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Introduction of base line and receiving sterile culture of sugar sorghum (Sorghum saccharatum (L.) Pers.)

Наведено результати введення вихідного матеріалу та отримання стерильної культури сорго цукрового, його польову схожість та життєздатність насіння різних сортів та фракцій. Встановлено, що сходи сорго цукрового на ділянках із висівом крупної фракції насіння з'являються раніше порівняно з дрібним насінням. Найістотніша різниця в часі появи сходів спостерігається між великим і дрібним насінням, в той час як між середнім та великим відмінність у появі сходів менш контрастна.
Стійку життєздатність мають рослини, вирощені з великих та середніх фракцій насіння. Досліджено вплив різних хімічних реагентів і експозицій на вихід стерильних і життєздатних експлантів. Найвищий відсоток стерильного та життєздатного насіння сорго було за використання розчину 35% білизни за експозиції 35–40 хвилин, що забезпечило стерильних – 100% та життєздатних – 93,0% експлантів.
Найвищий відсоток стерильних і життєздатних міжвузлів сорго отримано за експозиції 35–40 хвилин та 0,2% розчину сулеми.

Ключові слова:
сорго, вихідний матеріал, насіння, стерилізація, життєздатність, експланти.

Introduction. Considering the importance of production of alternative types of biofuel that can be obtained from agricultural bioenergy feedstock, it is necessary to develop breeding of energy crops. In the world of breeding practice for acceleration of its productivity use biotechnological methods which allow not only to reproduce and store the materials created by traditional methods, but also to obtain new forms based on the advanced achievements of biotechnology [1, 2]. Therefore, the goal of the research was to establish the influence of the size and the weight of sugar sorghum seeds on its sowing properties and to develop introduction of the base line and its sterilization by using different explants.

Materials and technique of researches. The research were carried out in Sector of Culture of Tissues and Plants in vitro of Department of Genetics and Cytology and Sector of Seed Farming of Biopower Cultures of Institute of Bioenergy Crops and Sugar Beet NAAS of Ukraine.

Experiments with seeds were made on standard and generally accepted scientific techniques [3, 4].

As source material for receiving sterile culture used seeds and internodes of hybrids of sugar sorghum: Medovy, Nectarny, Sylosny 42. Cultivation was carried out in the thermal areas at temperature of 24 ± 2 °C, illumination of 3000–4000 lux, relative humidity of 65–70% and the photoperiod – 16 hours.

Sterilization of explants was carried out by dichloride solution of mercury (mercuric chloride), aqueous solution of sodium hypochlorite, chloramines at various concentration and expositions.

Efficiency of sterilization defined: for 5–10 days percent of sterile explants, and for 10–14 days – viable.

Materials and tools, ware and nutrient mediums prepared according to the standard techniques [5, 6].

For further cloning of bines used modified solid nutrient medium by prescription of Murasihe and Skuga [7, 8].

The resulting digital material was processed according to conventional methods [8].

Results and discussion. The main condition for a successful receiving of the base line is the sterilization of plant objects consisting in killing of the fungal and bacterial spores on the external surface without damage of internal tissues. For this purpose use various sterilizing agents.

From the literature it is known that for superficial sterilization of plant tissues using compounds containing active chlorine (so-

dium hypochlorite, calcium hypochlorite, chloramines), mercury drugs (corrosive sublimate, diacid) and oxidizers (hydrogen peroxide, potassium permanganate), ethyl alcohol, rarely – the concentrated sulfuric acid, preparations of nitrate silver and antibiotics [1].

Type of sterilizing material, its concentration and duration of application depend on the density and sensitivity of tissues will be sterilized. The right choice of sterilizing material is that it has harmful affected all microorganisms and at the same time marginally damaged tissues. One more important condition is that sterilizing material should be easily removed from tissue by washing with distilled water or decompose. Otherwise there is a poisoning of tissues that negatively influences viability.

Seeds of sugar sorghum are different on form and color: oval, egg-shaped, barrel-shaped, rounded, elongated and another; in size – large (the mass of 1000 grains is more than 25–30 g), medium (mass of 1000 grains is 20–25 g) and small (the mass of 1000 grains is less than 15–20 g); on tunica color – white, orange, brown, cream and other colors and shades; on prevalence of skin – membranous and bare granular [4].

Data of phenological observations show that shoots in areas with a large fraction of sowing seeds there is usually earlier spire compared with small seeds. It should be noted that the most essential time difference of emergence of shoots is observed between big and small seeds, while between average and big distinction in emergence of shoots less contrast. In areas with big sowing seeds shoots appear for 2–3 days earlier compared to plots where sowed small fractions of seeds however further the difference of approach of fenophase is leveled.

Daily calculation of quantity of plants, came up within 7 days, from the date of emergence of shoots showed that large seeds germinate more intensively and for the 2nd day from the beginning of emergence of shoots, the quantity of the sprouted seeds reaches 64.3%, while for seeding of small seeds only 40%. The same tendency was observed and for the seventh day – field viability of seeds of small fraction was 34.6% lower. For seed mixture, field viability for the seventh day was 14.3% higher than than in small quantity and 20.3% less than seeding by large seeds (tab. 1).

Table 1

Dynamics of of germination and field emergence of seeds of sugar sorghum depending the mass of 1000 seeds, 2009–2012 rr.

Variant	Mass of 1000 seeds, г	Number of plants came up from early germination by days, %				
		the first	the second	the fifth	the seventh	full sprouts
Sowing: Mixture	17–20	40.2	53.1	59.2	61.4	85.7
big	25–30	57.6	64.3	74.3	81.7	90.3
average	20–25	51.4	58.6	67.1	68.6	89.1
small	15–20	14.3	40.0	42.9	47.1	79.1
HiP_{05}						4.6

To obtain high yields it is important not only receiving full-fledged shoots, but also providing conditions for preservation of plants by the end of the growing season in order to have the optimum number of plants per unit area. By results of researches it is established that the plants which have been grown up from big and average fractions of seeds have steady viability. Number of surviving plants by sowing seeds of large and medium-sized fractions is 89–94%, while in areas with sowing small seeds – no more than 84%, and a mixture – 86.1%.

By results of laboratory and field researches as the base line it was chosen seeds of sugar sorghum an average in size and yield of 2012 (fig. 1).

For sterilization we used: mercury dichloride, aqueous solution of sodium hypochlorite, chloramines on expositions of 20–25 and 35–40 minutes. In laminar camera shoots were transferred to the sterile bottles, filled with 0.2 and 0.4% solution of corrosive sublimate and closed lids. For better sterilizing flasks were placed on shaker. In 20–25 and 35–40 minutes solution of corrosive sublimate was poured into a special container and washed three times with sterile distilled water, changing the water every 15–20 minutes. Sterilized material was removed on filter paper, which sterilized in the autoclave under pressure of 1.5 atm for an hour and dried up in the dry-heat oven at 100 °C, cut off with sterile scalpel particles of tissues cuts that directly have contact with the sterilizing solution. Explan length 1.0–2.0 cm was planted on sterile environment for clonal microreproduction.

The results of the data indicate that at sterilization by corrosive sublimate at concentration of 0.4% and an exposition of 20–25 minutes it is provided 100% sterility of seeds and internodes, but there is no viable explants. Reducing the concentration of 0.2%, and increasing the exposition of 35–40 allowed to receive 100% sterile

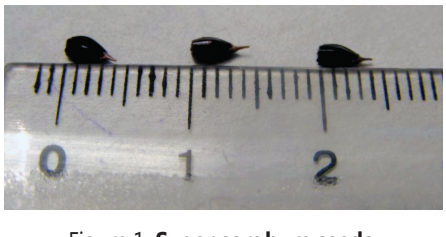

Figure 1. **Sugar sorghum seeds.**

Table 2

Effect of sterilization on the viability and sterility of explants

Sterilizing material	Concentration, %	Exposition, min.	Explants, %			
			sterile		viable	
			seed	internode	seed	internode
corrosive sublimate	0,4	20–25	100.0	100.0	–	–
	0,2	35–40	100.0	98.0	–	76.3
sodium hipochlorite	35	35–40	100.0	95.0	93.0	–
	45	20–25	87.6	63.2	–	–
Chloramine	35	35–40	61.4	50.1	–	–
	45	20–25	60–,5	45–6	–	–

and 76.3% viable internodes. By using sodium hypochlorite and chloramines it is not noted viable internodes and found a low percentage of sterile explants. It should be noted that the highest percentage of sterile and viable seeds of sorghum was when using sodium hypochlorite solution of 35% at exposition 35–40 minutes, which provided 100% sterile and 93.0% viable explants (Table 2).

Figure 2. **Sterile and sprouted sorghum seeds.**

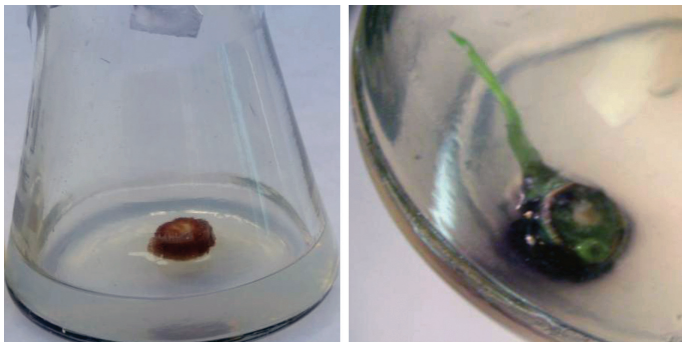

Figure 3. **Sterile and sprouted sorghum internodes.**

Conclusions. Seeds of sugar sorghum of large and medium fractions has field germination (68.6–81.7), so they are more valuable seed grain than seeds of small fraction.

The plants which have been grown up from large and average fractions of seeds have viability within 87–94% against 84%, grown up of small fraction and 86.1% – in control.

Should be used for sterilization of seeds of sugar sorghum 35% sodium hipochlorite solution on expositionfor of 45 minutes that provides receiving 92% of sterile and 86% of viable explants.

The highest percentage of sterile and viable internodes of sorghum it was obtained by exposition of 35–40 minutes and 0.2% of solution of corrosive sublimate.

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