Improvement of Crop Production by Means of a Storage Effect

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Abstract**—** *This study summarizes the results of 30 years of our experiments with Vicia faba L seeds. Our long-term practical observations of different Vicia faba L. cultivars points out the method useful for the higher yield of seeds in terms of their viability and thus higher crop production.*

Our experiments led to the following important findings regarding of seed viability:

1. *Individual and group variability of seeds;*
2. *Storage condition before germination; and*
3. *The condition of their germination.*

All these three influential conditions is possible to optimalize by method of storage effect described in this our report resulting in the improvement of crop production. This is especially important in case of seeds that are rare and/or expensive, i.e. seeds that are genetically modified or with rearranged karyotypes.

Keywords— seed color, higher germination, improvement of viability, higher crop production.

I. INTRODUCTION

Seeds have been studied for more than hundred years (cf. Murín 2001; Murín and Mičieta 2009). The first reports uncovered the relationship between the decline of their vitality and their storage conditions (Navaschin 1933; Cartledge and Blakeslee 1934, 1935; Stube 1935; Nichols 1942; D'Amato 1951; Murín 1961; Avanzi et al. 1969). Since respiration is the most marked manifestation of metabolism in stored seeds, it should also be considered. Rieger and Michaelis (1959) found that *V. faba* seeds are susceptible to the action of ethanol or other "automutagens" which can accumulate during the respiration of seeds stored over long period. Bewley and Black (1982, 1994) explored the relationship between the color of the testa and the dormancy and germination of wheat, as affected by the level of inhibitors (catechinins and their derivates) occurring in the testa. Floris and Anguillesi (1974) made a major contribution to the understanding of this external manifestation of the internal state of broad bean seeds when they reported on several biochemical and functional changes in aging seeds. Over the course of long storage, enzymes like catalase, peroxidase, cytochrome oxidase and decarboxylase display diminished activity, while the protein-synthesizing capacity of older seeds is lost in the process of germination. Furthermore, membrane permeability increases, resulting in reduced sugars and other metabolic products.

According Roos (1980) four factors must be considered in seed storage – time, temperature, relative humidity (seed moisture content) and a level of oxygen. With the exception of recalcitrant species, two factors – time and oxygen level, have very little effect on storability if the optimum seed moisture content and storage temperatures are observed. For example, Roberts and Ellis (1977) predicted the 95% survival of pea (*Pisum sativum* L.) seeds after 1,090 years of storage at -20°C and 5% seed moisture content. If the storage temperature is reduced further, the viability may be extended indefinitely. Attempts to prolong life of seeds during the storage were focused at the use of liquid nitrogen (LN2) as a storage medium with a temperature -196°C. At this temperature, presumably all biochemical activity is reduced to essentially zero. Thus the deteriorative changes noted above should be eliminated. According Babasaheb (2004), safe seed storage moisture should be less than 8%.

In 1981, King et al. reported that the survival of lemon (*Citrus limon* L.), lime (*C. aurantifolia* Swing.) and sour orange seeds (*C. aurantium* L.) was examined under a wide range of constant moisture contents and temperatures. Seed longevity was increased by decreasing the moisture content and temperature of the storage environment. Maximum viability was maintained in a combination of storage conditions including the lowest moisture content (5%) and lowest temperature (-20°C). The practicality of the dry storage of citrus seeds for genetic conservation was pointed out.

Bonner (1990) offered classification of stored seeds into four classes of storage characteristics: „'true orthodox' seeds can be stored for long periods at seed moisture contents of 5–10% and sub-freezing temperatures; 'sub-orthodox' seeds can be stored under the same conditions, but for shorter periods due to high lipid content or thin seed coats; 'temperate recalcitrant' seeds cannot be dried at all, but can be stored for 3–5 years at near-freezing temperatures; and 'tropical recalcitrant' seeds also cannot be dried, and they are killed by temperatures below 10–15°C."

Grilli et al. (1995) described the level of Poly (A) Polymerase as a significant marker of the viability of seeds during their long term storage. Also, during imbibition the production of the major organic volatiles, ethanol and acetaldehyde, depends greatly on the long term storage of the seeds (Górecki et al. 1992). Murthy et al. (2002) identified two primary biochemical reactions responsible for deterioration of seed vigour during long term storage – lipid peroxidation and non-enzymatic protein glycosylation reducing sugars. The PCR analysis of Chwedorzewska et al. (2002) led the authors to the conclusion that long term storage of seeds causing the loss of their viability also generates heritable changes in the preserved germplasm. On the other hand, antioxidant activity in stored seeds under different conditions (temperature and w.c.) is not related to seed viability (Merritt et al. 2003). However, Andreev et al. (2004) found that the loss of germination during the storage of rye seeds was accompanied by a decreased excision of chromatin loop domains. As Patrick and Stoddard (2010) stated, "the large seed size of the faba bean has enabled this species to be a model for studies of the molecular physiology of seed development."

The darkening of the testa of aging *V. faba* L. seeds and its manifestation has been a practical part of our work since 1988 (Murín 1988 a, b). Today we know that the color of the testa indicates the viability as well as the age of the seeds. Our goal was to study the relationship between the different storage conditions, the color of the testa of seeds and the viability of the seed samples.

II. MATERIALS AND METHODS

2.1 Seed samples

For our experiments we used sets of *V. faba* L. cv. Inovec seeds of the standard karyotype harvested in 1974 and also for each year from 1976 to 1982. Non-standard, rearranged ACB karyotype (Michaelis and Rieger 1971) were harvested in 1975 and 1982. The oldest cultivar Přerovský was from 1971.

The colors of the seeds were classified from A to U according to Fisher-Saller's scale in order to determine their individual variability. Originally designed for hair color, this scale was used for the first time by us because it registers a wide spectrum of brown hues. In the first experiment, a total of 1,419 broad bean seeds were examined in this way. The effect of storage time and conditions on seed coat color was also reported by Yousif et al. (2003) in their study of Australian adzuki beans.

2.2 Soaking and germination

The conditions for standard soaking and germination were altered during our experiments according to the knowledge we obtained in each experimental stage. Our optimal set involved the soaking of seeds in plastic jars that allowed their continual air-bubbling of distilled water. The seeds were then germinated in wet sawdust. The last six experiments were germinated and grown in intact material Perlite.

As *V. faba* is sensitive to hypoxia, we made the following arrangements to prevent higher sensitivity affecting the results of our experiments: a) better air circulation in the desiccators, which were not kept at 25 °C, but at laboratory room temperature; b) during soaking, we used 5% chloramine B to prevent microbe contamination of the seedlings which were treated for 30 min and then washed with distilled water; and c) the seeds were germinated in wet sawdust instead of wet cotton wool which does not permit the satisfactory respiration of seeds.

2.3 Storage

2.3.1 Storage conditions

All of the harvested *V. faba* seeds from our supply were stored at room temperature. Rearranged ACB karyotype was stored at 4 °C.

Twenty-two seed samples from eight countries, nineteen cultivars and nine harvests (from 1972 to1984) from the Seed Bank in Gatersleben, Germany, were divided into two groups (A and B). Groups A and B were stored in the seed bank at temperatures of -10 to -17 °C, and +14 to +20 °C respectively.

The seeds were then stored for 0 or 8 days at 25 ^{o}C above 600-mL sterile water at room temperature in the desiccators. Following treatment, washing and re-drying, half of the seed samples were allowed to germinate immediately and the root-tips were cut and put into fixation solution after two recovery times (48 h and 72 h). The other half of the seed samples were allowed to germinate after 8 days of storage.

2.3.2 Specific water content (w.c.)

After treatment and washing, the seeds were re-dried at 50% by heating for 2 h at 37 °C in a thermostat with a fan to obtain a specific w.c. Half of the seed samples were allowed to germinate immediately, and the root-tips were fixed after various recovery-times. The other half of the seed samples were stored for 8 days and then allowed to germinate.

2.3.3 Control of water content

For control of w.c. in seeds during the experiment, an extra sample of ten seeds was weighed before and after special drying (8 h at 105 °C) and calculated according to the formula 100 – (Yx100/X) = w.c., where X = weight before drying and Y = weight after drying.

2.4 Mutagen treatment

In one experiment of this series, *Vicia faba* seeds were first treated for 5 h with a dose of 2mm of methyl methanesulphonate (MMS, Merck) in distilled water at pH 4.8. After the mutagen treatment, the seeds were washed for 2 h in tap water to eliminate the mutagen residue.

2.5 Tests of vitality

The vitality and length of the roots (from sets of 35 seeds) were measured for precise time periods: 36h, 48 h, 72 h, 80 h and 96 h, 120 h, 144 h and 168 h if necessary.

Both groups of seeds from the Seed Bank in Gatersleben were tested periodically from 1991 until 1999 to record the viability and frequency of aberrant ana-telophases.

2.6 Cytological evaluations

For cytological evaluation we chose ana-telophases in accordance with other authors (Bezrukov and Lazarenko 2002). These mitotic figures are simpler to evaluate and thus allowed us to experiment with a large number of samples under different mutagen doses and recovery times. The mutagen-treated roots of seed samples were fixed in ethanol (1N): acetic acid (1N) in rate 3:1, squashed and stained by aceto-orcein. On average, 200 ana-telophases (50 in control) per recovery time were evaluated on the occurrence of fragments (F), bridges (B) or both (F+B).

2.7 Statistical methods

We used a standard Student's t-test to evaluate the SEM. All evaluations were conducted under blind conditions.

III. RESULTS AND DISCUSSION

3.1 Individual and Group Variability

V. faba L. seeds have been used for decades as an experimental model in cytological laboratories, and the biological characteristics of this genus are widely known. However, insufficient attention was paid to the darkening of seeds during their storage. A closer examination of this phenomenon bore striking implications for our research. Although the seeds seem to be the same, they express their individual and group variability by the colors of their testa.

In our first experiment, we studied the seeds' light and dark colors in relation to the years when they were harvested. Only seeds showing a definite color were evaluated; those of intermediate colors were excluded from our final evaluation. Table 1 and Fig. 1 show the gradual darkening of the seeds over an 11-year period.

TABLE 1
PROPORTION OF DARK AND LIGHT SEEDS IN THE COURSE OF AGING

Harvest (year and cultivar)	Proportion and color type of seeds		No. of seeds examined	No. and color type of seeds excluded
	light	dark		
1982, ACB	100%; ACB	0.00%	32	0
1981, Inovec	68.78%; A-B	12.14%; G-H	173	33; E, F
1980, Inovec	25.80%; B-C	20.00%; Q, R, S	155	84; F, G, H
1979, Inovec	17.05%; B-C	65.88%; Q, R, S	129	22; G, H
1978, Inovec	11.46%; C-E	71.97%; Q, R, S	157	26; L, M
1977, Inovec 1976, Inovec 1975, ACB 1971, Přerovský	5.48%; A, B, C 5.71%; C-E 7.05%; A-C 0.00%	70.54%; R, S, T 86.28%; S, T 85.47%; R, S, T 100%; U	146 175 241 211	35; E, F G 14; F, G 17; K-L 0

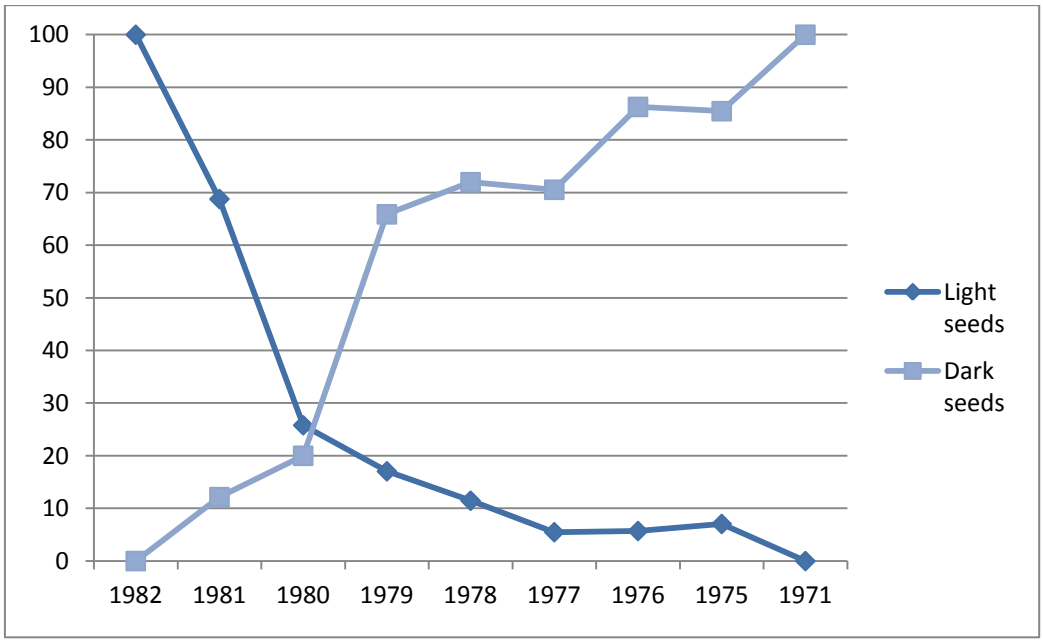

FIGURE 1: PROPORTION (%; ORDINATE) OF LIGHT AND DARK SEEDS IN DIFFERENT HARVESTS (ABSCISSA)

In a set of seven-year-old seeds (harvested in 1975) of the rearranged ACB karyotype that were stored in 4 ^{o}C instead of room temperature we observed a small deviation from the gradual darkening tendency. The higher the proportion of viable seeds and thus the better germination rate of this seed set confirmed the relationship between seed viability and storage conditions that was also stated by Michailov and Korytova (1971). We noted with interest the complete loss of viability of 9-year-old cv. Přerovský seeds and their uniformly dark color (among the darkest on the color scale – U).

In addition to confirming the irreversible tendency of seeds to darken in the course of their storage, these experiments suggested a possible difference in the viability and germinating capacity of seeds harvested in the same year, but differentiated by external darkening. Three differently arranged independent experiments (Tables 2 – 4) revealed a clear difference between dark and light seeds. It shows the importance of individual or inter-individual variability among seeds of the same age, externally manifested by darkening.

TABLE 2
COMPARISON OF GERMINATING CAPACITY OF LIGHT AND DARK SEEDS FROM VARIOUS HARVESTS.

Years of storage and color of seeds	No. of germinated seeds out of 16 after 96 hrs	168 hrs	Germination rate %
6 years, light	10	4	87.5
dark	0	2	12.5
7 years, light	7	6	81.2
(ABC) dark	0	6	37.5

TABLE 3
COMPARISON OF GERMINATING CAPACITY OF LIGHT AND DARK SEEDS FROM VARIOUS HARVESTS.

Years of storage and color of seeds	No. of germinated seeds out of 16 after 98 hrs	Germination rate %
4 years, light	14	87.5
dark	3	18.7
7 years, light	11	68.7
dark	3	18.7

TABLE 4
COMPARISON OF GERMINATING CAPACITY OF LIGHT AND DARK SEEDS FROM VARIOUS HARVESTS

Years of storage and color of seeds	No. of germinated seeds after 72 h	96 h	120 h	144 h	168 h	Germination rate %
3 years, light	9	5	0	1	0	93.75
dark	2	3	4	1	2	75.00
6 years, light	5	6	2	2	0	93.75
dark	0	0	0	0	2	12.50

The next two experiments aimed at chromosome aberrations of light (more viable) and dark (less viable) colored seeds. In the first of these experiments, we devoted most of our attention to the 1st-through-2nd mitosis (roots 12-20 mm long) and 3rd mitosis (roots 20-30 mm long). A total of 5 750 anaphases were examined. Table 5 illustrates the differences in the degree of chromosome aberration within a harvest (e.g., after 4 years of storage, between light $0.75 \pm 0.14\%$ and dark $2.30 \pm 1.18\%$ seeds), the gradual differentiation among harvests having been preserved (dark seeds 1979: $1.73 \pm 0.7\%$, dark seeds 1976: $6.00 \pm 0.7\%$).

TABLE 5
CHROMOSOME ABERRATIONS IN LIGHT AND DARK SEEDS IN THE FIRST MITOSES

Years of storage and color of seeds	Root length mm	Aberration rate % (No. of anaphases examined)				Total aberration rate %
		72 h	94 h	120 h	144 h	
4 years, light	12-20	2.0 (800)	1.5 (400)	0.0 (400)	1.0 (300)	1.13 ± 0.4
	20-30	0.8 (400)	1.0 (300)	0.5 (200)	n.d.	0.75 ± 0.1
dark	12-20	1.3 (300)	3.6 (300)	1.5 (300)	0.5 (200)	1.73 ± 0.7
	20-30	2.3 (300)	n.d.	n.d.	n.d.	2.30 ± 1.2
7 years, light	12-20	3.3 (700)	2.0 (200)	1.0 (200)	1.0 (150)	1.82 ± 0.5
	20-30	1.0 (250)	1.6 (300)	1.0 (150)	n.d.	1.20 ± 0.2
dark	12-20	n.d.	7.0 (200)	5.0 (200)	n.d.	6.00 ± 0.7
	20-30	n.d.	n.d.	n.d.	5.0 (150)	5.00 ± 0.0

n. d. = not detected

Examining seeds stored for 4 and 7 years, we found that the differences within a seed set were more marked in older seeds, while dark seeds were similarly differentiated in the early mitotic cycles. In an additional experiment, we confirmed this tendency in later mitoses as well. After 98 h of germination, 30 root-tips containing 3,000 anaphases were examined. The root-length varied from 28 to 84 mm depending on the individual variability, while in most roots it was about 50 mm. In light seeds of both harvests (Table 6), anaphases were evaluated separately at a root-length of 28-34 mm (3rd mitotic cycle) and of 44-57 mm (4th mitotic cycle).

TABLE 6
CHROMOSOME ABERRATIONS IN LIGHT AND DARK SEEDS IN LATER MITOSES

Years of storage and color type of seeds	Aberration rate %		
	In roots long		
	28-34 mm	44-57 mm	Total
4 years, light	0.0	1.2 ± 0.37	0.66 ± 0.3
dark	2.60 ± 0.5	n.d.	2.60 ± 0.5
7 years, light	1.5 ± 0.82	0.5 ± 0.82	1.00 ± 0.3
dark	2.00 ± 1.2	n.d.	2.00 ± 1.2

Three hundred anaphases were examined in each test.

Table 7 shows some difference in the aberration rate between light and dark seeds of the same harvest, while the aberration rate among seeds of the same color harvested in different years was almost the same. This shows a clear manifestation of viability dependent at individual variability of seeds.

TABLE 7
COMPARISON OF CHROMOSOME ABERRATION RATE WITH GERMINATION RATE OF SEEDS STORED FOR 4 AND 7 YEARS.

Years of storage and color type of seeds	Aberration rate %	Germination rate %
4 years, light	0.66 ± 0.3	87.5
dark	2.60 ± 0.5	18.7
7 years, light	1.00 ± 0.3	68.7
dark	2.00 ± 1.2	18.7

Three hundred anaphases were examined in each test.

This finding is in accordance with already reported changes in the aberration rate of cells in seeds during their long-term storage (Avanzi et al. 1969; Sevov et al. 1973; Cebrat 1977; Murín 1988a). The comparison of data presented in Tables 1 – 4 confirms this conclusion in relation to seed viability and chromosome damage in root-tip cells (Tables 5 – 7). In the present case, the external signs of reduced viability (darkening) were manifested, irrespective of their age, with almost corresponding consequences for genetic and physiological damage. The differences observed between whole seed harvests (indiscernible among individual seeds) could thus be explained by a decreasing proportion of viable seeds and an increasing number of less viable (dark) seeds in the same set as a result of long-term storage. In *V. faba* seeds, this process could be followed from the 100% proportion of light seeds in the youngest set of one-year-old seeds (with the highest germination rate and lowest aberration rate) to the complete mortality of a seed set after 9 years of storage when the proportion of dark seeds had reached 100%.

To confirm these results, once again new experiments were designed (Tables 8 – 9). Two experiments involved 1-year old seeds selected by their color (Table 8), and two experiments involved 5-year old seeds with and without the mutagen treatment (Table 9). This time they were not germinating and growing in sawdust, but in intact material Perlite.

TABLE 8

ONE-YEAR OLD SEEDS SELECTED BY THEIR COLOR TESTED IN VITALITY. GERMINATION AND CHROMOSOMAL ABERRATIONS IN THEIR ROOT TIPS.

		72 h			96 h			120 h	
	Length of roots (mm)	**Germi-nation (%)**	**Chrom. ab. (%)**	**Length of roots (mm)**	**Germi-nation (%)**	**Chrom. ab. (%)**	**Length of roots (mm)**	**Germi-nation (%)**	**Chrom. ab. (%)**
Light seeds	32.97	100.0	1	37.77	100	2	39.92	100	0.5
Dark seeds	22.15	87.5	2	26.12	90	1	27.62	90	1.5

TABLE 9

FIVE-YEAR OLD SEEDS WITHOUT AND AFTER TREATMENT FOR 5 H WITH DOSE OF 2mM OF METHYL METHANESULPHONATE (MMS. MERCK)SELECTED BY THEIR COLOR TESTED IN VITALITY. GERMINATION AND CHROMOSOMAL ABERRATIONS IN THEIR ROOT TIPS.

		72 h			96 h			120 h	
	Length of roots (mm)	**Germi-nation (%)**	**Chrom. ab. (%)**	**Length of roots (mm)**	**Germi-nation (%)**	**Chrom. ab. (%)**	**Length of roots (mm)**	**Germi-nation (%)**	**Chrom. ab. (%)**
Light seeds	32.67	100	2.5	37.03	100	1.00	38.0	100	0.9
Dark seeds	21.40	87	4.5	23.43	87	5.57	24.8	87	1.0
		72 h			96 h			120 h	
MMS. Merck	Length of roots (mm)	Germi-nation (%)	Chrom. ab. (%)	Length of roots (mm)	Germi-nation (%)	Chrom. ab. (%)	Length of roots (mm)	Germi-nation (%)	Chrom. ab. (%)
Light seeds	18.47	93.5	12.18	20.87	93.5	25.18	21.47	93.5	14.31
Dark seeds	13.70	66.5	8.50	15.60	70.0	17.79	15.87	70.0	25.04

The summarized results are found in Table 10 and confirm all of the previous findings and shows higher sensitivity of dark colored individuals in vitality, germination and chromosomal aberrations in their root tips. However, we also found some disturbances in this tendency in comparison with light colored individuals in chromosomal aberrations in recovery times of 72 h and 96 h.

TABLE 10

COMPARISON OF THE PREVIOUS RESULTS FROM TABLES 8-9 SHOWING AN AVERAGE IN ALL TESTED PARAMETERS (LENGTH OF ROOTS. GERMINATION AND C.A.) WITH SEM BETWEEN 1-YEAR OLD SEEDS (LINES 1-2). 5-YEAR OLD SEEDS (LINES 3-4) AND 5-YEAR OLD SEEDS TREATED BY MUTAGEN (LINES 5-6)

	72 h			96 h			120 h		
	Length of roots (mm)	**Germi-nation (%)**	**Chrom. ab. (%)**	**Length of roots (mm)**	**Germi-nation (%)**	**Chrom. ab. (%)**	**Length of roots (mm)**	**Germi-nation (%)**	**Chrom. ab. (%)**
Light seeds	32.98±0.38	100.0±0.00	1.00±0.00	37.78±0.63	100.0±0.00	2.00±1.00	39.93±0.08	100.0±0.00	0.50±0.50
Dark seeds	22.15±1.80	87.5±2.50	2.00±0.00	26.13±3.23	90.0±0.00	1.00±0.00	27.63±4.48	90.0±0.00	1.50±0.50
Light seeds	32.67±0.20	100.0±0.00	2.50±0.50	37.03±2.70	100.0±0.00	1.00±0.00	38.00±3.47	100.0±0.00	0.90±0.90
Dark seeds	21.40±6.13	87.0±0.00	4.50±0.50	23.44±5.17	87.0±0.00	5.57±1.57	24.80±5.27	87.0±0.00	1.00±0.00
Light seeds	18.47±3.00	93.5±6.50	12.8±5.18	20.87±4.60	93.5±6.50	25.8±11.18	21.47±4.80	93.5±6.50	14.31±1.37
Dark seeds	13.70±5.10	66.5±3.50	8.50±1.50	15.60±5.13	70.0±10.0	17.79±10.79	15.87±5.27	70.0±10.00	25.04±10.90

3.2 Condition of seed storage before germination

Table 11 shows the spectrum of the tested samples of *V. faba* L. seeds from the Seed Bank in Gatersleben. It is interesting to note that although the temperature used for group A was expected to be in favor of the longer survival of *the V. faba* L. seeds in the case of *A. manglesii* and *M. tetragona*, Merritt et al. (2003) we observed the opposite effect of storage at a temperature of -18 ^{o}C in comparison with higher storage temperatures, contrary to the findings of other authors mentioned before. According Murthy et al. (2002), another extreme temperature for storage is above 40 ^{o}C. The darkening of testa was also confirmed for cv. Fiesta (Nasar-Abbaset et al., 2009).

TABLE 11
SPECTRUM OF THE TESTED SAMPLES OF *V. FABA* L. SEEDS AND THEIR CONDITIONS (– SHOWS ZERO VIABILITY).

Year	Cultivar	Country of origin	A	B
1972	Féverole du Gers	France	+	-
1975	Maly	Italy	+	-
1977	Parvin	Great Britain	+	+
1978	Milión	Czechoslovakia	+	+
	Diana	Czechoslovakia	+	-
	Kronberger	Germany	+	+
	tennis Murat	Ethiopia	+	+
1979	Féverole du Gers	France	+	-
	tennis Murat	Ethiopia	+	+
	Latvija	USSR	+	+
	Mazur No.18	Poland	+	+
	DornburgerAckerb.	Germany	+	+
		Great Britain	+	-
1980	Romana	Italy	+	+
	Maly	Italy	+	-
	Přerovský	Czechoslovakia	+	+
1981	Dire Dawa	Ethiopia	+	+
	Skorospelka	USSR	+	+
	Banská	USSR	+	+
1982	Equina	Italy	+	+
	tennis Murat	Ethiopia	-	+
1984	Maly	Italy	+	+

Both groups were tested periodically over nine years (1991 – 1999). The first evaluated parameter to be checked was seed vitality (i.e., their germinating capacity) according to their color; the difference is especially evident for summarized data obtained for the 1978-1984 harvests (Table 12).

TABLE 12
GERMINATION OF LIGHT AND DARK SEEDS FROM 22 DIFFERENT SAMPLESOF 17 CULTIVARS FROM 9 COUNTRIES IN CONSEQUENT YEARS.

Year of harvest	Light seeds					Dark seeds				
	1991	1992	1996	1997	1999	1991	1992	1996	1997	1999
1972	98.0	95.0	27.3	50.0	55.0	n.d.	n.d.	n.d.	n.d.	n.d.
1975	82.0	90.0	41.7	70.0	30.0	n.d.	n.d.	n.d.	n.d.	n.d.
1977	94.0	70.0	70.0	73.3	45.0	0.0	0.0	0.0	0.0	5.00
1978	100.0	96.3	95.8	97.5	92.8	100.0	27.5	23.3	10.0	18.3
1979	98.5	92.5	81.6	94.6	63.4	97.6	26.0	36.0	16.7	12.5
1980	92.0	83.3	74.5	51.1	32.4	92.0	43.3	30.0	4.4	7.5
1981	90.6	93.3	86.6	71.9	52.5	90.6	40.0	30.0	35.5	20.0
1982	95.0	75.0	73.3	80.0	40.0	95.0	50.0	13.3	20.0	10.0
1984	86.0	90.0	50.0	55.6	15.0	89.0	47.5	10.0	40.0	5.0
Total 1978-84	**93.7** ±2.13	**88.4** ±3.23	**76.9** ±6.39	**75.1** ±7.89	**49.3** ±11.03	**94.0** ±1.74	**39.0** ±4.14	**23.7** ±4.19	**21.1** ±5.74	**12.2** ±2.43

When confirming the weaker vitality of darker seeds stored in less favorable conditions, we were interested in the aberration rate as expressed by the summarized data in Table 13.

TABLE 13
SUMMARY OF ABERRATIONS PER YEAR.

Year of harvest		Light seeds			Dark seeds	
	1992	1996	1997	1992	1996	1997
∑ 1972	0.33 ± 0.0	4.81 ± 3.2	1.17 ± 1.2	n.d.	n.d.	n.d.
∑ 1975	0.66 ± 0.0	1.65 ± 4.9	1.51 ± 1.0	n.d.	n.d.	n.d.
∑ 1977	0.33 ± 0.0	1.00 ± 0.0	n.d.	0.00 ± 0.0	0.00 ± 0.0	n.d.
∑ 1978	0.49 ± 0.6	1.00 ± 3.3	0.08 ± 0.8	1.22 ± 0.2	1.10 ± 1.3	0.00 ± 0.0
∑ 1979	0.39 ± 0.4	0.00 ± 0.0	0.47 ± 1.2	3.26 ± 1.8	4.65 ± 2.4	0.19 ± 1.3
∑ 1980	0.44 ± 0.6	0.25 ± 0.9	1.04 ± 1.2	2.44 ± 1.2	0.25 ± 0.9	1.00 ± 1.0
∑ 1981	0.33 ± 0.6	0.57 ± 1.1	0.25 + 1.3	2.22 ± 0.8	0.00 ± 0.0	0.92 ± 1.9
∑ 1982	0.00 ± 0.0	0.00 ± 0.0	2.85 ± 0.0	4.66 ± 0.0	1.00 ± 0.0	0.00 ± 0.0
∑ 1984	1.00 ± 0.0	1.23 ± 4.9	1.58 ± 0.8	0.00 ± 0.0	3.08 ± 2.5	n.d.

n. d. = not detected

As noted, the aberration frequency was not very high in old seeds for significant results. Therefore, the most important tendencies in the evaluations from the years 1991, 1992, 1996, 1997 and 1999 best expressed themselves in the viability measured by the percentage of germination after 96 hours. The summarized results are shown in Table 14.

TABLE 14
AVERAGE GERMINATION IN % OF LIGHT AND DARK SEEDS FOR ALL EVALUATION TIMES. YEARS OF HARVEST AND CULTIVARS.

Light seeds			
	day 1	day 2	day 3
∑ 46.4 ± 24.6	32.4 ± 20.6	49.2 ± 21.7	56.8 ± 24.8
Dark seeds			
	day 1	day 2	day 3
∑ 11.7 ± 7.0	7.00 ± 2.5	11.7 ± 6.5	13.1 ± 7.70

The difference is significant, although it is affected by a wide range of germination of light seeds, which confirms our earlier results from a lesser number of Czechoslovak cultivars except in the case of the parameter of the root growth (Table 15.).

TABLE 15
AVERAGE ROOT LENGTH OF LIGHT AND DARK SEEDS FOR ALL EVALUATION TIMES. YEARS OF HARVEST AND CULTIVARS IN CM.

Light seeds			
	day 1	day 2	day 3
Σ 2.3 ± 1.5 cm	0.7 ± 0.12	2.2 ± 0.59	4.0 ± 0.80
Dark seeds			
	day 1	day 2	day 3
Σ 1.9 ± 1.5cm	0.5 ± 0.08	1.4 ± 0.76	2.9 ± 1.65

This tendency was also confirmed in the case of the aberrant ana-telophases, where in the range of 0.0 – 3.66 % SEM was 0.0 – 2.42 %, thus rendering all results non-significant. There was no provable difference between the light and dark seeds or even between cultivars or years of harvest.

All of these results support our previous conclusions and findings (Murín et al 2007). In all of the evaluated parameters, we have observed that despite the great difference in the two basic storage conditions and the time between the harvest years and the evaluation frequency (17 years in the case of the oldest year), germination was the only provable parameter. This confirms the possibility of the practical use of this interesting manifestation of different storage conditions.

3.3 Experimental Storage Effect

The basic observations mentioned in the introduction regarding various plant species were later supplemented by attempts to demonstrate a relationship between the aging of seeds and the mode of their storage and sensitivity to the action of chemicals (e.g. Avanzi et al. 1969). Interesting results were obtained due to the effects of different moisture on stored seeds in the course of the presumed repair mechanisms in plant cells and the corresponding recovery effects after the action of alkylating agents (Gichner and Gaul 1971, Gichner and Velemínský 1973).

Following the method of the last mentioned authors in a series of experiments (Murín and Mičieta 2001), described in detail in our recent report (Murín and Mičieta 2014), we found the experimental "storage effect" to be a universal method for recovering and enhancing seed viability for higher crop production.

Seeds stored for a long time and impacted by significant chromosome damage by a chosen mutagen can recover by this method. It means that their experimental storage under the defined conditions for 8-days resulted in 3 to 4 times lower frequency of chromosomal aberration in root tips and a significantly higher viability (Tables 16 and 17, Figures 2 and 3). Even 12-year old seeds after experimental storage showed viability comparable with 2-year old seeds without storage. This prolongation of the G-1 phase causes a decrease in damage caused by long term storage and other stresses up to a condition similar to that of young undamaged seeds. According to our experience (Murín and Mičieta 1997), the prolongation of the storage period for more than 8 days has no greater effect, i.e. the "storage effect" is limited probably due to the limited source of repair enzymes stored in dormant seeds.

TABLE 16
GERMINATION AND CHARACTER OF ABERRATIONS (F-FRAGMENTS. B-BRIDGES. F+B) IN ROOT TIP CELLS OF 2. 6 AND 12-YEAR OLD *V. FABA* SEEDS AFTER 0 DAYS OF EXPERIMENTAL STORAGE.

Years	MMS in mM	Germination (in %)	No. of cells scored 48h/72h	No. of aberrant cells 48 h recovery time F	B	F+B	72h F	B	F+B
	0	77.0	150/120	2	1	0	1	3	0
2	3	90.0	155/140	17	6	3	8	1	1
	6	87.0	150/130	24	3	4	11	9	2
	0	57.0	210/140	40	7	12	15	6	3
6	3	50.0	250/90	40	11	16	11	3	3
	6	77.0	250/150	57	26	18	35	4	4
	0	19.4	40/70	7	2	2	11	1	0
12	3	17.4	25/15	1	6	2	4	2	2
	6	13.0	60/25	10	16	20	14	2	2

TABLE 17

GERMINATION AND CHARACTER OF ABERRATIONS (F-FRAGMENTS. B-BRIDGES. F+B) IN ROOT TIP CELLS OF 2. 6 AND 12-YEAR OLD *V. FABA* SEEDS AFTER 8 DAYS OF EXPERIMENTAL STORAGE.

Years	MMS in mM	Germination (in %)	No. of cells scored 48h/7h	No. of aberrant cells recovery time 48 h F	B	F+B	72h F	B	F+B
	0	89.6	270/150	7	1	0	4	1	0
2	3	94.0	260/150	3	0	0	3	1	0
	6	86.0	290/150	7	0	1	0	2	0
	0	87.5	300/160	2	1	0	0	0	0
6	3	88.6	300/150	8	1	0	2	0	0
	6	82.4	300/150	6	2	0	3	1	0
	0	60.0	230/150	18	5	2	4	1	0
12	3	46.0	205/150	8	6	0	11	1	1
	6	72.0	180/150	12	4	1	2	2	2

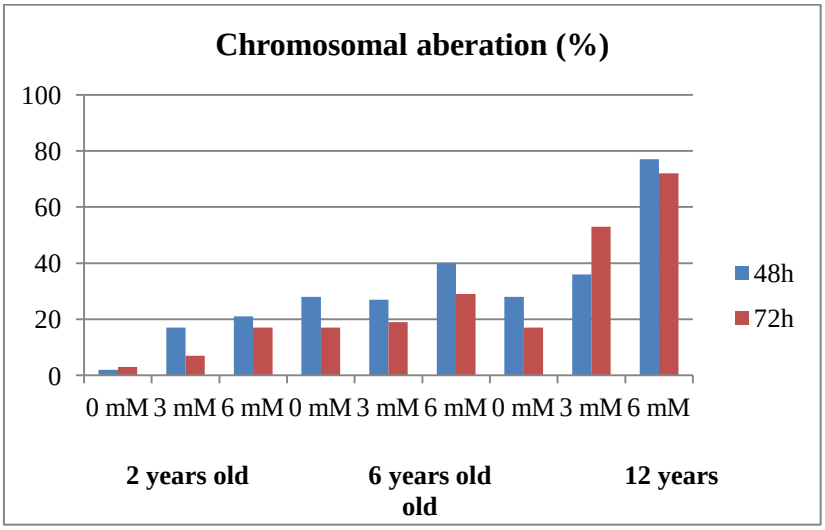

FIGURE 2. FREQUENCY OF CHROMOSOME ABERRATIONS ON DAY 0 OF EXPERIMENTAL STORAGE.

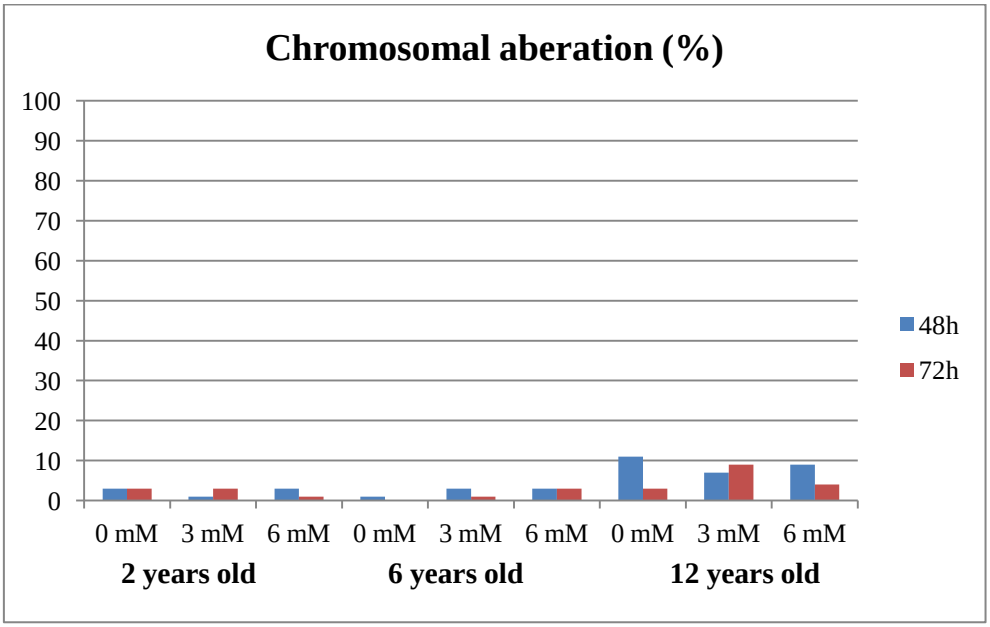

FIGURE 3. FREQUENCY OF CHROMOSOME ABERRATIONS ON DAY 8 OF EXPERIMENTAL STORAGE.

IV. CONCLUSION

In addition to the theoretical ramifications of our experiments, our work may also have practical implications in three fields:

1. As pointed out by Čupič et al. (2005), widely used agro-technical plant seeds, as in the case of Alfalfa (*Medicago sativa* L.), are often stored for years after harvest, which influences their germination energy, germination, rate of abnormal sprouts and dead seeds. This can be easily repaired by the "storage effect" with an interrupted germination period under the described conditions causing the prolongation of the G-1 phase with a significant increase of vitality of seeds treated this way. Consequently, it will lead to an improvement of their crop production that is most important in the case of seeds that are genetically modified or rearranged (see ACB karyotype seeds used in our experiments).
2. Our findings may be very helpful to seed banks worldwide. The regular checking of the viability of seeds according to germination leads to irreversible losses of stored seeds, while a simple visual test based at seed color would provide the same answer with no loss of material. Moreover, such a test could be conducted in sealed ampules, thus not interfering with the storage conditions in the particular seed bank. By using "storage effect" these seeds can be revitalised (or rejuvenalised) and stored further with the possibility of long-term survival of the seeds in seed bank.
3. Finally, with the above mentioned "storage effect" the amount of yield of viable seeds can be significantly recovered and by this method to prolong of their useful survival in the particular agricultural supply. Just at the example of *Vicia faba* beans it could cause a significant economical improvement as its seeds are distributed in more than 55 countries when 4.56 million tons of dry grains are produced in the harvested area of 2.56 million ha yearly.

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