ORIGINAL PAPER

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Quality of Soybean (*Glycine max L.*) and Fodder Pea (*Pisum arvense L.*) Seeds After Five Years Hermetic Storage

Abstract: In the laboratory of the Faculty of Agriculture in Osijek (CROATIA), statistically highly significant correlations were found between storage longevity (five researched years 1993-1997) and seed germinability of soybean and fodder pea seeds. Seeds of the above mentioned species were stored in hermetic glass containers at air temperature of 20°C and relative humidity 65%. Moisture level in stored seeds was 9%. The study showed that sprouting energy of soybean and fodder pea seeds was in negative correlation with storage longevity. Sprouting energy achieved the greatest values before seed storage (soybeans 90.50% and fodder peas 94.00%). After 5 years hermetic storage, sprouting energy achieved the smallest values (soybeans 13,25% and fodder peas 74.25%). The differences in sprouting energy found between the years were statistically highly significant (P<0.01).

Similar results were achieved by studying of seed germinability; the largest values of seed germinability were of the seeds before storage (95.75%). After 5 years hermetic storage, seed germinability achieved the smallest values (soybeans 15.50% and fodder peas 80.75%). The differences in seed germinability found between years were statistically highly significant (P<0.01).

According to the obtained results, seeds of fodder peas had greater values of sprouting energy and germinability than soybean seeds. The differences found between the species were statistically highly significant for sprouting energy (P<0.01) and significant for germinability (P<0.05).

Key words: Vigour, Viability, Seed, Hermetic storage, Cultivar, Soybean (*Glycine max L.*), Fodder peas (*Pisum arvense L.*), Sprouting energy, Germinability, Quality, Croatia.

نوعية بذور فول الصويا (*Glycine max L.*) والبازلاء الرعوية (*Pisum arvense L.*) بعد خمس سنوات من التخزين

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المستخلص: في مختبر كلية الزراعة فى أوسي جيك (كرواتيا) تم التوصل إلى علاقة توافقية معنوية لطول فترة التخزين (خمس سنوات من البحث 1993-1997) وقدرة الإنبات لبذور فول الصويا وبذور البازيلاء الرعوية. لقد تم تخزين البذور المذكورة أعلاه في عبوات زجاجية على درجة حرارة 20 مئوية ورطوبة نسبية مقدارها 65%. وقد كان مستوى نسبة الرطوبة في البذور المخزونة 9%. وقد أظهرت الدراسة أن قدرة التفرغ في بذور فول الصويا والبازلاء الرعوية متوافقة توافقاً سلبياً مع طول فترة التخزين. إن قدرة الإنبات قد حصلت أعلى قيمة قبل التخزين (بذور الصويا 90..5% ، البازلاء الرعوية 94%). بعد خمس سنوات من التخزين، أظهرت قدرة التفرع أما قيمة (فول الصويا 24.13% ، البازلاء الرعوية 74٪). وقد وجد أن الاختلاف في قدرة التفرع بين سنوات التخزين كان ذا قيمة معنوية إحصائية عالية (P<.0^01) نتائج مماثلة تم الحصول عليها من دراسة إنبات البذور، أعلى قيم من إنبات البذور قبل التخزين (97.75%) بعد خمس سنوات من التخزين إنبات البذور أصبح أقل ما يكون (فول الصويا 15.5٪ ، البازلاء الرعوية (80^75%). إن الاختلاف في إنبات البذور بين السنوات المختلفة وجد أيضاً معنوياً درجة عالية من الناحية الإحصائية .(P>0.0I) بناءاً على النتائج المتحصل عليها وجد أن بذور البازلاء الرعوية لها أعلى قيم من الناحية الإنباتية وقدرة التفرع من بذور الصويا. وقد وجد أن الاختلافات بين النوعين كانت من الناحية الإحصائية لها قيمة معنوية بدرجة عالية لقدرة التفرع (P>0.0I) ومعنوية لإنبات البذور (P>0.0 5).

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Introduction

Soybean and fodder peas are important agricultural species in world agriculture. Due to the significant importance for human and animal nutrition these species are sown on large land areas and it is crucial to assure enough seeds for sowing. Seeds must have good quality characteristics such as sprouting energy and germinability. High quality characteristics are especially important when the seeds from one season are to be used for sowing after a few years. Seed damage during processing may also have a direct or indirect influence on seed vigour and germination. Insects and rodents are the main causes of damage to stored bean and maize (Vazquezarista, *et al.* 1995). Many scientists have concluded that the seed storage environment has a significant influence on seed longevity (Sanchez, *et al.* 1993; Tang and Sokhansanj, 1993; Owen and Pill, 1994; Sanhewe and Ellis, 1996 and Ellis, *et al.* 1996).

Humidity and temperature had a notable effect on viability; ability decreased with increased storage time (Khoroshailov and Zhukova, 1978; Ellis, *et al.* 1980; Nienhuis and Baltjes, 1985; Flood, 1986; Momonoki and Momonoki,. 1987; Murariu, *et al.* 1994 and Specht, *et al.* 1997).

Conn and Deck (1995) concluded that less than 1% of wild oat and foxtail barley seeds were viable after 3-7 years of storage in mesh bags at 15 cm deep in soil, but more than 7 years were required for loss of all viability. Tekrony, *et al.* (1993) concluded that temperature and soybean seed moisture during storage have significant influence on germination rate. Sung and Chiu (1995), after research on soybean seeds, stated that achieved results indicated aging inhibited seed germination and enhanced lipid peroxidation at both 5°C and 25°C, but with more rapid seed deterioration and a greater extent of lipid peroxidation at the latter temperature. According to an investigation by Zanakis, *et al.* (1993) on soybeans, the results confirmed that the seed viability equation of Ellis, *et al.* (1980) provides a framework within which the seed longevity of different genotypes can be compared. Bingham, *et al.* (1994) concluded that the rate of radicule and coleoptile extension was more sensitive to aging than germination; all aging treatments reduced the extension rate of coleoptiles and radicules, but only the longer treatments influenced germination significantly. Under conditions of accelerated aging of soybean seeds (36°C and 75% RH), fluorescence of soluble proteins accumulated, which was closely correlated with the loss of seed germinability and vigor (Sun and Leopold, 1995).

Therefore, the aim of our program is to determine the influence of seed storage length under controlled conditions on sprouting energy and germinability.

Material and Methods

The study was conducted during five years (1993-1997) using two species. Species one; "Tisa" (soybean cultivar, basic seed) was selected in Croatia. This cultivar belongs to later I maturity group (I-II). The thousand kernel weight is 170 g. Optimal plant density is 550,000 plants per hectare. Plant height is 110 cm and the seed has 22% oil and 38% protein in dry matter. Sowing time is the third decade of April with a sowing rate of 600 germinable seeds/m^2. Species two; "Maksimirski bijeli" (winter fodder pea cultivar, basic seed) was made in Croatia. Plant height is 100-110 cm and the seed has 32% protein in dry matter. This cultivar has an average seed yield 2,5 t/ha and the thousand kernel weight is 210 g.

Samples of cleaned and processed seeds of the researched species were put in hermetically sealed glass containers with a volume of 500 ml. Three quarters of the container volume were filled with seed mass. Seed moisture level during storage was 9%, air temperature 20°C and relative air humidity 65%. After five years of storage, the species were examined for quality (sprouting energy and germinability) by the standard method of examination, ISTA (1985). Filter paper was used as a sprouting base. Sprouting energy was determined after 5 days of sprouting and germinability after 8 days (for both species). The results were subjected to variance analysis.

Results and Discussion

Sprouting energy

The results of the influence of soybean and fodder pea storage on sprouting energy are shown in Table 1. From the results it is obvious that seed storage caused a decrease in sprouting energy in the two studied species. Prior to storage, the highest values of sprouting energy were found in fodder peas (94.50%) and the lowest in soybean seed (90.50%). After five years of storage, sprouting energy decreased in the two studied species and the highest values achieved were with fodder peas (74.25%) and the lowest with soybean seed (13.25%). Differences in sprouting energy between years of storage are statistically highly significant ($P<0.01$). The greatest decrease of sprouting energy was in soybean seed (decreasing 77.00%). Differences in sprouting energy found between the researched species are statistically highly significant ($P<0.01$).

Table 1. Sprouting energy of soybeans and fodder peas seeds before and after five years of hermetic storage

Sprouting energy before storage (%)					
Species	Replications				Average
Soybean	90.00	91.00	90.00	91.00	90.50
Fodder peas	95.00	95.00	94.00	94.00	94.50
Average	92.50	93.00	92.00	92.50	92.50

Sprouting energy after 5 years of hermetic storage (%)					
	Replications				Average
Soybeans	12.00	13.00	14.00	14.00	13.25
Fodder peas	75.00	74.00	73.00	75.00	74.25
Average	43.50	43.50	43.50	44.50	43.75

Effect A (Years)	92.50 (Before storage)	43.75 (After 5 years)
Effect B (Species)	51.88 (Soybeans)	84.38 (Fodder peas)

	F test	LSD test	
Years (A)	13689.001**	$LSD_{0,05}$ = 1.0270	$LSD_{0,01}$ = 1.8852
Species (B)	6084.000**	$LSD_{0,05}$ = 1.1169	$LSD_{0,01}$ = 1.6920
A x B	4678.560**	$LSD_{0,05}$ = 1.4501	$LSD_{0,01}$ = 2.3109

Germinability

Similar results were achieved by studying the influence of seed storage on germinability, these are shown in Table 2. Seed storage caused a decrease in germinability in all studied species after the five year program. Before storage, soybean seed had higher germinability (96.25%) than fodder pea seed (95.25%). After five years of storage, germinability decreased in all studied species and the highest values were achieved with fodder peas (80.75%) and the lowest in soybean seed (15.50%). Differences in germinability between years of storage are statistically highly significant ($P<0.01$). Similar results were achieved for sprouting energy; the highest decrease of germinability was found in soybean seeds (decreasing by 80.75%).

Szabo and Viranyi (1971) according to results of ten years storage on 37 species, concluded that the best retention of viability was found in legumes, while vegetable and oil crops showed rapid loss of germination. Cereals occupied an intermediate position.

The decrease of sprouting energy and germinability is probably the consequence of growth inhibitors with phenolic origin. The denaturation and coagulation of protein molecules during storage may have brought about a degeneration of enzymes important for sprouting (Kastori, 1984).

Many scientists have found that long storage caused seed quality loss and lower percentage of germinability (Kastori, 1984; Kolak, 1994; Morenomartinez, *et al.* 1994; Guberac, *et al.* 1997 and Martincic and Guberac 1998).

Mills and Woods (1994) carried out a sutdy on field peas (*Pisum sativum L.*) and white beans (*Phaseolus vulgaris L.*) and concluded that beans suffered severe germination loss under less extreme conditions than peas. Pandey (1996) stated that field peas seeds, hermetically stored at 2% MC, did not show any loss of viability after 2,190 days.

Alyahya (1995) conducted a study where resistant and susceptible corn hybrids were stored for 50 days at 22% moisture content in storage units of 1 kg capacity at 26°C. The hybrids were tested by measuring the dry matters loss which was obtained by determining the amount of carbon dioxide evolved from respiration of stored grains. This measure indicates the deterioration rate of grain in storage. This study indicated that the loss rate in the stored grain weight increased with increasing

Table 2. Germinability of soybean and fodder pea seeds before and after five years of hermetic storage

Germinability before storage (%)					
Species	Replications				Average
Soybeans	97.00	95.00	96.00	97.00	96.25
Fodder peas	96.00	94.00	96.00	95.00	95.25
Average	96.50	94.50	96.00	96.00	95.75

Germinability after 5 years of hermetic storage (%)					
	Replications				Average
Soybeans	14.00	16.00	14.00	18.00	15.50
Fodder peas	80.00	81.00	82.00	80.00	80.75
Average	47.00	48.50	48.00	49.00	48.12

Effect A (Years)	95.75 (Before storage)	48.13 (After 5 years)
Effect B (Species)	55.88 (Soybeans)	88.00 (Fodder peas)

	F test	LSD test	
Years (A)	4785.527**	$LSD_{0,05}$ = 2.3754	$LSD_{0,01}$ = 4.3604
Species (B)	2177.439**	$LSD_{0,05}$ = 1.6089	$LSD_{0,01}$ = 2.4373
A x B	2315.110**	$LSD_{0,05}$ = 2.7208	$LSD_{0,01}$ = 4.5197

storage time. All tests indicated decrease of seed germination in the resistant corn hybrid to be significantly less than in the susceptible corn hybrid. Opoku and Gamble, 1995, after investigation on barley, concluded that after eight months warm germination decreased (3%), whereas cold germination decreased more (6%).

The temperature of storage and relative air humidity are very important for seed longevity. Relative air humidity and high temperature of storage are in negative correlation with seed longevity and germinability (Jansen and Ison, 1994; Alsadon, *et al.* 1995; Depaula, *et al.* 1996; Vanpijlen, *et al.* 1995 and Guberac, *et al.* 1997).

On the basis of the results it can be concluded that hermetic seed storage in glass containers and controlled temperature as well as air humidity conditions can preserve germinability at a high level in fodder peas (after five years of hermetic storage 80.75%). Soybeans rapidly lost sprouting energy and germinability after five years of storage (average level of 15.50%).

Conclusions

On the basis of the results of research conducted at the Faculty of Agriculture in Osijek (Croatia) on soybean and fodder pea seeds stored for five years, the following can be concluded:

1. Sprouting energy of the two studied species decreased with storage years. Therefore, sprouting energy was the highest at the beginning of storage (average 92.50%) and the lowest at the end of the storage (average 43.75%). The differences in sprouting energy found between the investigated years are statistically highly significant ($P<0.01$).

2. Decreases of sprouting energy in studied species ranged from 20.25% (fodder peas) to 77.25% (soybeans). The differences in sprouting energy are found to be statistically highly significant ($P<0.01$).

3. Germinability was decreased with storage. The highest values were at the beginning of the storage period (average 95.75%) and the lowest

at the end of the storage period (average 48.12%). The differences in germinability are considered to be statistically highly significant (P<0.01).

4. Decreases of germinability ranged from 14.50% (fodder peas) to 80.75% (soybean). The differences in germinability between the two studied species were statistically highly significant (P<0.01).

The results of this research show that hermetically stored fodder peas preserve a satisfactory percentage of sprouting energy and germinability and could be safely used for sowing.

Acknowledgements

This research has been funded by the Science and Technology Ministry of the Republic of Croatia (project 079-109). We thank our students Marija Besek and Ivica Jozic at the Faculty of Agriculture in Osijek for helping during the investigation.

References:

Alyahya, S.A. (1995) Losses of corn in the storage. *Arab Gulf Journal of Scientific Research* **13** (1): 199-212.

Alsadon, A., Yule, L.J. and **Powell, A.A.** (1995) Influence of seed aging on the germination, vigour and emergence in module trays of tomato and cucumber seeds. *Seed Science and Technology* **23** (3): 665-672.

Bingham, I.J., Harris, A. and **Macdonald, L.** (1994) A comparative study of radicle and coleoptile extension in maize seedlings from aged and unaged seed. *Seed Science and Technology* **2** (1): 127-139.

Conn, J.S. and **Deck, R.E.** (1995) Seed viability and dormancy of 17 weed species after 97 years of burial in Alaska. *Weed Science* **43** (4): 583-585.

Depaula, M., Perezotaola, M., Darder, M., Torres, M., Frutos, G. and **Martinezhonduvilla, C.J.** (1996) Function of the ascorbate-glutathione cycle in aged sunflower seeds. *Physiologia Plantarum* **96** (4): 543-550.

Ellis, R.H., Roberts, E.H., Whitehead, J. (1980) A new, more economic and accurate approach to monitoring the viability of accessions during storage in seed banks. *Plant Genetic Resources Newsletter* **41:** 3-18.

Ellis, R.H., Honh, T.D., Astley, D., Pinnegar, A.E. and **Kraak, H.L.** (1996) Survival of dry and ultra-dry seeds of carrot, groundnut, lettuce, oilseed rape, and onion during five years hermetic storage at two temperatures. *Seed Science and Technology* **24** (2): 347-358.

Flood, R.J. (1986) Investigation on old seed samples. *J. of the National Institute of Agricultural Botany* **17** (2): 257-266.

Guberac, V., Banaj, D. and **Horvat, D.** (1997) Kakvoca sjemena lucerne i stocnog graska nakon pet godina hermetickog skladistenja. (Quality of Alfa-alfa (Medicago sativa L.) and fodder pea (*Pisum arvense L.*) seeds after five years hermetic storage). (In Croatian) *Sjemenarstvo* **14** (97): 5-6, 309-315.

ISTA (1985) *International rules for seed testing*. Vol. **13**, No. 2.

Jansen, P.I. and **Ison, R.L.** (1994) Hydration-dehydration and subsequent storage effects on seed of the self-regenerating annuals *Trifolium balansae* and *T. resupinatum*. *Seed Science and Technology* **22** **(3):** 435-447.

Kastori, R. (1984) Fiziologija semena. (Seed physiology). *In: Matica srpska.* (In Serbian) Novi Sad, Yugoslavia.

Khoroshailov, N.G. Zhukova, N.V. (1978) Long-term storage of seeds of VIR (All Union Institute of Plant Industry, Leningrad) world collection. *Byulleten Vsesoyuznogo ordena Lenina i ordena Druzhby Narodov Nauchno issledovatel'skogo Instituta imeni N.I. Vavilova* **77:** 9-19.

Kolak, I. (1994) Sjemenarstvo ratarskih i krmnih kultura. (Seed science of arable and forage crops). *In: Nakladni zavod Globus.* (In Croatian) Zagreb, Croatia.

Martincic, J. and **Guberac, V.** (1998) Effect of cereal seed storage interval on germinability. *7th Proceedings of International Working Conference on Stored-product Protection (IWCSPP),* Beijing-CHINA, 14-19.X 1998, **2:** 1642-1646.

Mills, J.T. and **Woods, S.M.** (1994) Factors affecting storage life of farm-stored field peas (*Pisum sativum L.*) and white beans (*Phaseolus vulgaris L.*). *J. of Stored Product Research* **30** (3): 215-226.

Momonoki, T. and **Momonoki, Y.S.** (1987) Estimation of germinability of gramineous and leguminous seeds in long-term storage by means of peroxidase activity and TTC reduction. *Japan Agricultural Research Quarterly* **20** (4): 296-301.

Morenomartinez, E., Vazquezbadillo, M.E., Navarretemaya, R. and **Ramirezgonzales, J.** (1994) Seed viability of different varieties of bean (*Phaseolus vulgaris L.*) stored under low and high relative humidity. *Seed Science and Technology* **22** (2): 195-202.

Murariu, D., Cristea, M., Fartais, L., Drochioiu, G., Raibuh, A. and **Murariu, M.** (1994) Effect of temperature and humidity on seed viability in some plant species stored in the gene bank at Suceava. *Analele Institutului de Cercetari Pentru Cereale si Plante Tehnice, Fundulea* **61:** 57-64.

Nienhius, K.H. Baltjes, H.J. (1985) Seed storage and germination in testing varieties for distinctness, uniformity and stability. *Seed Science and Technology* **13** (1): 19-25.

Opoku, G. and **Gamble, E.E.** (1995) Storability of seeds of normal and naked types of oac kippen barley. *Plant Varieties and Seeds* **8** (3): 197-205.

Owen, P.L. and **Pill, W.G.** (1994) Germination of osmotically primed asparagus and tomato seeds after storage up to 3 months. *J. of the American Society for Horticultural Science* **119** (3): 636-641.

Pandey, D.K. (1996) A siutable liqued preservative for enhancing longevity of orthodox seeds. *Scientia Horticultura* **66** (1-2): 1-8.

Sanchez, V.M., Sundstrom, F.J., Mcclure, G.N. and **Lang, N.S.** (1993) Fruit maturity, storage and postharvest maturation treatments affect bell pepper (*Capsium annuum L.*) seed quality. *Scientia Horticulturae* **54** (3): 191-201.

Sanhewe, A.J. and **Ellis, R.H.** (1996) Seed development and maturation in *Phaseolus vulgaris*. 2. Post-harvest longevity in air-dry storage. *J. of Experimental Botany* **47** (300): 959-965.

Specht, C.E., Keller, E.R.J., Freytag, U., Hammer, K. and **Borner, A.** (1997) Survey of seed germinability after long-term storage in the Gatersleben genebank. *Plant Genetic Resources Newsletter* **111:** 64-68.

Sun, W.Q. and **Leopold, A.C.** (1995) The maillard reaction and oxidative stress during ageing of soybean seeds. *Physiologia Plantarum* **94** (1): 94-104.

Sung, J.M. and **Chiu, C.C.** (1995) Lipid peroxidation and peroxide-scavenging enzymes of naturally aged soybean seed. *Plant Science* **110** (1): 45-52.

Szabo, L. and **Viranyi, S.** (1971) Germination data on seeds of different crops after storage in unconditioned enviroment. *Agrobotanika* **12:** 15-20.

Tang, J. and **Sokhansanj, S.** (1993) Drying parameter effects on lentil seed viability. *Transactions of the ASAE* **36** (3): 855-861.

Tekrony, D.M., Nelson, C., Egli, D.B. and **White, G.M.** (1993) Predicting soybean seed germination during warehouse storage. *Seed Science and Technology* **21** (1): 127-137.

Vanpijlen, J.G., Kraak, H.L., Bino, R.J. and **Devos, C.H.R.** (1995) Effects of aging and osmopriming on germination characteristics and chromosome aberrations of tomato (*Lycopersicum esculentum* Mill) seeds. *Seed Science and Technology* **23** (3): 823-830.

Vazquezarista, M., Ramirezflores, A. and **Blancolabra, A.** (1995) Maize and bean storage and their use by rural farmers in a central state of Mexico. *Journal of Stored Product Research* **31** (4): 325-333.

Zanakis, G.N., Ellis, R.H. and **Summerfield, R.J.** (1993) Response of seed longevity to moisture content in three genotypes of soybean (*Glycine max*). *Experimental Agriculture* **29** (4): 449-459.

(Received 27/09/1999, in revised form 27/01/2001)