Valuable agronomic traits of chufa (*Cyperus esculentus* L.) accessions from the VIR collection: methods of preparing nodules for long-term storage

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N. G. KON’KOVA*, G. F. SAFINA

N.I. Vavilov All-Russian Institute of Plant Genetic Resources,
42, 44 Bolshaya Morskaya Street,
St. Petersburg 190000, Russia
* n.konkova@vir.nw.ru

**Background.** Chufa is one of the most promising rare oil-seed crops. The issues of preserving and obtaining high-quality seed material are very important. The development of long-term genebank storage techniques for chufa nodules is a pressing task.

**Materials and methods.** We studied 18 chufa germplasm accessions of various origin. The field study was conducted in 2010–2012 under the environmental conditions of Krasnodar Territory, Russia. Protein and oil content was analyzed according to the guidelines on the methods of quality control and safety of bioactive food additives (R 4.1.1672-03,..., 2004); vitamin E according to the guidelines on the methods of analyzing the quality and safety of food products (Skurikhin, Tutelyan, 1987); fatty acid composition in line with IUPAC (1979) and GOST R 512677-2006, using an IIRGS chromatograph.

**Results and conclusions.** The study of valuable agronomic characters in chufa accessions showed that the height of plants varied from 49 to 69 cm; the number of nodules per plant from 80 to 110 pcs; the weight of nodules per plant from 18 to 49 g; the weight of nodules per plot (1 m²) from 101 to 393 g. Their oil content varied from 13.1 to 21.06 g/100 g, and protein content from 6 to 10%. Chufa accessions contained large amount of vitamin E: 19.23 to 35.23 mg/100 g. The content of saturated fatty acids in chufa oil was 17.75 to 20.99%, monounsaturated fatty acids from 68.21 to 71.55%, and polyunsaturated fatty acids from 9.94 to 12.17%. A technique of preparing chufa nodules for long-term storage was developed. The optimal time to assess germination energy is four days, with 11 days for germination percentage. Storage of chufa nodules for three years at a temperature of +20°C causes a decrease in their germination by 15–16%. When stored under low positive (+4°C) or negative temperatures (−18°C and −196°C), their germination percentage remained unchanged.

**Key words:** yield, oil content, protein, tocopherol, fatty acids, germination percentage, germination energy.

**Хозяйственно ценные признаки образцов чуфы (*Cyperus esculentus* L.) из коллекции ВИР: методика подготовки клубеньков к длительному хранению

**Н. Г. КОНЬКОВА*, Г. Ф. САФИНА**

Федеральный исследовательский центр Всероссийский институт генетических ресурсов растений имени Н.И. Вавилова, 190000 Россия, г. Санкт-Петербург, ул. Б. Морская, 42, 44
* nkonkova@vir.nw.ru

**Актуальность.** Чуфа является перспективной малораспространенной масличной культурой. Поэтому вопросы получения высококачественного посевного материала приобретают большое значение. Актуальной является разработка методики длительного хранения клубеньков чуфы в генбанках растительных ресурсов.

**Материалы и методы.** Материалом для исследования послужили 18 образцов чуфы различного происхождения. Полевое изучение проводилось в 2010–2012 годах в условиях Краснодарского края Российской Федерации. Содержание белка и масла проанализировано в соответствии с руководством по контролю и безопасности биологически активных пищевых добавок Р 4.1.1672-03, 2004, витамина Е — методами анализа качества и безопасности пищевых продуктов. Анализ жирно-кислотного состава — IUPAC (1979), ГОСТ Р 512677-2006, с помощью хроматографа IIRGS 5300. Влажность и влажность клубеньков — по методикам, рекомендуемым для семян сельскохозяйственных культур (ГОСТ 12038-84 и ГОСТ 12041-82).

**Результаты и заключение.** Изучение хозяйственно ценных признаков чуфы показало, что высота растений изменялась в пределах от 49 до 69 см, количество клубеньков одного растения — от 80 до 110 штук, вес клубеньков — от 18 до 49 г, вес клубеньков с делинки (1 м²) — от 101 до 393 г. Содержание масла варьировалось от 13,1 до 21,06 г/100 г, белка — от 6 до 10%, витамина Е — от 19,23 до 35,23 мг/100 г. Показатель насыщенных жирных кислот в масле клубеньков чуфы — от 17,75 до 20,99%, мононенасыщенных жирных кислот — от 68,21 до 71,55%, полиненасыщенных жирных кислот — от 9,94 до 12,17%. Оптимальное время для определения энергии прорастания — четыре дня, влажности — одиннадцать. Хранение клубеньков чуфы в течение трех лет при температуре +20°C вызывает снижение их всхожести на 15–16%. При хранении в условиях низких положительных (+4°C) и отрицательных температур (−18°C и −196°C) всхожесть остается неизменной.

**Ключевые слова:** урожайность, масличность, белок, токоферол, жирные кислоты, всхожесть, энергия прорастания.
Introduction

*Cyperus esculentus* L. is a perennial herbaceous plant of the Cyperaceae family, which naturally grows in the Mediterranean countries and in Africa, along the banks of the Nile. The nodules of this plant are considered one of the earliest food sources known to mankind. It is recognized that they were cultivated by the ancient Egyptians, starting from 5000 BC (Allouh et al., 2015). The *C. esculentus* nodules are commonly known by several names, such as chufa, ground almonds, and tiger nut. Chufa is cultivated in some European countries: the Netherlands, Switzerland, Germany, Poland, Hungary, as well as in some African countries: Mali, Benin and Ivory Coast, as an oilseed and nut-bearing plant. It is grown on an industrial scale in Spain.

In agriculture, it is cultivated as an annual plant. *C. esculentus* is the only cultivated species of the genus Cyperus. Its valuable nutritional qualities are determined by the content of proteins, oil, carbohydrates and micronutrients in the nodules (Nizova, Kon’kova, 2008; Codina-Torrella et al., 2014). Micronutrients include potassium, phosphorus, magnesium, calcium, sodium, iron, zinc, copper, and vitamin C (Suleiman et al., 2018). Chufa oil is used for food purposes. Oleic acid dominates in the oil composition of chufa: its content is comparable to that in olive oil (Arafat et al., 2009). Chufa oil also contains considerable amounts of essential fatty acids, phytosterols, phospholipids, fat-soluble vitamins, and tocopherols (Ezeh et al., 2014; Shakhova et al., 2014). According to the data produced by M. A. Allouh et al. (2015), chufa consumption increases the level of testosterone in human blood. The experiments by C. Imo et al. (2019) showed that even ethanol extracts from chufa did not produce an expressed negative effect on animal liver function. Using chufa as a natural preservative in confectionery, bakery, meat and other food industries is considered quite promising (Kuznetsova et al., 2019; Glotova et al., 2016). Studying the possibility of isolating such natural bioactive compounds with their subsequent use as preservatives to prolong the shelf life of natural products is a promising trend for the development of food industry. Whole-ground chufa nodules were reported to have an extensive shelf life, despite their significant oil content. The research performed by I. V. Bobreneva and A. A. Baioumy (2019) ascertained the possibility of using chufa as a dietary supplement for preventing and normalizing cardiovascular diseases and confirmed its potential for the food industry in increasing the shelf life and nutritional value of various types of meat products. The optimal amount of chufa in the composition of meat products is 5% (Bobreneva, Baioumy, 2019).

The carbohydrate composition in chufa favors its use in the diet of patients suffering from metabolic syndrome, including diabetes mellitus (Sabiu et al., 2017). Due to its unique properties, chufa is an excellent object for inclusion in the list of Biological and Technical Life Support Systems as the main link that regenerates food, water and air in closed space systems (Motorin et al., 2009; Shklavtsova et al., 2014).

Chufa is also widely used for producing the traditional Spanish drink *horchata* de chufa (Butova et al., 2019). It is a white-colored refreshing drink with a pleasant taste. The production of *horchata* has great economic importance for the food industry in Spain, especially for Valencia, the main region of chufa cultivation. About 90% of the chufa harvest is used for making *horchata*, with an annual consumption of up to 50 million liters. *Horchata* production has an estimated retail value of 60 million euros per year (Roselló-Soto et al., 2018a; Roselló-Soto et al., 2018b).

Analyzing physical and chemical properties of chufa oil, such as density, viscosity, or acid, iodine and cetane values, shows that chufa oil can be used for biodiesel production (Sidhouhonde et al., 2018). Chufa flowers rarely, forming small seeds, so it is propagated vegetatively, with nodules developed on underground shoots from special swellings, in the end of the tillering stage. Considering great practical interest in this crop, the problem of its preservation in plant genebanks is very important. Plant genetic resources are preserved in genebanks usually as seeds, but in the case of chufa it is quite difficult to obtain seeds, especially under northern latitudes, so it seemed relevant to explore the possibility of chufa germplasm conservation in the form of nodules, as the propagules of chufa plants. With this in view, the purpose of this study was to study valuable agronomic characteristics of chufa nodules and develop methods for determining nodule germination and preparation for long-term storage under the conditions of a genebank, including the analysis of the impact of different storage temperatures on germination.

Materials and methods

Eighteen chufa accessions of various origin from the VIR collection served as the target material for the study. The field study was conducted in 2010–2012 in the environments of Krasnodar Territory, Russian Federation (Kuban Experiment Station of VIR). Protein and oil content was measured according to the guidelines on the methods of quality control and safety of bioactive food additives (R 4.1.1672-03…, 2004). The fatty acid composition analysis was performed in accordance with standard methods (IUPAC…, 1979; GOST R 512677-2006…, 2007). The composition of fatty acid methyl esters (FAME) was studied using the CARLO ERBA STRUMENTAZIONE IRIRGC 5300 chromatograph (Agilent Technologies, USA). Vitamin E content was measured in accordance with the guidelines on the methods of analyzing the quality and safety of food products (Skurikhin, Tutelyan, 1987).

To assess germination, the nodules were sprouted up in wet paper rolls in a thermostat at a temperature of +25°C. Methods for determining germination percentage basically conformed to those prescribed for large seeds in the Interstate Standards (GOST 12038-84…, 2016). Moisture content in the nodules was measured according to the Interstate Standards (GOST 12041-82…, 2004). Prior to their placement for storage, the nodules were dried according to the technique recommended for the FAO genebanks (Genebank Standards…, 2014) in the drying room at a temperature of +18…+20°C and a relative humidity (RH) of 10–12%. Then, the nodules were hermetically packed in vacuum-laminated foil bags, 25 nodules per bag. Since there is currently no method for storing chufa nodules, sample packages (several packages for each variant) were stored under different temperatures (+20°C, +4°C, –18°C, and –196°C).

Results and discussion

The study of valuable agronomic traits in chufa accessions showed that the height of plants varied within the range from 49 to 69 cm, the number of nodules per plant from 80 to 110 pieces, the weight of nodules per plant from 18 to 49 g, and the weight of nodules per plot (1 m²) from 101 to 393 g. In terms of yield, nine accessions exceeded the average: k-1 (Russia) with 116%; k-2 (Russia) with 114%; k-7 (Poland) with 101%; k-10 (Bulgaria) with 114%; k-12 (Benin) with 113%; k-21 (Belarus) with 107%;
k-14, k-16 and k-17 (Ivory Coast) with 103%, 111% and 190%, respectively. The weight of 1000 nodules was higher than the reference in the following 9 accessions: k-1 (144%), k-2 (153%), k-7 (106%), k-9 (108%), k-10 (144%), k-15 (125%), k-17 (163%), k-21 (144%), and k-23 (102%). The best earliness was observed in k-12 from Benin: it matured earlier than the average schedule (118 days). The accession with the latest maturation was k-20 from Ukraine, with the growing season of 127 days (Table 1).

The analysis showed that oil content in chufa accessions varied from 13.1 to 21.06 g/100 g, and protein content from 6 to 10% (Table 2). The following accessions were identified for the highest oil content: k-13 (Germany) with 20.5 g/100 g of the product, k-21 (Belarus) with 21.01 g/100 g, and k-11 (Mali) with 21.06 g/100 g. Protein content in chufa nodules is higher than in starchy root crops, such as cassava or sweet potato, being comparable with that in cereals, such as rice and sorghum (Suleiman et al., 2018). The following accessions were identified as the best in terms of protein content in nodules: k-18 (Ivory Coast) with 6.75%, k-11 (Mali) with 6.76%, and k-9 (Bulgaria) with 6.79%. Chufa oil contains large amount of vitamin E: according to our data, the range of its variability was 19.23 to 35.23 mg/100 g of oil. Nutritional and physical properties of oil largely depend on the amount and composition of tocopherols (vitamin E) – bioactive compounds that increase the oil’s nutritional value. Moreover, tocopherols are natural inhibitors of highly non-limiting fatty acids and other easily oxidizable substances (Ezeh et al., 2014). The following accessions were identified for their high total vitamin E content: k-7 (Poland) with 35.23 mg/100 g, k-11 (Mali) with 26.54 mg/100 g, and k-19 (Ivory Coast) with 26.35 mg/100 g.

Table 1. Valuable agronomic traits of chufa accessions from the VIR collection (2010–2012)

| VIR catalogue No. / № по каталогу ВИР | Origin / Происхождение | Growing season / Вегетационный период | Yield / Урожайность | Weight of 1000 nodules / Масса 1000 клубеньков |
|----------------------------------|------------------------|-----------------------------------|---------------------|-----------------------------------------------|
|                                 | Days / дни | ± to the average / ± к ср. | g/m² / г/м² | % of the average / % к ср. | g /г | % of the reference / % к ст. |
| 1 Russia / Россия                | 121        | 0                              | 240               | 116                                          | 340  | 144                                          |
| 2 Russia / Россия                | 120        | −1                             | 237               | 114                                          | 360  | 153                                          |
| 7 Poland / Польша               | 121        | 0                              | 210               | 101                                          | 250  | 106                                          |
| 8 Bulgaria / Болгария            | 122        | +1                             | 186               | 90                                           | 150  | 64                                           |
| 9 Bulgaria / Болгария            | 122        | +1                             | 203               | 98                                           | 255  | 108                                          |
| 10 Bulgaria / Болгария           | 123        | +2                             | 236               | 114                                          | 340  | 144                                          |
| 11 Mali / Мали                  | 119        | −2                             | 195               | 94                                           | 235  | 100                                          |
| 12 Benin / Бенин                 | 118        | −3                             | 234               | 113                                          | 150  | 64                                           |
| 13 Germany / Германия            | 122        | +1                             | 155               | 75                                           | 130  | 55                                           |
| 14 Ivory Coast / Кот-д’Ивуар     | 122        | +1                             | 214               | 103                                          | 170  | 72                                           |
| 15 Ivory Coast / Кот-д’Ивуар     | 122        | +1                             | 204               | 99                                           | 295  | 125                                          |
| 16 Ivory Coast / Кот-д’Ивуар     | 124        | +3                             | 230               | 111                                          | 160  | 68                                           |
| 17 Ivory Coast / Кот-д’Ивуар     | 123        | +2                             | 393               | 190                                          | 385  | 163                                          |
| 19 Russia / Россия               | 122        | +1                             | 203               | 98                                           | 190  | 81                                           |
| 20 Ukraine / Украина             | 127        | +6                             | 101               | 49                                           | 170  | 72                                           |
| 21 Belarus / Беларусь            | 121        | 0                              | 221               | 107                                          | 270  | 144                                          |
| 23 France / Франция             | 125        | +4                             | 146               | 71                                           | 240  | 102                                          |
| 25 Ukraine / Украина             | 125        | +4                             | 111               | 54                                           | 165  | 70                                           |
The fatty acid composition of chufa oil is characterized by high content of oleic acid. In the course of our research, k-20 (Ukraine) was identified for the highest content of oleic acid (71.29%). Palmitic acid content in the studied accessions varied from 12.86% to 14.53%, stearic acid from 2.83% to 5.78%, linoleic acid from 9.72% to 11.93%, linolenic acid from 0.15% to 0.29%, and arachidic acid from 0.39% to 0.80% (Table 3).

The content of saturated fatty acids (SFA) varied from 17.75 to 20.99%. The lowest SFA content was observed in the accessions: k-7 (Poland) with 17.75%, and k-20 (Ukraine) with 17.89%. Chufa oil is a valuable source of monounsaturated fatty acids (MUFA) (Mohdaly, 2019). The MUFA content ranged from 68.21 to 71.55%. The highest content was identified in the accessions: k-20 (Ukraine) with 71.55%, and k-13 (Germany) with 70.76%. The content of polyunsaturated fatty acids (PUFA) varied from 9.94 to 12.17%; the highest percentage was observed in the accessions: k-10 (Bulgaria) with 12.17%, and k-14 (Ivory Coast) with 12.07% (Table 4).

Individual accessions were selected according to a set of valuable agronomic traits: k-7 (Poland) for its yield, 1000 nodule weight, oleic and linoleic acid contents, high content of vitamin E, and low SFA content; k-9 (Bulgaria) for its 1000 nodule weight, and high protein content; k-11 (Mali) for

Table 2. Oil, protein and vitamin E content in the nodules of chufa accessions from the VIR collection

| VIR catalogue No. / № по каталогу ВИР | Origin / Происхождение | Oil, g/100 g of the product / Масло, г/100 г продукта | Vitamin E, mg/100 g of oil / Витамин Е, мг/100 г масла | Protein, % / Белок, % |
|--------------------------------------|------------------------|--------------------------------------------------|-------------------------------------------------|---------------------|
| 1                                    | Russia / Россия         | 18.73                                            | 25.84                                           | 5.85                |
| 2                                    | Russia / Россия         | 17.21                                            | 25.06                                           | 6.52                |
| 7                                    | Poland / Польша         | 18.42                                            | 35.23                                           | 6.40                |
| 8                                    | Bulgaria / Болгария     | 18.35                                            | 24.37                                           | 6.49                |
| 9                                    | Bulgaria / Болгария     | 18.22                                            | 22.34                                           | 6.79                |
| 10                                   | Bulgaria / Болгария     | 17.24                                            | 25.00                                           | 6.69                |
| 11                                   | Mali / Мали             | 21.06                                            | 26.54                                           | 6.76                |
| 12                                   | Benin / Бенин           | 20.26                                            | 25.40                                           | 4.17                |
| 13                                   | Germany / Германия      | 20.50                                            | 23.07                                           | 5.66                |
| 14                                   | Ivory Coast / Кот-д’Ивуар| 14.51                                            | 23.95                                           | 4.54                |
| 15                                   | Ivory Coast / Кот-д’Ивуар| 15.25                                            | 24.15                                           | 5.60                |
| 16                                   | Ivory Coast / Кот-д’Ивуар| 13.21                                            | 24.90                                           | 5.84                |
| 17                                   | Ivory Coast / Кот-д’Ивуар| 14.11                                            | 24.00                                           | 5.59                |
| 18                                   | Ivory Coast / Кот-д’Ивуар| 14.56                                            | 23.85                                           | 6.75                |
| 19                                   | Ivory Coast / Кот-д’Ивуар| 19.77                                            | 26.35                                           | 5.62                |
| 20                                   | Ukraine / Украина       | 17.41                                            | 19.23                                           | 5.33                |
| 21                                   | Belarus / Беларусь       | 21.01                                            | 24.32                                           | 4.56                |
| 23                                   | France / Франция        | 20.15                                            | 24.90                                           | 6.30                |
high oil, protein, vitamin E and oleic acid contents; k-12 (Benin) for earliness, yield, and oleic acid content; and k-13 (Germany) for oleic acid and MUFA content. Our research showed that the oil, protein and total vitamin E contents were different in accessions of African and European origin (Codina-Torrella et al. 2014), which was confirmed by the studies where the metabolomic method had been applied to reveal differences in the geographical origin of chufa (Rubert et al., 2018). In European accessions, the oil content ranged from 17.21 to 21.05 g/100 g of the product, protein from 6.53% to 11.21%, and vitamin E from 22.34 to 35.23 mg/100 g of oil. African accessions contained 13.21 to 20.06 g/100 g of oil, and 5.32% to 7.04% of protein, while their vitamin E level varied from 23.85 to 26.54 mg/100 g of oil. No significant differences in the composition of fatty acids were found between the two groups of accessions.

Table 3. Fatty acid composition in the nodules of chufa accessions from the VIR collection, % of the total (C)

| VIR catalogue No. / № по каталогу ВИР | Origin / Происхождение | C 14:0 | C 16:0 | C 16:1 | C 18:0 | C 18:1 | C 18:2 | C 18:3 | C 20:0 |
|-------------------------------------|-------------------------|-------|-------|-------|-------|-------|-------|-------|-------|
| 1 Russia / Россия                   | 0.15                    | 14.28 | 0.28  | 3.87  | 69.40 | 11.09 | 0.29  | 0.64  |
| 2 Russia / Россия                   | 0.14                    | 14.29 | 0.30  | 5.35  | 68.15 | 10.98 | 0.24  | 0.55  |
| 7 Poland / Польша                   | 0.15                    | 14.14 | 0.27  | 2.83  | 70.14 | 11.62 | 0.22  | 0.63  |
| 8 Bulgaria / Болгария               | 0.13                    | 13.79 | 0.28  | 4.60  | 69.23 | 11.10 | 0.22  | 0.66  |
| 9 Bulgaria / Болгария               | 0.14                    | 13.92 | 0.28  | 5.13  | 67.93 | 11.70 | 0.26  | 0.64  |
| 10 Bulgaria / Болгария              | 0.13                    | 13.87 | 0.33  | 4.88  | 68.11 | 11.93 | 0.24  | 0.51  |
| 11 Mali / Мали                      | 0.12                    | 13.16 | 0.25  | 4.76  | 69.89 | 10.90 | 0.24  | 0.67  |
| 12 Benin / Бенин                    | 0.14                    | 12.86 | 0.21  | 5.37  | 70.23 | 10.17 | 0.21  | 0.80  |
| 13 Germany / Германия               | 0.15                    | 13.61 | 0.30  | 4.87  | 70.46 | 9.72  | 0.22  | 0.67  |
| 14 Ivory Coast / Кот-д’Ивуар         | 0.03                    | 14.26 | 0.41  | 4.68  | 67.97 | 11.85 | 0.22  | 0.57  |
| 15 Ivory Coast / Кот-д’Ивуар         | 0.15                    | 14.49 | 0.38  | 4.39  | 68.29 | 11.53 | 0.22  | 0.54  |
| 16 Ivory Coast / Кот-д’Ивуар         | 0.17                    | 14.37 | 0.22  | 4.48  | 69.73 | 10.46 | 0.18  | 0.39  |
| 17 Ivory Coast / Кот-д’Ивуар         | 0.19                    | 14.12 | 0.32  | 4.85  | 69.60 | 10.00 | 0.15  | 0.77  |
| 18 Ivory Coast / Кот-д’Ивуар         | 0.14                    | 13.95 | 0.32  | 4.56  | 68.78 | 11.43 | 0.24  | 0.58  |
| 19 Ivory Coast / Кот-д’Ивуар         | 0.13                    | 14.36 | 0.37  | 4.08  | 69.23 | 11.02 | 0.21  | 0.59  |
| 20 Ukraine / Украина                | 0.12                    | 14.04 | 0.26  | 3.18  | 71.29 | 10.36 | 0.20  | 0.55  |
| 21 Belarus / Беларусь                | 0.14                    | 14.53 | 0.31  | 5.78  | 67.98 | 10.52 | 0.21  | 0.54  |
| 23 France / Франция                 | 0.13                    | 14.44 | 0.28  | 5.69  | 68.57 | 10.10 | 0.25  | 0.53  |
Table 4. The content of saturated and unsaturated fatty acids in the oil extracted from the nodules of chufa accessions from the VIR collection

| VIR catalogue No. / № по каталогу ВИР | Origin / Происхождение | ∑ SFA / ∑ НЖК | ∑ MUFA / ∑ МНЖК | ∑ PUFA / ∑ ПНЖК |
|-------------------------------------|-------------------------|--------------|----------------|----------------|
| 1 Russia / Россия                   | 18.94                   | 69.68        | 11.38          |
| 2 Russia / Россия                   | 20.33                   | 68.45        | 11.22          |
| 7 Poland / Польша                  | 17.75                   | 70.41        | 11.84          |
| 8 Bulgaria / Болгария              | 19.18                   | 65.91        | 11.32          |
| 9 Bulgaria / Болгария              | 19.83                   | 68.21        | 11.96          |
| 10 Bulgaria / Болгария             | 19.39                   | 68.44        | 12.17          |
| 11 Mali / Мали                     | 18.71                   | 70.14        | 11.14          |
| 12 Benin / Бенин                   | 19.17                   | 70.44        | 10.38          |
| 13 Germany / Германия              | 19.3                    | 70.76        | 9.94           |
| 14 Ivory Coast / Кот-д’Ивуар       | 19.54                   | 68.38        | 12.07          |
| 15 Ivory Coast / Кот-д’Ивуар       | 19.57                   | 68.67        | 11.75          |
| 16 Ivory Coast / Кот-д’Ивуар       | 19.41                   | 69.95        | 10.64          |
| 17 Ivory Coast / Кот-д’Ивуар       | 19.93                   | 69.92        | 10.15          |
| 18 Ivory Coast / Кот-д’Ивуар       | 19.23                   | 69.10        | 11.67          |
| 19 Ivory Coast / Кот-д’Ивуар       | 19.16                   | 69.6         | 11.23          |
| 20 Ukraine / Украина              | 17.89                   | 71.55        | 10.56          |
| 21 Belarus / Беларусь              | 20.99                   | 68.29        | 11.23          |
| 23 France / Франция               | 20.79                   | 68.85        | 10.35          |

The nodule sprouting technique was tested on one of the studied accessions: k-14 in the VIR catalogue (Ivory Coast). During the process of germination, the emergence of shoots and roots was observed. Germination energy was assessed on the shoots and roots that reached the nodule size. Assessment of germination percentage took into account robust shoots (from one to three), twice or more times longer than the nodule, with well-developed adventitious roots (Figure 1, 2).

Germination percentage was assessed on the 7th and 11th days. The results showed that seven days were not enough to assess germination, because new full-fledged seedlings continued to appear after that. The optimal time for determining the germination energy was found to be 4 days, and germination percentage 11 days. The initial values of germination energy and percentage were 20.0 ± 2.5% and 80.0 ± 2.5%, respectively. After measuring the germination percentage, the seedlings were planted into pots with soil for greenhouses (turf: 91.7%, limestone flour: 7.1%, NPK fertilizer: 1.2%) and left for further growth to make sure that they would grow into full-fledged plants (Figure 3).

Then the plantlets were transplanted into the open ground. The analysis of yield was made in the end of the growing season (Figure 4).

Nodule preservation experiments were carried out using the example of k-14, an accession from Ivory Coast. One of the requirements for successful long-term storage of plant germplasm is low moisture content. Before the start of the experiments, nodules had moisture content of 5.4%. For storage, the nodules were dried in a drying room at a temperature of +18...+20°C and a relative humidity (RH) of 10–12%. In the process of drying they were periodically weighed and their moisture content was measured. After drying in a drying room for three months, the weight of the nodules became constant at 4.2–4.4% moisture content. Germination percentage before and after drying remained unchanged (80 ± 2.5%). The nodules prepared in this way were hermetically packed in vacuum-laminated foil bags and stored for a long time at different temperatures: +20°C, +4°C, -18°C, and -196°C (in liquid nitrogen) for further research. After three-year storage under different temperature conditions, their germination energy and germination percentage were tested. The experiments were performed in four replications. It was shown that the germination energy, regardless of the storage option, averaged 4.5 ± 2.9%. Germination percentage of nodules stored at +20°C decreased by approximately 15–16%. Under low positive (+4°C) and negative temperatures (-18°C and -196°C), germination percentage remained unchanged (Figure 5). A decrease in germination energy in all storage options may be explained by the drying of nodules before placement for storage and, accordingly, by the slowdown of the initial stage in their germination process.

Thus, even short-term storage of chufa nodules at room temperature adversely affects their germination. Further experiments should demonstrate what time schedules and temper-
Fig. 1. Four-day-old sprouts from chufa nodules (assessment of germination energy)
Рис. 1. Четырехдневные проростки клубеньков чуфы (оценка энергии прорастания)

Fig. 2. Eleven-day-old sprouts from chufa nodules (germination percentage assessment)
Рис. 2. Одиннадцатидневные проростки клубеньков чуфы (оценка всхожести)

Fig. 3. Chufa in the greenhouse (Pushkin and Pavlovsk Laboratories of VIR)
Рис. 3. Чуфа в теплице (Научно-производственная база «Пушкинские и Павловские лаборатории ВИР»)
temperatures are most suitable for the long-term storage of chufa nodules.

Currently, one of the urgent problems for both Europe and Russia is to improve nutrition patterns of the population and increase the share of natural and environmentally friendly products in the daily diet (Konarev et al., 2019; Sabo et al., 2018). The main problem of such products is their short shelf life, while the use of chemical preservatives not only negates the benefits of a natural product, but also harms the human organism. This is especially typical for perishable products.

To solve this problem and increase the shelf life of natural products, it is promising to use plants with natural antioxidant potential, such as chufa. The main components in the antioxidant system of chufa nodules are tocopherols, mainly γ-tocopherol and α-tocopherol (Yeboah et al., 2012). The use of chufa accessions with a high content of vitamin E (k-7, k-11 and k-19) is promising for the development of this trend in the food industry.

The key factors for successful long-term storage of chufa nodules are humidity and temperature patterns. High moisture and high drying temperatures reduce the content of protein, oil and fiber in chufa nodules (Omale et al., 2020).

A comprehensive study of C. esculentus requires performing molecular genetic studies aimed at obtaining maximum sizes of chufa nodules in order to optimize the processing technology. It is also necessary to develop a biotechnological method for producing a natural preservative from chufa and conduct medical research into the effect of chufa products on carbohydrate and lipid metabolism among patients with diabetes, metabolic syndrome, and obesity.
Conclusion

The results of our research show that the chufa collection accessions manifest significant genetic diversity in their biochemical composition. Chufa accessions have been selected for a set of valuable agronomic features. The selected accessions can be used as source material in breeding programs.

A technique was developed for measuring germination percentage, and preparation for long-term storage was carried out. The optimal age of the shoots from chufa nodules to assess their germination energy was found to be four days, with eleven days for germination percentage assessment. It was shown that three-year storage of chufa nodules at +20°C caused a decrease in their germination by 15–16%. When stored at low positive (+4°C) or negative temperatures (–18°C and –196°C), their germination percentage remained unchanged.

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ORCID

Конькова Н.Г. https://orcid.org/0000-0002-4920-3904

Сафина Г.Ф. https://orcid.org/0000-0002-8566-0192

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