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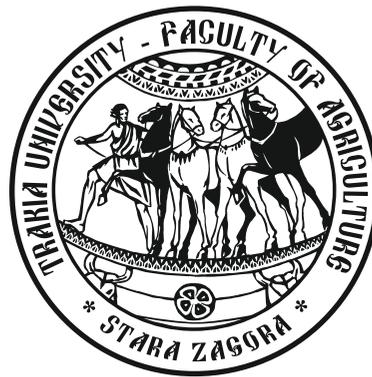
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## Drying of seeds from common wheat (*Triticum aestivum* L.) by using Silica gel for ex situ storage

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**Abstract.** One of the most widely used methods for maintenance of the biodiversity of the cultural plant species is their preservation under ex situ conditions. This major approach is related to storage of accessions at organism level in genetic stock centers by reducing the metabolite activity of the seeds as a result of low moisture content and low temperature. Seed moisture is one of the main indices for the storage ability of seeds. When working with a small number of samples in long term storage collections, a suitable method for reduction of moisture is the use of desiccant Silica Gel. The aim of this study was to develop a practically applicable system for drying winter wheat seeds by using Silica Gel for the purposes of an ex situ working collection of common wheat, following the variations of moisture throughout the entire process of dehydration. Seeds from three contemporary cultivars of DAI were used, Aglika, Enola and Pryaspa, which were grown in 2012. The experiment was designed at three different volumes of grain:desiccant ratios: 1 part grain to 0.5 parts silica gel (1:0.5), 1 part grain to 1 part silica gel (1:1), and 1 part grain to 1.5 parts silica gel. Each cultivar was involved in the above design, in three replications for each variant. Moisture content in seeds was determined by a weight method. The first reading was done on the 30<sup>th</sup> day, the second and third – at 20 day intervals. Two-factor dispersion analysis and variation analysis were applied for statistical processing of the data with the help of the software XLSTAT Pro ver. 7.5.2. The lowest moisture content at the end of the experiment was determined for cultivar Enola: 5.2 % at ratio 1:1.5 grain seeds/silica gel. It was found that at variant 1:1.5 seeds/silica gel the lowest moisture levels were reached and moisture reduction was the fastest. A practically applicable and economically efficient methodology was developed for drying according to which the variant 1:0.5 was the most suitable for the purposes of long-term storage of winter wheat.

**Keywords:** common wheat, desiccant, drying, storage of embryo plasm

### Introduction

One of the most common methods for maintenance of biodiversity of cultural plants is their preservation under ex situ conditions. This main approach is related to maintenance of accessions at organism level in seed gene pools and is based on creating artificial conditions for lower metabolic activity of the seeds, most often as a result of low moisture content and low temperature (Cohen et al., 1991).

The availability and management of ex situ collections associated to the breeding programs helps to preserve valuable embryo plasm for a long period of time at minimum losses of genetic material, labor costs and reproduction investments (Stoyanova, 1994, 2001; Chamurlyisky, 2012). The entire process is characterized with control of some parameters related to the viability of the stored accessions, namely germination energy, germinating capacity and moisture.

Moisture in seeds is one of the main indices for their storage ability. The ability of seeds to survive drying and freezing according to the classification of Roberts (Roberts, 1973) is the factor which determines if they are "storageable" (orthodox) or "difficult to store" (recalcitrant). When the accessions come in for storage under controlled conditions, it is necessary to achieve optimum levels of moisture content, applying various methods of drying for this purpose. One of the most common methods is sorption drying. It is practically applied in the approved storage system at the Bulgarian National Genetic Stock Center and the researches have shown that it can be considered a suitable regime for treatment of common wheat seeds (Stoyanova, 1987, 1990). Such a method of drying,

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however, is economically justifiable when working with large-scale gene pools. When working with a small number of samples in working collections for long-term storage, other methods of drying are more suitable according to literary sources (Gómez-Campo, 2007; Kameswara et al., 2006), such as using Silica gel desiccant\*.

The aim of this investigation was to develop an applicable system for drying of common wheat seeds by using silica gel in ex situ working collection of cereal crops, monitoring the variation of moisture throughout the entire process of dehydration.

### Material and methods

Seeds from three common winter cultivars extensively used in wheat production at Dobrudzha Agricultural Institute (DAI) were used: Aglika, Enola and Pryaspa, which were grown in 2012. The investigation was carried out under laboratory conditions at DAI.

The experiment was designed with three different volume ratios of grain and desiccant: 1 part grain to 0.5 parts silica gel (1/0.5); 1 part grain to one part silica gel (1/1), and 1 part grain to 1.5 parts silica gel (1/1.5). For easier practical application volume ratio was used instead of weight ratio. We worked with /Blaugel – CAS-7631/, which guaranteed approximately equal weight and shape of the granules in each sample. Each cultivar was involved according to the designs described above, in three replications for each variant.

The seeds were placed in preliminary scaled glass vessels and were covered with the desiccant, which was enwrapped in small bags of breathable material to avoid mixing of grains and granules.

\* - It is accepted in literature to use the term *desiccant* to refer to drying of seeds for the purposes of ex situ storage

Then the glass vessels were closed as tightly as possible. Moisture content in seeds was determined by the weight method (BGS 601-85) and was calculated by the formula:

$$S = \frac{(M2 - M3)}{(M2 - M1)} \cdot 100$$

where S was moisture content in %; M1 – weight of glass (g); M2 – weight of glass and contents before drying (g); M3 – weight of glass and contents after drying (g).

Moisture content in grains is measured simultaneously in two replications, according to the methodology. The difference between the results from the two replications should not exceed 0.2 %. If it is greater, the analysis should be repeated. In this particular investigation, however, to determine moisture content, seeds were taken only once per glass vessels, because taking two samples from each vessel would reduce the number of seeds used. Each variant at the respective ratios was considered a replication. Measurements were taken at regular intervals.

The initial moisture at placing the seeds in the glass vessels was marked with (S<sub>H</sub>). The first reading was done on the 30<sup>th</sup> day (S<sub>1</sub>), the second and the third – at intervals of 20 days (S<sub>2</sub> and S<sub>3</sub>). A control measurement was done after another 20-day interval. Two-factor dispersion analysis and variation analysis were applied for statistical processing of data using XLSTAT Pro ver. 7.5.2.

## Results

The results from the investigation are presented in Table 1. The average initial seed moisture at the beginning of the drying process of all three cultivars was 11.7 %, the lowest value being measured in cultivar Enola (11.4%). These results are justifiable since the experiment was not done immediately after harvesting. The duration of the entire process of drying was 70 days. The lowest moisture content was measured in cultivar Enola (5.2%) at ratio 1/1.5 grain/silica gel, obtained after the end of the investigation. The highest moisture percentage was observed in cultivar Aglika (at ratio 1/1, 6.48%). The standard error of the experiment was 0.14%. Based on this and on the mean values of moisture content at the respective ratios, the final effect of the drying process was differentiated. The data from variants 1/0.5 and 1/1 were significantly higher than the result from variant 1/1.5 according to Frideman's test (K Paired Samples Comparison). Between 1/0.5 and 1/1 the differences were not significant.

The results from the two-factor dispersion analysis showed that the measured values of moisture can be correctly analyzed (Table 2). The duration of storage and the influence of silica gel on the seeds is the factor with the highest effect on the duration process describing 97,1% of variations. The volume ratio among the seeds and silica gel as a factor has an insufficient effect – 1,7%.

A clearer idea for the process of seed drying is given by the results presented in Table 3. It presents the reduction of moisture

**Table 1.** Results from moisture measurements after drying with silica gel

Variety	S <sub>H</sub>	1/0.5			1/1			1/1.5			
		S <sub>1</sub>	S <sub>2</sub>	S <sub>3</sub>	S <sub>1</sub>	S <sub>2</sub>	S <sub>3</sub>	S <sub>1</sub>	S <sub>2</sub>	S <sub>3</sub>	
Aglika	11.77	8.39	6.12	5.82	7.88	6.61	6.36	7.09	5.98	5.49	
	11.71	8.30	6.21	5.87	7.79	6.59	6.31	6.99	5.85	5.70	
	11.78	8.26	6.26	5.69	7.96	6.51	6.48	6.89	5.96	5.71	
Enola	11.41	7.69	6.33	5.98	7.28	6.33	6.16	6.49	5.91	5.38	
	11.38	7.79	6.40	5.89	7.14	6.48	6.09	6.65	5.81	5.20	
	11.44	7.64	6.45	5.92	7.18	6.45	5.99	6.44	5.99	5.27	
Pryaspa	11.94	8.23	6.66	6.27	7.62	6.30	6.08	6.74	5.99	5.56	
	11.90	8.31	6.78	6.22	7.55	6.42	6.08	6.77	6.08	5.53	
	11.87	8.33	6.59	6.19	7.69	6.47	6.26	6.54	5.90	5.63	
Average	11.69		6.48ns				6.74ns				6.06**
Standard Error										0.14	

\*\* – p ≤ 0.05; \* – p ≤ 0.1; n.s – non-significant

Sign. Level α=0.05

**Table 2.** Results from the two-factor dispersion analysis

Source of Variation	SS	df	MS	F	P-value	F crit	%
Ratio seeds/silica gel	9.798	8	1.225	213.93	0.000	2.070	1.7
Duration of drying (days)	572.192	3	190.731	33315.54	0.000	2.732	97.1
R*D	6.626	24	0.276	48.23	0.000	1.669	1.1
Error	0.412	72	0.006				0.1
Total	589.029	107					

**Table 3.** Absolute and relative values of reduced moisture content

Measuring	Variety	1/0.5		1/1		1/1.5	
		Difference to S <sub>H</sub>	%	Difference to S <sub>H</sub>	%	Difference to S <sub>H</sub>	%
S1	Aglika	-3.45	29.34	-3.89	33.08	-4.78	40.59
	Enola	-3.71	32.47	-4.21	36.92	-4.89	42.81
	Pryaspa	-3.65	30.54	-4.32	36.16	-5.26	44.02
S2	Aglika	-5.51	47.06	-5.14	43.88	-5.78	49.36
	Enola	-4.99	43.86	-4.96	43.59	-5.48	48.14
	Pryaspa	-5.22	43.87	-5.50	46.24	-5.91	49.65
S3	Aglika	-5.98	50.80	-5.39	45.80	-6.14	52.15
	Enola	-5.51	48.19	-5.36	46.87	-6.16	53.84
	Pryaspa	-5.64	47.52	-5.74	48.30	-6.30	53.07
Average		-4.85	41.52	-4.95	42.32	-5.63	48.18

according to the initial percentage expressed in absolute and relative values. Considering the already mentioned lowest result from Table 2, cultivar Enola reduced its moisture by 53.8 % in comparison to the initial value, which, expressed in absolute value, means this cultivar marked a decrease of 6.2 points for 70 days at ratio 1/1.5 seeds/silica gel. As early as the first measurement after day 30, moisture in the seeds of cultivar Pryaspa was reduced by 44.02 % at variant 1/1.5. The average decrease of the values for the three ratios varied from 4.9 to 5.6 % in comparison to the initial values.

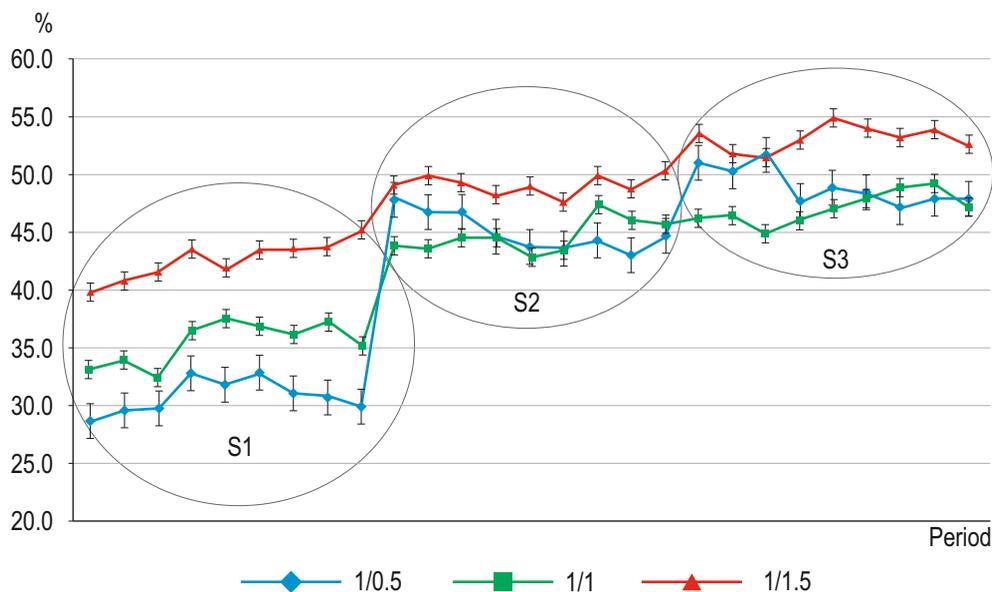
## Discussion

The dynamics of the entire process of drying is expressed graphically in Figure 1. The absolute values of moisture decrease in grain are presented on the Y-axis. In this case the role of the cultivar is ignored to focus on the changes depending on the specific ratio of amount of seeds with silica gel. The segment of the axis indicating the standard error in each variant was different depending on the

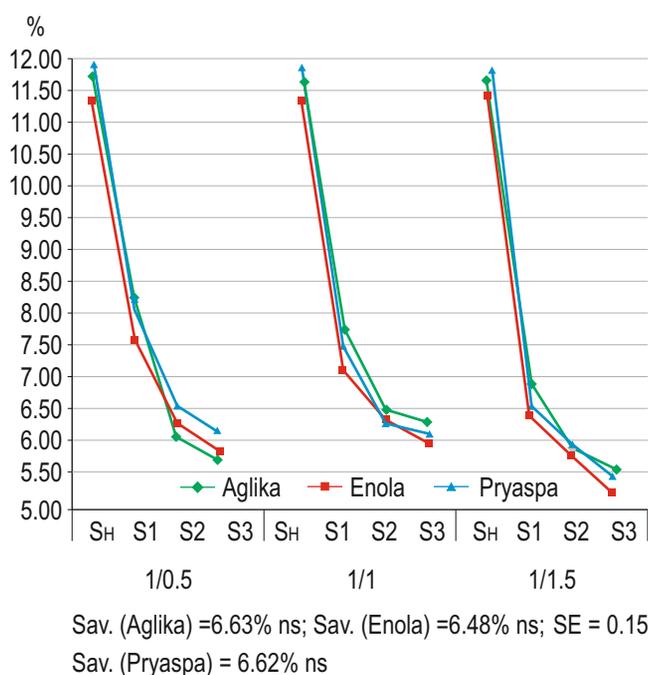
variation of the respective measurement. The overlaps of the error segments show there is no significant difference between the values of the respective ratios. This also clearly shows the distinction between variants 1/0.5 and 1/1, on one hand, and 1/1.5, on the other.

After the initial stage (S1) of drying, in the first two variants moisture was released more slowly than in variant 1/1.5, where moisture reduction occurred faster, the difference being almost 10 %. In the second stage of measurement (S2), moisture release in variant 1/1.5 was already more even, while in the other two variants a sharp change in the process of drying was observed. Twenty days after the first reading of 1/0.5, there already was a difference of 20 %. The conclusion can be made that the less the amount of silica gel, the more time is necessary to reach lower moisture content of seeds. At stage S3 the values varied considerably less and the differences with the previous measurement were much less significant.

The dynamics of drying of grain as influenced by the cultivar can be followed on Figure 2. The curves describing this process dynamics for each cultivar were similar for all three seeds/silica gel ratios. The differences between the mean values of the specific genotypes were not significant. The conclusion can be drawn that



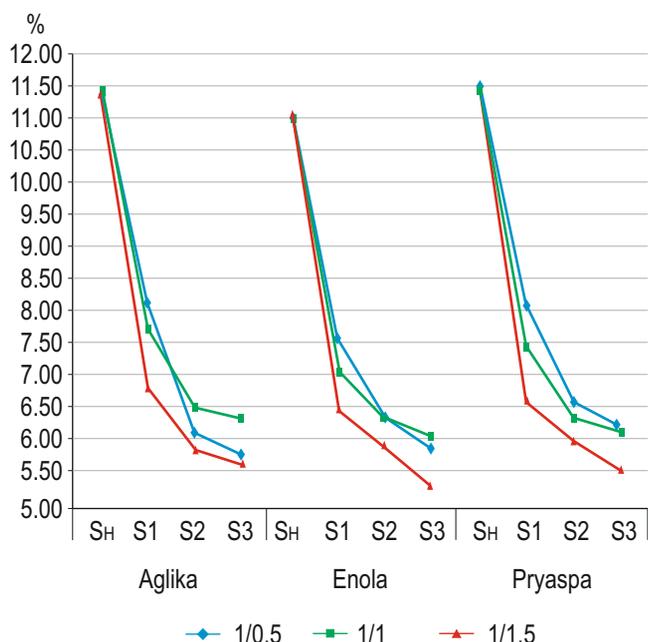
**Figure 1.** Dynamics of moisture variation under the effect of silica gel during the entire period of drying



**Figure 2.** Effect of the genotype on drying at different speeds/silica gel ratios

among the factors chosen for this experiment, the cultivar did not have a significant effect on moisture reduction. Therefore, in a practically applicable system of drying of wheat seeds the factor seeds/desiccant ratio is the most important. This research should be further developed in the future involving a greater number of cultivars to get a better idea on the possible significant effect of the genotype of the dynamics of the drying process.

Figure 3 shows also the effect of the ratios on the three cultivars used in this investigation. As early as the first measurement on the 30<sup>th</sup> day, moisture levels were determined in all variants which were close to the requirements for *ex situ* storage of common wheat. The most successful were the results obtained at the second measurement (after 50 days) for ratios 1/0.5 and 1/1, where average



**Figure 3.** Effect of the ratio on the drying process of different cultivars

moisture levels of 6.8 % were reached, while at ratio 1/1.5 in some replications values below 6 % were obtained; in such a situation dehydration may damage the viability of seeds. There are investigations in this respect showing that values below this level are not suitable for the purposes of long-term storage (Stoyanova et al., 2007). Optimum seed moisture of the seeds for this crop is within 6.35 - 6.05 % (Desheva and Stoyanova, 2012).

The analyses carried out undoubtedly showed that the use of silica gel as a method of seed drying is entirely applicable for such purposes. There are publications (Ondier and Siebenmorgen, 2010) giving similar results in other crops (rice). The researches of Kameswara et al. (2006) also provide evidence that the best ratio between seeds and desiccant is 1/1, and to shorten the time of drying a higher amount of silica gel is recommended. Our investigation showed that for the purposes of the working collection under conditions of long-term storage where a comparatively small number of accessions is involved, mainly of cereal crops, the most suitable variants are ratios 1/0.5 and 1/1. Although the period of drying was longer (50 days) than in variant 1/1.5 (30 days), the results obtained were indicative. It can be concluded that variant 1/0.5 is also suitable because there were no significant differences to variant 1/1; this variant is also economically more advantageous because the expenses for silica gel are lower.

## Conclusion

The lowest moisture content values were obtained at drying with desiccant at ratio 1/1.5 seeds/silica gel. In variant 1/1.5 seeds/silica gel the fastest reduction of moisture was observed. It was found in the selected accessions that the genotype did not have significant effect on the process of drying. In working collections where a comparatively small number of samples are dealt with, ratios 1/0.5 and 1/1 are justifiable in spite of the longer dehydration process.

A practically applicable and economically justified methodology of drying was developed, in which the use of variant 1/0.5 seeds/silica gel was the most suitable for the purposes of long-term storage of winter wheat.

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**The manuscript** should be structured as follows: Title, Names of authors and affiliation address, Abstract, List of keywords, Introduction, Material and methods, Results, Discussion, Conclusion, Acknowledgements (if any), References, Tables, Figures.

**The title** needs to be as concise and informative about the nature of research. It should be written with small letter /bold, 14/ without any abbreviations.

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**Keywords:** Up to maximum of 5 keywords should be selected not repeating the title but giving the essence of study.

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**Results** are presented in understandable

tables and figures, accompanied by the statistical parameters needed for the evaluation. Data from tables and figures should not be repeated in the text.

**Tables** should be as simple and as few as possible. Each table should have its own explanatory title and to be typed on a separate page. They should be outside the main body of the text and an indication should be given where it should be inserted.

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**Discussion:** The objective of this section is to indicate the scientific significance of the study. By comparing the results and conclusions of other scientists the contribution of the study for expanding or modifying existing knowledge is pointed out clearly and convincingly to the reader.

**Conclusion:** The most important consequences for the science and practice resulting from the conducted research should be summarized in a few sentences. The conclusions shouldn't be numbered and no new paragraphs be used. Contributions are the core of conclusions.

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**Todorov N and Mitev J**, 1995. Effect of level of feeding during dry period, and body condition score on reproductive performance in dairy cows. IX<sup>th</sup> International Conference on Production Diseases in Farm Animals, Sept. 11 – 14, Berlin, Germany, p. 302 (Abstr.).

### Thesis:

**Penkov D**, 2008. Estimation of metabolic energy and true digestibility of amino acids of some feeds in experiments with muscovy duck (*Carina moshata*, L). Thesis for DSc. Agrarian University, Plovdiv, 314 pp.

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### Ethics

Studies performed on experimental animals should be carried out according to internationally recognized guidelines for animal welfare. That should be clearly described in the respective section "Material and methods".

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