Creation of new high-sugar forms of sweet sorghum as a source of raw materials for the production of bioethanol in the conditions of Submontane Crimea

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Abstract. This work displays the results of the research aimed at the creation of high-sugar forms of sweet sorghum. During 2019 and 2020 samples of sweet sorghum from the collections of N. I. Vavilov All-Russian Institute of Genetic Resources of Plants and the Agrotechnological Academy FSAEI HE “V. I. Vernadsky Crimean Federal University” were studied, new hybrids were derived. Of the particular interest are the high-sugar forms. The juice of sorghum stems contains sugars that can easily compete in quality with those of sugar beet. The plant can be used to produce alcohol and biofuel.

1. Introduction
Sweet sorghum (Sorghum saccharatum – (J.) Pers.) is an agricultural plant that received its name because of the concentration of soluble sugars (fructose, glucose, saccharose) in its stem juice [1-7]. Sorghum is a multi-purpose plant the potential of which is exploited in three main areas: as animal feed, in the production of sugar syrup, and as a source of biofuel.

The following factors allow the use of the plant as biofuel:

- Its short growth cycle (around four months);
- Easy seed multiplication;
- High potential for completely mechanized production;
- The use of sugars from the plant stem to receive starch from grains as raw material for the ethanol-blended fuel;
- Highly efficient use of water and nutrients;
- The possibility to use of the byproducts (pulp and feed) to generate energy;
- High adaptability to various sorts of environments [1].

This plant grows in tropical and subtropical areas as well as in moderate climate zones. Sorghum does not require irrigation or high rainfall. The plant can accumulate up to 12-20% of sugars in the stem juice [8]. Because of its origin and species diversity, sorghum allows receiving stable high-yielding crops of grains and the green mass even in the hottest and most drought-affected regions of...
the world, unlike other agricultural plants. This fact makes sorghum one of the leading grain-fodder and food crops. Sweet sorghum is cultivated in about 100 countries on more than 44 million hectares. Upon the availability of a large number of studies aimed at the production of biofuel, sorghum is mostly cultivated to produce syrups, animal feed and grains. In the United States and Europe this plant is grown in industrial quantities for the production of ethanol that is further mixed with fossil fuels [5]. In the last decade studies aimed at the generation of high-sugar forms of sorghum have started to develop in Russia again. The interest in cultivating sweet sorghum in the conditions of the Crimea is increasing in view of the fact that this plant can be cultivated in drought-affected southern regions where growing sugar beet is either unprofitable or impossible [3; 9]. Studies aimed at the generation of new high-sugar lines, varieties and hybrids of sweet sorghum are now being conducted in the Crimea.

2. Materials and methods

The hybrids of sweet sorghum obtained when crossing sweet sorghum varieties of different geographic origin from the collection of N. I. Vavilov All-Russian Institute of Genetic Resources of Plants and the Agrotechnological Academy FSAEI HE “V. I. Vernadsky Crimean Federal University” were selected for the research. All in all, 115 varieties, lines and hybrids $F_1$ of sweet sorghum were studied. Out of them, 10 forms were selected for this work: Pamyati Shepelya, Krymskoe 15, Early Fulgar, Iskra 2 S x Early Fulgar ($F_1$), (Iskra 2 S x GOS11)S x Early Fulgar ($F_1$), Prosvet 1/1, (Korichnevaya 11 S x GOS 11)S x Prosvet 1/1 ($F_1$), PNS 2-13, (Korichnevaya 11 S x GOS 11)S x PNS 2-13 ($F_1$), Iskra 2 S x PNS 2-13 ($F_1$).

The experiment was conducted in three replications in 2019-2020 on the experimental field of the Academy, on two-line plots of 14 m$^2$ each. Growth rate studies and development phenological observations were carried out during the vegetative period; biometric measurements were taken before the harvesting. During the middle dough stage, the measurements of sugar content of stem juice were taken. It is during this period that the plants accumulate the largest amount of sugars. The juice was extracted from the stems at the level of the third or fourth internode from five plants with isolated panicles.

The sugar content of sorghum stem juice in the field was determined using a refractometer. The concentration of sugar (Brix) in a refractometer is measured on the scale of 0 ° to 30 °Bx, where 1°Bx = 1% of sugars, which indicates the presence of 1 g of dry matter in 100 ml of juice [8].

The determination of dry matter and hygroscopic moisture content was carried out at the Department of Plant-grower of the Agrotechnological Academy FSAEI HE “V. I. Vernadsky Crimean Federal University” using methods for determination of dry matter content (GOST 31640-2012). The determination of crude ash, crude fiber and crude protein content was conducted in a certified laboratory in accordance with GOST 32933-2014, GOST 13496.4-2019 and GOST 31675-2012. The data received were processed using the analysis-of-variance method by Dospekhov [4].

3. Results

Our research was aimed at obtaining new high-sugar varieties and hybrids of sweet sorghum in the conditions of Submontane Crimea. The aims were the following:

- Creating new $F_1$ hybrids using hybridization method;
- Obtaining new self-pollinated forms of sorghum with high content of sugar in the stem juice using multiple inbreeding method.

As a result of the research, the forms showed ambiguous results that could be associated with the weather conditions (figure 1) [6]. With the lack of water after a warm winter, the seedlings were sparse and weak in 2019 whereas the heavy rainfall after the seedling growth in June 2019 and May
2020 resulted in soil washout. This situation led to the long organogenesis period of sweet sorghum. It lasted until May 23-25 in comparison to a good year, when the planting takes place on May 4-7, and the seedlings appear in 7-8 days, on May 11-15.

Great attention should be given to the evaluation of samples by the length of the vegetation period, the necessity of which was pointed out by N.I. Vavilov and N.N. Kuleshov in the 1930-s. Late ripening forms of sweet sorghum give higher yields in the dry conditions of the Crimea. Because of the longer vegetation period, late ripening forms can get the rainfall of September.

The varieties and hybrids of sweet sorghum can be subdivided into the following groups basing on the length of the vegetation period: early ripening forms (96-100 days) – (Korichnevaya 11 S x GOS 11)S x Prosvet 1/1, middle-early forms (101-105 days) – Pamyati Shepelya, (Korichnevaya 11 S x GOS 11)S x PNS 2-13, Early Fulgar, Prosvet 1/1, PNS 2-13, and mid-ripening forms (106-110 days) – Early Fulgar, Iskra 2 S x Early Fulgar, Iskra 2 S x PNS 2-13, (Iskra 2 S x GOS11)S x Early Fulgar. The latest ripening form is Krymskoe 15 (115 days) (table 1).

| Names of varieties and hybrids | Plant height, cm | Panicle length, cm | Leaf, cm | Nodes, items | Stem diameter, cm |
|-------------------------------|------------------|--------------------|----------|--------------|------------------|
| Pamyati Shepelya              | 101              | 211.6              | 23.4     | 55.3         | 11               | 1.6              |
| Early Fulgar                 | 106              | 160.4              | 24.7     | 62.4         | 8.0              | 11               | 2.0              |
| Iskra 2 S x Early Fulgar      | 107              | 158.0              | 24.0     | 60.7         | 7.3              | 10               | 2.0              |
| (Iskra 2 S x GOS11)S x Early Fulgar Prosvet 1/1 | 109 | 161.9 | 24.5 | 57.1 | 8.1 | 9 | 2.0 |
| (Korichnevaya 11 S x GOS 11)S x Prosvet 1/1 | 105 | 132.3 | 12.7 | 66.2 | 6.3 | 9 | 1.9 |
| PNS 2-13 | 100 | 180.7 | 19.3 | 62.7 | 7.3 | 12 | 2.0 |
| Iskra 2 S x PNS 2-13 | 103 | 201.6 | 28.0 | 59.6 | 5.9 | 9 | 1.6 |
| PNS 2-13 | 102 | 171.0 | 27.5 | 61.4 | 6.7 | 10 | 1.9 |
| Krymskoe 15 | 108 | 170.0 | 27.3 | 55.0 | 6.9 | 9 | 1.6 |

Table 1. Morpho-biological characteristics of varieties and hybrids of sweet sorghum (average for 2019-2020).
Plant height is quite an important characteristic. It is positively correlated with anther characteristic, the yielding capacity of the green mass. In this case the plant height should not exceed 2.5 m as higher plants complicate combine harvesting of the ensilage. According to the results of a two-year research, the varieties with the highest plants are: Pamyati Shepelya – 211.6 cm, Krymskoe 15 –207.7 cm, PNS 2-13 – 201.6 cm. The majority of the samples are medium-grown plants (200-151 cm): Iskra 2 S x Early Fulgar – 158.0 cm, (Iskra 2 S x GOS11)S x Early Fulgar – 161.9 cm, (Korichnevaya 11 S x GOS 11)S x Prosvet 1/1 – 180.7 cm, (Korichnevaya 11 S x GOS 11)S x PNS 2-13 – 171.0 cm.

Grain panicles of sweet sorghum are not used as raw material for sugar syrup, but they can be used in the production of animal feed, biofuel, in technical industry, etc. Most varieties and hybrids have middle-length panicles (21-30 cm) – for example, Pamyati Shepelya, Krymskoe 15, Iskra 2 S x Early Fulgar, (Iskra 2 S x GOS11)S x Early Fulgar. Prosvet 1/1 and (Korichnevaya 11 S x GOS 11)S x Prosvet 1/1 formed the panicles that were less than 20 cm long.

Leaf size affects the photosynthetic activity of sweet sorghum plants. The samples with the biggest leaves included Early Fulgar (62.4 х 8.0 cm) and Krymskoe 15 (64.4 х 9.1 cm). The number of nodes on the stem varied between 9 and 12 items, with Krymskoe 15 variety exceeding this indicator (14 nodes). The varieties with the biggest stem diameter included Early Fulgar, Iskra 2 S x Early Fulgar, (Iskra 2 S x GOS11)S x Early Fulgar – 2.0 cm, and Krymskoe 15 – 2.2 cm.

The results of the studies showed that in 2019-2020 hybrids and varieties had different stem juice sugar content. In 2019 it varied between 13.3 % ((Iskra 2 S x GOS11)S x Early Fulgar) and 22.7 % (Prosvet 1/1), and in 2020 – between 11.2 % ((Iskra 2 S x GOS11)S x Early Fulgar) and 18.3% ((Korichnevaya 11 S x GOS 11)S x Prosvet 1/1) (figure 2).

As a result of the research, were identified two forms with the highest content of sugars: simple hybrid F₁ Iskra 2 S x PNS 2-13 and three-line hybrid F₁ (Korichnevaya 11 S x GOS 11)S x Prosvet 1/1. Besides, the stem juice of such varieties as Pamyati Shepelya, Prosvet 1/1, PNS 2-13 and Krymskoe 15 contains more than 15% of sugars.

In spite of the dry conditions during 2019-2020, sweet sorghum showed quite a high level of shoot yielding capacity. On average, the indicator varied between 31.6 and 50.0 tons per hectare (t/ha) during these two years. The highest shoot yield was observed in Krymskoye 15 (50.0 t/ha), (Iskra 2 S x GOS11)S x Early Fulgar (44.6 t/ha), Pamyati Shepelya (43.4 t/ha) (table 2).
Table 2. Yielding capacity and calculated sugar yield of sweet sorghum crops (average data per 2019-2020).

| Indicator | Pamyati Shepelya | Early Fulgar | Iskra 2 S x Early Fulgar | GOS 11 S x Early Fulgar | Prosvet 1/1 | (Korichneva 11 S x GOS 11 S) x Prosvet 1/1 | PNS 2-13 | (Korichneva 11 S x GOS 11 S) x PNS 2-13 | Iskra 2 S x PNS 2-13 | Krymskoe 15 |
|-----------|-----------------|--------------|-------------------------|-------------------------|------------|-------------------------------------------|---------|------------------------------------------|----------------------|-------------|
| Shoot yielding capacity, t/ha | 43.4 | 37.2 | 39.3 | 44.6 | 32.3 | 34.0 | 31.6 | 38.6 | 42.2 | 50.0 |
| Grain yield in panicles, t/ha | 7.7 | 7.7 | 8.4 | 9.4 | 5.5 | 6.7 | 5.7 | 8.0 | 8.7 | 8.3 |
| Grain yield, t/ha | 2.5 | 2.6 | 3.1 | 3.4 | 2.0 | 2.6 | 2.3 | 3.1 | 3.2 | 3.0 |
| Stem yield, t/ha | 28.6 | 24.1 | 25.2 | 27.4 | 20.6 | 23.2 | 19.6 | 24.9 | 27.7 | 34.6 |
| Juice content, t/ha | 21.5 | 18.1 | 18.9 | 20.6 | 15.4 | 17.4 | 14.7 | 18.7 | 20.7 | 25.9 |
| Juice yield after the extraction, t/ha | 17.1 | 14.4 | 15.1 | 16.4 | 12.3 | 13.9 | 11.8 | 14.9 | 16.6 | 20.7 |
| Sugar content of stem juice, % | 16.7 | 14.6 | 14.4 | 12.5 | 19.5 | 18.3 | 18.5 | 13.1 | 15.9 | 15.5 |
| Sugar yield, t/ha | 2.9 | 2.1 | 2.2 | 2.0 | 2.4 | 2.5 | 2.2 | 1.9 | 2.6 | 3.2 |
| Alcohol yield, dal/ha | 160.9 | 118.1 | 121.8 | 114.7 | 134.4 | 142.9 | 122.6 | 109.2 | 147.8 | 180.3 |

Stem juice contains the highest amount of sugars. Thus, stem content in the shoot yield structure of sweet sorghum is of paramount importance. It should be noted that high yield of stems per hectare is characteristic for the varieties and hybrids with high shoot yield indices: Krymskoye 15 (34.6 t/ha), (Iskra 2 S x GOS11)S x Early Fulga (27.4 t/ha), Pamyati Shepelya (28.6 t/ha).

According to research results, the samples studied provided 1.9 - 3.2 t/ha of sugar yield. It is also worth noting that, unlike beet sugar, the sugar obtained from sweet sorghum does not crystalize as it contains glucose and fructose as well as saccharose. The obtained syrup can be used for the production of bioethanol as well as in food industry. The varieties with the highest alcohol yield are Krymskoye 15 (180.3 dal/ha) and Pamyati Shepelya (160.9 dal/ha).

However, sweet sorghum grain can be used for the production of bioethanol as well as the sugar of this plant due to its high starch content. With the grain yielding capacity of 3.0 t/ha of Krymskoe 15, the starch content of which makes 69%, the alcohol yield equaled 123.2 dal/ha (41.1 dal/1 ton of raw material). For Pamyati Shepelya, the grain yielding capacity made 2.5 t/ha, starch content equaled 62%, and alcohol yield made 92.4 dal/ha (36.9 dal/1 ton of raw material) (table 3).

Table 3. Estimate indicators of alcohol yield of sweet sorghum grain (average data per 2019-2020).

| Variety name | Grain yielding capacity, t/ha | Amount of fermentable carbohydrates, t/ha | Alcohol yield, dal/ha | Alcohol yield, dal/1 ton of raw material |
|--------------|-------------------------------|------------------------------------------|----------------------|------------------------------------------|
| Krymskoe 15  | 3.0                           | 2.0                                      | 123.2                | 41.1                                     |
| Pamyati Shepelya | 2.5                           | 1.5                                      | 92.4                 | 36.9                                     |
The sorghum bagasse left after the extraction also has an enormous energetic potential: it contains more than two thirds of the whole energy reserve. Bagasse can be used to produce bioethanol, biobutanol, fuel briquets and pellets, biogas, etc.

Sweet sorghum is more than just raw material for the production of sugar and bioethanol: it can also be used as feed for agricultural animals. The determination of crude ash, crude protein and crude fiber in the dry matter of sweet sorghum biomass allows determining the feed qualities of the plant (table 4).

| Variety, hybrid                     | Hygroscopic moisture (%) | Air dry matter (%) | Crude ash (%) | Crude protein (%) | Crude fibre (%) |
|-------------------------------------|--------------------------|-------------------|---------------|------------------|-----------------|
| Pamyati Shepelya                   | 14.93                    | 85.07             | 3.96          | 5.75             | 20.92           |
| Early Fulgar                       | 11.39                    | 88.61             | 4.57          | 8.88             | 16.88           |
| Iskra 2 S x Early Fulgar           | 18.41                    | 81.59             | 5.08          | 10.06            | 13.03           |
| (Iskra 2 S x GOS11) x Early Fulgar | 9.45                     | 90.55             | 5.66          | 10.00            | 16.76           |
| Prosvet 1/1                         | 17.82                    | 82.18             | 3.21          | 7.50             | 15.00           |
| (Korichnevaya 11 S x GOS 11) x Prosvet 1/1 | 13.00                | 87.00             | 3.60          | 7.75             | 14.13           |
| PNS 2-13                            | 14.36                    | 85.64             | 6.13          | 7.50             | 17.71           |
| (Korichnevaya 11 S x GOS 11) x PNS 2-13 | 14.85                 | 85.15             | 5.60          | 10.63            | 17.30           |
| Iskra 2 S x PNS 2-13                | 13.43                    | 86.57             | 4.86          | 12.25            | 12.22           |

When developing animal diets, what is usually taken into consideration is not the moisture but the air dry matter determined as the difference between 100% and moisture. It has been determined that the air dry matter weight content in sorghum plants varied between 81.6 % (Iskra 2 S x Early Fulgar) and 90.6 % ((Iskra 2 S x GOS11) x Early Fulgar).

The dry matter fraction called crude protein has the highest indicator. Proteins consisting of amino acids make the major part of crude protein. The higher this indicator is, the higher the feeding value of sorghum feed is. The chemical composition of such hybrids as Iskra 2 S x Early Fulgar, (Iskra 2 S x GOS11) x Early Fulgar, (Korichnevaya 11 S x GOS 11) x PNS 2-13, Iskra 2 S x PNS 2-13 contain the highest amount of crude protein (10.0 % - 12.25 %).

Crude fiber plays the role of energy source in animal diet, and provides normal digestive processes. Crude fiber accounts for 12.22 % (Iskra 2 S x PNS 2-13) to 20.92 % (Pamyati Shepelya) in sorghum cultures.

Crude ash consists of the oxides and salts of the mineral element feed contained in the dry matter, and the inclusions of sand, clay and unburned particles of coal. Such varieties as Prosvet 1/1 and PNS 2-13 contained the lowest amount of crude ash (3.21 % and 6.13% respectively).

**4. Discussion**

Sweet sorghum is a source of animal feed, sugar and biofuel. Its high yielding capacity and elevated concentration of sugars in the stem juice enables us to use this plant to obtain larger amounts of sugar and alcohol.

In spite of the great benefits of sorghum cultures, the crop areas of this plant in the Crimea are still inconsiderable. One of the main reasons of that is poorly managed seed industry, insufficient development of zone and sort agricultural equipment necessary to grow this plant on non-irrigated lands.

The widespread use of sorghum cultures allows improving the production of sugars in the drought-affected southern regions of our country, including the Crimea.

The specialists of the Agrotechnological Academy FSAEI HE “V. I. Vernadsky Crimean Federal University” pay a lot of attention to sweet sorghum selection. It is intended that further studies be aimed at improving genetic selection methods, and creating new varieties and hybrids of sorghum.
basing on the cytoplasmic male sterility with a complex of valuable morphological and agricultural characteristics, high feed qualities, high crop yielding capacity and elevated concentration of sugars in the stem juice in the conditions of Submontane Crimea.

5. Conclusion
The following conclusions were drawn as a result of the research conducted:

- The analysis of concentration of sugars in the stem juice of sweet sorghum in 2019-2020 demonstrated the effectiveness of targeted selection for sugar content. The following forms were singled out as a result of selection work aimed at the creation of high-sugar sweet sorghum varieties: simple hybrid F1 Iskra 2 S x PNS 2-13, three-line hybrid F1 (Korichnevaya 11 S x GOS 11) S x Prosvet 1/1, and such varieties as Pamyati Shepelya, Prosvet 1/1, PNS 2-13 and Krymskoe 15 (sugar content of the stem juice more than 15%);
- Samples with high crop yielding capacity have the maximal stem output per hectare: Krymskoe 15 (34.6 t/ha), (Iskra 2 S x GOS11)S x Early Fulga (27.4 t/ha), Pamyati Shepelya (28.6 t/ha);
- Sweet sorghum sugar syrup can be used in food industry as well as in the production of ethanol. Krymskoe 15 and Pamyati Shepelya, the varieties with high sugar output (3.2 and 2.9 t/ha respectively), are characterized by the maximal output of alcohol (180.3 dal/ha and 160.9 dal/ha respectively);
- Sweet sorghum grain can also be used as raw material for the production of bioethanol. On average, in 2019-2020 the grain yielding capacity of Krymskoe 15 (starch content 69%) equaled 3.0 t/ha with the alcohol output equaling 123.2 dal/ha (41.1 dal/ton of raw material). For Pamyati Shepelya (starch content 62%), the alcohol output made 92.4 dal/ha (36.9 dal/ton of raw material) with the yielding capacity of this variety equaling 2.5 t/ha;
- Sweet sorghum is a source of animal feed. The chemical analysis of the air-dry matter of sorghum sprouts allowed determining that the varieties with the highest feeding value are the ones with high protein levels (10.0 % - 12.25 %): Iskra 2 S x Early Fulgar, (Iskra 2 S x GOS11)S x Early Fulgar, (Korichnevaya 11 S x GOS 11) S x PNS 2-13, Iskra 2 S x PNS 2-13.

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