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METHODS FOR APOZYGOSIS INDUCTION IN SUGAR BEET IN ENVIRONMENT OF BREEDING AND GREENHOUSE COMPLEX

The article surveys methods for induction of apozygosis in pollensterile sugar beet breeding lines in environment of breeding and greenhouse complex. Shown is a methodology approach to obtain sterile and dialyflower lines with apozygosis seed formation. The necessity of stressors (pollenless mode and contrast of temperatures during flowering) to induce this phenomenon in plants is established. Determined are methods for apozygosis identification.
Keywords: *pollenless mode; apozygosis; contrasting temperature; mixoploidy; sugar beet*

Introduction. In the evolutionary aspect unsexual reproduction of many plants, which occurs under certain conditions, is a form of preservation of the species. This phenomenon, known as "apomixis" from the Greek *apo* – without and *mixis* – mixing, attracts attention of geneticists and biologists who study various forms of microevolution in nature. In the scientific sources the term "apomixis", "agamospermous" and "apozygosis" are synonymous, they are used to refer to pollenless (one-parent) method of producing seeds. Apomixis is a way to seed reproduction without fertilization, in which the embryo develops from gametophyte cells under various changes of the sexual process. This phenomenon is widespread in nature and is of great importance for the evolution development [6,11].

One of the important problems in crop breeding is the creation of new varieties and hybrids, which are characterized by useful productive signs and valuable biological features. To reduce time for their creation is still the priority. At the present days, breeding development is impossible without new techniques and new processes that enable to breed rapidly a variety of genotypes, which can be drawn as initial material with new signs [1]. Pollenless method of reproduction involves changes in sexual mode of seed reproduction, opening up new possibilities in the breeding process of sugar beet both at haloid and dihaploid levels. This method of seed reproduction aimed at expanding the gene pool of pollensterile lines, simplify and cost reduce of hybrid breeding program [7]. Thus, usage of apozygosis seed reproduction method is an important act to breed a new initial material.

The goal of the experiment was to determine complex of factors that motivate apozygosis induction in pollen-sterile in sugar beet lines.

Materials and methods. The experiment was performed in Yaltushkiv experimental breeding station EBS IBCSB NAAS in breeding and greenhouse complex during 2009 - 2012. As initial materials were used 150 diploid sugar beet lines with CMS of different origin. These were self-pollinating lines of deep inbreeding, which were in isolation, and simple sterile hybrids that pollinated with non-complementary O-type lines.

In order to speed up the breeding process the materials grew in breeding and greenhouse complex under "from seed to seed" cycle [6].

Sugar beet seeds were sown to the prepared soil in August following the plan stipulated in Table 1. Soil was the main substrate for cultivation of sugar beet plants in greenhouse. The soil was prepared in advance in a specially designated plot and stored in piles. To fill the plots we have to follow such proportions: 40% of soil, 30% of humus, 20% of manure and 10% of sand. In addition, before filling plots, it is necessary to fertilize soil (per 100 kg of soil): KH_4NO_3 (34%) in amount of 40 – 50 g, superphosphate (19%) in amount of 100 - 110 g, potassium salt (40%) in amount of 30 - 40 g.

Table 1

Breeding materials sowing plan in the greenhouse

№	Breeding number	Plant number	Lines sowed
1	11-136 CMS	1	10
		2	20
2	11-133 CMS	1	30
		2	10

After sowing the seeds, watering was performed with soil moisture in amount up to 60-70% of the total capacity. Watering in greenhouse complexes was provided through semi-automatic drencher.

Due to the small volume of soil for long-term cultivation of sugar beets the plants require mineral nutrients. For this purpose, we need to provide periodic dressing, which is being determined under growth intensity and development of leaf apparatus [2].

To provide the complete development cycle "from seed to seed" sowing in greenhouse was carried out in August in order to get complete plants before the frosts. Taking into account the small number of apozygosis seeds, sowing was carried out manually, planting seeds with a distance of 5 cm in the row. Records were conducted under seed similarity sign at greenhouse conditions according to State Standard of Ukraine DSTU 2292-93 (GOST 22617.2-94) [3].

Tending crops in greenhouse consisted of: watering on regular and timely basis, loosening the soil surface, removing dry leaves and weeds, preventing proliferation of pests and diseases. Seed embryos should be tied up in time. [5]

Results. We applied four methods for induction of apozygosis mode of reproduction and its identification, such as:

Pollenless mode method in breeding and greenhouse complex. Determination of sterility and monogermity was performed in phase of developed buds. The pollenless mode method should be used to select apozygosis plants. Specific symbols are used to mark apozygosis reproduction generation. The letter "A" indicates that the seed progeny was obtained through apozygosis method, while the index indicates the generation number [12]. The method of individual selection was used for apozygosis progeny growing.

Pollenless mode method includes sugar beet breeding with CMS-0 phenotype in isolated soil boxes at breeding and greenhouse complex. Phenotype of each plant was determined by pollen signs in May of next year before disclosure of flowers. Only the plants with completely sterile pollen (phenotype CMS-0) from others were used for reproduction. Plant identification under phenotypes was performed throughout the period of flowering. Plants of mosaic phenotype and sterile sign variability, which have half-fertile pollen on lateral shoots, were removed classifying as fertile. Classification of plants was carried out by Owen [4].

Method of contrasting temperatures during flowering. Another stress factor that causes apozygosis is temperature mode, which is well regulated in breeding and greenhouse complex. Because the adaptive abilities of sugar beet as a crop are high, i.e. plants tolerate both increase and decrease in temperature during flowering, we used as stress factor their contrast (difference between day and night temperatures) [9].

The temperature rises in average from 38^0C to 51^0C in daylight hours and decreases from 20^0C to 24^0C in night hours (Fig. 1). According to our data the contrast in temperature in pollenless mode induces apozygosis embryo development.

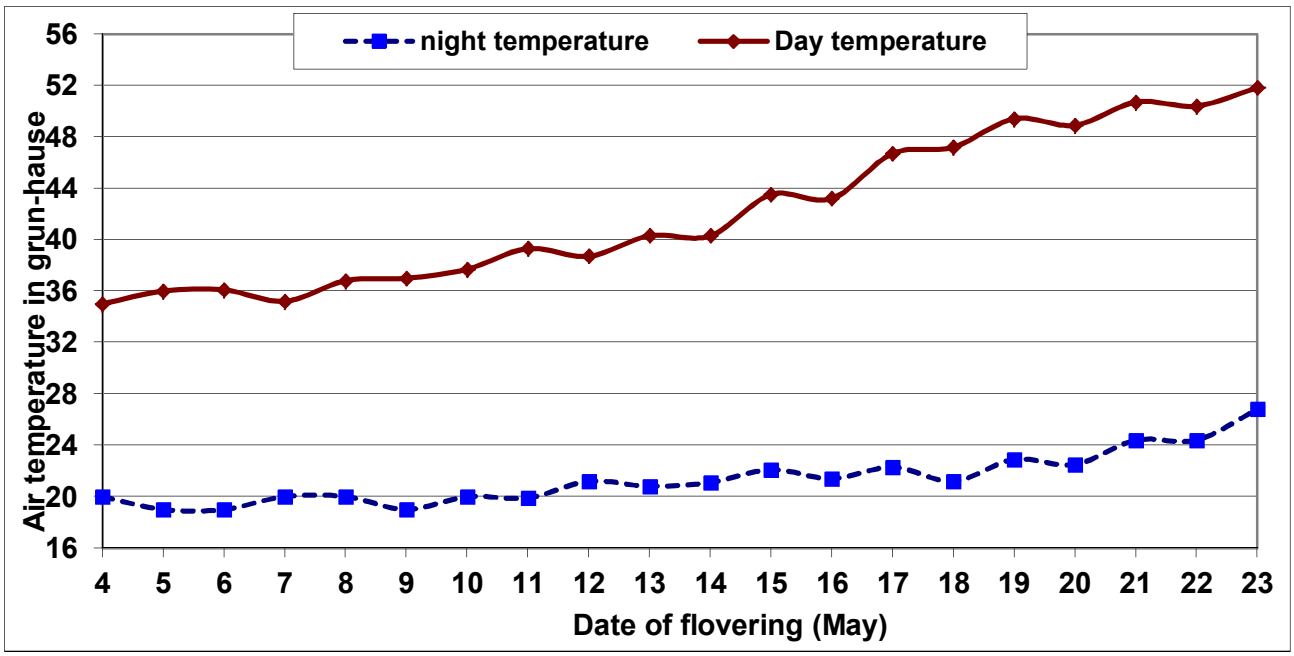

Fig. 1. Temperature control in breeding and greenhouse complex

Offspring, which produced over 5 g of seeds, was remained for repeated reproduction. 62 samples out of 150 CMS lines set seeds after four reproduction cycles (Fig. 2).

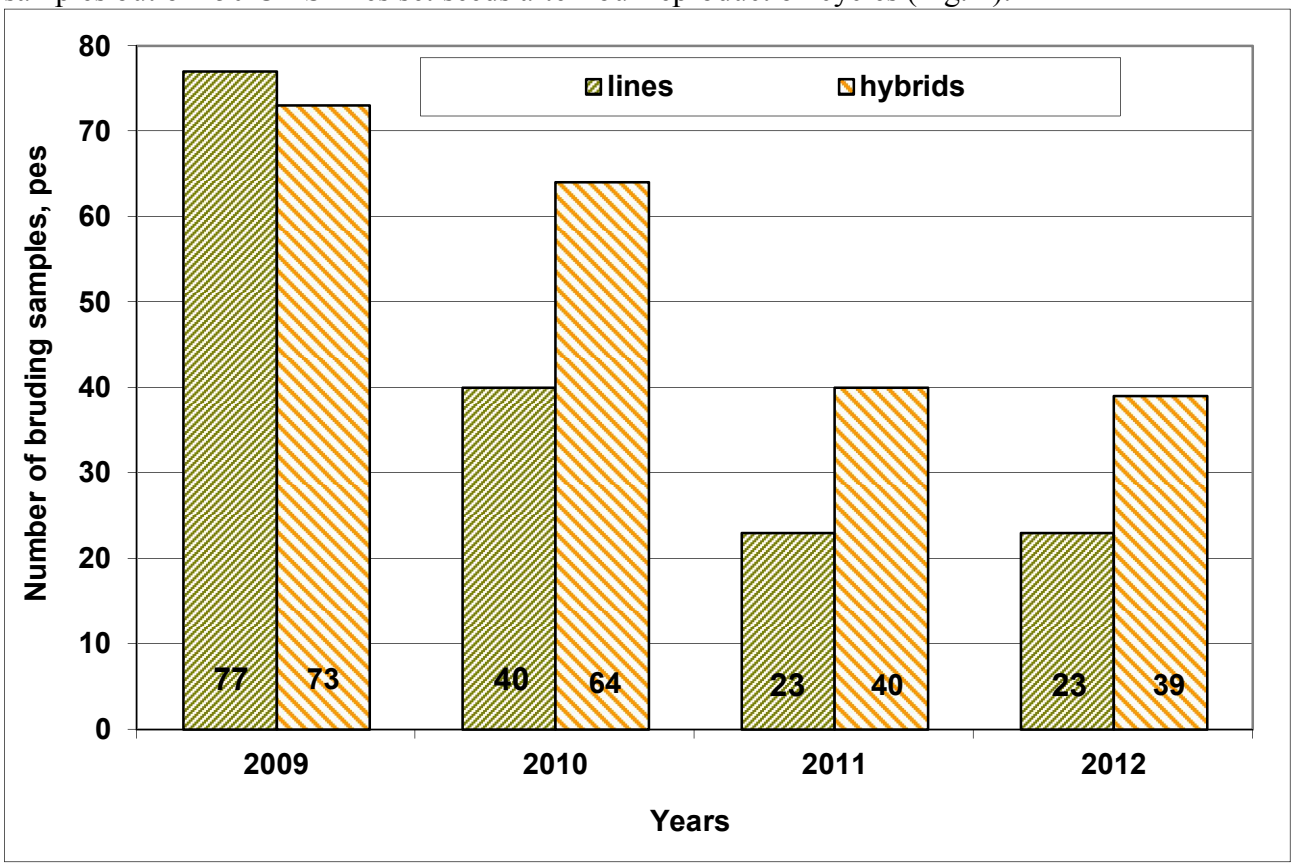

Fig. 2. Breeding numbers that were used in the breeding process (2009-2012)

Embryological method was used to confirm apozygosis and determine its type. For this purpose flowering was marked in sterile line breeding materials obtained by pollenless mode as well as E. Shyriaeva's accelerated method of determining development of sugar beet embryos [10].

Apozygosis reproduction method is confirmed by embryo development delay as well as deviation in female gametophyte development (Fig. 3, 4, 5, 6).

Fig. 3 depicts polyembryony phenomenon as an example. Fig. 4 depicts embryo development at "sphere" stage.

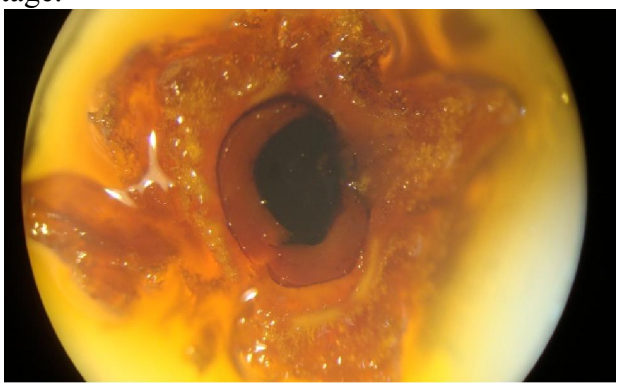

Fig. 3. The development of two embryos at one time (polyembryony) on 28th day after fixation

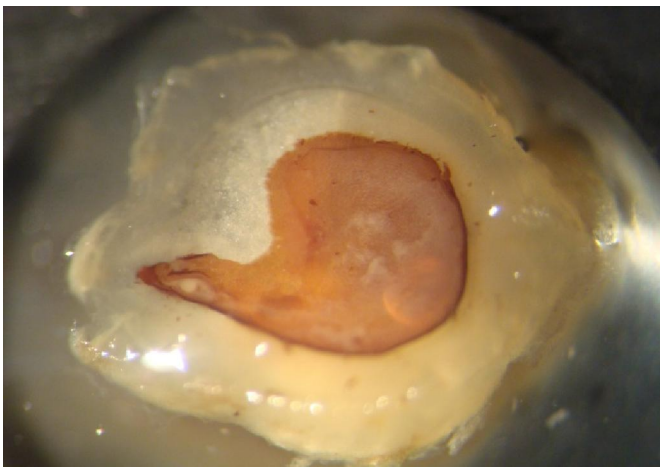

Fig. 4. The development of the embryo at the " sphere " stage

The embryo development from nucelus cells and integument tissues is shown in Fig. 5 and 6.

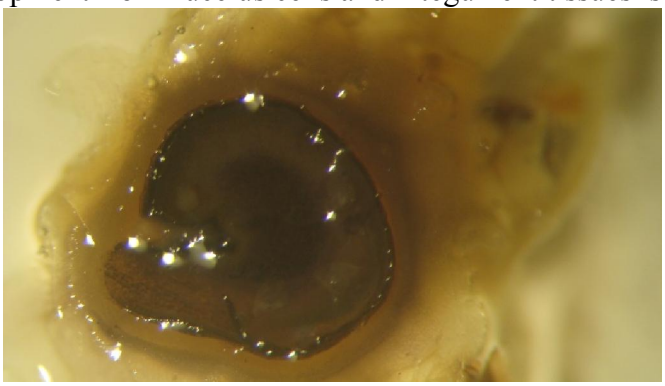

Fig. 5. Development of apozygosis embryo formed from nucelus cells

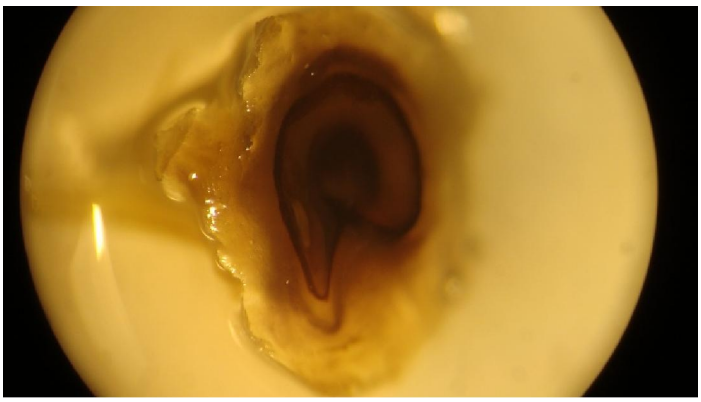

Fig. 6. Development of apozygosis embryo from integument tissue

Method of cell population mixoploidy determining in sugar beet with apozygosis. Alongside with pollenless and temperature modes, cell population mixoploidy is also the factor of apozygosis identification in sugar beet.

Ploidy analysis using cytological methods includes: fixation, chromosome reduction, staining. Positive result depends on both the material quality and possibility to select metaphase, a specific stage of the cell cycle division. Often metaphase chromosomes are located not in the same plane. A new method of flow cytometry using «Partec» automatic ploid analyzer simplifies the experiment. We did not use active division of cells (apical merisystems) for the experiment, but easy for analysis sugar beet leaf cell population.

To determine ploidy level of new apomictic materials labeling of breeding material was performed as well selected young leaves with petioles. Strips of parchment paper with the specified number were tied to petioles with thin string or thread. If necessary, the test samples were transported in moistened filter paper packages. Living tissue of upper and middle part of the leaf (1-2см^2) was used to prepare nucleus suspension. The tissue was grinded up by means of blade (avoiding tissue crushing) in Petri dishes with 0.5 ml of buffer "F" (IBCSB). When leaf grinded, 0.5 ml of fluorochrome DAPI (Partec, Germany) and 1 ml of buffer "F" were added to Petri dish (IBCSB). The mixture was kept still for 5 minutes at room temperature in Petri dishes and filtered through nylon filter to clean the nuclei of large cell fragments and leaf remnants. Measurement of fluorescence intensity and the number of nuclei in 1 cm^3 of the solution was carried out in "Partec" cytometer [7]. Test tubes with cell suspension were connected to the electrodes and ploidy level was determined under histograms. The data were put into the table (Table 2).

Table 2

Ploidy of apomict plants grown in greenhouse

No	Breeding number	Plant (bush) number	Number of tested plants	2x	3x	4x	x; 2x; 4x;	2x; 4x;8x;
1	12-136 CMS	1	30	30				
		2	30	30				
2	12-138 CMS	3	30	20		5	2	3
		4	30	15	1	10	3	1

Notes: **x; 2x; 4x; mixoploid population with the average rate of fluorescence intensity in channels; 50; 100; 200;*

***2x; 4x; 8x; mixoploid population with the average rate of fluorescence intensity in channel; 100, 200, 400.*

According to our data, ploidy level stabilized after the fourth generation. Lines of 90-100% combination of sterility, flower separating and diploid genome level signs were used in subsequent breeding practice.

Conclusions. According to results of our research, we discovered that for induction of apozygosis in sugar beet MS-lines is necessary to create a combination of the following stress factors: pollenless mode and corresponding temperature regime during flowering. Identification of A_1 plants with apozygosis reproduction method should be done on the basis of cell population analysis in ploidy by cytophotometric method using AP «Partec» under embryological control.

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Анотація

Яцева О.А.

Методи індукції апозиготії цукрових буряків в умовах селекційно-тепличного комплексу

Розглядаються методи індукції апозиготичного розмноження у пилкостерильних ліній цукрових буряків у селекційно-тепличному комплексі. Визначено методичні підходи для отримання стерильних та роздільноквіткових ліній з апозиготичним зав'язуванням насіння. Встановлено необхідність поєднання стресових факторів (без пилковий режим і контрастність температур під час цвітіння) для індукції цього явища. Визначено способи ідентифікації апозиготії.

Ключові слова: *безпилковий режим, апозиготія, контрастні температури, міксоплоїдія, цукрові буряки*

Аннотация

Яцева О.А.

Методы индукции апозиготии сахарной свеклы в условиях селекционно-тепличного комплекса

Рассматриваются методы индукции апозиготического размножения в пыльцестерильных линий сахарной свеклы в селекционно-тепличном комплексе. Определены методические подходы для получения стерильных и раздельноцветковых линий с апозиготическим завязыванием семян. Установлена необходимость сочетания стрессовых факторов (безпыльцевойрежим и контрастность температур во время цветения) для индукции этого явления. Определены способы идентификации апозиготии.

Ключевые слова: *беспыльцевой режим, апозиготия, контрастные температуры, миксоплоидия, сахарная свекла*