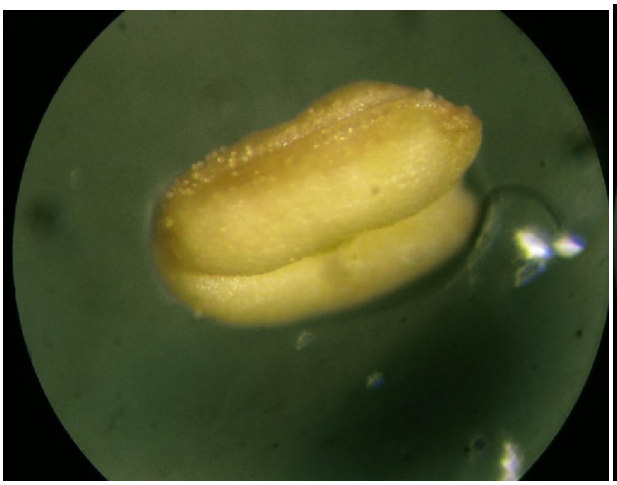

Fig. 1. Anther of sugar beet in vitro

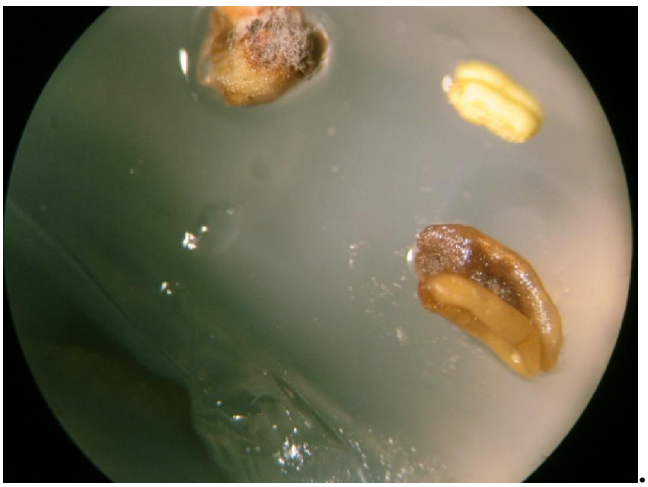

Fig.2. Proliferation of callus

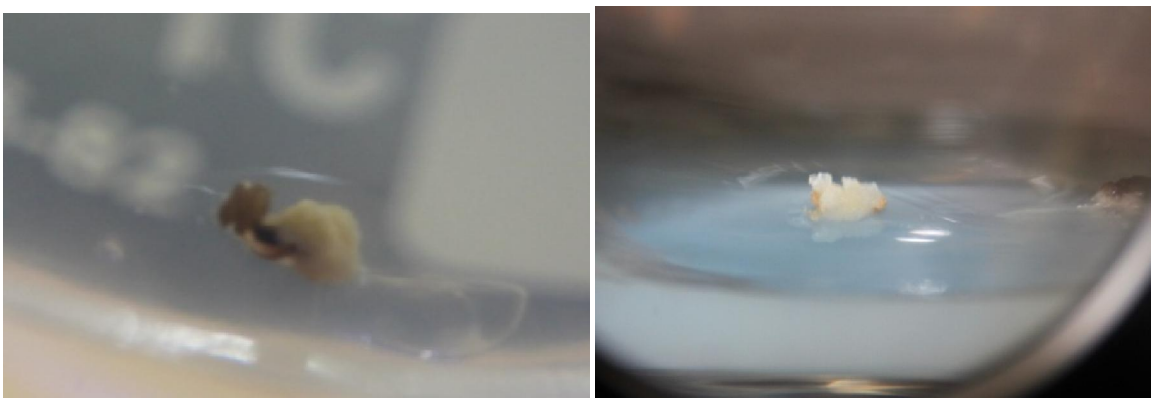

Fig. 3, 4 . Types of primary androgenic callus.

Depending on the stage of cultivation obtained callus been conventionally divided into primary, secondary and tertiary . Primary callus, formed by culturing anthers in the absence of light in an incubator after the transfer in terms of the culture room with lights on for 18 hours, took on a more saturated color and were increasing in size. After a certain period of time (1-3 weeks) to ensure the continued growth and development, callus transplanted into a second series of nourishing environments, which contributed to an increase in the size callus and change the color and structure. Passivation of calluses and third series of environments helped further differentiate callus color and structure and the presence of primary morphogenetic structures - primary roots and buds. Studies have shown that at different stages of cultivation calluses under various active ingredients in the composition of nourishing environments were obtained secondary (Fig. 5 , 6) and tertiary calluses (Fig. 7, 8). Differentiation of callus occurred not only in size, texture - solid , loose , semi-solid ; color - white, green, brown, and colorful; fabric structure - homogeneous, heterogeneous; the structure of the surface - tuberous, granular, nodular, smooth; depending on the ability to further morphogenesis – morphogenetic and nonmorphogenetic. Morphogenetic with green meristematic centers, roots, shoots and leaves rosette (Fig. 9, 10)

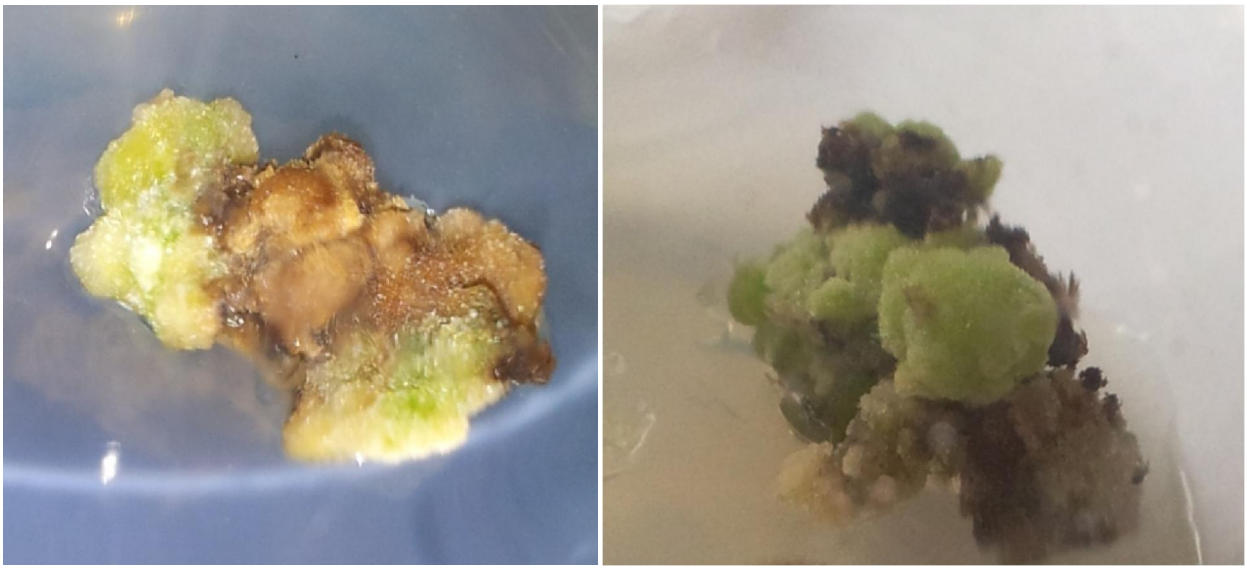

Fig. 5, 6. Types of secondary androgenic calluses

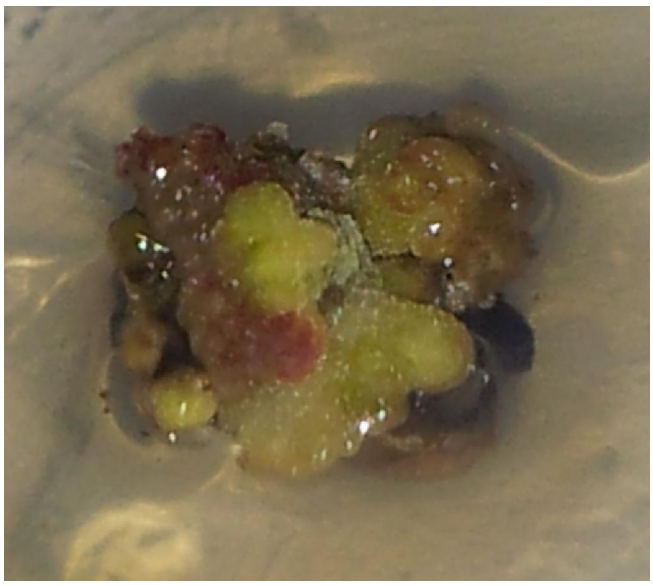
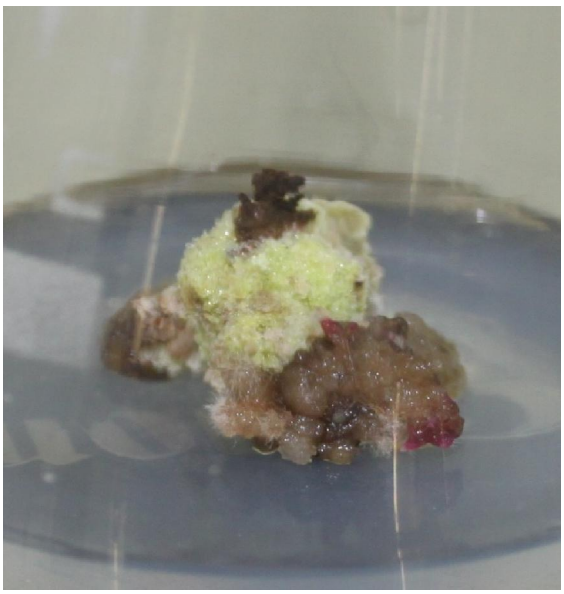

Fig. 7, 8. Types of secondary androgenic calluses

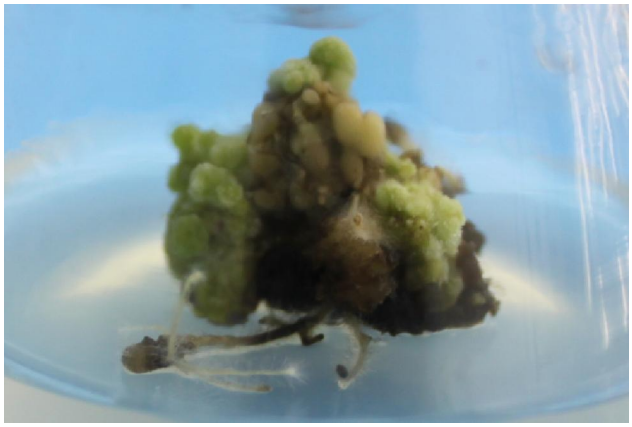
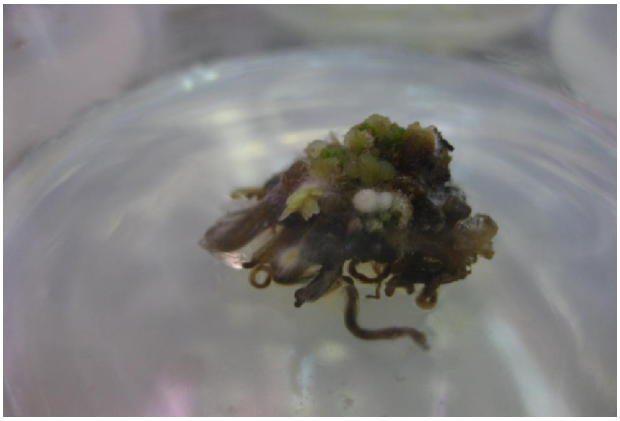

Fig. 9, 10. Types of thirds androgenic calluses (morphogenic)

Conclusions.

1. The studies developed the basic elements of the indirect androgeneses of sugar beet in culture in vitro, in particular, the composition of nourishing environments for culturing anthers, proliferation of callus and obtain its various modifications in color, structure.

2. Determined that the content and value growth regulators (auxin - cytokinins) is crucial to get callus and stimulate their morphogenic activity.

3. Depending on the ratio of growth regulators and other components of the nutrient (amino acids , vitamins, sugars, etc.) obtained callus differed in color, chlorophyll content, homo- or heterogeneity of structure, the presence of meristematic centers and capacity for morphogenic meristems and organogenesis.

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Анотація

Гонтаренко С.М., Герасименко Г.М.

Андрогенні калуси цукрових буряків

В статті наведений спосіб отримання андрогенних калусів цукрових буряків шляхом культивування пиляків в умовах in vitro. Розглянуті основні фактори, що впливають на процес індукції андрогенезу, зокрема склад живильних середовищ для культивування пиляків, проліферації калусу. Показано, що саме вміст та співвідношення регуляторів росту (ауксини – цитокініни) є визначальним для отримання калусів та стимуляції їх морфогенної активності.

Ключові слова: *цукрові буряки, культура in vitro, пиляк, калус, андрогенез*

Аннотация

Гонтаренко С.Н., Герасименко А.Н.

Андрогенные каллусы сахарной свеклы

В статье представлен способ получения андрогенных каллусов сахарной свеклы при культивировании пыльников в условиях in vitro. Рассмотрены основные факторы, влияющие на процесс индукции андрогенеза, в частности состав питательных сред для культивирования пыльников, пролиферации каллусов. Показано, что именно содержание и соотношение регуляторов роста (ауксины - цитокинины) является определяющим для получения каллуса и стимуляции их морфогенного активности.

Ключевые слова: *сахарная свекла, культура in vitro, пыльник, каллус, андрогенез*