# HUNGARIAN UNIVERSITY OF AGRICULTURE AND LIFE SCIENCES

Doctoral School of Environmental Sciences


# NEW METHODS FOR THE TESTING OF ARABLE CROP GENE BANK COLLECTIONS


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Doctoral theses


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## 1. BACKGROUND OF THE WORK AND OBJECTIVES

Without plants humans could not survive on Earth. We need to conserve every single plant species in order to maintain the complex processes of life on the planet and to supply humankind with food. Besides the preservation of cultivated plants the conservation of wild plant species is also essential for our survival. Consequently, these two categories shall not be separated, and related conservation activities need to be performed simultaneously in order to complement each other. Besides conservation sustainable use is also a key issue, since this is the only way for long term conservation. According to the Convention on Biological Diversity, sustainable use means "the use of components of biological diversity in a way and at a rate that does not lead to the long-term decline of biological diversity, thereby maintaining its potential to meet the needs and aspirations of present and future generations" (UN 1992).

The role and the work of gene banks are becoming more important year by year. Due to the destruction of wild plant species and the withdrawn of some of the cultivated plants from production the importance of gene bank based conservation is increasing all over the world. The main tasks of plant gene banks include the collection, conservation, documentation, reproduction and dissemination of plant genetic resources.

The Food and Agriculture Organization of the United Nations (FAO) drew attention to the decreasing diversity of plant genetic resources for food and agriculture through several publications and issues in the last decades. According to the supplied data arrived to FAO and the estimates of the organization more than 75% of the varieties used in agriculture has been withdrawn from production in the last 100 years (FAO 2004, SoWPGR-2 2010).

In order to stop and reverse this process several international objectives have been set in the last three decades. In 2012 we created the Hungarian national strategy on the conservation of plant genetic resources for food and agriculture up to 2020 with my colleagues according to these objectives in the former Ministry of Rural Development. However the deadline for performing the actions set in strategy ended in 2020, its vision is still applicable today: "The long term preservation of the current diversity of Hungarian plant and microorganism genetic resources for food (our national treasures) without any genetic damages, revealing their real economic value as far as possible, spreading their sustainable use in their natural environment, and facilitating their utilization in research, educational and national breeding activities" (VM 2013).

The gene bank located in Tápiószele has an outstanding role in gene bank conservation activities in Hungary. Besides safeguarding the largest Hungarian gene bank collection, the institute also performs national coordination tasks in the field of plant gene conservation. It was founded more than 60 years ago, and its collections are continuously growing and expanding (NBGK 2019). Today the institute deals with the gene bank conservation of all plant groups including crop wild relatives (Baktay 2016) and also the species of the Hungarian wild flora (Peti et al. 2015). I have been directing the gene bank for 10 years during which period I gained a huge amount of experience in the field of plant gene conservation both from the point of professional policy and practical aspects.

During this time I realized that gene conservation tasks – that is practical gene bank work – and scientific research activities on gene bank stored genetic resources are widely different. In spite of the fact that we gained a huge amount of new information on the different gene bank accessions during the conservation activities, it cannot or just partly can be regarded as scientific result. Consequently, gene banks are not scientific research institutes. This is a very important fact to consider when evaluating collections, since gene bank stored genetic resources are primarily not basic materials for research but the basis of agriculture in the past, the present and the future. Of course, it is also true that every research result on gene bank stored genetic resources helps the process of gene conservation. Especially if it connects to practical gene bank activities or provides information on physiological characteristics which are essential for the success of conservation efforts.

Conserving the remained diversity of cultivated plants native to the Carpathian Basin and their wild relatives survived at the different sites (in natural plant communities, floodplain orchards, enclosed gardens, home gardens, small farms, gene bank collections and plantations, base material collections for breeding and botanical gardens) and analysing the conserved accessions are important and complex tasks requiring extensive research, analyses and national and international cooperation. In order to reach such goals co-sciences and other branches of science also must be involved in gene conservation through joint research, development and innovation activities in the future.

Problems related to the physiological status of gene bank stored genetic resources or variety identification issues cover a significant share of the diverse research topics to be dealt with.

Accordingly, I set the following goals during my research activity:

- developing a brand new method for the testing of gene bank stored seeds,
- creating a special thermal camera prototype for seed testing,
- using the thermal camera for testing the viability of gene bank stored seeds,
- using the thermal camera for the identification of the different varieties during the analysis of gene bank stored seeds.

## 2. MATERIALS AND METHODS

### 2.1. Selection of the species to be tested

Due to the nature of thermal camera imaging, during the selection of the species it was an important aspect to test seeds of different size and shape from those species well represented in the gene bank. Furthermore, we focused on those species having significant gene bank collections, and being important in the agricultural sector of Hungary. During the first measuring activities they proved to be well measurable and adaptable to the measuring method and system. Accordingly, the gene bank accessions of the following species were involved in the thermal camera analyses (Table 1):

Table 1: List of the tested gene bank accessions

| Institutional ID | Species | Hungarian name of the species | Name of the variety | Year |
|---|---|---|---|---|
| RCAT003611 | Triticum aestivum L. | Közönséges búza | Martonvásári 12 (Mv. 12) (Mv. 06-79) | 1979 |
| RCAT014479 | Zea mays L. | Kukorica | Florentini 8 soros (T-372) | 1978 |
| RCAT014988 | Zea mays L. | Kukorica | Valticka | |
| RCAT014640 | Zea mays L. | Kukorica | Valticka | |
| RCAT017513 | Panicum miliaceum L. | Termesztett köles | Tápiószentmártoni tf. | 1987 |
| RCAT017703 | Phaseolus vulgaris L. | Veteménybab | GH-162. | |
| RCAT017757 | Phaseolus vulgaris L. | Veteménybab | The Prince | 1962 |
| RCAT017817 | Phaseolus vulgaris L. | Veteménybab | Extender | 1963 |
| RCAT018955 | Phaseolus vulgaris L. | Veteménybab | Prinsa | 1957 |
| RCAT067091 | Phaseolus vulgaris L. | Veteménybab | Mezőnagymihályi tf. | 1994 |
| RCAT019270 | Phaseolus vulgaris L. | Veteménybab | Magyi tf. ("Helmeci bab") | |
| RCAT019866 | Phaseolus vulgaris L. | Veteménybab | Bodrogolaszi tf. | 1960 |
| RCAT024077 | Cicer arietinum L. | Csicseriborsó | FLIP 81-39 | 1982 |
| RCAT024574 | Cicer arietinum L. | Csicseriborsó | Békéscsabai tf. | 1987 |
| RCAT038201 | Helianthus annuus L. | Közönséges napraforgó | | 1989 |
| RCAT038505 | Helianthus annuus L. | Közönséges napraforgó | Nagykállói tf. | 1990 |
| RCAT055754 | Helianthus annuus L. | Közönséges napraforgó | Egreskátai tf. | 2002 |
| RCAT072087 | Triticum monococcum L. | Alakor | (tf.) FAZEKAS-2970 | 1998 |
| RCAT072092 | Triticum monococcum L. | Alakor | (tf.) JANICS-2400 | 1998 |
| RCAT002725 | Triticum monococcum L. | Alakor | | |

In the case of sunflower we searched for gene bank accessions from which full and empty seeds were also available. From maize, einkorn and common bean we tested accession also having discarded gene bank samples beyond the healthy ones. Furthermore, in the case of maize it was also important to have a significant number of gene bank accessions (open-pollinated varieties) in order to be able to test enough samples besides the hybrid varieties.

### 2.2. Method of seed preparation

The seeds are part of the collections of the National Centre for Biodiversity and Gene Conservation. Accordingly, all samples are stored in cooled seed storage rooms after being dried. In the case of fresh materials seeds are dried before testing at the temperature of 10–25°C by keeping a relative humidity level of 10–15% in a drying chamber or with the help of desiccants. This way seeds reach a moisture content of 3–7% depending on the species. In the next step seed accessions to be tested are either cooled to -18°C ± 3°C as long as possible – but at least for 24 hours – in closed containers, or are simply put on the special 2850 × 1800 mm large testing tray in a freezer in "no frost" mode without any covering in order to prevent the settling and the freezing of moisture on the surface of the seeds.

### 2.3. Method of seed germination

Although thermal camera tests aim at the non-invasive acquisition of information on the given seed, during the development of the method and the testing of viability we germinated every single seed according to the relevant standards in order to see if the results of thermal camera imaging really match with that of germination tests.

### 2.4. Presentation of the used thermal cameras

We used special custom-built thermal cameras for the thermal imaging tests (Table 2 and 3).

Table 2: Technical parameters of the thermal camera used between 2016 and 2019

| **Custom-built thermal camera system No. I. for special gene bank seed tests** (from 2016 to 2019): | |
|---|---|
| Type of sensor: | uncooled FPA microbolometer |
| Zoom: | ×1 |
| Pixel count: | 384 × 288 |
| Built-in imaging devices: | thermal camera (16 bit/pixel) |

| | |
|---|---|
| Outputs: | USB |
| Spectral sensitivity: | min. 50mK@300K, 50Hz |
| Measuring corrections: | automatic |
| Power supply: | accumulator and external adapter (230V AC, 50Hz) |
| Moisture content during operation: | 10–95%, non-condensing |
| Measuring range: | from $-30^0$C to $+1000^0$C |
| Spectral range: | 8–14 mikron |

Table 3: Technical parameters of the thermal camera used from 2020

| **Custom-built thermal camera system No. I. for special gene bank seed tests** (from 2016 to 2019) (Figure 1): | |
|---|---|
| Type of sensor: | uncooled FPA microbolometer |
| Zoom: | ×1, ×2 (digital zoom with processing software) |
| Pixel count: | 640 × 480 |
| Built-in imaging devices: | thermal camera (16 bit/pixel) |
| Outputs: | USB, external display connection |
| Spectral sensitivity: | min. 30mK@300K, 50Hz |
| Measuring corrections: | automatic (external temperature, distance, relative humidity level) |
| Power supply: | external adapter (230V AC, 50Hz) |
| Moisture content during operation: | 10–95%, non-condensing |
| Measuring range: | from $-30^0$C to $+1000^0$C |
| Spectral range: | 8–14 mikron |

Figure 1: The custom-built thermal camera used since 2020

Both thermal cameras are camera systems developed in Hungary. They use unique solutions enabling for accurate and reliable data collection required by imaging when testing seeds. Due to its custom development its thermal sensitivity is twice as better as that of the commercial camera systems, reaching 25 mK. Accordingly, very small temperature differences can be also detected, which is essential for the imaging of the structural elements of the seed having different temperature levels and that of rapid temperature fluctuations. The measuring range is also scalable, so measuring can be carried out from -50°C to +100°C according to the objective of the test. Furthermore, the angle of view can be set based on the size of the objects to be tested. This way the different sized seeds – ranging from a few millimetres to even more centimetres – can be tested by using the same camera system, so no other recording systems are needed during the analyses.

## 2.5. Digital methods and software used during the thermal imaging of seeds

The processing and the assessment of the raw images made by the thermal camera include several steps. The first and most important step is the retrieval of the files made by the camera. This is carried out with the help of the software called IRPlayer 4.0. IRPlayer is a kind of software handling the unique file format of the thermal camera developed for special laboratory measuring by Hexium Ltd. The program handles both the visual data made by the camera during the laboratory tests and those created by the external

control software. From the files having the extension of ".idsf" the program can display intensity values pixel by pixel or even absolute temperature data. Supported file formats for data saving: .bmp, .tif, .jpg, .raw, .cvs, .avi, .xml.

After this the program called Lumi IDSF 5.42 is used for the analysis of the accurate measuring results. This software can measure the intensity of batched digital images according to on built-in (e.g. Y'709, L1 or L2) or freely definable (custom) functions.

During processing the results of measuring designation is carried out on the whole surface of the seeds, and not only certain points are selected. The total area measured by the camera is 640 × 480 pixels, and the designated area is about 10% of this surface (depending on the species). The software measures the average intensity of pixels belonging to the designated area. In the images this means 1400 pixels by seed on the average. Intensity can be interpreted as the average of the emitted energy. This measured value is the digital value of energy emission per pixel.

# 3. RESULTS

## 3.1. Developing a new method for the thermal imaging of gene bank stored seeds

### 3.1.1. Elaboration of the process of the analysis, preparation of the seeds, measuring environment

There is no standard method for the thermal camera analysis of seeds in Hungary or internationally. Consequently, I could not use any existing method during my thermal camera based measuring activities. Therefore I had to elaborate a brand new method for the thermal imaging of seeds stored in gene bank collections. This subchapter presents the developed methodology which is one of my most important research results in practice, since a new measuring method has been elaborated for the thermal camera based measuring of seeds.

There is very limited experience on seed testing carried out by thermal imaging in the world. Available observations confirm that special circumstances are needed in order to get correct and accurate results when testing seeds.
During measuring it quickly turned out that the relative humidity level of the surrounding air (meaning the direct vicinity of the seeds) is a crucial factor affecting the results.
In order to provide special measuring environment we need a special laboratory. For correct measuring a relative humidity level of 70–90% is needed, which should be constant, if possible. Accordingly, temperature level should be 16°C ± 4°C. The temperature and the humidity levels of the measuring laboratory need to be regulated by special equipment. Preventing the development of draught is crucial, since it would negatively affect the accuracy of measuring.
A measuring site with constant humidity level can be provided in two ways: either through a site having a naturally high humidity level, or by a laboratory where constant high humidity level is generated artificially. Since we did not have such an artificial infrastructure, we looked for a site having a naturally high humidity level where measuring activities could be carried out together with the cooling of the seeds.
The National Centre for Biodiversity and Gene Conservation operates two cooled seed storage rooms for the preservation of safety duplicates in one of the abandoned mine passages located at the Esztramos Mountain in the territory of the Aggtelek National Park. The natural humidity level of the mine passage can be considered constant, and usually reaches 70%. As a result, measuring activities took place in the mine passage of the Esztramos

Mountain. Humidity level constantly exceeded 70%, and the cooled seed storage rooms operating at the temperature level of -20°C proved to be suitable for keeping the seeds cooled.

Measuring activities are carried out according to the following method:

- selection of plant species and varieties
- selection of gene bank accession
- selection of gene bank sample
- selection of seeds
- cooling of dried seeds (-20°C) on the special grid developed for such purpose
- putting cooled seeds under the camera together with the grid
- imaging of seed samples in the visible light and also the thermal spectrum
- conversion of raw images, designation of seeds, assessment of recordings

During the development of the method we found that the type of tray used for putting the seeds in the field of view of the thermal camera and the exact positioning of the seeds are crucial factors when performing measuring activities. We tested 7 different seed holding media during the development of the method. Those materials which cannot insulate well (that is are too effective heat conductors) are not suitable for making the tray. According to our recordings, these materials include:

- unglazed ceramics
- glazed ceramics
- glass
- foamed PVC (Palfoam™)
- modelling clay
- wood

During our recordings we found that cardboard had a smaller heat capacity, therefore it had only a minimal impact on the temperature change (warming up) of seeds placed on it and also on the reliability of the results on physiological characteristics derived from the temperature change of seeds. Based on the empirical analyses, the duration of the measuring was short, which confirmed that cardboard is a suitable material for making the seed holding tray and that it has the heat capacity required by the measuring.

In the case of cardboard altogether 4 × 5 pieces of maize seeds could be placed separately on the holder dimensioned to the image cut-out based on the distance of the thermal camera and the platen. The cardboard was developed in a way that the individual seeds could be fixed with the help of a physically separated grid (Figure 2).
It is very important to place only that many seeds in the field of view of the camera which are visible in the image, and they also need to be properly spaced in order to avoid the unwanted disturbance of their neighbours during warming up.

Figure 2: Thermal imaging of seeds placed on a cardboard in the mine passage of the Esztramos Mountain

Seeds must be put in the freezer on a “tray”, and only its edge (rim) is allowed to be touched when placing it on the platen in order not to warm up the seeds with the heat emitted by our fingers. It is advised to use gloves during the process of measuring. Seeds must not be touched before measuring. If the seeds need to be arranged, it must be done by using a clip.

Seeds are kept in the -20°C cold freezer for at least 24 hours before the measuring. It is important to prevent the freezing of moisture on the surface of the seeds, since this affects the measuring process, and leads to false results.

After placing the seeds under the field of view of the thermal camera, changes start, which are well detectable also in the visible light spectrum. Warming up seeds show significant and characteristic changes about in the first 20 minutes – depending on the sample –, and then the pace of warming slows down. The most intensive warming phase can be detected about in the first 5 minutes. During measuring we observed 3 separate phases, which can be witnessed in the case of all species (Figure 3). The sample reaches the level of the external temperature by a monotonic function increasing asymptotically.

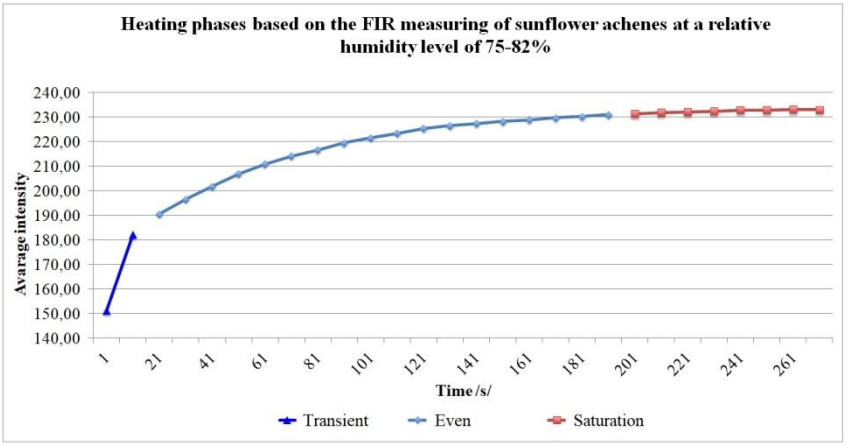


Figure 3: The three separate warming up phases detected during the thermal camera based measuring of seeds

I. **Transient phase** – This is the initial phase of warming up where the warming process of the seeds starts. The length of this phase varies by accessions. It usually lasts for 4–10 seconds.

II. **Even warming up phase** – The length of this phase varies by species and probably by variety. The level of warming up can be measured. It can be shorter or longer, usually lasting for 150–300 seconds.

III. **Saturation phase** – In this phase warming up increases, which can be measured. The data of the line fitted to this part (gradient, point of intersection, standard deviation) can be characteristic of the individual accessions.

Expected effects appear when the temperature difference is the highest between the air and the seed, therefore seeds should be put under the field of view of the camera from the freezer as fast as possible.

### 3.1.2. Creating a special laboratory for thermal imaging

In the framework of the Thematic Excellence Program won by the National Centre for Biodiversity and Gene Conservation we could create a special laboratory exclusively used for carrying out thermal camera based measuring activities. The laboratory is inside a converted shipping container, which is totally independent and separate from any buildings (Figure 4 and 5).

Figure 4: Special laboratory created for thermal imaging in the site of the National Centre for Biodiversity and Gene Conservation in Tápiószele

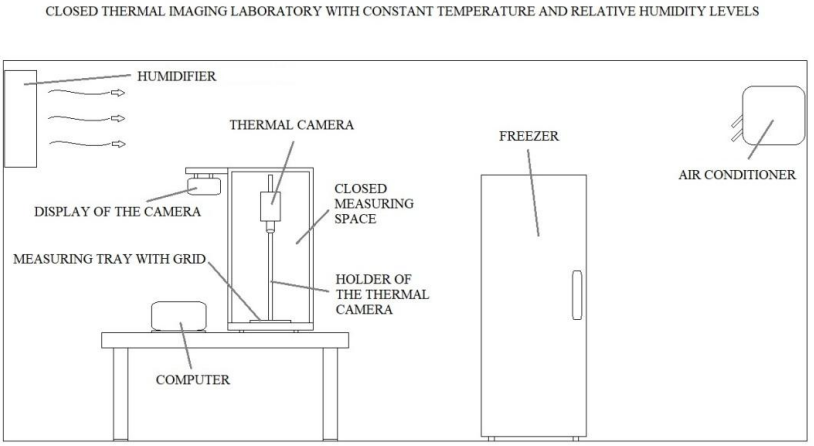


Figure 5: Schematic layout of the special laboratory created for thermal imaging

In order to provide special measuring environment we need a special laboratory. For correct measuring a relative humidity level of 70–90% is needed, which should be constant, if possible. Accordingly, temperature

level should be 16°C ± 4°C. The temperature and the humidity level of the measuring laboratory need to be regulated by special equipment. Preventing the development of draught is crucial, since it would negatively affect the accuracy of measuring.
The camera and the space used for measuring are enclosed in a draught-free "cage" with transparent walls, having the dimensions of 50 cm × 60 cm × 117 cm (width × depth × height). It is fixed with a 49 × 49 cm large door for inserting the seed accessions to be measured, which can be tightly closed in order to exclude any external disturbances (e.g. the development of draught), which could affect the results of measuring. The humidity and the temperature levels of the closed measuring space are the same as that of the laboratory.
Seeds are not damaged during the process of measuring, so they can be further analysed, measured again, or can be used for other gene bank/agricultural purposes (e.g. multiplication). If dried again the seeds can be further stored under cooled conditions. Accordingly, one single seed can be measured even several times.

### 3.2. Differentiation of empty and full sunflower achenes

Based on the developed method empty and full sunflower achenes could be unambiguously differentiated with the help of the thermal camera according to the results gained after the assessment of the thermal images of empty and full sunflower achenes.
In the case of the empty achenes the curve of the initial transient phase is steeper since there is air inside the achene instead of the seed. The second even warming up phase virtually does not exist in the case of empty seeds, while it is well observable when investigating full seeds. The curve of the third saturation phase is steeper in the case of full seeds. Figure 6 shows that the average intensity curves of full and empty seeds clearly differentiate from each other in relation to time.

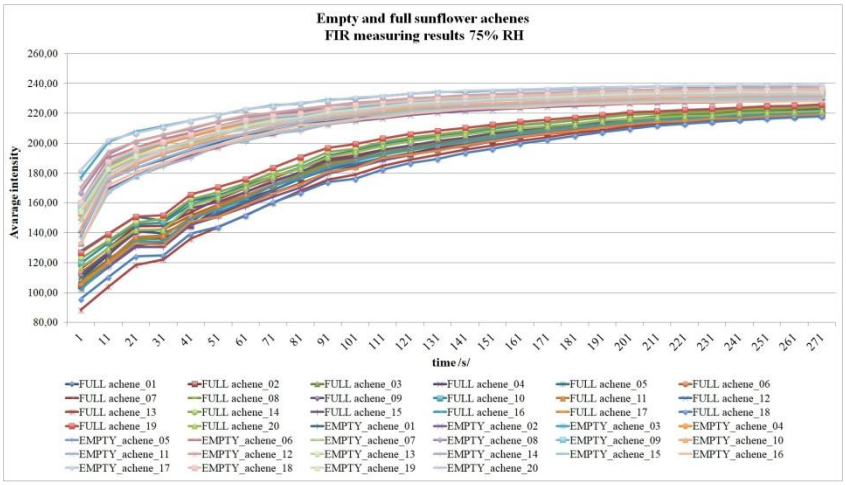


Figure 6: Warming up curves of full and empty sunflower achenes according to the results of thermal camera based measuring. Each curve shows the warming up of a single sunflower achene through the average intensity value of a given time. The curves clearly show that the pace of warming up is totally different in the case of the full and the empty achenes, especially in the middle even phase. Consequently, full and empty achenes can be unambiguously differentiated from each other based on the curves.

### 3.3. Testing seed viability by thermal imaging

When starting the thermal imaging of seeds our primarily goal was to acquire information on the viability of seeds through the results of measuring. Consequently, we were searching for correlation between the results of measuring and the viability of the individual seeds already from the beginning of our research. In order to be able to assess the results of our measuring, every single seed included in the research has been germinated, and the results of the germination tests were compared to that of thermal imaging.

In 2016 we tested 100-100 seeds of common bean, einkorn and maize gene bank accessions under proper circumstances in the mine passage of the Esztramos Mountain. From the 100 seeds of the gene bank accessions 50 were considered discarded from gene bank points of view, while the remaining 50 seeds were thought to be viable (based on the germination tests) in the case of all the three species. After performing the thermal camera based measuring activities seeds have been germinated according to the relevant standards.

The results of germination verified our preliminary expectations: discarded seeds really did not germinate, while “normal” seeds germinated according

to the gene bank protocol (reaching the germination ratio of 85% compared to the value measured before storage).
The average intensity curves of the individual seeds recorded by thermal imaging really have a different run in relation to time in the case of “discarded” and “normal” seeds (Figure 7).

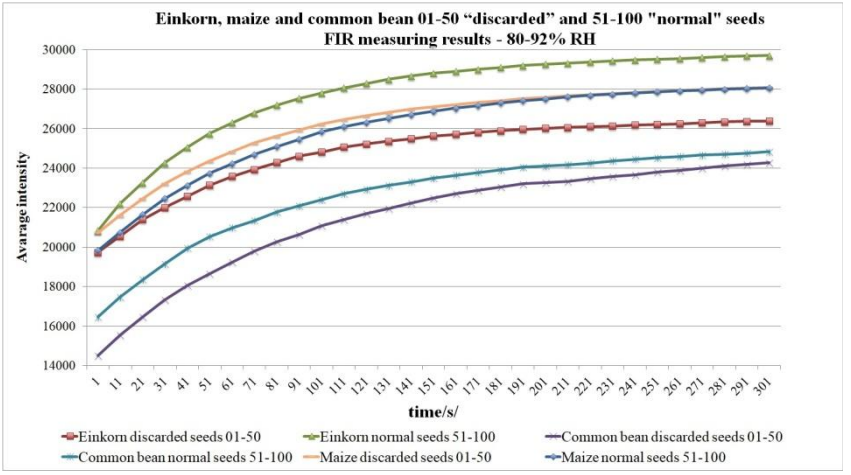


Figure 7: Measuring results of einkorn, maize and common bean

We also examined in the case of all the three species if the seeds categorized non-germinating but “normal” by the measuring results of the thermal camera based analyses differentiate from the really germinating “normal” seeds or not. Figure 8 shows that germinating seeds do really differentiate from those categorized non-germinating but “normal” during the thermal imaging based measuring activities. The largest distance between the two curves can be seen in the second even warming up phase in the case of all the three species.

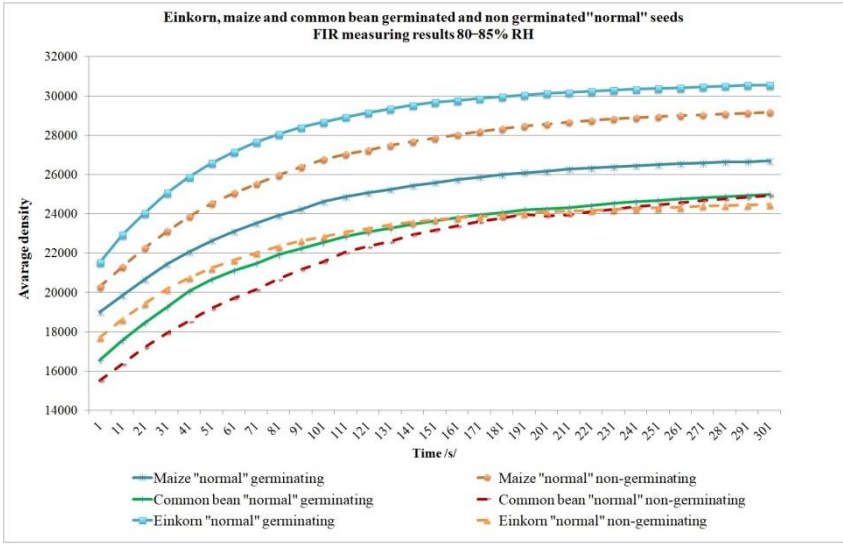


Figure 8: Comparison of the measuring results of “normal” einkorn, maize and common bean seeds with the results of germination. The average intensity of non-germinating seeds in relation to time (marked with dashed line) unambiguously differentiates from the average intensity of the germinating seeds (marked with normal line) in the case of all the three species.

### 3.4. Differentiation of maize varieties by thermal imaging

At the moment, different plant varieties can be differentiated with absolute certainty only through morphological assessment based on international descriptors and/or by DNA analysis. Seeds are lost in the case of both testing methods. Seeds can be used for the identification of the different varieties only in exceptional cases. Since it turned out already at the beginning of our thermal camera based measuring activities that the different varieties of the same species showed different pictures, it needed to be investigated if thermal imaging can be used for the differentiation of varieties or not. It happened many times that differences could be observed already on the display of the camera, but the different varieties showed different pictures also during the assessments of the records (even if they focused on other issues not directly on the differentiation of the varieties).

I measured 40 inbred hybrid maize varieties and 40 maize accessions from the gene bank which can be considered open-pollinated by analysing 20 seeds from each variety. The goal of the measuring was to differentiate the maize varieties of the gene bank from the hybrid ones. The thermal imaging of seeds took about 10 minutes, after this period no significant fluctuations could be observed in the process of change. Measuring activities have been carried out by using the new thermal camera under proper circumstances in

the special laboratory of the National Centre for Biodiversity and Gene Conservation including the varieties listed in the following Table 4:

Table 4: Names of the maize varieties involved in the thermal camera based analyses

| **Analysed maize varieties** | |
|---|---|
| **Gene bank accession** | **Hybrid** |
| 8 soros sárga fillér | DKC3972 |
| Bánkúti | DKC4351 |
| Csemege téli | DKC4670 |
| F korai sárga lófogú | P0412 |
| Fehér fillér | P8816 |
| Kék főznivaló | P9009 |
| Sárga lófogú 030821 | P9978 |
| Sárga lófogú 031412 | SU REPLIX |
| Sárga simaszemű | SY ORPHEUS 251 |
| Sárgásfehér fillér 031413 | SY ZEPHIR 147176 |

The two groups clearly differentiate from each other (Figure 9). The transient phases can be differentiated, and the characteristics of the different variety groups are clearly visible. Average intensity values significantly differ from each other, which means that any unknown maize variety can be sorted into one of the groups (hybrid or open-pollinated) with the help of the thermal camera.

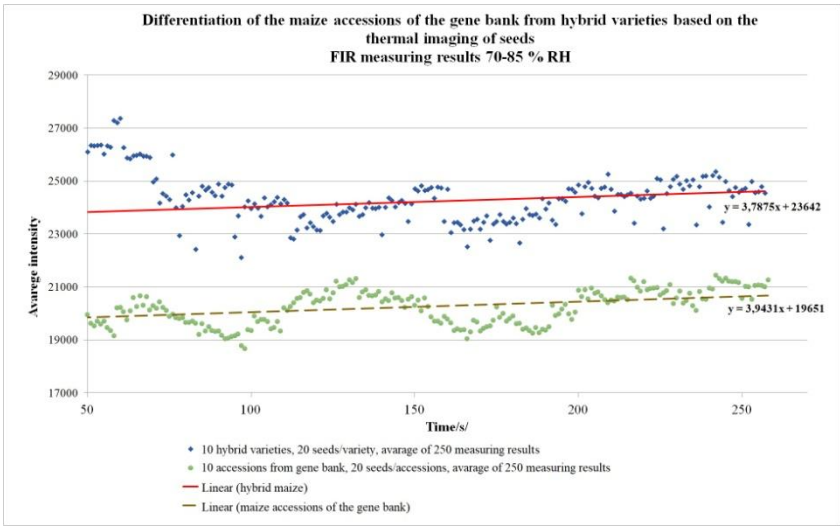


Figure 9: Differentiation of the maize accessions of the gene bank from hybrid varieties based on the thermal imaging of seeds. The average intensity of the seeds of hybrid maize varieties (marked with blue dots) unambiguously differentiates from that of gene bank stored maize accessions between the 50$^{th}$ and 250$^{th}$ second of the measuring.

## 4. CONCLUSIONS AND SUGGESTIONS

Today the conservation of genetic resources is a highly important task all over the world, and gene bank collections are expected to become even more important both for agriculture and nature conservation in the future. Consequently, every piece of information on the conserved genetinc materials has a great significance. During my research work I developed a brand new measuring method for the thermal camera based analysis of the seeds of gene bank accessions. With the help of this method a huge amount of new information can be gained without the destruction of the analysed seed, which was not available before.

Thanks to the possibilities provided by digital image processing a special processing method has been developed by which – after objective software aided data processing – the images of thermal camera based analyses can be used for gaining information on the viability of seeds and for the differentiation of varieties. Measuirng activities have been carried out by involving more than 10 plant speices, but the method could be used in the case of almost all plant species according to our measurings. Consequently, it could be applied for the majority of the seed collections irrespectively of the shape or the tissue structure of the seeds.

Since thermal camera based measuring activities can be performed quickly from the point of germination, physiological processes necessary for germination are not likely to start. In the future, it is advisable to investigate exactly which physiological processes start in the first 400 seconds – also measured by the thermal camera –, and which processes take place in the cells of the analysed seeds during this period of time.

In the case of sunflower empty and full achenes could be certainly differentiated from each other with the help of the thermal camera. Such results reveal that the thermal camera can be used for detecting the presence of air in seeds. Consequently, it can show if the seed/fruit contains air or even pests also in the case of other species. As a result, it is advisable to investigate the applicability of thermal camera analyes from the point of plant protection in the future.

As a result of the detailed elaboration of the method a special laboratory has been developed in which seeds and other living organisms and tissues can be analysed under specific circumstances by using a custom-built thermal camera. The laboratory provides the necessary circumstances for the application of the method. The built thermal camera prototype can be used for measuring seeds in the developed grid system, therefore further measuring activities also can be carried out. The gene bank located in Tápiószele safeguards more than 56000 gene bank accessions of about 1200 plant species, this way further species, varieties, clones or even breeding

lines can be measured. As a result, we can gain new information on the preserved seeds, which helps the success of their conservation.
Further examinations are required to reveal which other species could be investigated with this method, and to decide if the existing thermal camera is suitable for the analysis of the seeds of a specific species or not. Probably, the size of seeds will be the most important parameter defining the suitability of the measuring method, since very small seeds warm up quickly, and may reach or approximate the temperature level of the measuring space already before being put in it. I recommend to thoroughly measure the seeds of different plant species in order to gain as much information on the possibilities offered by thermal camera based analyses as possible.
I recommend to measure the seeds of all measurable plant species and to catalogue the gained data in a database. For this reason, as many germination test results should be attached and compared to that of thermal camera based measuring as possible. After this, the results of germination tests – carried out according to the gene bank protocol – could be complemented with that of thermal imaging by using the created database in order to collect as many data and information for the gene bank as possible. This could be easily put in practice in the gene bank if the seeds of the gene bank accessions selected for germination testing were first analysed by the thermal camera and then were germinated in the standard way. Finally, the results of thermal imaging could be compared to that of the starndard germination test the way already presented in the thesis.
In the case of the most important cultivated plant species of Hungary I recommend to carry out measuring activities on the different varieties. If all varieties of the National List of Varieties were recorded in the database, individual varieties could be easily identified with the help of thermal imaging.
Following further developments and the elaboration of special software, data analysis could become automated with the help of artificial intelligence by using the gathered data and the databases. Consequently, results would be available fast and easy.
In addition, I recommend extending the range of measuring to other living organisms and tissues, since differences are also expected to be observed in this case based on the thermal images and their information content. For this end tissues and organisms surviving freezing without any damages need to be searched.

## 5. NEW SCIENTIFIC RESULTS

1. New information on the possibilities offered by the thermal camera based analysis of seeds is a new result both in Hungary and internationally. New information on the individual species/varieties gained through thermal camera based measuring is unique, since no similar measurings have been published before. According to the results it can be stated that the thermal camera and the method are suitable for the analysis of seeds.

2. I elaborated the method of measuring, which had been improved through the series of measuring activities carried out for long years. With the presented method the warming up of gene bank stored seeds can be tracked from -18 ± 3°C to 16°C by using a thermal camera. By developing this new method a truly non-invasive method has been created, as these analyses can be carried out on the same seeds even several times provided that they are dried and put in the base storage room again after each measuring activity.

3. After analysing the results of thermal camera based measuring germinating and non-germniating seeds could be differentiated in the case of maize, einkorn and common bean.

4. After analysing the results of thermal camera based measuring empty and full sunflower achenes could be unambiguously differentiated from each other.

5. With the help of the thermal camera hybrid maize varieties and the open-pollinated gene bank accessions of maize could be differentiated from each other.

6. I created a laboratory providing special circumstances required by the measuring, which can be used for the thermal imaging of seeds and other living organisms or tissues. As a result of my research work a special custom-built thermal camera has been developed for the thermal imaging based analysis of seeds.

## 6. RELEVANT SCIENTIFIC PUBLICATIONS

### 1. Proofread full text scientific publication published (approved to be published) in scientific journals

### 1.1. In foreign language journal with impact factor (according to WEB OF Science)

#### 1.1.1. Published in Hungary

Peti, E.**,** Schellenberger, J., Németh, G., Málnási Csizmadia, G., Oláh, I., Török, K., Czóbel, Sz., **Baktay,** B. (2016): Presentation of the HUSEED wild – a seed weight and germination database of the Pannonian flora – through analysing life forms and social behaviour types. Applied Ecology and Environmental Research 15(1): 225–244. Print ISSN: 1589 1623, Online ISSN: 1785 0037

### 1.2. In foreign language journal without impact factor

#### 1.2.1. Published in Hungary

Török P., Tóth E., Tóth K., Valkó O., Deák B., Kelbert B., Bálint P., Radócz Sz. , Kelemen A., Sonkoly J., Miglécz T., Matus G., Takács A., Molnár V. A., Süveges K., Papp L. , Papp L. jr., Tóth Z., **Baktay B**., Málnási Csizmadia G., Oláh I., Peti E**.,** Schellenberger J., Szalkovszki O, Kiss R, Tóthmérész B.(2016): New measurements of thousand-seed weights of species in the Pannonian Flora. Acta Botanica Hungarica 58(1-2). p.187–198. Print ISSN: 0236-6495, Online ISSN: 1588-2578

### 1.3. In Hungarian in Hungarian journal without impact factor

A Pannon Magbank program (2010–2014) maggyűjtési, tárolási, előzetes életképesség vizsgálati eredményei és módszerei / Peti Erzsébet ; Málnási Csizmadia Gábor ; Oláh Imre ; Schellenberger Judit ; Török Katalin ; Halász Krisztián ; **Baktay Borbála** In: Természetvédelmi közlemények. – (2015) 21., p. 215–231.

### 2. Full text professional informative publication or study published (approved to be published) in professional journals

**Peti Erzsébet;** Málnási Csizmadia Gábor; Oláh Imre; Schellenberger Judit; **Baktay Borbála**; Török Katalin; Halász Krisztián; (2015): Éltető gyűjtemény – A Pannon Magbank, Természetbúvár p. 11–13.

Ponicsánné Gyovai Ágnes, Kollár Zsuzsanna, Peti Erzsébet, Horváth Balázs, Oláh Imre, Szalkovszki Ottó, **Baktay Borbála** (2013): Tájfajták a Zempléni-hegységben, a 2013-2014-es gyűjtőút program első állomásának tapasztalatai, Tájökológiai Lapok 11. évf. 2. szám, Gödöllő

Oláh Gábor, Dikasz Endre, Kristó Attila, Málnási-Csizmadia Gábor, Szalkovszki Ottó, **Baktay Borbála**: Gyűjtőút a Nagy-Fátrában és Dél-Baranyában magyar-szlovák kétoldalú együttműködés keretében. In: Botanikai Közlemények, 2016. 103. köt. 2. füzet, pp: 227–236

## 3. Proofread book/lecture note (section) (in printed format or by electronic data medium), informative book

### 3.1. Book writing in foreign language

**B. Baktay**, A. Simon: (2016) Hungarian Strategies for the Conservation of Crop Wild Relative and Landrace Diversity. In: Enhancing crop genepool use: capturing wild relative and landrace diversity for crop improvement / edited by Nigel Maxted, M. Ehsan Dulloo, Brian V. Ford-Lloyd. Boston, MA: CABI, p. 318–325

## 4. Publications published in Congress Publications (in printed format or by electronic data medium – only if the publication is authenticated by ISBN, ISSN or by other means)

### 4.1. One-page summary in foreign language or in Hungarian based on a given lecture or a presented poster published in an edited scientific journal or its special issue

Peti Erzsébet, Málnási Csizmadia Gábor, Oláh Imre, Schellenberger Judit, Török Katalin, Halász Krisztián & **Baktay Borbála** (2016): Seed biology and morphology investigations on Pannon Seed Bank collection and possible applicability of the results./A Pannon Magbank gyűjteményének magbiológiai és morfológiai vizsgálatai és azok felhasználási lehetőségei. „XI. Aktuális flóra- és vegetációkutatás a Kárpát-medencében nemzetközi konferencia, Budapest, 2016. február 12–14., absztrakt kötet p. 209. (ISBN 978-963-9877-25-2).

Peti Erzsébet; Málnási Csizmadia Gábor.; Oláh Imre, Schellenberger Judit; **Baktay Borbála**; Török Katalin; Halász Krisztián (2014): A gyűjtéstől a hűtőtárolókig: a pannon magbank program gyakorlati tapasztalatainak összefoglalása. „II. Fenntartható fejlődés a Kárpát-medencében”/ Through collecting to storages: summary of the practical experiences related to

Pannon Seedbank project. 11-12. December, 2014, Budapest, pp. 43–44. (ISBN 978-963-269-455-9)

Peti Erzsébet – Málnási Csizmadia Gábor – Oláh Imre – Schellenberger Judit – Veres Emese –**Baktay Borbála** (2015): Rózsafélék (*Rosaceae*) néhány fajának ex situ megőrzése és vizsgálata a Pannon Magbankban [Ex-situ Conservation and Investigation of Some *Rosaceae* Species in Pannon Seedbank] – In: Kerényi-Nagy, V.,Szirmai, O., Helyes, L., L., Penksza, K., Neményi, A. (eds.) „1st Rose- and hawthornconference in Carpathian Basin" international conference, Gödöllő, Hungary, 2015.05.30., Proceedings-Book, pp. 244–246. (ISBN: 978-963-269-479-5)

Peti Erzsébet; Málnási Csizmadia Gábor, Oláh Imre, Schellenberger Judit; Halász Krisztián, Török Katalin; **Baktay Borbála** (2014): Results of the Pannon Seedbank projekt [A Pannon Magbank LIFE+ Program eredményei] – In: Zimmermann, Z., Szabó G. (eds.) „II. Sustainable development int he Carpathian Basin˘international conference, Budapest, Hungary, 2014. 12.11.-12., Book of Abstracts, pp. 122–123. (ISBN 978-963-269-455-9)

## 5. Publications published in Congress Publications (in printed format or by electronic data medium – non-authenticated publications)

### 5.1. One-page summary in foreign language or in Hungarian

**Baktay Borbála** (2014): Hungarian strategies for the conservation of crop wild relative (CWR) and landrace (LR) diversity. ENHANCED GENEPOOL UTILIZATION – Capturing wild relative and landrace diversity for crop improvement. 16-20 June 2014. Cambridge. Book of abstracts pp. 40–41.

Berke József, Bánáti Hajnalka, **Baktay Borbála**, Szalkovszki Ottó, Szabó Rita, Takács Eszter, Darvas Béla és Gyulai Ferenc (2014): Hőfelvétel-alapú vizsgálati módszerrel való elkülönítés lehetőségei MON 810-es Bt-kukorica fajtacsoport utódmagvain. IV. Ökotoxikológiai konferencia. 2014. november 21. Budapest. Abstracts/Összefoglalók pp. 7–8.

Peti Erzsébet; Málnási Csizmadia Gábor, Oláh Imre, Schellenberger Judit; **Baktay Borbála**; Török Katalin; Halász Krisztián (2014): A gyűjtéstől a hűtőtárolókig: a pannon magbank program gyakorlati tapasztalatainak összefoglalása. „II. Fenntartható fejlődés a Kárpát-medencében" 2014. december 11-12., Budapest, Abstracts/Összefoglalók pp.44.

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