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Study on seed quality testing of Russian dandelion

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Background. Kok-saghyz, or Russian dandelion, is one of the three most promising natural rubber plants in the world. This study examined the seed quality of kok-saghyz from different sources with reference to the methods in the International Rules for Seed Testing.

Materials and methods. A kok-saghyz accession from the VIR collection (k-445) and an accession of American origin were analyzed. The study was carried out in 2018–2020 under the conditions of a field experiment at the experiment station in Harbin (45.592729°N, 126.581668°E).

Results. Sampling principles and methods were established, and the seed quality of kok-saghyz was thoroughly evaluated. A seed purity test showed above 80% of pure seeds for both accessions. The thousand-seed weight was approximately 0.5 g when measured by the hundred-seed method. The moisture content was about 5.4% according to the low-temperature drying technique. The time required for reaching a natural imbibition rate was 2.5 h. The authenticity test demonstrated that koksaghyz seeds can be distinguished from those of other *Taraxacum* spp. according to their appearance. The health test found 4 types of mold inside and outside the kok-saghyz seeds.

Conclusion. This study provides technical support for kok-saghyz seed quality testing, and offers recommendations for kok-saghyz seed trade.

Keywords: rubber plant, kok-saghyz, seed quality, seed testing regulations

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ИЗУЧЕНИЕ И ИСПОЛЬЗОВАНИЕ ГЕНЕТИЧЕСКИХ РЕСУРСОВ РАСТЕНИЙ

Научная статья

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Изучение качества семян русского одуванчика

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Актуальность. Кок-сагыз, или русский одуванчик, входит в тройку самых перспективных каучуконосов в мире. В этом исследовании изучалось качество семян кок-сагыза из разных источников со ссылкой на методы, изложенные в Международных правилах тестирования семян.

Материалы и методы. Объектом исследования служили образец кок-сагыза из коллекции ВИР (к-445) и образец американского происхождения. Исследования проводили в 2018–2020 гг. в условиях полевого опыта экспериментальной станции в Харбине (45.592729°с. ш., 126.581668°в. д.).

Результаты. В ходе исследования определили принципы и методы отбора проб, а также провели всестороннюю оценку качества семян кок-сагыза. Анализ чистоты показал, что чистота семян обоих образцов кок-сагыза превышает 80%. Вес 1000 семян равен примерно 0,5 г, определялся с помощью метода 100 семян. Содержание влаги, измеренное методом низкотемпературной сушки, составляет около 5,4%. Время, необходимое для достижения естественной скорости насыщения семян влагой, составило 2,5 ч. Проверка на подлинность показала, что семена кок-сагыза можно отличить от других видов рода *Тагахасит* по внешним характеристикам. В ходе проверки на грибковые заболевания обнаружили четыре типа плесени внутри и снаружи семян кок-сагыза.

Заключение. Данное исследование обеспечивает техническую поддержку технологии оценки качества семян коксагыза, а также предоставляет рекомендации по оценке материала для последующей коммерческой реализации семян кок-сагыза.

Ключевые слова: каучуконосное растение, кок-сагыз, качество семян, правила проведения испытаний

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Introduction

Russian dandelion (*Taraxacum kok-saghyz* Rodin) is a perennial herbaceous plant of the genus *Taraxacum* of the Asteraceae family. It is one of the three most excellent natural rubber plants in the world. Its roots contain 2.89 to 27.89% of natural rubber (Whaley, Bowen, 1947; Luo, 1951; Shen et al., 2018; Shen et al., 2019), and also 25 to 40% of inulin (Polhamus, 1962). The intensive growth and yield of kok-saghyz is greatly influenced by the frequency of watering and fertilization (Shen, 2021; Shen et al., 2023; Zhang et al., 2023). The leaves can be used in pharmacy, for tea manufacturing, and as anti-inflammatory feed additives (Molinu et al., 2019). Koksaghyz may also serve as a model plant to study the mechanism of natural rubber synthesis (Wu et al., 2024). Therefore, it possesses important scientific and practical values.

Seed testing refers to the inspection and measurement of seed quality, that is, authenticity, purity, cleanness, germination rate, viability, seed health, moisture content, and 1000-seed weight. Agricultural production, commodity exchange, economic and trade activities can be guided on the basis of seed quality information. Through seed testing, it is possible to select high-quality seeds for sowing, eliminate or reduce the risk of seedling shortage and yield reduction due to seed quality, control the spread and damage of harmful weeds, ensure normal growth of crops and increase yields, which is of great significance to kok-saghyz production (Hu, 2015).

In 1924, the International Seed Testing Association (ISTA) was established. In 1931, with the joint efforts of ISTA members and Dr. Franck, ISTA promulgated the first version of the International Rules for Seed Testing. After the 1990s, ISTA basically followed the principle of conducting minor revisions to the rules every three years and comprehensive revisions ev-

nology of the International Rules for Seed Testing and combined with their own specific conditions. Since the 1980s, China has formulated and promulgated a number of national standards, such as the Crop Seed Inspection Procedures, Forage Seed Inspection Procedures, and Vegetable Seed Quality Standards. With the development of agriculture, China revised and promoted regulations on food crops (cereals and beans), industrial crops (fibers and oilseeds), and cucurbits (melons, cabbage, and leafy vegetables) in the 1990s. These standards have played a role in improving the quality of the country's crop seeds and effectively guaranteed the safety of food production and industrial crop cultivation.

This study presents the results of a research on the inspection methods for kok-saghyz seed quality, based on the guidelines prescribed by the International Rules for Seed Testing (ISTA, 2020), and provides technical support for the seed quality classification technology. In order to promote kok-saghyz cultivation and increase rubber production, it is important to establish seed quality standards for Russian dandelion and provide a reference for kok-saghyz seed trade.

Materials and methods

Kok-saghyz fruits are achenes. Since the peel and seeds are tightly connected and difficult to separate, dandelion fruits are generally called seeds, so the kok-saghyz seeds are actually their fruits in the context of this study.

We planted a kok-saghyz accession imported from Russia (k-445) and the Buckeye accession from the United States in different locations with different cultivation patterns, and then used the collected seeds at different times as experimental materials. There were four replications in total. The relevant information is as follows (Table 1).

 Table 1. Experimental materials of kok-saghyz seeds

 Таблица 1. Экспериментальные материалы по семенам кок-сагыза

Sources	Harvest sites	Harvest coordinates (BD09)	Cultivation patterns	Harvesting dates	Weight, g
k-445 (collected in 2018)	New Industrial Zone in Harbin	126.5717, 45.5832	In the field	July – September	19.47
k-445 (collected in 2019)	New Industrial Zone in Harbin	126.5717, 45.5832	In the field	July – September	22.66
k-445 (collected in 2020)	Institute of Natural Resources and Ecology	126.6526, 45.7184	Potted	July – September	36.54
Buckeye (collected in 2020)	Institute of Natural Resources and Ecology	126.6526, 45.7184	Potted	July – September	17.52

ery six years. Nowadays, the International Rules for Seed Testing have been cited by the International Seed Quality Certification System of the Organization for Economic Cooperation and Development (OECD) (ISTA, 1996), and become a guideline that must be followed by the international seed trade and circulation; it is generally adopted by countries around the world. The International Rules for Seed Testing have a total of 50 basic standards and inspection specifications (ISTA, 2020).

Except for a few countries that directly implement the rules, most countries in the world have formulated national seed inspection regulations based on the standardized tech-

The seed purity analysis was made for all four batches, the health test analyzed the batches collected in 2018 and 2020, and the other tests were performed for the Russian seed batch harvested in 2018 and the American seed batch harvested in 2020.

Experimental methods

The seed testing process can be divided into three parts: sampling, testing, and result reporting. The testing procedure starts with taking samples from the seed batch to form primary samples and mixed samples to perform cleanness, natural imbibition, germination, weight, purity, health, and au-

thenticity tests. Since no dormancy phenomenon was observed in kok-saghyz seeds and the seeds were small, no viability testing experiment was conducted within this study.

According to the ISTA regulations, seed lots need to have a specified maximum and minimum weight. Seed size is a significant factor influencing the seed lot weight, but it is also affected by whether the seed is for agricultural or horticultural use. The maximum number of seeds in a seed batch is 1×109 (i.e., 10,000 units, with each unit containing 100,000 seeds) (Hu, 2015). Since the 1000-seed weight of kok-saghyz is 0.45-0.70 g, the maximum weight of a seed batch (including various coating materials or films) shall not exceed 1000 kg, the error shall not exceed 5%, and the minimum weight shall not be less than 30 g (according to the recommendations for *Taraxacum officinale* in the International Rules for Seed Testing, the 1000-seed weight of ordinary dandelion is higher than that of kok-saghyz, so the minimum weight is no less than 30 g). The maximum number of seeds in a seed batch is 1×109 (Luo, 1951 1), and the 1000-seed weight of kok-saghyz is generally 0.5 g. The maximum weight of a seed batch is 500 kg, and the error is not allowed to exceed 5%.

According to the ISTA regulations, when the container of a seed batch is 1, the number of initial samples taken is not less than 3 (Polhamus, 1962). In this experiment, 4 primary samples were taken from each seed lot, and they were sampled using spoons at different positions. After mixing, a composite sample was formed, and then random samples were taken from it to form a working sample. The "freehand halving method" is used to separate the experimental sample from the initial sample (Whaley, Bowen, 1947).

Purity analysis

The sample for purity analysis contained at least 25,000 seeds. The 1000-seed weight of kok-saghyz was calculated as 0.5 g. Therefore, the seeds weight for a purity analysis should be at least 12.5 g. Each seed lot undergoes 3 replications, the average value is taken.

Net seed weight percentage: (net seed weight) / (net seed weight + impurity weight + other seed weight) \times 100%.

The seed morphology method was used to identify koksaghyz seeds and impurities. According to the description in the Flora of China, Russian dandelion achenes are light brown, (2-)2.5-3.5 mm long, with many small spines on the upper 1/3-1/2, and the remaining part has small tubercles.

With the help of a magnifying glass, sieve, scalpel, etc., without damaging the germination ability of the seeds, the sample was separated into three components: clean seeds, other plant seeds, and general impurities on the purity analysis table, based on the obvious characteristics of the seeds. Weighed separately in grams.

Natural imbibition

Clean seed samples were taken from the seed lots from Russia and the United States, respectively. We took about $0.2~\rm g$ of each sample, repeated 3 times, soaked in tap water at room temperature, taking them out every half hour, wiping dry, and weighing until constant weight. At this time, the seed imbibition rate was 100%.

Weight measurement

Eight portions of 100 kok-saghyz seeds were manually counted, weighed individually, and the coefficient of variation was calculated. If the coefficient of variation is less than 4.0, the 1000-seed weight can be calculated on the basis of the actual measurement results. If the coefficient of variation is

higher than 4, 8 more replications should be weighed, and the standard deviation of the 16 replications should be calculated. For any replications that show more than twofold difference from the mean, the standard deviation will be ignored. Finally, the values of 100 seeds for each replication will be ignored. The average weight multiplied by 10 is the measured seed weight (Luo, 1951).

Weight of 1,000 seeds = weight of 100 seeds / number of replications of 100 seeds \times 10.

Authenticity verification

We used the seed appearance morphology method to identify 364 seeds. We observed the surface morphology of kok-saghyz seeds under an Olympus SZX7 microscope (20×), including color, shape, spines, beak base, etc., and then took pictures using ImageJ 1.53q (Polhamus, 1962) to measure seed length, width and beak base length. Then a fixed-distance plant key table was established to distinguish koksaghyz from other common dandelion plants in Northeast China on the basis of seed characteristics.

Moisture content assessment

Using the low constant temperature drying method, koksaghyz seeds were dried in an oven at 103°C for 17 h to constant weight, and then the seed moisture content was calculated (Luo, 1951).

Health testing

The washing test method was used in this study to check for pathogenic spores or pathogenic nematodes attached to the seed surface, as well as the culture method to check for pathogenic bacteria inside kok-saghyz seeds (Hu, 2015).

Calculation of the spore load on the seed surface is to be done according to the following formula:

$$N = \frac{n_1 \times n_2 \times n_3}{n_4},$$

where N is the spore load per gram of seeds; \mathbf{n}_1 is the average number of spores per the field of view; \mathbf{n}_2 is the number of fields of view on the cover glass area; \mathbf{n}_3 is the number of drops in 1 mL of water; \mathbf{n}_4 is the weight of the test sample.

After taking pictures with a microscope, the number of spores was calculated using the ImageJ (1.53q) software. We used the software to open the picture file taken by the microscope, clicked the menu Image-Type-8-bit, turned the picture into black and white, then clicked Process-Find Maxima..., entered the appropriate number in the Prominence box, and checked Exclude edge maxima and Preview point selection, selected the output type as count, adjusted the appropriate number of Prominence based on the comparison between the number of spores and the number of markers in the picture, and finally clicked OK to automatically calculate the number of spores and save the result as an MS Excel file.

We used the culture method to count the fungal species, isolation frequency, and bacterial load inside kok-saghyz seeds. The formula is as follows:

Isolation frequency (%) = number of occurrences of a certain isolate / total number of occurrences of isolates \times 100.

Bacteria-carrying rate (%) = total number of bacteria-carrying seeds / total number of tested seeds \times 100.

Fungal identification:

The isolated fungi were purified, examined under microscope, and transferred to tubes for storage. Identification was based on fungal culture traits and morphological characteristics, and with reference to relevant fungal identification tools.

Results and analysis

Purity analysis

The analysis of seed purity showed that the gain and loss rates of all seed batches was less than 5%, so its results were valid. Among the samples, Russian seeds collected in 2020 (Fig. 1, A) had the highest proportion of pure seeds, 95.19%, and American seeds collected in 2020 (Fig. 1, C) had the lowest share, 81.47% (Table 2, see Fig. 1).

Natural imbibition

The weight of the Russian and American seeds increased fastest in the first 0.5 h after absorbing water, and then gradually slowed down. When the seeds absorbed water for 2.5 h, the weight of the seeds no longer increased significantly (α = 0.05), so it is considered that the seed swelling rate reaches 100% at this time. So, when the seeds germinate, they need to be soaked in water for at least 2.5 hours. The 1000-seed weight of the American seeds was similar to that of the Russian seeds, but their weight after water absorption was always significantly higher than that of the Russian seeds (Fig. 2, Fig. 3).

Weight measurement

The average weight of 100 Russian seeds was found to be 0.0486 g, and the coefficient of variation was 0.11, i.e., less than 4, so the 1000-seed weight was estimated as 0.486 g. The average weight of 100 American seeds was 0.0478 g, and its coefficient of variation was 0.05 (less than 4), so the 1000-seed was 0.478 g, which was slightly lower than that of the Russian seeds (Fig. 4).

Authenticity verification

Kok-saghyz seeds are in fact achenes, described as light brown, gray-brown, or dark brown. They have diverse shapes: oblong, oblanceolate, or obovate. They are 1.4 to 4.2 mm long, and 0.3 to 1.2 mm wide. The surface consists of 11 to 16 longitudinal ribs and grooves; the upper 1/3 to 1/2 has many small spines, and the remaining part has small or no tumorlike protrusions. The base of the beak is 1.0 to 1.8 mm long. The ratio of beak base to achene length is 1:2 to 1:3, and there are fewer spines at the beak base (Table 3).

Kok-saghyz can be distinguished from other species of the genus *Taraxacum* in Northeast China (*T. mongolicum, T. liao-*

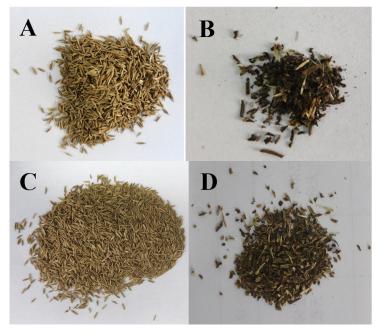


Fig. 1. Purity analysis of kok-saghyz seeds (A and B are pure seeds and inert matter of the Russian kok-saghyz; C and D are pure seeds and inert matter of American origin)

Рис. 1. Анализ чистоты семян кок-сагыза (A, B – чистые российские семена и инертные вещества кок-сагыза; C, D – чистые американские семена и инертные вещества)

Table 2. Purity analysis of kok-saghyz seeds Таблица 2. Анализ чистоты семян кок-сагыза

Seed source	Total sample weight, g	Inert matter weight, g	Weight of pure seeds, g	Weight of other seeds, g	Total weight after the analysis, g	Loss rate after the analysis, g	Pure seeds, %
Russia (2018)	21.74	1.21	20.29	0.00	21.50	0.01	93.35
Russia (2019)	34.98	3.56	30.97	0.00	23.12	0.01	88.51
Russia (2020)	13.14	0.63	12.35	0.00	12.98	1.20	95.19
U.S. (2020)	13.16	2.43	10.64	0.00	13.07	0.01	81.47



Fig. 2. Experiment on water absorption of kok-saghyz seeds
Рис. 2. Эксперимент по водопоглощению семян кок-сагыза

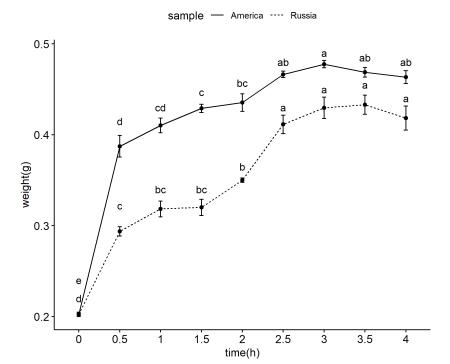


Fig. 3. Kok-saghyz seed weights at different times after water absorption Рис. 3. Масса семян кок-сагыза в разное время после впитывания воды

tugense, *T. formosanum, T. ohwianum, T. urbanum, T. antungense, T. asiaticum, T. asiaticum* var. *lonchophyllum*) according to the length of the seed, the number of spines at the base of the beak, and the ratio of the base of the beak to the length of the seed (Wu et al., 2011).

Moisture content assessment

The three repeated moisture content levels were 5.2%, 5.2%, and 5.5%, respectively, so the average value was 5.4%.

Health testing

Seed coat fungal spores

The spore count was determined under a microscope. The counting methodology is described in the Materials and Methods section. A cover glass with the following parameters was used: length × width is 18×18 mm, area is 324 mm²; 400x microscope diameter is 0.375 mm, i.e., a circle with the radius of 0.187 mm and the area of 0.11 mm², i.e., the number of fields of view on the coverslip is 324/0.11 = 2945. Calculation of the spore load was performed twice and repeated as N1 and N2, respectively, as follows:

 $N1 = n1 \times n2 \times n3/n4$

 $N1 = 5810 \times 2945 \times 20/1 = 293 \times 10^{12}$

 $N2 = 6103 \times 2945 \times 20/1 = 359 \times 10^{12}$

The average number of fungal spores on the epidermis of 1 g of kok-saghyz seeds was 326×10^{12} (Fig. 5).

Mold was found in the Russian seeds of kok-saghyz collected in 2018, with concatenated spores (inside and on the surface of the seeds) (Fig. 6, Fig. 7).

The fungi were found to belong to the Aspergillaceae family of the subclass Euascoma. According to the color, 4 types were identified, namely: blue mold, yellow mold, red mold, and white mold. The isolation frequencies were 59.8%, 2.6%, 2.1%, and 1.5%, respectively. Two types of molds were detected on the Russian seeds collected in 2019, namely blue mold and yellow mold, with isolation frequencies of 86.0% and 1.8%, respectively (Table 4).

Discussion

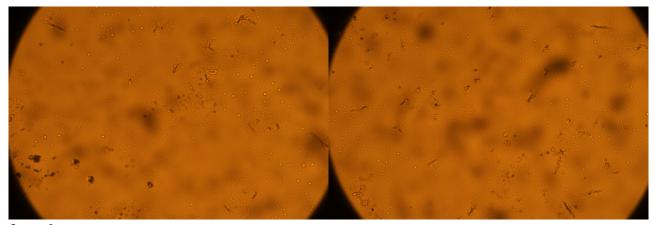
Kok-saghyz seeds have no dormancy phenomenon; their seeds are small, with a 1000-seed weight of only about 0.5 g. It is difficult to directly observe the staining of the embryos with the naked eye. When there are no experimental conditions, the viability measurement can be ignored, and the germination rate represents the viability of the seeds. Therefore, crop seed inspection procedures should determine what to do on the basis of seed characteristics and experimental conditions.



Fig. 4. Thousand-seed weight of kok-saghyz seeds Рис. 4. Масса 1000 семян кок-сагыза

Table 3. Description statistics of the length and width of kok-saghyz seeds Таблица 3. Описательная статистика длины и ширины семян кок-сагыза

Parameter	Average	Mid-range	Maximum	Minimum	Std.	CV
Seed length	2.85	2.80	4.23	1.38	0.56	0.20
Seed width	0.70	0.67	1.17	0.30	0.19	0.27



fungal spores

Fig. 5. Fungal spores on the surface of kok-saghyz seeds (400 x) Рис. 5. Споры грибов на поверхности семян кок-сагыза (400 x)

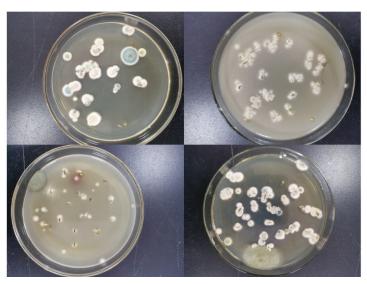


Fig. 6. Identification of fungi inside kok-saghyz seeds using the culture method Рис. 6. Идентификация грибов в семенах кок-сагыза методом культивирования

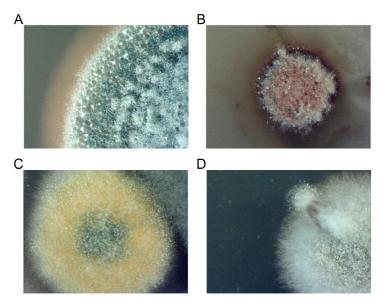


Fig. 7. Four types of mold on the surface of kok-saghyz seeds (A: blue, B: red, C: yellow, D: white)

Рис. 7. Четыре типа плесени на поверхности семян кок-сагыза (A: синяя, B: красная, C: желтая, D: белая)

We did not find any other plant seeds during the authenticity test of kok-saghyz seeds, especially seeds from the genus *Taraxacum*. Previous studies had found that kok-saghyz would hybridize with other related dandelion plants, resulting in impure seeds (Whaley, Bowen, 1947). Therefore, any related dandelion weeds need to be removed during kok-saghyz cultivation. However, we did not find hybrid seeds, and we need to continue observations in the future cultivation practice.

The 1000-seed weight of kok-saghyz samples from Russia was higher than that of American ones. Generally speaking, the seed size is a component of seed quality that affects crop performance. Large seeds have higher seedling survival and establishment rates (Luo, 1951), indicating that Russian seeds have greater plant vigor. In actual cultivation, we also found that kok-saghyz plants from Russia had a higher survival rate and greater biomass. Therefore, in order to increase the yield of kok-saghyz, selecting large seed germplasm sources and increasing the seed size and weight should be among the main research trends.

The pure seed rates in the kok-saghyz seed batches collected from different sources and in different years were also different, as it is common for the purity rates of seed lots from different sources for many other crops (Polhamus, 1962; AIST, 1996; Dan, 2006; Hu, 2015; ISTA, 2020), which may be associated with seed maturity and harvesting methods.

The natural imbibition rate of kok-saghyz seeds from Russia and the United States was 2.5 h, which shows that the natural imbibition time of kok-saghyz seeds from different sources is consistent. It also shows that when conducting kok-saghyz seed germination experiments, the seeds need to be soaked in water for at least 2.5 h.

By establishing a fixed-distance key table of seed characteristics, kok-saghyz can be distinguished from other common dandelion plants in Northeast China. It provides an additional method for people to identify dandelion plants. This method can be extended to all dandelion plants in the future.

Different molds were detected in kok-saghyz seeds harvested from the same environment in different years. Seeds stored for a long time contain more molds, which may be

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Table 4. Isolation frequencies for fungi on the surface of kok-saghyz seeds

Таблица 4. Частота выделения грибов на поверхности семян кок-сагыза

Year of seed harvesting	Types of fungi	Isolation rate		
2018	Blue mold	59.8%		
2018	Yellow mold	2.6%		
2018	Red mold	2.1%		
2018	White mold	1.5%		
2019	Blue mold	86.0%		
2019	Yellow mold	1.8%		

caused by the storage environment. Due to limited funds, when testing the seed health of kok-saghyz, those molds were classified according to their color, but more precise classification requires gene sequencing to be confirmed.

Conclusion

This article establishes kok-saghyz seed inspection procedures, stipulates sampling principles and methods, offers the patterns of the seed purity analysis, 1000-seed weight and moisture content assessments, and health testing techniques. The time for kok-saghyz seeds to naturally swell and reach imbibition is identified. A method for the checkup of kok-saghyz seed authenticity is provided. A fixed-distance key table of seed characteristics is recommended to compare kok-saghyz with other common dandelions in Northeast China.

The maximum weight of a batch of kok-saghyz seeds is prescribed to be 500 kg, the error is not allowed to exceed 5%, the minimum weight should be no less than 30 g, and the number of initial samples taken is not to be less than 3.

The purity analysis of kok-saghyz seeds requires at least 12.5 g. Each seed batch is replicated three times, and the average value is taken. The pure seed ratio of all kok-saghyz seeds was above 80%.

The minimum time for the natural imbibition rate of koksaghyz seeds from Russia and the United States to reach 100% was 2.5 h. Therefore, kok-saghyz seeds need to be soaked in water for at least 2.5 h before germination experiments.

The 1000-seed weight of kok-saghyz samples can be accurately calculated by using the 100 seed method, which is repeated 8 times and takes 100 seeds for each replication. The 1000-seed weight of kok-saghyz from Russia was $0.486~\rm g$, and that of American origin was $0.478~\rm g$.

The characteristics of kok-saghyz seeds are as follows: 1.4 to 4.2 mm in length, 0.3 to 1.2 mm in width, 11 to 16 longitudinal ribs on the surface, many small spines in the upper 1/3 to 1/2, and small knob-like protrusions or protrusions in the remaining part. There are no tumor-like protrusions, the beak base length is 1.0–1.8 mm, the beak length is 5–6 mm, and the ratio of beak base to achene length is 1:2-1:3. Kok-saghyz can be distinguished from other common dandelion species in Northeast China on the basis of fruit length, number of spines at the base of the beak, and ratio of beak base to fruit length.

The moisture content in kok-saghyz seeds can be measured using the low-temperature drying method; the average moisture content was 5.4%.

The washing test method detected that the average number of fungal spores contained in 1 g of kok-saghyz seed coat was 326×10^{12} .

The culture method helped to find that there were two types of mold inside the kok-saghyz seeds, blue and yellow mold. As the storage time increased, yellow and red mold were observed, blue mold being the most common. The isolation frequency for the seeds harvested in 2018 was 86.0%. In 2017, blue mold decreased, with an isolation frequency of 59.8%.

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