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OIL CONTENT IN CHICKPEA SEEDS OF THE NATIONAL COLLECTION OF UKRAINE

Abstract: Chickpea (*Cicer arietinum* L.) is an important legume crop grown and consumed worldwide. Oil content in chickpea seeds ranges from 4 % to 7 % according to various data sources. Considering the interest of breeders in this issue, as well as for the purpose of inventory of the presented chickpea genetic material in the collection of the National Center for Plant Genetic Resources of Ukraine, these studies were carried out. Field experiments were carried out in 2016–2018 in the scientific crop rotation of the Plant Production Institute n.a. V. Ya. Yuriev of NAAS (Kharkiv, Ukraine). 43 samples (21 - *kabuli* type and 22 - *desi* type) of different ecological and geographical origin were studied. Oil content in chickpea seeds was determined using gravimetric method of S. V. Rushkovsky (Yermakov, 1987) in the laboratory for genetics, biotechnology and quality of the Plant Production Institute n.a. V. Ya. Yuriev of NAAS. On average, over the years of study, in the *kabuli* type accessions, the oil content level in the seeds made 7.08 %; for accessions - 6.05 %. The range of variability of this trait for the *kabuli* chickpea ranged from 5.22 % to 8.69 %, and for *desi* - from 4.40 % to 7.26 %. A low variability of the studied trait was noted for both the *kabuli* ($V = 6.88-15.04$ %) and for *desi* ($V = 8.98-14.15$ %) chickpea cultivars. The advantage in terms of oil content in seeds, regardless of the growing conditions, was retained for the *kabuli* type. The accessions with the maximum level of the “oil content in seeds” trait manifestation were selected as “reference” for each type: for *kabuli* - variety Pamyat (Ukraine) - 7.95 %, for *desi* - Yarina (Ukraine) - 7.13 %. The best oil-bearing chickpea samples can be used in specialized programs to create new genotypes with a higher oil content in seeds.

Keywords: legumes, *Cicer arietinum* L., National Center for Plant Genetic Resources of Ukraine, selection, sample, *kabuli* morphotype, *desi* morphotype, oil content in seeds

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СОДЕРЖАНИЕ МАСЛА В СЕМЕНАХ НУТА НАЦИОНАЛЬНОЙ КОЛЛЕКЦИИ УКРАИНЫ

Аннотация: Нут (*Cicer arietinum* L.) является важной зернобобовой культурой, которую выращивают и потребляют во всем мире. Содержание масла в семенах нута по различным данным колеблется от 4 до 7 %. Учитывая интерес к данному вопросу со стороны селекционеров, а также с целью инвентаризации представленного генетического материала нута в коллекции Национального центра генетических ресурсов растений Украины были проведены настоящие исследования. Полевые опыты были проведены в 2016–2018 гг. в научном севообороте Института растениеводства им. В. Я. Юрьева (Харьков, Украина). Исследовали 43 образца (21 тип *kabuli* и 22 типа – *desi*) разного эколого-географического происхождения. Содержания масла в семенах нута определяли гравиметрическим методом С. В. Рушковского (Ермаков, 1987) в лаборатории генетики, биотехнологии и качества Института растениеводства им. В. Я. Юрьева НААН. В среднем за годы изучения у образцов морфотипа *kabuli* содержание масла в семенах составило 7,08 %; у образцов типа *desi* – 6,05 %. Диапазон изменчивости данного признака для морфотипа *kabuli* колебался от 5,22 до 8,69 %, а у *desi* – от 4,40 до 7,26 %. Отмечена невысокая вариативность изучаемого признака как для морфотипа *kabuli* ($V = 6,88-15,04$ %), так и для *desi* ($V = 8,98-14,15$ %). Преимущество по содержанию масла в семенах, независимо от условий вегетации, сохранялось за морфотипом *kabuli*. В качестве «эталонных» для каждого из морфотипов выделены образцы с максимальным уровнем проявления признака «содержанием масла в семенах»: для морфотипа *kabuli* – сорт Память (Украина) – 7,95 %, для *desi* – сорт Ярина (Украина) – 7,13 %. Лучшие по масличности образцы нута могут быть использованы в специализированных программах для создания новых генотипов с повышенным содержанием жира в семенах.

Ключевые слова: зернобобовые культуры, *Cicer arietinum* L., Национальный центр генетических ресурсов растений Украины, селекция, образец, морфотип *kabuli*, морфотип *desi*, содержанием масла в семенах

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Introduction. Chickpea (*Cicer arietinum* L.) is an important grain legume, which is grown and consumed worldwide and will gain in importance with climate change. The production and consumption of chickpea is steadily increasing. Ranked third in the world for production among legumes, chickpea is an important source of protein in densely populated but poor regions of Asia and Africa [1]. Two market chickpea types: desi and kabuli, are grown in the world, differing in the morphological characteristics of seeds. About 75 % of world chickpea production is Desi, angular and dark. They are grown mainly in Asia and Ethiopia. Kabuli seeds are beige in color and round in shape. They are usually grown in the Mediterranean and Mexico [15].

It is a good source of carbohydrates and proteins. The presence of lipids in chickpea seeds increases their nutritional value. Among legumes, peanut (*Arachis hypogaea* L.), soybean (*Glycine max* (L.) Merr.) and winged bean (*Psophocarpus tetragonolobus* (L.) DC.) are distinguished due to lipid content, which is 49.7 %, 21.3 % and 16.8 %, respectively [2]. Most of the legume seeds contained a low level of oil content, from 1.7 % in horse bean (*Vicia faba* L.), to 4.5 % in kidney bean (*Phaseolus coccineus* L.) [3]. As different sources claim, the oil content in chickpea seeds varies from 4 % to 7 %; of the total amount of fatty acids in chickpea oil, saturated fatty acids account for 13 %, monounsaturated fatty acids - for 20 %, and polyunsaturated ones – for 67 %. Among the unsaturated fatty acids, linoleic (43.29 %) and oleic (21.84 %) prevail in chickpea seeds, and palmitic (9.22 %) prevail among the saturated fatty acids [2, 4, 5, 6]. The content of these fatty acids can vary significantly depending on weather conditions. However, the influence of the genotype and genotype-environment interactions are equally important [7]. The chickpea oil contains B-sitosterol, campesterol and stigmasterol, which are important sterols [8]. There is a significant negative correlation between the oil content and content of phytin phosphorus compounds with anti-nutritional properties [9]. An increase in the oil content in the seeds of legume crops by breeding work significantly improves their nutritional quality. Thus, in pea, using the current genetic diversity, breeders have achieved a level of oil content in seeds of 7.7 %, while in the most common smooth-seeded varieties its level does not exceed 2.0 % [10]. The study of the national collection of genetic resources allows you to expand the range of use of genetic material in special breeding. So, in peas, genetically determined natural starch-modifying mutations are associated with the content and quality of oil [11].

Among the most widely used legumes in Ukraine (pea, common bean, chickpea and lentil), chickpea are characterized by the highest oil content, the lowest insoluble fiber and the absence of soluble dietary fiber [12].

The potential of chickpea for healthy nutrition is especially high. Polyunsaturated fatty acids (such as linoleic acid) actively reduce serum cholesterol; monounsaturated ones (for example, oleic acid) do not have an independent effect on the level of serum cholesterol, as saturated fatty acids (for example, palmitic acid) play the main role in the metabolism of this substance, increasing its level. The presence of polyunsaturated fatty acids creates the conditions for synthesis of prostaglandins, which prevent alpha lipoprotein (HDL) cholesterol from concentrating on of blood vessel walls [5]. For example, chickpea is included in special diets both for prophylaxis and for treatment of cardiovascular diseases, type 2 diabetes, diseases of the digestive system, and some types of cancer [8]. The specific chemical composition of chickpea (carbohydrates - 48 %; protein - 28 %; fat - 4.5 %) is most suitable for the production of meat and vegetable precooked foods. Patty cakes containing 20-25 % of chickpea flour have been developed for elderly nutrition [4].

Chickpea has become the main ingredient in follow-on formula for baby food that meets WHO / FAO complementary feeding requirements as well as EU rules for such follow-on formula with a minimum addition of oils, minerals and vitamins. Chickpeas have been used as a major source of carbohydrates and protein, making such formula more economical and affordable for low-income countries without compromising nutritional quality [13].

Adding chickpea flour to foods such as bread, snacks and chips increases their nutritional value by enriching them with protein and lowering anti-nutrients such as acrylamide [14].

Publications by researchers from India and Pakistan, countries where chickpea is the basis of the diet, dominate in the literature on the issue. In Ukraine, studies of the oil content in chickpea seeds are episodic [16, 17], and the core chickpea collection of the National Center for Plant Genetic Resources of Ukraine (NCPGRU) was not evaluated for this trait. Given the interest in this issue from breeders and

consumers, as well as for the purpose of inventorying the available genetic material of chickpea from the NCPGRU's core collection, this study was designed and conducted. There were also issues of a scale for assessing the oil content in seeds, identification of check accessions for further studies and sources of high oil content in seeds for breeding, which became our objectives.

Material and Methods. In 2016–2018, the oil content in chickpea seeds from the NCPGRU's core collection was analyzed for the first time. In 2016, 24 accessions were studied 12 *kabuli* (with light seeds) and *desi* (with dark seeds) accessions of different eco-geographical origin. These accessions have been investigated at the NCPGRU for many years, and some of them were previously chosen as check accessions for different valuable traits, such as yield capacity, resistance to ascochytirosis, seed size, early ripening, high protein content, cooking property, etc. [18]. In 2017–2018, the assortment of the studied accessions was expanded to 43 accessions (21 belonged to the *kabuli*-type and 22 - to the *desi*-type) due to local accessions of different origin.

According to the State Standard for identifying sources of valuable traits in the NCPGRU's collections, accessions whose values are by $\geq 15\%$ higher than the average across the sample are distinguished as sources¹.

The field experiments were carried out in the scientific crop rotation of the Plant Production Institute named after V.Ya. Yuriev, Kharkiv, Ukraine in 2016–2018. The forecrop was winter wheat. The field experiments were conducted in accordance with the “Guidelines for Studying the Genetic Resources of Grain Legumes” (2016)².

The oil content in chickpea seeds was determined by S.V. Rushkovsky's gravimetric method³ in the Laboratory of Genetics, Biotechnology and Quality of the Plant Production Institute of named after V.Ya. Yuriev of NAAS.

The weather conditions during the vegetation periods of the 3 study years varied significantly. 2016 was characterized by waterlogging during the chickpea flowering and ripening periods (June–August): the precipitation amount was 143.7 mm, which is by 60.6 % higher than the multi-year average for this period, while both 2017 and 2018 were hot and dry, with 43 % and 60.9 % of rainfall related to the multi-year average, respectively. In 2018, the average daytime temperature in June–August reached 29.6–35.5 °C and was accompanied by heavy rains during the seed setting and filling. Of the 3 study years, 2016 was unfavorable for the chickpea growth and development, and 2017 and 2018 were closer to the optimum for this crop, although they were marked by high temperature and drought.

Results: The 2016 results showed that the average oil content across the sample of chickpea seeds was 7.72 %, ranging 6.79 to 8.57 % in the *kabuli* accessions and 5.02 to 7.26 % in the *desi* ones (Table 1), which is significantly higher than the values reported by other researchers. For example, Shah et al. reported that the oil content in chickpea seeds from Pakistan was 3.54 % [22]; in Avola et al.'s publication it was 4.36 % for Italian accessions [23].

Seven out of the 12 *kabuli* accessions investigated in 2016 had an oil content within the average across the test sample (7.16 %), and in 2 accessions the values exceeded the average by more than 15 %: Ukrainian cultivars Dniprovskiyi Vysokoroslyi (8.27 %) and Smachnyi (8.52 %). As to the *desi*-type, 3 accessions had the intermediate contents: CDC Anna (7.26 %), Krasnokutskiy 123 (7.21 %) and Kolo-ryt (7.11 %). None of the *desi* accessions exceeded the average by more than 15 %.

The three-year study (2016–2018) of the oil content in chickpea seeds in the conditions of the Eastern Forest-Steppe of Ukraine showed that in this test sample the average was 7.08 % and 6.05 % across the *kabuli* and *desi* accessions, respectively (Table 2).

The oil content range in chickpea seeds over the study years was 5.22 % to 8.69 % and 4.40 % to 7.26 % for the *kabuli* and *desi* types, respectively. The oil content in seeds across the entire sample ranged 4.40 % to 8.69 %. It should be noted that in 2017 and 2018, compared with 2016, the oil content range in seeds expanded significantly, which can be attributed both to an increase in the number of accessions in the

¹ State Standard of Ukraine 7066.2009. Genetic resources of plants. Terms and definitions. Kyiv, Derzhspozhyvstandart Ukrainy, 2010. (in Ukrainian).

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³ Ermakov A. I. (ed.). Methods of biochemical studies on plants. 3rd ed. Leningrad, Agropromizdat Publ., 1987. 430 p. (in Russian).

Table 1. Oil Content in Chickpea Seeds Harvested in 2016

No in the National Catalogue of Ukraine	Name	Country of origin	Oil content, %	No in the National Catalogue of Ukraine	Name	Country of origin	Oil content, %
<i>Rabuli</i>				<i>Desi</i>			
UD0500417	Smachnyi	Ukraine	8.52	UD0500723	CDC Anna	Canada	7.26
UD0500444	Dniprovskiy Vysokoroslyi	Ukraine	8.27	UD0500101	Krasnokutskiy 123	Russia	7.21
UD0500736	Pamiat	Ukraine	8.18	UD0500429	Koloryt	Ukraine	7.18
UD0502113	Jamila	Azerbaijan	8.04	UD0501172	KP 3990	Ukraine	6.93
UD0500424	Rozanna	Ukraine	6.81	UD0500425	Aleksandryt	Ukraine	6.81
UD0501194	Dobrobut	Ukraine	7.91	UD0501164	Pehas	Ukraine	6.67
UD0500762	Zavolzhiyskiy	Russia	7.85	UD0500495	E 100	Greece	6.64
UD0500864	Flip 99-55C	Siria	7.70	UD0500719	Sovkhoznny 14	Russia	6.56
UD0500196	–	Azerbaijan	7.66	UD0500494	R 919	Russia	6.55
UD0501200	Slobozhanskyi	Ukraine	7.56	UD0500422	Cicer rotundum	Czech Republic	6.42
UD0502093	–	Ukraine	7.42	UD0500263	–	Ukraine	6.29
UD0500689	Skorospelka	Russia	6.79	UD0500022	–	Georgia	5.02
Mean			7.72	Mean			6.60
Coefficient of variation, %			6.88	Coefficient of variation, %			8.98
Variance			0.28	Variance			0.36

Table 2. Oil Content in Chickpea Seeds of the Two Types

Year	Oil Content, %											
	<i>Kabuli</i>				<i>Desi</i>				<i>Total</i>			
	Min	Max	Mean	CV, %	Min	Max	Mean	CV, %	Min	Max	Mean	CV, %
2016	6.79	8.52	7.73	6.88	5.02	7.26	6.63	8.98	5.02	8.52	7.18	10.98
2017	5.74	8.69	6.89	10.98	4.70	7.20	6.04	12.26	4.70	8.69	6.43	13.26
2018	5.22	8.65	6.62	15.04	4.40	7.07	5.47	14.15	4.40	8.65	5.99	17.41
Mean			7.08				6.05				6.53	

sample and to a possible influence of the plant vegetation conditions. Thus, in 2016 the oil co-tent was for the *kabuli*-type was 6.79 % - 8.52 %, and in 2017 and 2018 - 5.74 % - 8.69 % and 5.22 – 8.65 %, respectively. The same trend was noted for the *desi*-type: in 2016 the oil content amounted to 5.02 % - 7.26 %, and in 2017 and 2018 this parameter was in the range of 4.70 - 7.20 % and 4.40 % - 7.07 %, respectively.

Analysis of the 2016 data gave a low coefficient of variation of the “oil content in seeds” trait in the *kabuli* (CV = 6.88 %) and *desi* (CV = 8.98 %) accessions, which can be attributed either to a high homogeneity of the sample or to a consequence of its small size and effects of the weather conditions. Avola et al. (2012) noted that the oil content varied slightly in different accessions, but increased significantly during seed cooking [23]. Ukrainian researchers also reported a weak variability in the trait [17]. When the size of the sample increased to 43 accessions in 2017 – 2018, the coefficient of variation rose for the both types, but it did not fall outside of the average limits.

Working with genetic resources, one should evaluate large numbers of collection accessions, which requires a scale with a variability range of the trait under investigation. To develop such a scale, we reviewed literature data published by researchers from different countries (Table 3).

The minimum oil content in chickpea seeds was noted in Pakistan (3.54 % [22]) and in Italy (4.36 % [23]). The maximum was 8.00 % in Ukraine [16] and 9.01 % in Argentina [32]. Within this range, the scale gradations were calculated for assessing the oil content in chickpea seeds from the NCPGRU's core collection: Very low - below 5.00 %; Low - 5.00-5.99 %; Moderate - 6.00-6.99 %; High - 7.00-8.00 %; Very high - above 8.00 %.

After developing the scale, it became possible to evaluate chickpea accessions of the core collection of the National Center for Plant Genetic Resources of Ukraine for the oil content (Fig. 1).

Table 3. Oil Content in Chickpea Seeds from Different Countries (according to published data)

Country	Oil content, %		Reference
	<i>Rabuli</i>	<i>Desi</i>	
Ukraine	7.3 - 8.0	6.5	[16]
Ukraine	5.8		[17]
Italy (Sicily)	4.36	-	[23]
Italy (Sicily)	6	-	[24]
Makedonia	4.44 - 5.16	-	[25]
Turkey	4.45 - 6.11	-	[26]
Ethiopia	-	5.88	[27]
Pakistan	3.54	-	[22]
Pakistan	4.5	5.7	[28]
Pakistan	-	5.88 – 6.87	[29]
India	-	4.18 – 4.92	[30]
Canada	5,5	3,6	[31]
Argentina	5,68 – 9,01	-	[32]

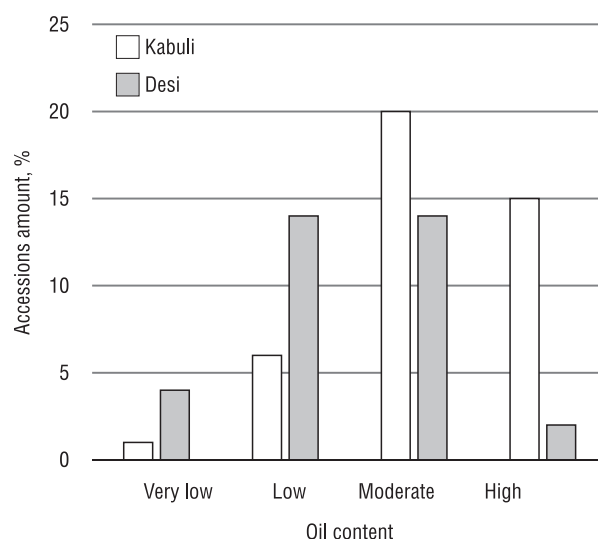


Fig. 1. Comparison of Chickpea Types According to the Oil Content in Seeds Scale

As a result, the accessions under investigation were categorized according to this scale. It was found that among the *kabuli* accessions, accessions with a moderate (10 accessions, 48 % of the entire *kabuli* sample) and high (8 accessions; 36 %) oil content in seeds were overwhelming. As to the *desi*-type, accessions with a low and moderate oil content in seeds were the most numerous (9 accessions of each type; 41 %).

The high stability of the trait under investigation allowed us to identify sources of high oil content in seeds. These accessions can be used in specialized breeding programs to create new genotypes with high oil content in chickpea seeds. T. M. Shah noted a more conspicuous increase in oil level when parental pairs of different morphological types (*kabuli* and *desi*) are crossed than when accessions belonging to the same morphological type are crossed [28]. Therefore, selection of accessions - sources of high oil content belonging to different types is a pre-requisite for further breeding to improve the nutritional qualities of chickpea.

A two-year study of accessions is sufficient to identify sources of high oil content in seeds. In 2017–2018, seeds of the *kabuli* accessions contained 5.78 % - 7.95 % (mean = 6.95 %) of oil. A high oil content (7.00 - 7.99 %) was recorded for 10 test *kabuli* accessions (47.62 %), which we recognized as sources of high oil content. These are Pamiat (Ukraine), NEC 2184 (Iran), UD0502111 (Russia) and others. There were no accessions with very high (above 8.00 %) oil content. The maximum oil level during the two study years was observed in Pamiat (Ukraine; 7.95 %), NEC 2184 (Iran; 7.79 %) and UD0502111 (Russia; 7.77 %).

As to the *desi*-type, the average oil content for the two study years was 5.89 %. A high oil level was only observed in 1 accession - Yaryna (Ukraine) - 7.13 %. The most of the *desi* accessions had a moderate (40.91 % of the sample) or a low (50 %) content of oil. Among the studied *desi* accessions,

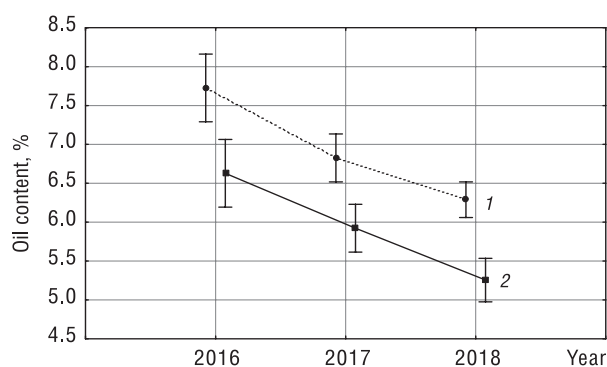


Fig. 2. Oil Content in Chickpea Seeds Depending the Year Conditions (1 - *kabuli*; 2 - *desi*). Oil content (%), Year, Type, LSM means, Hypothesis decomposition, Vertical bars denote 95 % confidence intervals

2 accessions with high oil content in seeds were identified: Yaryna (7.13 %) and Koloryt (6.91 %); the both cultivars have been bred in Ukraine and can be sources of this trait in the *desi*-type.

The accessions with the maximum expression of the trait of interest were selected as check accessions for their morphological types: cultivar Pamiat (Ukraine) for the *kabuli*-type and cultivar Yaryna (Ukraine) for the *desi*-type.

Unidirectional and similar fluctuations in the oil content were noted in both *kabuli* and *desi* accessions, depending on the year conditions (Fig. 2).

We found that the *kabuli*-type is superior to the *desi*-type in terms of oil content in seeds, regardless of the growing conditions.

Conclusions. Thus, our studies showed that the chickpea accessions of the NCPGRU's collection had a high oil content in seeds, which increases their value for breeding programs to develop new food cultivars for a balanced diet.

Valuable sources of high oil content in seeds (Pamiat (Ukraine), NEC 2184 (Iran) and UD0502111 (Russia) belonging to the *kabuli* type as well as Yaryna and Koloryt (Ukraine) belonging to the *desi*-type were identified. We recommend including them in breeding programs to develop new chickpea cultivars.

We experimentally developed the scale that is recommended for classification of chickpea accessions by oil content in seeds.

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