DOI: 10.22620/agrisci.2010.03.005

ВЛИЯНИЕ НА СУШЕНЕТО НА СЕМЕНАТА ВЪРХУ ЖИЗНЕНОСТТА ИМ ПРИ ДЪЛГОСРОЧНО СЪХРАНЕНИЕ В НАЦИОНАЛНАТА ГЕНБАНКА В БЪЛГАРИЯ
EFFECT OF SEED DRYING ON SEED VIABILITY UNDER LONG-TERM STORAGE CONDITIONS IN THE BULGARIAN NATIONAL GENEBANK

Сийка Стоянова
Siyka Stoyanova

Институт по растителни генетични ресурси „К. Малков" – Садово
Institute of Plant Genetic Resources – Sadovo, Bulgaria

E-mail: s_stoyanova@gbg.bg

Резюме

Изследвани са семенни образци, които са съхранявани повече от 20 години в генбанка. Обсъжда се увреждането на семената в резултат на ниското съдържане на вода в тях, което варира в зависимост от използваните методи за сушене. Определянето на оптимално влагосъдържание се основава на сравняването на положителния и отрицателния ефект от съхранението, като се използва промяната в кълняемостта на семената след периода на съхранение в зависимост от тяхната влажност. Въз основа на резултатите е определена „оптимална" стойност за влагосъдържание в семената за съхранение при -18°C. Семената с по-високо съдържание на мазнини оцеляват по-добре с влажност под 4%. Семената от бобови растителни видове са по-чувствителни към съхранението в сухо състояние и оптималната влажност в тях варира между 6,5% и 7,5%. При зърнено-житните тази стойност е между 6,1% и 7,2%.

Abstract

Seed accessions stored for more than 20 years in the genebank were evaluated. Seed deterioration was discussed in relation to the low seed moisture content which varies in relation to the drying methods used. The determination of the optimal seed moisture content was based on comparisons between the positive and negative effects of storage using the rate of seed germination after storage depending on the seed moisture content. On the basis of the observed results, the optimum seed moisture for storage at –18°C was suggested. Seeds with high amount of lipids survived better seed moisture content lower than 4%. Leguminous seeds were more sensitive to dry storage, and optimum seed moisture varied between 6.5% and 7.5%. In cereals this value was between 6.1% and 7.2%.

Ключови думи: генбанка, съхранение, семена, влажност.
Key words: genebank, storage, seed, moisture.

INTRODUCTION

The special ability of seeds to survive in a state of low water content and low temperature is a striking biological phenomenon. There are two main objectives in the storage of genetic stocks under genebank conditions: first, seeds need to be stored for a long period in order to avoid costs, complications and risks of growing plants for seed regeneration at frequent intervals; secondly, the stocks held under long-term storage show the minimum amount of alteration (Roberts, 1975). A number of empirical equations to predict longevity have been devised, with the most important one developed by Ellis and Roberts (1980a). This viability equation has widely proven its validity (Ellis & Roberts, 1980b; Ellis et al., 1989; Dickie et al., 1990). Our previous studies show that seeds of 207 plant species could survive in the genebank for more than 10 years with a minimal amount of viability changes (Stoyanova, 2001). Using viability equations in this research a grouping of plant species is presented according to the predicted safe storage life which varies from several decades to hundreds of years.

The present study aims to shed more light on understanding the practical work in the genebanks and thus to provide a scientific basis in the creation of monitoring protocols for seed storage in genebanks.

MATERIALS AND METHODS

Seed accessions from nine plant species (*Glycine max* Merr., *Pisum sativum* L., *Vicia sativa* L., *Triticum aestivum* L., *Zea mays* L., *Avena sativa* L., *Helianthus annuus* L., *Lactuca sativa* L., *Sesamum indicum* L.) stored for about

20 years in the genebank are evaluated. The control batch in this study is selected on the basis of fixed storage time for seed accessions: 7300 to 7310 days from the storage beginning. Seed germination before storage is determined (ISTA 1985), using the recommendations for work in the genebanks (Ellis et al., 1885a, b; Hanson, 1985). Seed germination tests after time of storage are carried out after re-humidification and pre-treatment of seeds, as described before (Stoyanova, 2001). Seed moisture content is determined using oven method of ISTA (1985) for a reduced sample (about 1–3g per accession). Genebank storage is carried out at –18°C in closed glass containers. The information for seed accessions in storage is maintained as ACCESS-database.

RESULTS AND DISCUSSION

Over the past years, a hypothesis was promoted where the authors suggest that the limiting factor in ageing of dry germplasm is the availability of water for chemical reactions (Vertucci and Leopold, 1987; Vertucci and Roos, 1993; Walters and Engels, 1998). From the practical point of view three main subjects could be discussed with respect to seed moisture and maintenance: re-humidification of dry seeds, optimal seed moisture and variation in seed moisture limits between crops.

Leguminous seeds need special attention, as described, with a shorter life span (Ellis et al., 1989; Specht et al., 1997, 1998; Stoyanova, 2001). As known, these seeds are more sensitive to injury during water imbibition before germination (Tittel, 1979; Ellis et al., 1990a, b).

In evaluation of three leguminous species (*Pisum sativum, Glycine max* and *Vicia sativa*) after more than ten years of storage in the genebank the variation of seed moisture content (SMC) and seed germination capacity (SGC) is determined. SMC varies as follows: soy-bean between 4.5% and 7.0% w.b.; pea between 6.1% and 7.6% w.b.; vetch between 6.3% and 7.9% w.b. (Table 1). The lower SMC is not automatically associated with better SGC.

Table 1. Effect of seed moisture content on germination rate of leguminous seeds preserved for more than 20 years in the National genebank at -18°C

Seed moisture content (wb) (%)	Acc[1]	Accessions preserved without changes	Reduction in seed germination rate with 5%	Reduction in seed germination rate from 5% to 10%	Odd results (reduction more than 10%)
Glycine max Merr. – 672 accessions evaluated					
< 5.2 4.80 ± 0.84[3]	76	17 (0.224)[2]	7 (0.092)	40 (0.526)	12 (0.158)
5.5–6.5 5.82 ± 1.15	350	191 (0.546)	61 (0.174)	71 (0.203)	27 (0.077)
> 6.5 6.70 ± 0.44	246	112 (0.455)	56 (0.228)	48 (0.195)	30 (0.122)
Pisum sativum L. – 260 accessions evaluated					
< 6.5 6.3 ± 1.4	107	78 (0.730)	7 (0.065)	10 (0.093)	12 (0.112)
6.4–7.0 6.78 ± 1.54	60	52 (0.867)	5 (0.083)	2 (0.033)	1 (0.017)
> 7.0 7.4 ± 1.78	93	62 (0.667)	13 (0.140)	11 (0.118)	7 (0.075)
Vicia sativa L. – 660 accessions evaluated					
< 6.8 6.5 ± 0.84	479	359 (0.749)	42 (0.089)	51 (0.106)	27 (0.056)
6.7–7.4 7.3 ± 0.75	174	121 (0.695)	27 (0.155)	24 (0.143)	2 (0.007)
> 7.3 7.6 ± 1.35	7	5 (0.714)	0	1 (0.143)	1 (0.143)

[1]Number of accessions in the respective groups described by seed moisture content
[2]In brackets is presented the frequency of the observed cases
[3]Mean

Table 2. Effect of seed moisture content on germination rate of cereal seeds preserved for more than 20 years in the National genebank at -18°C

Seed moisture content (wb) (%)	Acc[1]	Accessions preserved without changes	Reduction in seed germination rate with 5%	Reduction in seed germination rate from 5 to 10%	Odd results (reduction more than 10%)
Triticum aestivum L. – 2535					
< 6.5 6.10 ± 0.44[3]	423	380 (0.898)[2]	15 (0.035)	19 (0.045)	9 (0.022)
6.4–7.4 7.22 ± 0.65	1348	1190 (0.883)	57 (0.042)	67 (0.049)	34 (0.025)
> 7.3 7.40 ± 0.64	768	631 (0.826)	98 (0.128)	20 (0.026)	19 (0.025)
Zea mays L. – 2337					
< 7.0 6.4 ± 0.82	586	518 (0.884)	17 (0.029)	51 (0.087)	0
6.9–7.6 7.25 ± 0.97	697	579 (0.831)	61 (0.087)	57 (0.082)	0
> 7.5 7.7 ± 0.89	1054	833 (0.790)	124 (0.118)	97 (0.0.092)	0
Avena sativa L. – 508					
< 6.0 5.6 ± 1.8	257	222 (0.864)	29 (0.113)	4 (0.015)	2 (0.008)
5.9–6.8 6.4 ± 1.75	112	105 (0.937)	7 (0.063)	0	0
> 6.7 7.4 ± 1.35	139	121 (0.870)	8 (0.058)	8 (0.058)	2 (0.014)

[1]Number of accessions in the respective groups described by seed moisture content
[2]In brackets is presented the frequency of the observed cases
[3]Mean

The effect of SMC on SGC here is detected comparing the category of reduction in seed germination rate. The relationship between viability decline and seed moisture is not linear. However it is obvious that SGC after genebank storage depends on SMC. Seed germination decline, is detected using ‘conditions for compatibility’, as suggested by ISTA, for statistical tolerance of data (ISTA 1985). This approach is described earlier (Stoyanova, 2001) and has been used by other authors (Chen et al., 2003). On the basis of SMC, groups of cases are created (Table 1). The frequency of cases without changes, or with minimal changes, after storage is accepted as ‘good results’. On the basis of this approach the minimal rate of odd results for *Glycine max* seeds is observed when SMC is about 5.8% (5.5%–6.5%). When SMC in soy-bean is lower than 5.2% the rate of good results is significantly reduced. Similar analyses are carried out with *Pisum sativum* (Table 1). Comparing the value of ‘good results’ for pea seeds, the appropriate moisture content could be suggested about 6.8% (6.5%–7.0%). When pea seeds are stored with SMC lower than 6.5%, the rate of odd results is increased. Because of narrow scopes in seed moisture, vetch (*Vicia sativa*) seeds are evaluated on two levels of SMC (about 6.5% and about 7.3%). However it should be indicated that the higher SMC (about 7.3%) is associated with a lower rate of odd results. On the other hand, hard seeds are observed in a large number of accessions as a result of drying and storage. However it is obvious that odd results increase when seed moisture is reduced to 6.5%.

Cereal seeds are known with a longer life span. As described earlier the predicted ‘safe storage time’ for wheat, maize and oat are respectively: 194.39, 235.75 and 345.55 years (Stoyanova, 2001).The rate of SGC, in relation to SMC, under genebank storage is presented in Table 2. Wheat seeds are best preserved when SMC is about 6.10%. Very close to these results is the effect of 7.22% seed moisture in wheat. However if SMC is about 7.40%, a higher rate of germination

Table 3. Effect of seed moisture content on germination rate of oily seeds preserved for more than 20 years in the National genebank at -18°C

Seed moisture content (wb) (%)	Acc[1]	Accessions preserved without changes	Reduction in seed germination rate with 5%	Reduction in seed germination rate from 5% to 10%	Odd results (reduction more than 10%)
Helianthus annuus L. – 241					
< 4.1 3.72 ± 0.22[3]	83	83 (1.000)[2]	0	0	0
4.0–4.5 4.25 ± 0.625	55	54 (0.982)	1 (0.018)	0	0
> 4.5 5.10 ± 0.34	103	96 (0.932)	3 (0.029)	1 (0.010)	3 (0.029)
Lactuca sativa L. – 130					
< 4.0 3.52 ± 0.16	21	18 (0.856)	1 (0.048)	1 (0.048)	1 (0.048)
3.9–4.8 4.31 ± 0.22	63	27 (0.429)	8 (0.127)	8 (0.127)	20 (0.317)
> 4.7 5.20 ± 0.15	46	16 (0.349)	3 (0.065)	5 (0.108)	22 (0.478)
Sesamum indicum L. - 175					
< 3.0 2.25 ± 0.46	5	5 (1.000)	0	0	0
3.0–3.9 3.46 ± 0.43	31	30 (0.967)	1 (0.023)	0	0
> 6.7 7.4 ± 1.35	139	128 (0.921)	8 (0.058)	1 (0.007)	2 (0.014)

[1]Number of accessions in the respective group described by seed moisture content
[2]In brackets is presented the frequency of the observed cases
[3]Mean

decline is observed. The wide scale of accessions studied illustrate that seed moisture of wheat, associated with lower damage, is about 6.1%–7.2%. Maize accessions are evaluated in three groups, divided according to the average SMC. The results show a minimal rate of changes in the seed germination when seed moisture varies about 6.4%–7.25%. Because of large variation between maize accessions by size and by chemical composition, the observed deviation is explicable. Oat seed accessions are compared in three groups by SMC. Best results for preservation of SGC are detected when seed moisture is about 6.4%.

Oily seeds are described as more tolerant to low seed moisture, however the species possess different seed longevity under cold and dry storage. (Ellis et al., 1989). As presented before the predicted 'safe storage life' of seeds under genebank conditions for sunflower, lettuce is respectively: 20.94 years, 58.28 years and 604.00 years (Stoyanova, 2001). The changes in seed germination after more than 12 years of storage are compared in relation to the seed moisture (Table 3). Minimum changes in SGC are observed when seed moisture level is lower than 3 or 4%, respectively: 3.7% for *H.annuus,* 3.5% for *L. sativa* and 2.2% for *S. indicum*.

One of the important approaches in this study is the positive effect of re-humidification and pre-treatment of seeds before to be set for germination. All accessions have been pre-treated according our previous studies (Stoyanova, 2001) and that is the reason for reduced seed damages after long term storage in cool and dry environment. The application of seed pre-treatment in the genebank practice needs to be highlighted. When seed control tests are carried out without re-storing of water in dry seeds and appropriate pre-treatment, the observed seed germination capacity could lead to the wrong prediction of storage life. Furthermore, if dry seeds are sown in the field, that could provoke significant damage and loss of material because of liquid water injuries. We suggest re-humidification, pre-chilling of humidified seeds and chemical pre-treatment as successful practices for seeds after long-term genebank storage.

CONCLUSIONS

The storage life of seeds under genebank conditions depends on seed moisture, however the low seed moisture is not necessarily associated with high longevity. Under genebank conditions the optimum seed moisture for leguminous (6.5%–7.5%) and cereal seeds (6.1%–7.2%) is higher than that for oily seeds (2.2%–3.7%).

The minimum deteriorative effect of storage in relation to seed moisture could be achieved using both approaches: optimum seed moisture for drying before storage and appropriate re-humidification/pre-treatment of seeds after storage as a 'good genebank practice' that leads to limiting of damages and errors.

Acknowledgement: This study was partially supported by the National Science Found at The Ministry of Education and Science in Bulgaria, Project БК - 06/08.

REFERENCES

Chen, X., X. Lu, C. Cui, H. Chen, and H. Wang, 2003. Monitoring of sorghum (Sorghum vulgare) seed viability after a decade of storage in the National Genebank of China. – PGR Newsletter, **136**: 54–57.

Dickie, J. B., R. H. Ellis, H. L. Kraak, K. Ryders, and P. B. Tompsett ,1990. Temperature and storage longevity. – Annals of Botany, **65**: 197–204.

Ellis, R. H., and E. H. Roberts, 1980a. Improved equations for the prediction of seed longevity. – Annals of Botany, **45**: 13–30.

Ellis, R. H., and E. H. Roberts. 1980b. The influence of temperature and moisture on seed viability period in barley (Hordeum distichum L.). – Annals of Botany, **45**: 31–37.

Ellis, R. H., T. D. Hong, and E. H. Roberts, 1989. Handbook for Genebanks, Vol.I, IBPGR, Rome.

Ellis, R. H., T. D. Hong, and E. H. Roberts, 1989. A comparison of the low moisture content limit to the logarithmic relation between seed moisture content and longevity in twelve species. – Annals of Botany, **63**: 601–611.

Ellis, R. H., T. D. Hong, and E. H. Roberts, 1990a. Moisture content and the longevity of seeds of Phaseolus vulgaris. – Annals of Botany, **66**: 341–348.

Ellis, R. H., T. D. Hong, and E. H. Roberts, 1990b. Effect of moisture content and method of rehydration on the susceptibility of pea seeds to inhibition damage. – Seed Science and Technology, **18**: 131–137.

Hanson, J., 1985. Practical Manuals for Genebanks. IBPGR, Rome.

ISTA, 1985. International rules for seed testing. – Seed Science and Technology, **13**: 299–355.

Roberts, E. H., 1975. Problems of long-term storage of seed and pollen for genetic resources conservation. – In: Frankel O.H., Hawkes J.G (eds): Crop Genetic Resources for Today and Tomorrow, Cambridge University Press, Cambridge, 269–293.

Specht, C. E., U. Freytag, K. Hammer, and A. Boerner, 1998. Survey of germinability after long-term storage in the Gatersleben genebank (Part 2). – PGR Newsletter, **111**: 64–68.

Specht, C. E., E. R. J. Keller, U. Freytag, K. Hammer, and A. Boerner, 1997. Survey of germinability after long-term storage in the Gatersleben genebank. – PGR Newsletter, **115**: 39–43.

Stoyanova, S. D., 2001. Ex situ conservation in the Bulgarian genebank. I. Effect of storage. – PGR Newsletter, **128**: 68–76.

Tittel, C., 1979. Die Trocknung von Leguminosensamen fuer die Langzeitlagerung. – Kulturpflanze, **27**: 175–180.

Vertucci, C. W., and A. C. Leopold, 1987. The relationship between water binding and desiccation tolerance in tissues. – Plant Physiology, **85**: 232–238.

Vertucci, C. W., E. E. Roos, 1993. Seed storage, temperature and relative humidity. – Seed Science Research, **3**: 215–216.

Walters, C., and J. Engels, 1998. The effect of storing seeds under extremely dry conditions. – Seed Science Research, **1**: 3–8.

Статията е приета на 6.04.2010 г.
Рецензент – проф. дсн Дияна Светлева
E-mail: svetleva @yahoo.com