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Unexpected Aftereffects of Low Dose γ-irradiation on Quality of Individual Dry Pea Seeds

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Abstract Time-dependent aftereffect induced by γ-radiation in dry pea seeds at non-lethal 0.19–10 Gy doses is considered. By using phosphorescence at room temperature (RTP), seed lot was fractionated to strong and weak seeds producing normal and abnormal seedlings, respectively, and to dead seeds. After 0.19 Gy γ-radiation, strong seeds transformed to weak seeds during two months, whereas seeds after 7–10 Gy became weak during 2–4 days that was accompanied by doubling glucose content and decreasing seed hydration. This indicated non-enzymatic hydrolysis of carbohydrates apparently caused by γ-induced activation of water molecules. At 7–10 Gy, an unexpected back transition of weak seeds to "improved" seeds occurred, producing normal seedlings. This "improvement" is due to glucose decrease to strong- seed level presumably via reaction between free glucose carbonyl and protein amino group. "Improvement" is transient; these seeds gradually die apparently due to end products of amino-carbonyl reaction (Amadori-Maillard products). Therefore, two effects of indirect non-target action of γ-radiation on dry seeds at low doses were suggested: non-enzymatic hydrolysis of carbohydrates and then amino-carbonyl reaction. They result at first in transition of strong to weak seeds and then of weak to "improved" seeds, response time being dependent on γ-radiation dose.

Keywords *Pisum sativum*, Seed Quality, Weak Seeds, "Improved" Seeds, Glucose Accumulation, Non-enzymatic Hydrolysis, Amino-carbonyl Reaction, Radiation Aftereffect

1. Introduction

The effects of γ-irradiation on dry seeds depend on the dose applied. Such dose dependence is species-specific. At high doses, seeds die, whereas the effects of low doses vary from their absence to stimulation of seed germination [1-3]. The discrimination of low and high doses depends on the criteria applied by the researchers.

Usually, seed radicle protrusion is measured to indicate any action applied to seeds. However, it is insufficient for the evaluation of seed quality because the fate of germinated seeds is not taken into the account. Not only seed germination but also successful seedling development from these seeds is the prerequisites of favorable plant growth. For this reason, International Seed Testing Association [4] has introduced the criterion of seed quality, i.e. number of seedlings lacking any morphological modifications, to characterize the quality of seed lot. The routine technique is to germinate a seed sample and then to estimate the number of normal seedlings for the evaluation of whole seed lot. Much more rapid and non-destructive method was worked out in our laboratory permitting to avoid the germination procedure and evaluate seed quality in each air-dry seed.

This method represents the measuring of Room Temperature Phosphorescence (RTP) in individual seeds. According to various RTP values, a seed lot can be subdivided to some fractions studied in terms of seed viability, electrolyte leakage, germination and seedling development [5, 6]. The fraction called "strong" seeds represents viable seeds characterized by very weak electrolyte leakage and production of normal well-developed seedlings. Dry seeds belonging to the fraction of "week" seeds are also viable, their electrolyte leakage is twofold higher; after germination they produce seedlings with morphological abnormalities. Some viable "weak" seeds do not germinate because they suffocated during imbibition due to hypoxia [7,8]. In many cases, the fraction of dead seeds was recorded too. It consisted of dead seeds characterized by the highest electrolyte leakage; they never will germinate at all. Strong seeds are typical of any seed lot of high quality, whereas the weak seeds appear in response to some factors applied.

The advantage of this approach is that RTP measurements of dry seeds make it possible to evaluate not only the quality of individual seed, but also to predict the fate of the seedling, which will develop from this particular seed. Such approach was used for the analysis of seed quality during aging [6] and is suitable for studying the changes in seed quality after γ-irradiation of dry seeds. Many-fold successive RTP measurements of the same seed allowed us to evaluate the state of individual irradiated seed and its subsequent changes.

As the irradiated seeds were air-dry, they do not contain free water. For this reason, the participation of enzymatic processes in air-dry seeds is improbable. Therefore, the processes resulting in seed quality changes must be non-enzymatic. To avoid the possibility of enzyme activation by added water, the biophysical methods were applied for dry seed investigation. Non-enzymatic processes can be lipid peroxidation, carbohydrate hydrolysis and amino-carbonyl reactions [9].To elucidate the possible participation of non-enzymatic processes in irradiated seed behavior, we compared the strong, weak and dead seeds selected by RTP values.

The aim of this work was to study the processes determining the changes in quality of air-dry seeds after low doses of γ-irradiation.

2. Materials and Methods

2.1. Plant Material

Seeds of pea (*Pisum sativum* L., cv. Nemchinovskii-85) were stored at room temperature for three years. Germination percentage of seed lot was 80%. Visually undamaged seeds of uniform size and weight of 225 ± 25 mg were selected for experiments.

2.2. Germination and Radicle Emergence Testing

For each measurement, four replicates of 12 seeds were taken. Seed germination occurred at 20-22°C in glasses in filter paper rolls moistened with tap water. Radicle protrusion was recorded two days after imbibition start at the radicle length exceeding 4 mm. Seeds were defined as germinated if they produced normal seedlings after 5-day-long imbibition. Seedlings with morphological defects and various growth modifications were classified as abnormal seedlings according to ISTA Rules [4]. Viable seeds without radicle protrusion were defined as suffocated if they exhibited lysis after 5-day imbibition. Seeds lacking protruded radicles and remaining hard during imbibition were defined as dead seeds.

2.3. γ- Irradiation

A pea seed lot with 80% germination was γ-irradiated in the Joint Institute for Nuclear Research (Dubna, Russia). Seeds were irradiated at the doses of 3, 7, 10, 25, 50 and 100 Gy. The device "ROKUS-M" produced γ-rays at a dose strength of 0.913 Gy/min at the distance of 75 cm from seeds. For irradiation at the dose of 0.19 Gy, a ^{60}Co γ-quant source was used at the dose strength of 5.7 mGy/h at the distance of 80 cm. Control seeds were not irradiated.

2.4. RTP Measurements of Individual Air-dry Seeds

RTP of individual air-dry seeds was measured with a previously described device [8]. The system consisted of double-disk phosphoroscope continuously producing intermittent illumination of a seed with 6 ms-long flashes and 25 ms dark interval (a KGM-100 incandescent lamp, Russia, illuminance of 60 Klux). The phosphorescence signal was measured between recurrent flashes within 3-18 ms with a FEU-79 photomultiplier tube (Russia) and expressed in arbitrary units. The RTP measuring of each seed took about 2-3 s. Then each seed was placed into a numbered cell in order to follow further RTP changes in it. Basing on RTP values measured in individual seeds, the RTP distribution curves were plotted.

For characterizing the subsequent changes in γ-irradiated individual seeds, RTP levels were measured prior to irradiation (control seeds) as well as in the same irradiated seeds after 2, 4, 6, 18-20, 54-62 days or after five months. In seeds γ-irradiated at high doses (25, 50, 100 Gy), RTP was evaluated prior to irradiation and six days after it.

For further investigations, individual seeds were collected one by one according to their RTP levels close to the fraction maxima. Fraction I consisted of strong seeds with RTP values of 403 arb. un. (first maximum of seed distribution by RTP). Weak seeds of fraction II had RTP values of 60 ±3 arb. un.; "improved" seeds (see below) had RTP values of 35 3 arb. un, whereas dead seeds exhibited high RTP level of 85-95 arb. un.

2.5. Seed Water Content

Seed water content was measured in seed powder by a routine method at 105°C [4]. Moisture level in seeds was expressed as per cent of water per fresh weight (% fr.wt.).

2.6. Thermochemiluminescence (TCL)

TCL was used for detecting the products of lipid peroxidation and glucose content in air-dry seeds**.** The correctness of TCL application to POL product and glucose measurements was checked in numerous model experiments [6].

TCL was measured with a chemiluminometer operating in a single photon counting mode, which was developed at the Department of Biophysics of Moscow State University. The photomultiplier (FEU-85, Russia) detected photons in the visible spectral region; its output signal was recorded with a computer and processed with a dedicated program.

The powder sample of air-dry seeds (40 mg) was placed into a porcelain chamber and heated from room temperature to 190-200°C at a rate of 10°C/min. The sample temperature was determined with a microthermister installed into the chamber. The background level of emission from the chamber itself, or containing quartz sand instead of seed powder, ranged from 1 to 3 arb. un. The TCL level was recorded continuously and simultaneously with monitoring the sample temperature.

POL products. The light emission by dry seed powders observed within a temperature range of 50-110^{o}C was determined by the reaction of lipid peroxidation products (POL) with free terminal amino groups of proteins, i.e. by amino-carbonyl reaction [6]. TCL intensity indicates POL presence. POL values combine individual POLs having various maxima within the 50-110^{o}C temperature range.

Glucose content. Upon further heating of pea seed powder, a TCL peak appeared at 150°C. Within 110-170^{o}C, the light emission is determined by glycosylation, i.e. by interaction of glucose with free terminal amino groups of amino acids or proteins [6]. The TCL level at 150^{o}C was directly proportional to glucose content.

2.7. Statistical Treatment

Data in tables and figures represent mean values and their standard deviations. Total number of individual analyzed seeds was 790. Experiments were made in 3–5 replicates. Each point on thermograms is a mean value calculated from 30 measurements. The data obtained were statistically processed using Statistica 12.0 software. The data in tables and Fig. 3 demonstrated normal distribution. Statistical significance for differences between non-irradiated and irradiated seeds was calculated using the T-test. The changes were considered significant at $p<0.05$.

As to the comparison of frequency distributions in Figures 1 and 2 we isolated the data describing the distributions of strong seeds, and the sequences of data for each radiation dose were compared with normal distribution by using Kolmogorov-Smirnov test. The obtained values of significance level ($p<0.05$) indicate the certainty of the results.

3. Results

3.1. Changes in Seed Quality after γ-irradiation of Dry Pea Seeds

RTP analysis of irradiated seeds was used for the evaluation of seed quality changes. The RTP distributions of non-irradiated (curve 0 Gy) seeds and seeds irradiated at the doses of 3, 10 and 50 Gy are shown in Fig. 1.

Control seeds exhibited an almost unimodal distribution by RTP level with a maximum at 35–40 arb.un. (strong seeds) and small shoulder at 55-65 arb.un. (curve 0 Gy). The small shoulder was due to the presence of weak (aging) seeds. At low RTP values (20-60 arb. un.), there is no significant difference between control and 10Gy irradiated seeds, whereas the difference between them and seeds irradiated at 3 and 50 Gy is significant.

Among the seeds with RTP values of 20–50 arb.un., most seeds (80%) produced normal seedlings (Table 1). Therefore, these seeds can be considered strong seeds. From seeds characterized by RTP values exceeding 55 arb. un., abnormal seedlings will develop that indicates weak quality of these seeds.

Seed irradiation at 3 Gy resulted in the increase of a second maximum at 55–65 arb. un., in addition to the first maximum at 40 arb. un. (curve 3 Gy). The RTP distribution at 3 Gy became bimodal and showed partial seed redistribution from strong to weak seeds. Such irradiation induced a decrease in seed fraction producing normal seedlings and an increase in the number of seeds producing the abnormal seedlings and of suffocated seeds (Table 1).

After seed irradiation at 10 Gy, the distribution again became unimodal similarly to the distribution of control seeds, being only slightly shifted to lower RTP values (Fig. 1, curve 10 Gy). It seemed that a portion of seeds returned from weak to strong seeds. Such transition should increase the fraction of normal seedlings at the expense of the decreased number of suffocated seeds and seeds producing abnormal seedlings. Really, Table 1 shows the seed “improvement” (“invigoration”), i.e. some weak seeds became strong and could produce the normal seedlings.

At a higher irradiation dose of 50 Gy, RTP distribution radically changed, i.e. fraction of strong seeds diminished, but the fraction of weak seeds rose and a new fraction (dead seeds) appeared at 85–100 arb. un. (Fig. 1, curve 50 Gy). A similar effect was demonstrated by the data in Table 1. As the irradiation dose increased from 25 to 100 Gy, the fraction of normal seedlings from strong seeds sharply decreased, whereas the fraction of abnormal seedlings from weak seeds and the fraction of dead seeds increased (Table 1). At 100 Gy dose, the fraction of dead seeds amounted only to 42%, whereas the fraction of suffocated seeds sharply rose to 49%. Therefore, many (58%) imbibed seeds remained alive, but these seeds rarely produced normal seedlings (only 4%).

The number of living seeds was evaluated as the difference between the total seed number and number of dead seeds. According to this criterion, we considered the doses of 10 Gy and below it as low doses characterized by the lowest constant fraction of dead seeds. High doses of 25 Gy and more resulted in the rapid seed death.

Among low doses, the stimulation was observed only at 7–10 Gy, an unexpected phenomenon manifesting the “improvement” of weak seeds to the quality level of strong seeds producing normal seedlings.

In the next sections, the attention will be paid to the effects of dry seed γ-irradiation at low doses.

3.2. Aftereffects of Dry Seed γ-irradiation at Low Doses

The seeds irradiated at the doses of 0.19, 3 and 10 Gy were analyzed by RTP on the second and forth days after irradiation and later after two and five months. Their RTP distributions were compared with the control non-irradiated seeds (Fig. 2, a–c, curves 1).

Table 1. Germination and quality of pea seeds after γ-irradiation at various doses (% of total seed number). Data represent mean values and their standard deviations.

Dose, Gy	Radicle protrusion after two-day long imbibition	Seedlings*		Seeds without radicle protrusion*	
		normal	abnormal	alive, suffocated	dead
0	90±2	80±3	10±2	8±1	2±1
0.19	83±3	58±5*	25±4*	15±5	2±1
3	75±2*	45±5*	30±5*	23±6*	2±1
7	90±2	76±5	14±3	8±3	2±1
10	91±3	83±6	10±5	5±1	2±1
25	82±6	52±7*	30±5*	13±3	5±2
50	76±9*	12±3*	64±5*	16±7*	8±2
100	9±2*	4±2*	5±2*	49±3*	42±3*

Note: Seeds were taken six days after γ-irradiation and imbibed for five days

*Relevant differences (T-test, p=0.05) compared to non-irradiated seeds (0 Gy)

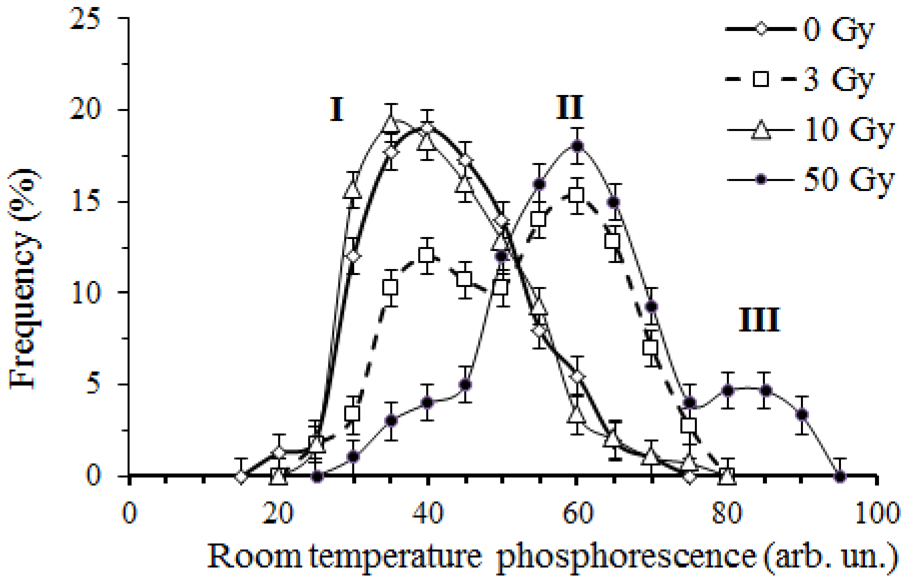


Figure 1. Room temperature phosphorescence distribution of air-dry seeds γ-irradiated at the doses of 0, 3, 10 and 50 Gy (six days after irradiation). I, II and III – strong, weak and dead seeds. Data represent mean values and their standard deviations.

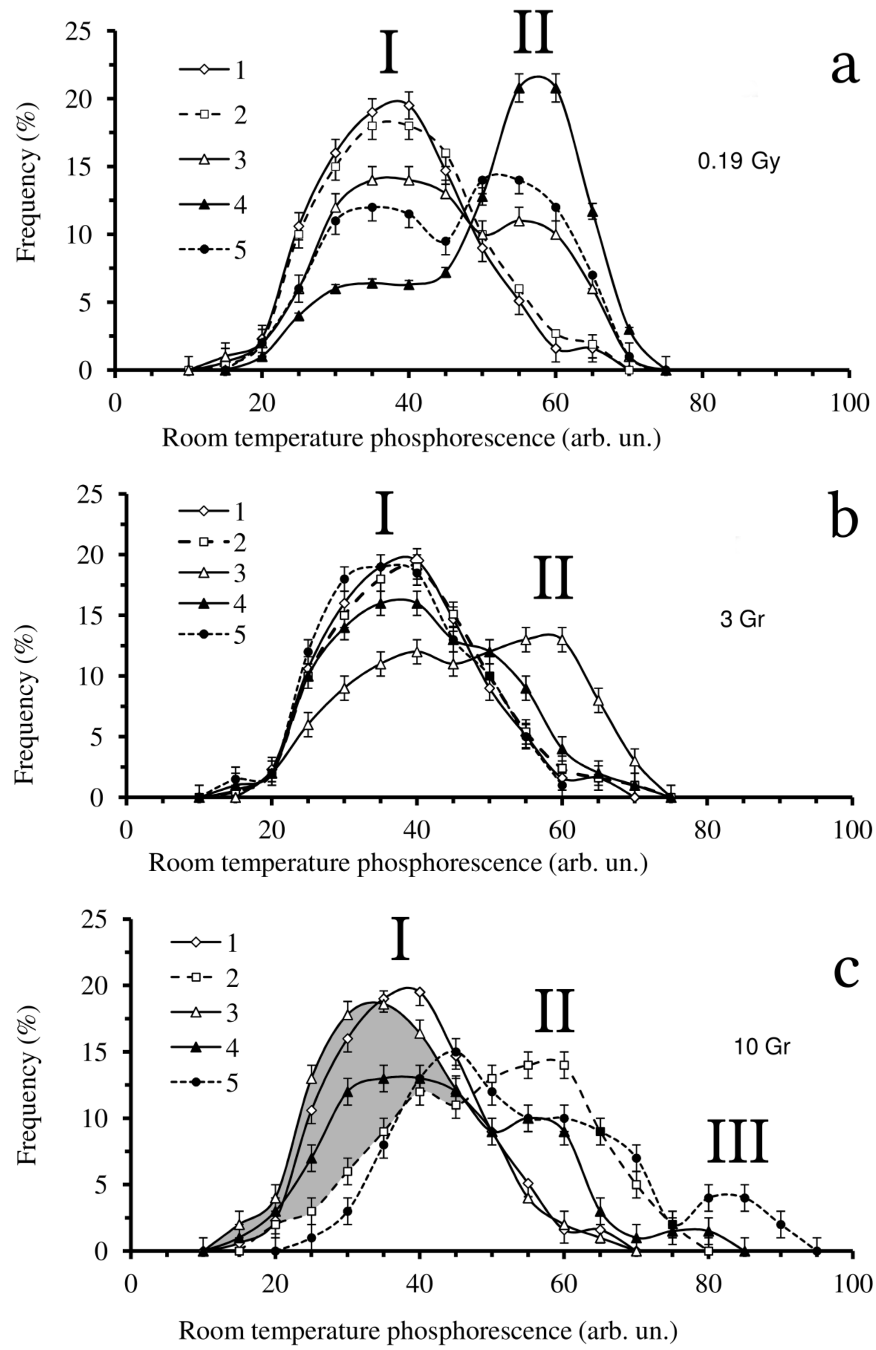


Figure 2. Distribution by RTP of air-dry pea seeds γ-irradiated at the doses of 0.19 Gy (**a**), 3 Gy (**b**) and 10 Gy (**c**). (1) – prior to irradiation; (2) – two days after irradiation; (3) – four days after irradiation; (4) – two months after irradiation; (5) five months after irradiation. **I, II** and **III** – strong, weak and dead seeds. Grey color indicates fraction of "improved" seeds formed from weak seeds. Data represent mean values and their standard deviations.

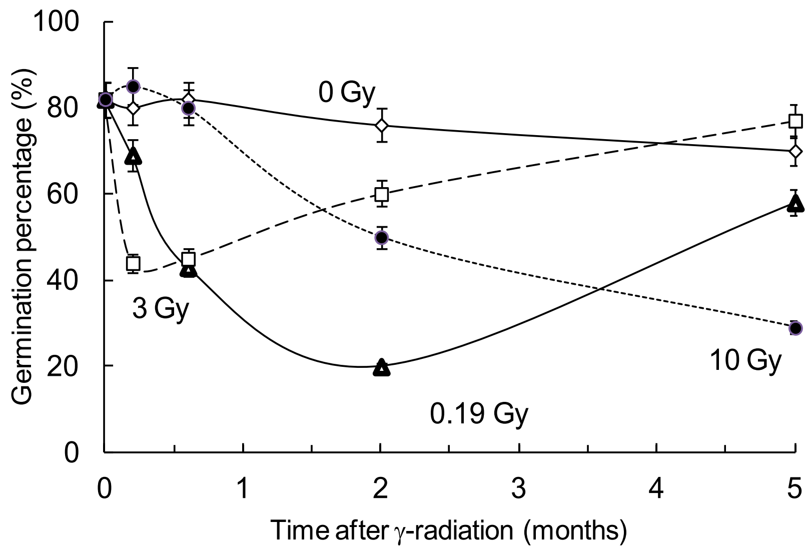


Figure 3. Influence of various γ-irradiation of dry seeds on the subsequent change in normal seedling number (%). Irradiation doses are shown near the curve. Data represent mean values and their standard deviations.

With time after irradiation, the RTP distribution gradually changed. Two days after 0.19 Gy-irradiation, the distribution almost did not differ (Fig. 2a, curve 2) from the distribution of seeds prior to irradiation. However, after next two days, the fraction of strong seeds declined and fraction of weak seeds significantly rose (Fig. 2a, curve 3). In the seeds stored for two months, a further decrease of strong seed percentage and increase of weak seed fraction occurred (curve 4).

Only five months after irradiation, a significant part of weak seeds returned to the fraction of strong seeds, but no new dead seeds appeared. Direct experiments with the germination of irradiated seeds confirmed the decline of normal seedling percentage from 80% to 20% (Fig. 3, curve 0.19 Gy) during two months. But after five months, the fraction of normal seedlings rose to 60%. Therefore, a long-term storage of 0.19 Gy γ-irradiated seeds resulted in the returning of a significant part of weak seeds to the fraction of strong seeds, i.e. the “improvement” of weak seeds.

Among control non-irradiated seeds stored for five months, the fraction producing normal seedlings decreased only by 10%, apparently due to the natural aging (Fig. 2, curve 0 Gy).

Seed irradiation at the dose of 3 Gy, like at the dose of 0.19 Gy, almost did not change the RTP distribution two days after treatment when compared with the distribution prior to treatment (Fig. 2b, curve 2). However, four days after irradiation, fraction of strong seeds decreased more significantly than in the case of 0.19 Gy, and a large fraction of weak seeds appeared. The unimodal distribution became bimodal (Fig. 2b, curve 3). Nevertheless, seed storage for two months after irradiation resulted in the returning of a significant portion of weak to strong seeds, i.e. in the appearance of “improved” seeds too.

When 3 Gy-irradiated seeds (Fig. 3, curve 3 Gy) germinated, the percentage of normal seedlings sharply decreased six days after irradiation and remained at this level to the 18th day. Then gradually the fraction of normal seedlings increased during two months after irradiation.

After five months, the germination of such seeds happened at the level of non-irradiated seeds.

For seeds irradiated at the dose of 10 Gy (Fig. 2c), a rapid transition of almost 50% strong seeds to weak state was observed during two days. But during the next two days, fraction of strong seeds rose by the returning of weak seeds to it, i.e. by the formation of “improved” seeds. The percentage of such “improved” seeds can be calculated in Fig.2c by the reduction of curve 2 values from curve 3 values in the region of 20–50 arb. un. RTP results corresponded to the direct measurement of seed germination (Fig. 3, curve 10 Gy). The seed fraction producing normal seedlings partly increased to the 5th day (Table 1).

During storage after irradiation, the percentage of normal seedlings decreased, being accompanied by an increasing fraction of abnormal seedlings and suffocated seeds. By two months after irradiation, dead seeds appeared (Fig. 2c, curve 4). By five months, the number of dead seeds increased.

3.3. Changes in γ-irradiated Dry Pea Seeds

3.3.1. Seed Moisture Content

Seeds of various fractions stored for 6 days after irradiation contained unequal amounts of water. Strong seeds, both non-irradiated and “improved”, contained 9.81 ± 0.01 and 9.84 ± 0.01 % of water, respectively (no significant difference, T-test at p=0.05). Weak and dead seeds had significantly lower water content compared to strong seeds (T-test, p=0.05) , namely 8.9 ± 0.01 and 8.02 ± 0.01 %, respectively. These data show that seed transition from strong to weak state and later to dead seeds was accompanied by partial water loss.

3.3.2. Lipid Peroxidation

To elucidate the possible participation of non-enzymatic processes in irradiated seed behavior, we compared selected strong, weak, “improved” and dead seeds by TCL method.

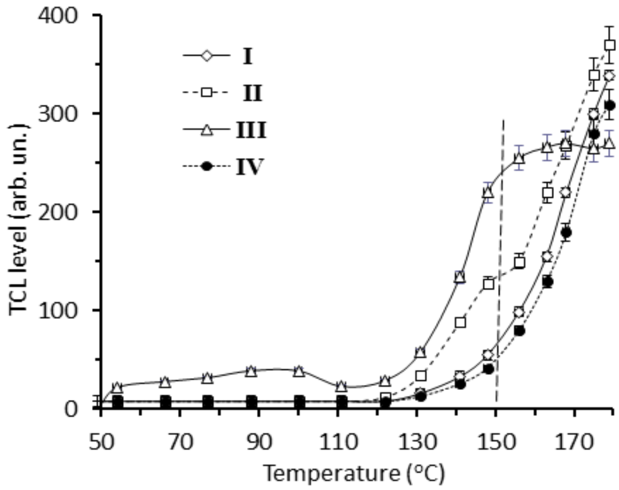
Intensity of TCL at 50–110^{o}C indicated the total amount of products of lipid peroxidation (POL). Fig. 4 shows that the TCL intensities of strong, weak and “improved” seeds were low and similar; they exceeded the background level by two or three times.

A clear TCL maximum at 50–110^{o}C was observed in seeds irradiated not at low but at high doses, for example at 100 Gy. Such values exceeded the background level by 10–20 times. This maximum is characteristic of the dead seeds.

3.3.3. Content of Glucose as a Product of Non-enzymatic Hydrolysis

Intensity of TCL within the range from 110^{o}C to 170^{o}C, with a maximum at 150^{o}C, was proportional to glucose content (Fig. 4). The TCL intensities in weak seeds were twofold greater than in strong seeds. The “improved” seeds produced TCL, which is a bit lower than that of strong seeds. Dead seeds were characterized by TCL exceeding strong seeds by 4-6 times.

The TCL data were expressed as glucose amounts (Table 2) and compared in relation to the dose and time after seed irradiation. Glucose contents in strong seeds were of 0.11 - 0.14 mg/g and were independent of the dose and time after irradiation (compared to non-irradiated seeds, T-test, p=0.05). The weak seeds always contained two-fold glucose levels and this difference was relevant (weak *vs* strong, T-test, p=0.05). In “improved” seeds returned from weak seeds, glucose decreased to the levels close to strong seeds. During the transition from strong to weak seeds, glucose was accumulated in weak seeds that indicated non-enzymatic carbohydrate hydrolysis.



I – strong non-irradiated seeds, II – weak seeds irradiated at the dose of 10 Gy, III – dead seeds irradiated at the dose of 100 Gy. IV – “improved” seeds irradiated at the dose of 10 Gy and returned to strong seeds. Dotted line at 150^{o}C shows TCL levels corresponding to glucose content in seed powders**.** The data are represented as means and standard deviations.

Figure 4. Representative thermograms of chemiluminescence of powders from dry seeds of various quality, Data represent mean values and their standard deviations

Table 2. Dynamics of glucose content in irradiated dry seeds (mg/g seed fr. wt)

Dose, Gy	Time after γ-irradiation, days	Strong seeds	Weak seeds	“Improved” seeds	Dead Seeds
	0	0.12 ±0.01	0.24±0.02	no seeds	no seeds
0	20	0.11±0.01	0.26±0.02	no seeds	no seeds
	54	0.12±0.01	0.26±0.02	no seeds	no seeds
	6	0.11±0.02	0.26±0.02	no seeds 0.10* 0.09±0.01	no seeds
0.19	18	0.13±0.02	0.28±0.02		no seeds
	56	0.11*	0.26±0.02		no seeds
	6	0.14±0.02	0.28±0.02	no seeds	no seeds
3	20	0.11±0.02*	0.26±0.02	0.10±0.01	no seeds
	54	no seeds	0.24±0.02	0.09±0.02	no seeds
	6	0.14±0.02	0.28±0.03	0.10±0.02	no seeds 0.72±0.06 0.78±0.07
7	20	0.13*	0.26±0.02	0.09±0.01	
	60	no seeds	0.28±0.02	0.10±0.02	
	6	0.12±0.02	0.29±0.03	0.09±0.01	no seeds 0.64±0.06 0.86±0.7
10	18	0.11*	0.26±0.03	0.10±0.01	
	54	no seeds	0.28±0.03	0.10±0.02	

Notes. For analyses, 4-5 seeds were taken. *only 1-2 strong or “improved” seeds were found in the samples of 48 seeds. Data are represented as means and standard deviation.

The differences between strong and weak seeds as well as between weak and "improved" seeds are significant at $p<0.05$, but there was no significant difference between strong and "improved" seeds.

4. Discussion

As follows from Table 1, γ-irradiated seeds exhibit different seed quality, non-linearly dependent on the radiation dose. After low-dose radiation, the percentages of germination and of normal seedlings declined while at a bit higher doses (7–10 Gy) these values were restored. At high doses, namely 25 Gy and more, decrease of germination percentage and deterioration of seed quality occurred more rapidly than at low doses and were accompanied by seed death.

Such result could be predicted from the seed distribution by RTP values measured independent of the measurements of seed germination (Fig. 1). After 3-Gy irradiation, a portion of strong seeds became weak seeds. This transition resulted in the increased number of abnormal seedlings and suffocated seeds. The 10Gy-irradiation returned some seeds from strong to weak state, and as a result the fraction of strong seeds enlarged while the fraction of weak seeds decreased. The number of normal seedlings rose. After 25 Gy-irradiation, dead seeds appeared.

The direct action of γ-radiation can result in water radiolysis, accumulation of reactive oxygen species and free radicals and in chromosome aberrations appearing later in germinating seeds. We did not expect any radiolysis effect, because irradiated dry seeds contain little free water (see section 3.3) representing the target for radiolysis, and because the exposures to ɣ -rays were very short. No accumulation of free radicals after γ-radiation at the doses below 10 Gy was observed (data not published). Such accumulation of free radicals occurs only at much higher doses exceeding 100 Gy [10]. Therefore, we suppose that observed decrease in water content during seed transition from strong to dead state is a result of γ-radiation effect on the non-enzymatic hydrolysis of oligosaccharides.

As to cytogenetic effects, the aberrant cells appear after seed germination, and their number does not exceed 5% even in 100 Gy-irradiated pea and onion seeds [11]**.** At low doses (below 0.5 Gy), their amount did not exceed 1% in pea and barley embryonic axes [12, 13]. The treatment with low doses permitted us not to focus the attention on these target effects of γ-radiation.

Therefore, at low doses the observed effects of γ-radiation on seed germination were not caused by direct radiation action and must be considered as indirect effects developing afterwards. They may manifest themselves only via non-enzymatic processes, because air-dry seeds are too poor in water for enzyme operation. Such processes include the oxidation of membrane lipids, hydrolysis of oligosaccharides and sucrose and glycosylation of proteins and amino acids [9]. The obtained results permitted us to elucidate the participation of these processes in the changes of seed quality after γ-radiation at low doses.

4.1. Lipid Peroxidation after γ-radiation of Dry Pea Seeds

Similar but low contents of POL products in non-irradiated and irradiated at the low dose of 10Gy seeds (Fig. 4) indicate that the process of lipid peroxidation is not responsible for the transition of strong to weak seeds. After high-dose γ-radiation, lipid peroxidation is intensive in dead seeds.

In the literature, significant lipid peroxidation was reported only at much higher doses exceeding 1kGy [14, 15]. At low doses applied here, no accumulation of additional POL products was observed.

4.2. Accumulation of Glucose as an Indicator of Non-enzymatic Carbohydrate Hydrolysis Due to γ-induced Water Activation

TCL at 150^{o}C showed almost twofold glucose accumulation in irradiated weak seeds in comparison with strong seeds (Fig. 4 and Table 2). The unexpected appearance of significant glucose amounts indicates the operation of non-enzymatic step-by-step hydrolysis of oligosaccharides and sucrose, similar to that occurring in aging seeds [6]. Each act of such non-enzymatic hydrolysis, of sucrose for example, proceeds with the participation of one water molecule bound to the surface of sucrose molecule; as a result, its H^+ and OH^- turn out to be incorporated into the hydrolysis products, namely glucose and fructose. Such water participation in the carbohydrate hydrolysis was confirmed by the measurements of water content in strong and weak seeds (section 3.3.1). Decrease in water content by 0.6-0.9% was really observed in weak seeds, in which glucose accumulation was recorded. At water content of 6-15%, amino-carbonyl reaction is most active [16].

It is tempting to suppose that water molecules activated by γ-radiation acquire higher mobility resulting in hydrolysis performance. Such water activation can result from the conversion of *para*-water molecules with antiparallel-oriented electron spin in hydrogen atoms to the *orto*-water molecules with parallel-oriented spins [17]. No glucose accumulation occurred in non-irradiated seeds. Apparently, the γ-radiation-induced activation of water molecules needs less energy expenditure than their radiolysis.

4.3. Transition from Strong to Weak Seeds after γ-radiation

The formation of weak seeds from strong seeds was clearly observed after seed γ-radiation at low dose of 0.19 Gy (Fig. 2a). The percentage of normal seedlings decreased by

22% during six days, and the fraction of abnormal and suffocated seeds increased (Table 1). Two months after 0.19 Gy-radiation, the percentage of normal seedlings declined to 20% (Fig. 2a). This effect developed slowly, whereas at higher doses it rapidly became evident. For example, at 10 Gy this transition embraced half of seeds during two days (Fig. 2c).

The transition of strong to weak seeds occurred in dry state. In weak seeds, it is visible in terms of glucose accumulation and water decrease. These observations permitted us to suppose that γ-induced transition of strong to weak seeds occurred via the carbohydrate hydrolysis driven by water activation and resulting in the appearance of very reactive glucose molecules dangerous for seed proteins (see next section). This mechanism represents the indirect non-target effect of seed irradiation at low doses. The deterioration of the quality of weak seeds will manifest itself later on, during seed imbibition and germination. Weak seeds imbibe much rapidly than strong seeds [18]. They earlier experience the respiration rise causing the hypoxia, like other rapidly imbibing legumes [19]. After radicle emergence, a post-hypoxic oxidative stress develops in these seeds resulting in DNA damage and morphological modifications of seedlings [8, 20].

4.4. Appearance of "improved" Seeds after γ-radiation

At 7-10 Gy, the percentage of normal seedlings unexpectedly increased while the fraction of abnormal seedlings reduced. Almost a half of seeds returned from weak to strong state and produced normal seedlings (Fig. 2c). They were named "improved", and their appearance was characteristic of these dose effects.

The appearance of "improved" seeds was accompanied with water content elevation up to the level typical of strong seeds (by 0.95%). In "improved" seeds, glucose previously formed in weak seeds by carbohydrate hydrolysis declined to the level of strong seeds (Table 2). Basing on these two specific features, namely utilization of glucose and formation of additional water molecules, the participation of non-enzymatic amino-carbonyl reaction (Maillard reaction) in seed back transition from weak to strong seeds was suggested.

In this reaction, glucose in linear state interacts by free carbonyl group with free amino group of protein producing glucosyl amine and water [21]. Such linear form of glucose molecule appeared in the course of non-enzymatic carbohydrate hydrolysis [22]. This form of glucose is chemically active, and its removal due to amino-carbonyl reaction from the metabolic sphere apparently provides the improvement of seed quality, i.e. back transition of weak to strong seeds.

The "improved" state of these seeds is a transient event; these seeds remained "improved" for 18-20 days, then gradually died (see the appearance of dead seeds, Table 2 or Fig. 3, curve 10 Gy). Such lethal effect can be caused by the further progress of amino-carbonyl reaction (protein glycosylation). After the subsequent Amadori rearrangement of Maillard products, the so-called "advanced glycosylation end products" are formed by cross-linking of some protein molecules [21]. Accumulation of these end products leads to protein deterioration and seed death. Such processes were described in aging seeds [9].

The appearance of "improved" seeds after 7-10 Gy radiation occurred too, but very slowly. For example, two months after γ-radiation at 0.19 Gy, almost all weak seeds became "improved" (note to Table 2) and within 2-5 months the number of normal seedlings increased (Fig. 3, curve 0.19 Gy).

It must be noted that the presence of "improved" seeds for some days will result in increasing germination percentage if seeds were sowed just at these days. This explains some observations concerning improvement of seed germination after ɣ-irradiation [3, 23].

5. Conclusions

The low-dose action of γ-radiation on dry pea seeds can be characterized as an indirect and non-target effect manifesting itself later as an aftereffect. Such aftereffect continued up to five months and non-linearly depended on the applied dose. This regularity was followed as seed fate up to germination of individual dry seeds exposed to γ-radiation. Radiation action was achieved via two mechanisms. At first, radiation caused the non-enzymatic carbohydrate hydrolysis resulting in the accumulation of glucose in linear form and seed transition from strong to weak state; such weak seeds could produce the abnormal seedlings. Then this glucose entered amino-carbonyl reaction with free amino groups of proteins and induced the transition of weak to "improved" seeds, from which the normal seedlings developed like from strong seeds. The life-span of "improved" seeds was rather short and they died due to further progress of amino-carbonyl reaction up to the production of toxic end Amadori-Maillard products. The response dynamics depended on the radiation dose. After γ-radiation at the doses of 0.19 -- 3.0 Gy, the deterioration developed very slowly and the seed response achieved the "improved" seed state during five months. Dose increase to 7–9 Gy resulted in the accumulation of dead from "improved" seeds by this time. Therefore, γ-radiation exerts dual aftereffect depending on the dose and time-dependent response progress, namely seed deterioration and transient "improvement" of seed quality.

Conflicts of Interest

There is no conflict of interest.

Contribution

All authors had equal contribution to this work.

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